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Genetic polymorphism of β-Lg gene in Iraqi buffalo using Polymerase Chain Reaction – Restriction Fragment Length Polymorphism

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ABSTRACT

This study was conducted on 50 buffaloes from Basrah Governorate, Iraq to study the genetic polymorphisms of the β-lactoglobulin gene in the local buffaloes. The lab examinations were conducted in the Genetic Engineering Laboratory, College of Agriculture, University of Basrah. DNA was extracted by kit (PureLink Genomic DNA kits, Invetrogen, USA). The results of this technique showed the appearance of the expected gene bundle of 252 base pairs from all Iraqi buffalo samples, as the primer interacted with all DNA isolates taken from the studied buffalo samples. Digestion technique with restriction enzymes (HaeIII) was used to detect the genetic polymorphisms of β-lactoglobulin gene, exhibiting two bands to all studied buffalo samples. Only the homozygous BB genotype has been shown.

Keywords: Genetic polymorphisms, β-lactoglobulin gene, PCR technique, Iraqi buffalo.
Article type: Research Article.

INTRODUCTION

One of the basic elements in determining the genetic forms of milk proteins is the use of electrophoresis (Cattaneo *et al.* 1996). Studies on the genetic polymorphisms of proteins have developed over time. Ng-Kwai-Hang (1998) summarized its most important goals by revealing the chemical properties of proteins, including milk and blood proteins, and some features of the similarity between these proteins, as well as clarifying the different relationships between different types of animals. Electrophoresis is used to detect genetic polymorphisms in milk proteins that result from the substitution of one amino acid for another or from the deletion of one or more amino acids. Gel electrophoresis, which displays several bands of various lengths, allows researchers to see even variation among genotypes (David & Deutch 1992). As it depends on the existence or lack of mutations, DNA analysis you could also examine the DNA. Point mutations and genetic DNA restructuring (Recombinant DNA - rDNA) are two of the most significant mutations that take place at the DNA level (Di Gregorio *et al.* 1991). In the case of point mutations, a single nucleotide is replaced, and in this case, differences between individuals are detected using restriction enzymes that cut DNA in specific regions, depending on the type of enzyme. As for the phenomenon of DNA organization, it includes the insertion or deletion of part of the genetic DNA fragments. The most common is the formation of sequential chains, and these can be individually amplified and observed using the Polymerase Chain Reaction (PCR) technique (Soller 1990). The phenomenon of genetic polymorphism in cattle has been well studied in recent years due to the close relationship between the genotypes of milk proteins and the important economic characteristics in cattle. Milk contains four main types of casein, namely, α S1-casein, α S2-casein, β-casein, κ-casein (Davies & Law 1987). The PCR-RFLP amplification is one of the first techniques used to identify genetic variation in DNA between two or more organisms (Bruns *et al.* 1991 & Strange 2003). It is also one of the most important techniques used in studies of the evolution of breeds with many organisms, as it has been used

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extensively in taxonomy. In this technique, a gene conserved on the target region is amplified and then the amplification product is digested by a specialized digestive enzyme to obtain the digested segments expressing that species (Chen 1992; Appel & Gordon 1995). The present study aimed at the use of milk protein polymorphisms in association with milk yield and milk component of Iraqi buffalo.

MATERIALS AND METHODS

DNA extraction

DNA was extracted from 50 buffaloes from Basrah Governorate in the Laboratory of Genetic Engineering, College of Agriculture, University of Basrah, Iraq. DNA was extracted by kit (PureLink Genomic DNA kits, Invetrogen, USA).

Measurement of the amount of DNA by the Nano Drop device

The amount of DNA was measured by a Nano Drop device prepared by the American company Thermo Scientific (Fig. 1) to identify the size of the genome (ng μL^{-1}). The presence of DNA was detected by electrophoresis technique in the presence of agarose gel at a concentration of 1%.

Fig. 1. The Nano Drop device, which is equipped with the American Thermo Scientific Company.

The materials for polymerase chain reaction technology were prepared and placed in a bowl containing pieces of ice for protecting from heat. The work was done in a sterile and clean place in a special PCR cabin, which contained UV rays to sterilize the micropipettes, tubes and tabs, taking into account wearing sterile medical gloves when working. The PCR reaction mixture was prepared in a 100 µL Eppendorf tube, the final concentration of the components was 25 µL, then the tubes were placed in a Snip centrifuge for 30 seconds. Table 1 shows the amounts of materials used in the reaction.

Table 1. Amounts of materials used in the PCR-RFLP techniques (microliters).

Chemical material	Master mix	Primer	DNA template	Distill water	Final volume
Volume	12.5	1*	5	6.5	25

* In the PCR-RFLP technique, two primers (Forward and Reverse) are used, and the amount of distilled water is reduced to 5.5 µL.

PCR-RFLP technique

Iraqi buffalo DNA was amplified using a specific primer for the β-lactoglobulin gene 5-GTC CTT GTG CTG GAC ACC GAC TAC A-3 and (forward) 5-CAG GAC ACC GGC TCC CGG TAT ATG A-3. The tubes were then placed in a thermo cycler, and the device's program was set according to Di Xi Ren *et al.* (2011) using the program for the initiator and according to the following steps, which are mentioned in Table 2.

Table 2. Phases of PCR reactions time, cycle numbers and temperature.

Phases	Temperature (℃)	Time (minutes)	No. of cycles
Primary denaturation	94	5.00	1
Denaturation	94	1.00	
Annealing	61	1.00	35
Elongation	72	1.00	
Final elongation	72	10.00	

Electrophoresis technique for amplification product

The electrophoresis technique was used on an agarose gel to determine the success of the DNA amplification process. The DNA Marker (100 base pairs) was used by mixing 1.5 µL of the molecular weight marker with 3.5 µL methyl orange. As for the rest of the pits, a PCR product of 5 µL was placed in it, the PCR product was passed on an agarose gel with a concentration of 2%, i.e., 0.5 g agarose was dissolved in 25 mL 1X TBE solution, with the addition of 1 µL ethidium bromide dye. Then, the electric current was fixed at 85 volts and 65 mA for 45 minutes. After completing migration, the gel was examined using a UV device to know the sizes of the bands in the gel.

Digestion by restriction enzyme

The digestion process was carried out by restriction enzymes, by cutting the gene of size (252 bp) with the help of (HaeIII) enzyme. The digestion mixture was prepared for the samples and mixed with a micropipette, then the enzyme was added to the mixture. So that, the final mixture became 20 µL, and then the samples were placed in a centrifuge for 5 seconds. Table 3 shows the concentrations and quantities of the materials used in the reaction.

Table 3. Amounts of materials used in the digestion process by the restriction enzyme (µL).

Id	Item	Quantity
1	DNA	7
2	Restriction Enzyme	1.5
3	10x Tango Buffer	2
4	BSA	0.5
5	Sterilized distill water	9

The samples were incubated in a water bath at a temperature of 37 °C for 3 hours. After the incubation period ended and the digestion process was completed, 3 µL of the product was taken and electrophoresis was carried out on a 2% agarose gel with a DNA marker (50 bp) placed to check the digestion process. After completing the electrophoresis process, the gel was examined with a UV device and the DNA bands were identified.

RESULTS AND DISCUSSION

Since the primers used in the experiment interacted with all of the DNA samples taken from the blood of the studied buffaloes, the results of this technique demonstrated the appearance of the expected bands of the desired gene, amounting to 252 base pairs, in all of the buffalo samples under study (Fig. 2). This confirmed that all of the tested isolates are from the buffalo.

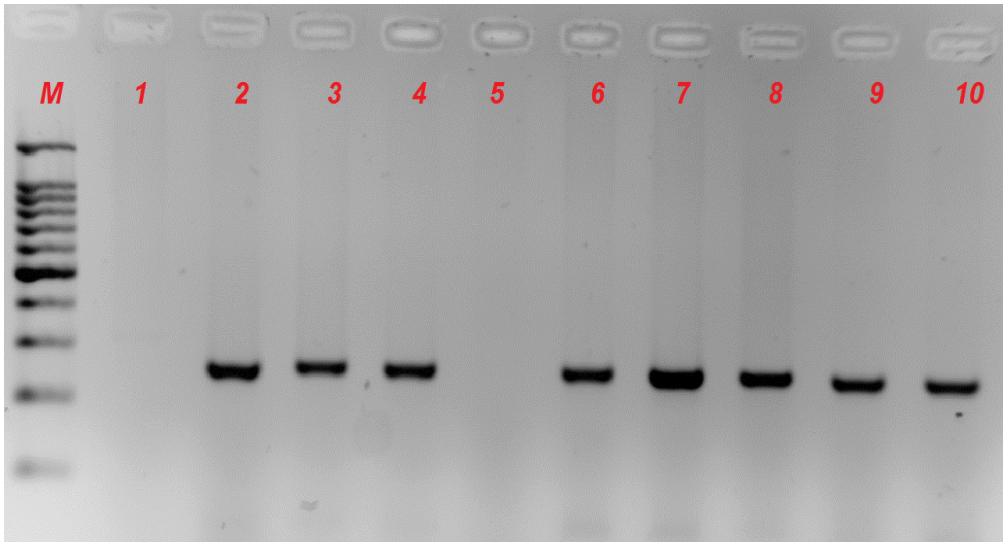

Fig. 2. Results of electrophoresis on a 2% agarose gel using the primer for the β-lactoglobulin gene, M. Molecular weight indicator (100 bp), (sample No. 1 and 5 are control samples and samples 2-4 and 6-10 represent the β-lactoglobulin gene).

PCR- RFLP technique

Digestion technique with categorical enzymes was used to detect the genetic polymorphisms of the gene β-lactoglobulin. Therefore, the amplification product was digested by the restriction enzyme HaeIII, which was

given in two packages to all studied buffalo samples (Fig. 3). This indicates that all studied samples belong to one genotype, which is BB. This result was consistent with Da Xi Ren *et al.* (2011), who indicated that the expected gene size of the Indian buffalo, using the same primers that was used in this study, as the gene size was 252 bp. If a genetic polymorphism of the β-lactoglobulin gene was found in the blood of local buffaloes, as electrophoresis examinations showed the presence of two clear bands indicating the presence of the genetic polymorphisms, however, all samples were similar genotype. The findings of the current study conflicted with those of Patel *et al.* (2007) and Kirimi *et al.* (2012), who discovered several alleles and three genotypes, AA, BB, and AB, respectively, with AB being the dominant genotype in a different investigation on the same gene but with a different primer (398 bp). Badola *et al.* (2003) and Soumi *et al.* (2006) obtained a single homozygous genotype. These results supported the present findings where there was only a single genotype using the same primer.

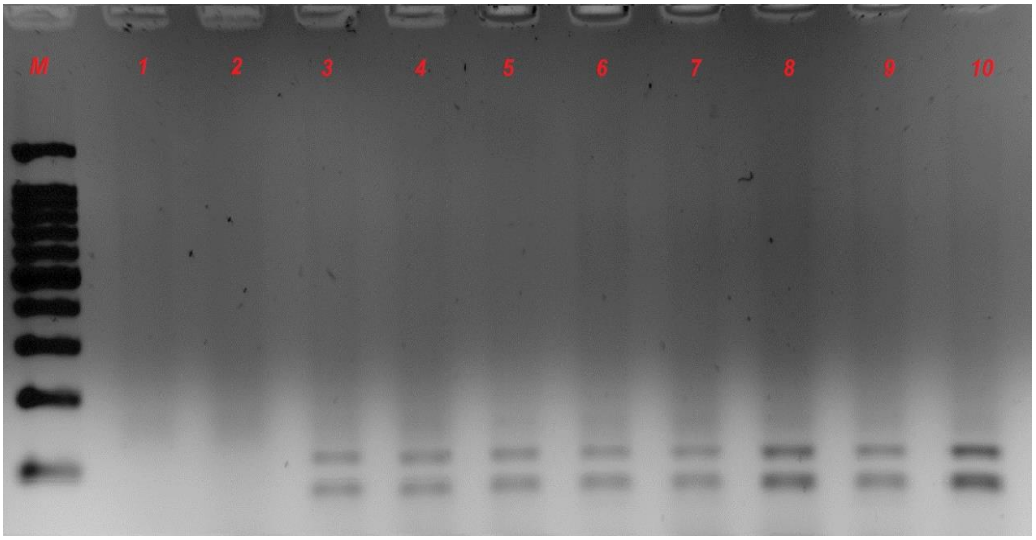

Fig. 3. Results of electrophoresis on a 2% agarose gel using HaeIII enzyme, M molecular weight marker (10 bp), sample No. 1 and 2 control, samples 3-10 homozygous genotype BB.

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