PCR-RFLP ANALYSIS OF BETA-LACTOGLOBULIN GENE IN MURRAH BUFFALOES

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ABSTRACT

PCR-RFLP analysis of beta-lactoglobulin gene locus was carried out on 110 DNA samples of Murrah buffaloes in the present study. A 262 bp fragment enclosing from exon IV to intron IV in b-lg gene was amplified with specific primers. All the 110 DNA samples resulted in 262 bp product on amplification. The PCR products were subjected for digestion with Pst1,EcoRI, HindIII and Hae III *enzyme. PCR products were not digested by* PstI, EcoRI *and* HindIII. *PCR products when digested with* HaeIII *enzyme resulted in monomorphic banding pattern in all the samples. Sequencing of PCR products also revealed no polymorphism (Gen Bank DQ340204) The DNA typing results of this study agreed completely with the milk protein typing of same buffalo milk samples for beta-lactoglobulin by PAGE, which revealed no polymorphism. PCR amplification and RFLP analysis presented in this study was found to be rapid and could be used as a valuable tool to investigate polymorphism at -lg locus directly at the DNA level without the milk samples of lactating females. One hundred and ten DNA samples of Murrah buffaloes examined in the present study revealed no polymorphism at b-lg gene locus.*

Key words: Beta-lactoglobulin, Murrah buffalo, Polymorphism

INTRODUCTION

Genetic polymorphisms are playing an increasingly important role as genetic markers in many fields of animal breeding. With the development of molecular genetic techniques it has become possible to establish a new class of gene markers based upon the variability at DNA sequence level. The discovery of RFLP generated renewed interest in the use of gene marker loci as an aid to selection programmes.

Milk protein genetic polymorphisms have evoked considerable research interest in recent years because of possible association between milk protein genotypes and economically important traits in dairy cattle. Milk protein genes such as k-casein and b- lactoglobulin are associated with milk production performance and have a major influence on the composition of milk and on the processing properties of milk (Ng-Kwai-Hang *et al*.,1990 Chung *et al.*,1994,Meignanalakshmi *et al.,* 2006).

The development of the PCR-RFLP technique to distinguish rapidly the genotypes of b-lg at the DNA level permits the determination of genotypes for both sexes of animals at any age (Meignanalakshmi *et al*,2001,Cengiz Elami *et al*., 2006).PCR-RFLP has been used by Satyanarayana *et al,* 2006 for

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genotyping beta-lactoglobulin in Sahiwal and Tharparkar catte breeds. Milk protein genes might be useful as genetic marker and is a promising alternative to the current methods of trait selection in dairy cattle breeding programmes. The work on milk proteins in buffalo is very limited. The objective of the present study was to amplify the b-lg gene locus and to find out polymorphism at b-lg gene locus by using RFLP in Murrah buffaloes.

MATERIALS AND METHODS

The present study was carried out on Murrah buffaloes maintained at Central cattle breeding farm, Alamadi, Tamilnadu. Individual blood samples of 5 ml each were collected from 110 Murrah buffaloes using 5 ml vacuitainer tubes containing EDTA from jugular vein and stored at 4^0C until processed.

Genomic DNA Isolation

Blood samples collected in a vacuitainer containing EDTA were transferred to a 15 ml centrifuge tube and centrifuged at 4000 rpm for 10 min and plasma was discarded leaving RBCs and WBCs. Two to three volumes of ice cold RBC lysis buffer (0.17M NH_4Cl) was added and kept on ice for complete lysis of RBCs. The leucocytes were spun down at 4000 rpm for 15 min and the supernatant containing lysed RBCs was discarded. If unlysed RBCs were present, RBC lysis buffer was added and the procedure was repeated till the WBC pellet was devoid of unlysed RBCs. Nine ml of TE buffer(10mM Tris HCI,0.1M EDTA,pH 8.0) was added to the WBC pellet and pellet was resuspended by vigorous vortexing. Seventy five ml of proteinase K(10 mg/ml), 0.5 ml of 0.5 M EDTA,pH 8.0 and 0.5 ml of 20% SDS were added, mixed well and incubated at 50^0C in a water bath for 3h with occasional shaking.

To the digested sample, 5 ml of saturated sodium chloride was added, vortexed and spun down at 5000 rpm for 15 min at room temperature. The supernatant was transferred to a sterile beaker and two volumes of 95% ice cold ethanol was added to the supernatant and DNA was spooled out on a glass rod, rinsed in 70% ethanol, dried and resuspended in 0.5 ml of TE buffer, pH 8.0 and stored at 4^0C.

Amplification of Genomic DNA at β-lg Locus by Polymerase chain reaction

Amplification of b-lg gene locus was carried out by using specific primers Meignanalakshmi *et al.(*2001)which amplified b-lg gene from exon IV to intron IV (enclosing 94 base pairs of exon IV and 168 base pair of intron IV) and resulted in 262 bp fragment in cattle. The same primers were used for amplifying b-lg gene in Murrah buffaloes.

The primers were obtained from Bangalore Genei Pvt. Ltd, Bangalore. The sequence of the forward and reverse primers are given below :

Primer I :
5' – GTCCTTGTGCTGGACACCGACTACA-3'

Primer II:
5' – CAGGACACCGGCTCCTGGTATATGA-3'

Reactions were carried out in 100ml volume. The reaction conditions and reagent concentrations were:100pmole of each primer,2.5 units of Taq.DNA polymerase,1X PCR buffer(10mM Tris – HCL, pH 9.0, 50 mM KCL and 1.5 mM $MgCl_2$), 150 mM of each dNTP and 50 ng of genomic DNA. After an initial denaturation of 3 min at 95^0C, 35 cycles were run on a Thermal Cycler (PTC 2000,MJ Research Inc.USA) each comprising 40 sec of denaturation at 95^oC, 40 sec of primer annealing at 64^0C, 30 sec of extension at 72^0C followed by a final extension for 10 min at 72^0C . PCR products were checked by electrophoresis on 2% Agarose gel in 1X TBE

buffer. 100bp ladder and 25bp ladder were used as molecular weight marker.After staining the gel with ethidium bromide, PCR products were visualized by UV- Transilluminator and photographed.

PCR-RFLP Analysis

Fifteen ml of the amplified DNA was digested with 20 units of *EcoRI, HindIII, PstI* and *Hae III* at 37^0C for 4 h. The restriction fragments were resolved by electrophoresis in 4% Agarose gel in 1X TBE buffer. 100 bp ladder was used as molecular weight marker. After staining the gel with ethidium bromide, fragments were visualized by UV transilluminator and photographed. All the PCR products were purified by using Qiagen PCR product purification columns and were sequenced in Genei.

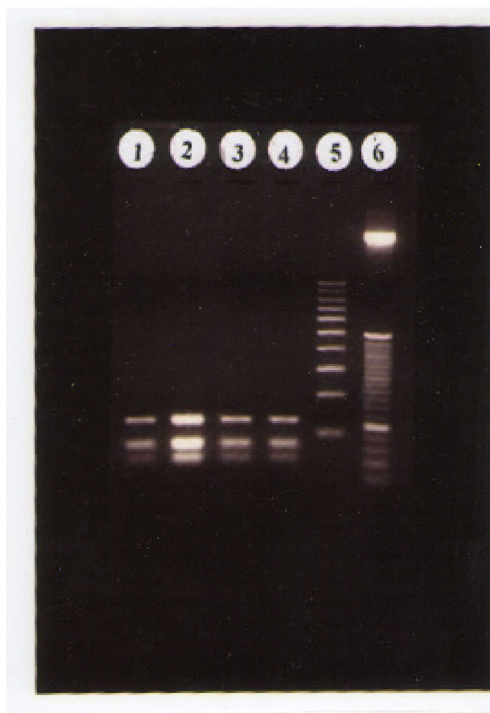
RESULTS AND DISCUSSION

Primers used for cattle (Meignanalakshmi *et al.,* 2001) were found to be suitable for amplifying b-lg gene in Murrah buffaloes ,which resulted in 262 bp fragment (Fig.1). Kim *et al*.,(1997) also reported that the amplified product with these primers was 262 bp in Hanwoo cattle. In the present study, all the 110 DNA samples of Murrah buffaloes gave the expected 262 bp fragment on amplification without any non specific DNA amplification. The PCR products were not digested by *PstI, EcoRI and Hind III*. The PCR product (262 bp fragment) of b-lg gene when digested with Hae III enzyme resulted in monomorphic banding pattern in Murrah buffaloes.All the PCR products(110 samples) on digestion with Hae III resulted in the same monomorphic banding pattern. (Fig.2). No polymorphism was found to be present in the b-lg gene locus of Murrah buffaloes in the present study. PCR product was sequenced (Sequence has been submitted to GenBank and have been assigned the accession number (**DQ340204**) PCR amplification and RFLP analysis of b-lg locus of 110 Murrah buffaloes revealed no polymorphism in the present study. The DNA typing results of this study agreed completely with the milk protein typing of same buffalo milk samples, which revealed the monomorphic banding pattern on PAGE (Meignanalakshmi and Mahalinganainar, 2007). This PCR-RFLP study can be used as a valuable tool to identify polymorphism at b-lg locus at any age of the animal irrespective of sex and eliminates the need for the milk of lactating females.

Fig.1.
Agarose gel electrophoresis of PCR products of beta-lactoglobulin gene in Murrah buffaloes.

Lane 1,2,3,4 and 5 : 262 bp PCR product of β-lg gene of Murrah buffaloes
Lane 6: Molecular Size marker - 100 bp ladder

Fig 2.
Agarose gel electrophoresis of *HaeIII* digested PCR products of beta-lactoglobulin gene in Murrah buffaloes.



Lane 1, 2 ,3 and 4 : *Hae III* digested PCR products of -lg gene in Murrah Buffaloes

Lane 5: Molecular size marker - 100 bp adder.

Lane 6: Molecular size marker- 25 bp ladder

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