

RFLP Analysis of Beta-Lactoglobulin Gene in Swamp and Murrah Buffaloes Using a Single Restriction Enzyme

Short Communication

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ABSTRACT

An attempt has been made to analyze the distribution of the beta-lactoglobulin genotype in swamp buffaloes and Murrah buffaloes utilizing polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Blood samples were taken from 50 swamp buffaloes and 50 Murrah buffaloes. The DNA was extracted by the phenol-chloroform method. The PCR-RFLP was performed using beta-lactoglobulin gene primers and a single restriction enzyme, *Hae* III. The enzyme digested products were separated by electrophoresis on 2.5% agarose gel. All the 100 DNA samples of swamp and Murrah buffaloes resulted in 398 bp product on amplifying beta-lactoglobulin gene. Those PCR products (398 bp fragment) were digested with *Hae* III produced only one type of restriction pattern yielding five fragments of 113, 99, 89, 73 and 24 bp. One hundred DNA samples of swamp and Murrah buffalo were examined in this study and revealed no polymorphism at the beta-lactoglobulin gene locus.

KEY WORDS beta-lactoglobulin genotyping, Murrah buffaloes, PCR-RFLP, polymorphism, swamp.

INTRODUCTION

Genetic polymorphism of milk proteins has evoked considerable research interest in recent years because of possible associations between milk protein genotypes and economically important traits in dairy cattle (Karimi *et al.* 2009; Ng-Kwai-Hang *et al.* 1984). Milk protein genes such as kappa-casein and beta-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.* 1992; Meignanalakshmi and Mahalinga Nainar, 2009). Beta-lactoglobulin is the most abundant whey protein found in ruminant milk (Badola *et al.* 2003). It is a molecule of 162 amino acids and occurs in different allelic forms (Meignanalakshmi and Mahalinga

Nainar, 2001). The development of the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique to distinguish rapidly the genotype of beta-lactoglobulin at the DNA level permits the determination of genotypes for both sexes of animals at any age (Meignanalakshmi and Mahalinga Nainar, 2009). PCR-RFLP using restriction enzyme-*Hae* III have been successfully genotyped polymorphism in cattle (Badola *et al.* 2003, 2004) and no polymorphism was found to be present in the beta-lactoglobulin gene locus of Murrah buffaloes in India (Meignanalakshmi and Mahalinga Nainar, 2009).

Earlier kappa-casein and beta-lactoglobulin genotypes in Brazilian *Bos indicus* and *Bubalus bubalis* were reported by Del Lama and Zago (1996). However, information on the beta-lactoglobulin genotype in Thailand Swamp buffalo

(*Bubalus bubalis*) and the Murrah buffalo (*Bubalus bubalis*), a rivertype dairy buffalo which is maintained on the first and only dairy buffalo farm in Thailand, is currently limited.

The objective of this study was to amplify the beta-lactoglobulin gene locus and to identify the genotype of beta-lactoglobulin in Swamp buffalo and Murrah buffalo, through the technique of PCR-RFLP from DNA isolated from blood samples.

MATERIALS AND METHODS

Fifty Swamp and fifty Murrah buffalo from a private farm in Cha Choeng Sao Province, were used in the study. Blood samples were collected via venupuncture of the jugular vein, using a 5 mL vacutainer containing EDTA 0.5 M pH 8. The tubes were maintained at -20 °C until used for DNA extraction.

DNA extraction

Genomic DNA was extracted from whole blood, using phenol chloroform extraction (Sambrook *et al.* 1989). The quality of genomic DNA was checked by taking the ratio of O.D. at 260 and 280 nm in the spectrophotometer. The sample reported an O.D. ratio between 1.7 and 1.9 which was considered good and suitable for the PCR study. The quantity of DNA was estimated by spectrophotometer taking O.D. value at 260 nm and kept at 4 °C until used (Badola *et al.* 2004).

PCR amplification

A 398 bp fragments of beta-lactoglobulin gene spanning over 104 bases of exon IV and 294 bases of intron IV was amplified using beta-lactoglobulin gene fragments as described by Badola *et al.* (2003) and forward and reverse primers: 5'-CGA GAA CAA AGT CCT TGT GCT-3' and 5'-CCG GTA ACA AAG GCT GTT AGA-3', respectively. The amplification reactions were conducted in a final volume of 25 µL, containing: 1 µL PCR buffer 10-X (200 mM (NH₄)₂ SO₄, 0.1% (v/v) Tween 20%, 750 mM Tris-HCl pH 8.8, Fermentus), 60 ng DNA, 10 nmol of each primer, dNTP 200 µM and 1 unit of *Taq* DNA polymerase. The reactions followed the sequence; 94 °C for 5 min (pre-denaturation) and 35 cycles of the sequence: 94 °C for 1 min, 56 °C for 1 min and 72 °C for 1 min and final extension at 72 °C for 10 min (Pipalia *et al.* 2001).

RFLP (restriction fragment length polymorphism) technique

After amplification, gene fragments were subjected to digestion by a restriction enzyme in a total volume at 20 µL (10 µL amplification product, 2 µL enzyme buffer, 2 µL

enzyme and 6 µL of reaction buffer) and incubated at 37 °C for 2 h. The digested product was electrophoresed in 2.5% (w/v) agarose gel at 80 V for 1 h. The gel was then stained with ethidium bromide (0.5 µg/mL) and documented under the gel documentation system. The size of DNA bands were determined from the gel documentation system, analyzed via 50 bp DNA ladder.

RESULTS AND DISCUSSION

The primers used for river line (Murrah and Bhadwari buffalo) and cattle (Badola *et al.* 2003) were found to be suitable for amplification of beta-lactoglobulin gene in swamp buffalo, resulting in a 398 bp fragment (Figure 1). In the present study, all 50 DNA samples of swamp and 50 samples from Murrah buffaloes produced the 398 bp fragment on amplification.

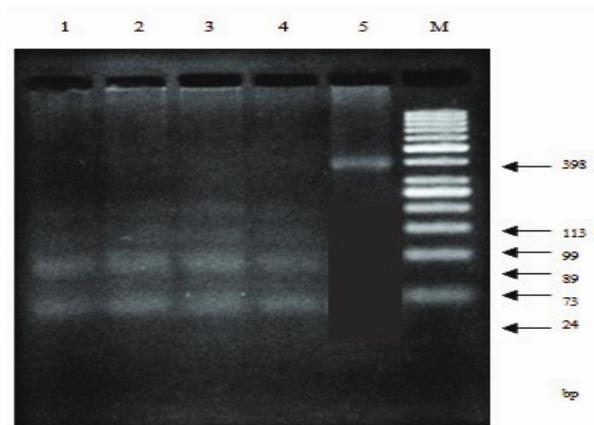


Figure 1 PCR-RFLP of beta-lactoglobulin gene polymorphism using *Hae* III restriction enzyme

Lane 1-4: *Hae* III digested PCR products of 113, 99, 89, 73 and 24 bp

Lane 5: 398 bp undigested PCR product

Lane M: 50 bp ladder marker

Our a little repetitive showed that primers used in this study were found to be suitable for amplifying beta-lactoglobulin gene in swamp buffaloes and Murrah buffaloes, which resulted in 398 bp fragment (Figure. 1). In both swamp and Murrah buffalo, the restriction analysis of 398 bp product of beta-lactoglobulin gene produced only one type of restriction pattern yielding five fragments of 113, 99, 89, 73 and 24 bp (Figure 1).

This pattern was similar to the pattern of buffalo (Murrah and Bhadawari) as reported by Badola *et al.* (2003). Only one kind of genotype was observed in buffalo depicting a highly conservative nature of the gene (Badola *et al.* 2003). Our results also corresponded to those reported in riverine buffalo (Murrah and Bhadwari) that the bubaline genome failed to produce any polymorphism for *Hae* III site (Badola *et al.* 2003).

The results have identified in excess of 99% homology between Murrah and Bhadwari buffalo. This suggests that there might be more than 99% homology in beta-lactoglobulin gene in swamp and Murrah buffalo although further confirmation is required. The phylogenetic tree analysis truly depicted the relatively higher distance between cattle and buffalo than between two buffalo breeds (Murrah and Bhadwari) (Badola *et al.* 2003). Furthermore, Meignanalakshmi and Mahalinga Nainar, (2009) reported that the DNA typing results of their study agreed completely with the milk protein typing of the same buffalo milk samples, which revealed the monomorphic banding pattern on PAGE.

The PCR-RFLP of these 50 swamp and 50 Murrah buffaloes were monomorphic for the beta-lactoglobulin gene.

Our results also corresponded to Meignanalakshmi and Mahalinga Nainar, (2009) in that no polymorphism appeared to be present in the beta-lactoglobulin gene locus of Murrah buffaloes. There was also no polymorphism observed in the buffalo populations in Brazil (Del Lama and Zago, 1996). In conclusion, this beta-lactoglobulin gene specific primer successfully amplified swamp and Murrah buffalo the beta-lactoglobulin gene. The *Hae* III -RFLP of swamp and Murrah buffalo beta-lactoglobulin gene failed to discover any evidence of polymorphism. This work has identified the homology in the genotype of beta-lactoglobulin in Swamp buffalo and Murrah buffalo.

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