ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 5 (4): 514-516, 2006
ISSN 1682-296X

The Characterization of *Hae*III Patterns in the Second Exon of the Buffalo MHC Class II DRB Gene

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Abstract: In this study, we used restriction endonuclease cleavage sites (*Hae*III) as tools to study polymorphisms in buffalo exon 2 of DRB gene. Our results showed that the animals with homozygous restriction patterns are present in high percentage (90%) compared with heterozygous restriction patterns (10%). The homozygous restriction pattern at 190 bp illustrated in 16 of 50 animals, the restriction pattern at 219 bp identified as homozygous site in 22 animals, while two tested animals had restriction pattern at 167 bp. The undigested fragment (284 bp) recorded in 5 animals refer to the absence of *Hae*III amino acid substitutions. The heterozygous sites at 190, 167 bp and 219, 190 bp were displayed with very low percentage in tested animals. The *Hae*III restriction site at 167 bp has correlated with Pro 56 amino acid which is involved in the formation of Antigen-Recognition Site (ARS) of MHC molecule. According to that, the buffalo MHC class II DRB genetic polymorphisms by *Hae*III revealed 6 genetic varients which is associated with animo acid substitutions.

Key words: Buffalo, major histocompatibility complex, PCR, RFLP

INTRODUCTION

Functional MHC class II are expressed as alpha: Beta dimeric receptors on the surface of antigen- presenting cells that are scanned by T-helper lymphocytes for presentation of foreign polypeptides against which an immune response is initiated (Rask *et al*., 1985). The functional molecule is formed by non-covalent association between an alpha and a beta chain peptide encoded by A and B genes, respectively. In both genes, exon 2 is functionally important and encodes amino acid associated with the Peptide-Binding Sites (PBS) in the first domains of the class II molecules.

The MHC class II genes are highly polymorphic and located either closely linked (as in human) or in two distinct chromosomal locations (as in cattle). Several methods have been used to characterize the polymorphism of MHC class II gene products and gene. Serology and one-dimensional isoelectric focusing (1. DIEF) has been used for studying protein polymorphism and restriction fragment length polymorphism (RFLP) and DNA sequencing elucidating the genetic organization and polymorphism of MHC class II genes (Sigurdardottir *et al*., 1991; Van Eijk *et al*., 1992; Amills *et al*., 1995, 1996; De *et al*., 2002; Sena *et al*., 2003).

In this study, we used PCR-RFLP method for amplifying the second exon of buffalo MHC class II DRB gene and detecting the characterization of *Hae*III restriction fragment length polymorphisms (RFLPs).

MATERIALS AND METHODS

Genomic DNA extraction: Genomic DNA was extracted from whole blood of 50 buffalo by phenol-chloroform method (John *et al*., 1991). Ten milliliter of blood taken on EDTA was mixed with 25 mL cold sucrose-triton and the volume was completed to 50 mL by autoclaved double distilled water. The solution was mixed well and the nuclear pellet was obtained by spin and discarding the supernatant. The nuclear pellet was suspended in lysis buffer with 20% sodium dodecyl sulfate and proteinase K and incubated overnight in a shaking water-bath at 37°C.

Nucleic acids were extracted once with phenol, saturated with Tris-EDTA (TE) buffer followed by extraction with phenol-chloroform-isoamyl alcohol (25:24:1) until there was no protein at the interface. This was followed by extraction with chloroform-isoamyl alcohol (24:1). To the final aqueous phase, 0.1 volume of 2.5 M Na acetate and 2.5 volume of cold 95% ethanol were added. The tubes were agitated gently to mix the liquids and a fluffy white ball of DNA was formed. The DNA was picked up with a heat-sealed Pasteur pipette and washed briefly in 70% ethanol.

The DNA was finally dissolved in an appropriate volume of 1X TE buffer. DNA concentrations were determined and diluted to the working concentration of 50 ng μL^{-1}, which is suitable for polymerase chain reaction.

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Polymerase Chain Reaction (PCR): The amplification of the second exon of the buffalo DRB gene was achieved using primers HL030: 5'- ATCCTCTCTCTGCAG CACATTTCC-3' and HL031: 5'- TTTAAATTCGCGCTCA CCTCGCCGCT-3'. A PCR cocktail consists of 1.0 µM upper and lower primers and 0.2 mM dNTPs, 10 mM Tris (pH 9), 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin (w/v), 0.1% Triton X-100 and 1.25 units of *Taq* polymerase. The cocktail was aliquot into tubes with 100 ng DNA of buffalo. The reaction ran in a Perkin Elmar apparatus. The reaction was cycled for 1 min at 94°C, 2 min at 60°C and 1 min at 72°C for 35 cycles.

RFLP and agarose gel electrophoresis: Twenty microliter of PCR product were digested with 10 units of *Hae*III in a final reaction volume 25 µL. The reaction mixture was incubated at 37°C in water bath over night. After restriction digestion, the restricted fragments were analyzed by electrophoresis on 2.5% agarose/1X TBE gel stained with ethidium bromide. The 100 bp ladder was used as molecular size marker. The bands were visualized under UV light and photographed with yellow filter on black and white film.

RESULTS AND DISCUSSION

The MHC class II DRB second exon in buffalo reported 22 sequencing alleles on the basis of SSCP patterns and nucleic acid sequence data. These 22 alleles were typed into 10 major types on the basis of their phylogenetic analysis and number of amino acid substitutions (De *et al*., 2002).

In this study, The PCR product amplified the buffalo MHC class II DRB gene containing a 284 bp which is homologous to cattle DRB 3 gene. We used restriction endonuclease cleavage sites (*Hae*III) as tools to study polymorphisms in buffalo exon 2 of DRB gene. Selection of the restriction enzyme depended on the sequence of buffalo MHC class II DRB gene. The *Hae*III restriction map of the DRB second exon (Fig. 1) is 4 polymorphic sites at 167, 171, 190 and 219 bp (Van Eijk *et al*., 1992; De *et al*., 2002).

Our tested animals showed high percentage of homozygous restriction patterns (90%) compared with heterozygous restriction patterns (10%) (Fig. 2 and 3). The homozygous restriction pattern at 190 bp illustrated in 16 of 50 animals, while the restriction pattern at 219 bp identified as homozygous site in 22 animals. Two tested animals had restriction pattern at 167 bp, while the undigested fragment (284 bp) recorded in 5 animals.

Our results revealed that the heterozygous sites at 190, 167 bp and 219, 190 bp were displayed in 2 and 3

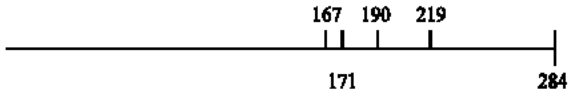

Fig. 1: Schematic representation of *Hae*III restriction sites of buffalo DRB second exon according to its sequence

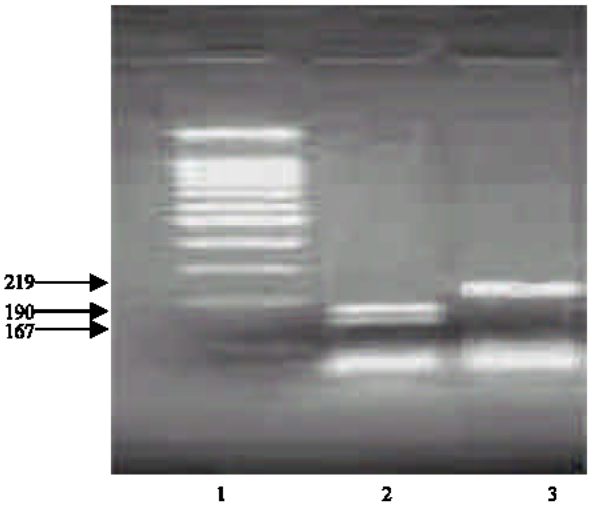

Fig. 2: DNA electrophoretic pattern obtained after digestion of PCR amplified buffalo MHC class II products with *Hae*III, Lane 1: 100 bp ladder marker, Lane 2: heterozygous restriction pattern at 190 and 167 bp, Lane 3: homozygous restriction pattern at 219 bp

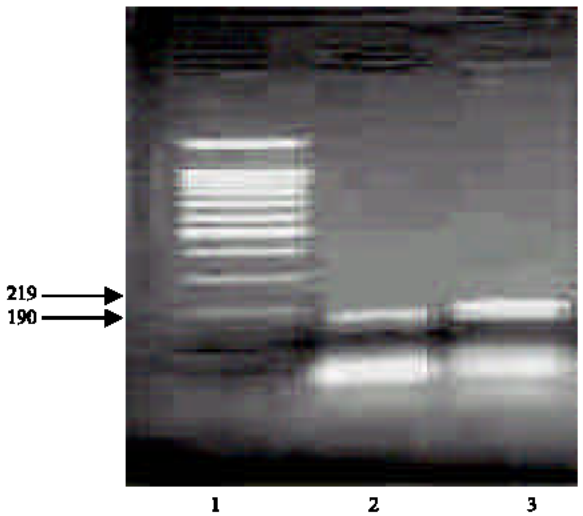

Fig. 3: DNA electrophoretic pattern obtained after digestion of PCR amplified buffalo MHC class II products with *Hae*III, Lane 1: 100 bp ladder marker, Lane 2: homozygous restriction pattern at 190 bp, Lane 3: hetertozygous restriction pattern at 219 and 190 bp

animals, respectively. These results illustrated that the buffalo MHC class II DRB has resembled restriction sites at 219 and 190 bp as in cattle (Van Eijk *et al*., 1992).

The homology sequence of buffalo MHC class II DRB gene and its close polymorphisms similarities to cattle DRB 3, found a resemblance between buffalo MHC class II DRB polymorphism and other different species like human, pig and goat (Gustaffson *et al*., 1990; Andersson *et al*., 1991; Amills *et al*., 1995).

The *Hae* III restriction site at 167 bp has correlated with Pro 56 amino acid which involved in the formation of Antigen-Recognition Site (ARS) of MHC molecule (Amills *et al*., 1996). This site recorded with low percentage 8% in our tested animals. Also, the presence of 5 animals carry the undigested sites of *Hae*III refers to the absence of *Hae*III amino acid substitutions. According to that, the buffalo MHC class II DRB genetic polymorphisms by *Hae*III revealed 6 genetic varients which is associated with animo acid substitutions.

In conclusion, the study of MHC polymorphism has interesting applications in the field of cell-mediated immunity and disease resistance. The possible existence of close associations between restriction sites and amino acid substitutions converts PCR-REFLP into a very powerful tool in the relating to specific amino acid substitutions at critical positions with disease resistance.

REFERENCES

Amills, M., O. Francino and A. Sanchez, 1995. Nested PCR allows the characterization of *Taq* I and *Pst* I RFLP in the 2nd exon of the caprine MHC class II gene. Vet. Immunol. Immunopathol., 48: 313-321.

Amills, M., O. Francino and A. Sanchez, 1996. A PCR-RFLP typing method for the caprine *MHC* class II DRB gene. Vet. Immunol. Immunopathol., 55: 255-260.

Andersson, L., S. Sigudardottir, C. Borsch and K. Gustafsson, 1991. Evolution of MHC polymorphism: Extensive sharing of polymorphic sequence motifs between human and bovine DRB alleles. Immunogenet., 31: 188-193.

De, S., R.K. Singh and G. Butchaiah, 2002. MHC-DRB exon 2 allele polymorphism in Indian river buffalo (*Bubalus bubalis*). Animal Gen., 33: 215-219.

Gustafsson, K., S. Germana, F. Hirsch, K. Pratt, G. LeuGuern and D.H. Sachs, 1990. Structure of miniature swine class II DRB genes: Conversion of hypervariable amino acid residues between distantly related mammalian species. Proc. Natl. Acad. Sci. USA, 87: 9798-9802.

John, S.W.M., G. Weitzner, R. Rozen and C.R. Scriver, 1991. A rapid procedure for extracting genomic DNA from leukocytes. Nucleic Acid Res., 19: 408-412.

Rask, L., K. Gutafsson, D. Larhammar, H. Ronne and P.A. Peterson, 1985. Generation of class II antigen polymorphism. Immunol. Rev., 84: 123-143.

Sena, L., M.P.C. Schneider, B. Brenig, R.L. Honeycutt and J.E. Womack, 2003. Polymorphisms in MHC-DRA and DRB alleles of water buffalo (*Bubalus bubalis*) reveal different features from cattle DR alleles. Anim. Gen., 34: 1-10.

Sigurdardottir, S., C. Borsch, K. Gustafsson and L. Andersson, 1991. Cloning and sequence analysis of 14 DRB alleles of the bovine major histocompatibility complex by using the polymerase chain reaction. Anim. Gen., 22: 199-209.

Van Eijk, M.J.T., J.A. Stewart-Haynes and H.A. Lewin, 1992. Extensive polymorphism of the BoLA-DRB 3 gene distinguished by PCR-RFLP. Anim. Gen., 23: 483-496.