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*Msp*I restriction enzyme evidence moderate polymorphism in the second exon of the *BoLA-DRB3* gene

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RESUMEN

Hasta el momento, se han descripto 53 alelos para el exón 2 del gen bovino *BoLA-DRB3* mediante la técnica de PCR-RFLP. Con el objetivo de analizar los posibles patrones de restricción para la enzima *Msp*I presentes en el exón 2 del gene *BoLA-DRB3*, se obtuvieron todas las secuencias de ADN correspondientes a dicho exón enviadas a la base de datos Genbank. Como resultado se observaron 2 sitios de restricción y 5 patrones diferentes para dicha enzima. Además, los patrones de restricción obtenidos a partir de la digestión del ADN genómico se compararon con los predichos a través del análisis de las secuencias reportadas en la base de datos. El uso de la enzima *Msp*I junto con *Bst*YI, *Hae*III y *Rsa*I podría ser de utilidad para el reconocimiento de ciertas secuencias que presenten un mismo alelo definido sobre la base de la combinación de los patrones de restricción, es decir, alelos definidos por PCR-RFLP, aumentando de esta manera la eficacia del método de tipificación diseñado por van Eijk *et al.* (1992). Aunque el número de patrones detectados por *Msp*I y *Bst*Y I es similar, los patrones de la primer enzima son más fáciles de identificar que los de la segunda. Por otra parte, *Msp*I utiliza el mismo buffer y temperatura de reacción que *Hae*III y *Rsa*I, simplificando las digestiones dobles. Finalmente, los costos de la tipificación podrían reducirse.

Palabras clave: *BoLA-DRB3*, métodos de tipificación, *Msp* I, polimorfismo.

ABSTRACT

At present, 53 PCR-RFLP alleles of the bovine *DRB3* exon 2 were reported. In order to analyse the putative *Msp*I restriction patterns present in the exon 2 of *BoLA-DRB3* gene, all available DNA sequences were retrieved from Genbank Data Library. In this analysis, two *Msp*I restriction sites and five restriction patterns were found. In addition, the observed restriction patterns obtained after *Msp*I digestion of genomic DNA were compared with those predicted from the *DRB3* DNA sequence analysis. The use of *Msp*I restriction enzymes together with *Bst*YI, *Hae*III and *Rsa*I could be useful in recognising some sequences differences in a single PCR-RFLP allele as defined by the previously used combination of enzyme, improving the robustness of this typing method designed by van Eijk *et al.* (1992). Despite the number of restriction patterns detected by *Msp*I and *Bst*YI are similar, the *Msp*I patterns are more easily identified than those of *Bst*YI. *Msp*I enzyme works well in the same reaction buffer and temperature as *Hae*III and *Rsa*I, thus simplifying double digestions. Finally, the costs may be reduced.

Keywords: *BoLA-DRB3*, typing methods, *Msp* I, polymorphism

Introduction

Several methods, such as alloantisera, restriction fragment length polymorphism (RFLP), microsatellites analysis, isoelectric focusing (IEF), DNA sequencing, sequence specific oligonucleotide (SSO)-PCR, and multi-primer target PCR have been used to investigate the genetic polymorphism of the *BoLA-DRB3* locus (Andersson *et al.*, 1986a; Andersson *et al.*, 1986b; Davies and Antczak, 1991; Davies *et al.*, 1992; Ellegren *et al.*, 1993; Aida *et al.*, 1995; Sitte *et al.*, 1995; Ledwidge *et al.*, 2001). In 1992, van Eijk *et al.*

differentiated thirty alleles of this locus using the PCR-RFLP method. An additional allele has been defined in the 5th International *BoLA* Workshop report (Davies *et al.*, 1994); and more recently, Gelhaus *et al.* (1995) and Maillard et al. (1999) added eleven and seven new *BoLA-DRB3* alleles defined by this technique, respectively. At present, 53 PCR-RFLP defined alleles (described by the combination of *Rsa*I, *Hae*III and *Bst*YI restriction enzymes) of the *DRB3* exon 2, were reported on the *BoLA* world-wide web site (http://www.projects.roslin.ac.uk/bola/drb3pcr.html).

In 2001, Díaz *et al.* demonstrated polymorphism for *Msp*I restriction enzyme in the second exon of *ELA-DRB* genes. This enzyme combined with *Rsa* I and *Hae* III was used to distinguish all *ELA-DRB* sequenced alleles.

The aims of this research are the study of the *Msp*I polymorphism in the *BoLA-DRB3* exon 2, and its putative use in PCR-RFLP for typing purposes.

MATERIALS AND METHODS

Analysis of bovine BoLA-DRB3 sequences

In order to analyse the putative *Msp*I restriction patterns present in the exon 2 of *BoLA-DRB3* gene, all available DNA sequences were retrieved from Genbank. Nowadays, 140 *BoLA-DRB3* sequences have been identified in different breeds, by sequencing genomic DNA, cDNA or cloned PCR products (Muggli-Cockett and Stone, 1989; Groenen *et al.*, 1990; Burke *et al.*, 1991; Sigurdardóttir *et al.*, 1991; Ammer *et al.*, 1992; Van Eijk *et al.*, 1992, 1999; Xu *et al.*, 1993; Davies *et al.*, 1994; Russell *et al.*, 1994; Aida *et al.*, 1995; Gelhaus *et al.*, 1995; Mikko and Andersson, 1995, 1997; Sitte *et al.*, 1995; Ballingall *et al.*, 1996; Fraser *et al.*, 1996; Maillard *et al.*, 1999, 2001; Mikko *et al.*, 1997; Park *et al.*, 1997; Damiani *et al.*, 1998; da Mota *et al.*, 1999; Takeshima *et al.*, 2001). The *Msp*I restriction patterns of each *BoLA-DRB3* sequence were deduced using Webcutter 2.0 program (Max Heiman copyright 1997).

Amplification of BoLA-DRB3 exon 2 by PCR

Genomic DNA was obtained from whole blood bovine samples using the DNAzol® (GibcoBRL®-Life Technologies, Rockville, MD, USA) technique as described by Cox (1968) and Ausubel *et al.* (1990). These animals were selected among samples previously typed using the method described by van Eijk *et al.* (1992). Amplifications of the second exon of *BoLA-DRB3* gene were performed by heminested-PCR, using the primers designed HL030, HL031 and HL032 (Van Eijk *et al.*, 1992).

RFLP analysis

Twelve microlitres of the amplification products, obtained from genomic DNA or recombinant plasmid DNA were digested at the temperature recommended by the suppliers with two and a half units of *Msp* I. Restriction fragments were resolved in 6% polyacrilamide minigels at 170 volts for 45 min, and stained with ethidium bromide (0.5 µg/ml). The restriction patterns observed under UV illumination were compared with those predicted by the *BoLA-DRB3* DNA sequence analysis.

Cloning of PCR Products

Restriction patterns were confirmed by cloning. To this end, PCR products were inserted into pGEM-T® Easy Vector System (Promega Corp., Madison, WI, USA) according to the supplier's protocol. *E. coli* competent cells (DH5αF'™, GibcoBRL-Life Technologies, Rockville, MD, USA) were transformed with the recombinant plasmid and selected by blue/white colour screening and standard ampicillin selection (Sambrook *et al.*, 1989).

Nomenclature for BoLA-DRB3 Msp I patterns detected by PCR-RFLP

In this paper, *Msp*I restriction patterns of *BoLA-DRB3* exon 2 defined by PCR-RFLP will be indicated by letters, according to Davies *et al.* (1997) and Russell *et al.* (1997).

Results

A total of 140 DNA sequences of *BoLA-DRB3*, obtained from GenBank, were analysed for the presence of *Msp*I restriction enzyme sites. In this analysis, two *Msp*I restriction sites and five restriction patterns were found (Figure 1). Although, five patterns have been identified, three patterns (from "a" to "c") are actually the result of combinations of the two polymorphic *Msp*I sites. The remaining two patterns (from "d" and "e") have restriction sites matching those of other patterns but are distinguished by the fragment length due to the presence of a deletion at nucleotide positions 178-180 (codon 65) (Figure 1).

*Msp*I restriction enzyme allowed the discrimination of more than one pattern in PCR-RFLP *DRB3* defined alleles 10, 13, 15, 22, 23 and 32, representing different nucleotide sequences. The theoretical results obtained with the analysis of *BoLA-DRB3* sequences were in accordance with the experimental results (Figures 1 and 2).

Figure 1.
*Msp*I restriction patterns of the BoLA-DRB3 exon 2 based on the analysis of the GeneBank DNA sequences. The line drawn on the top portion represents the 284 bp PCR product; « b» indicates the location of the restriction sites; «▼» indicates a three base pair deletion. Sizes for restriction fragments are indicated between restriction sites.

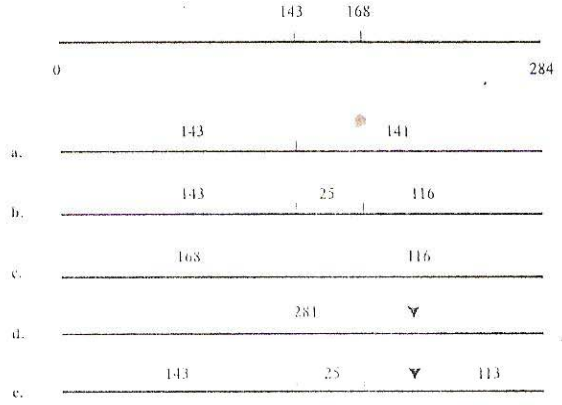
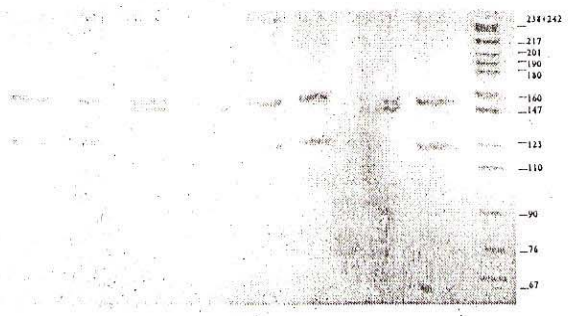


Figure 2.
Allelic patterns of *BoLA-DRB3* exon 2 obtained by digestion of recombinant plasmid DNA with *Msp*I. Allelic patterns for *Msp*I are as follows: Lane 1: b; lane 2: b; lane 3: a; lane 4: b; lane 5: b; lane 6: b; lane7: a; lane 8; b. Fragments smaller than 30 bp were not clearly resolved by the gel system used. An *Msp*I digest of pBR322 was used as size marker (lane 9).



Discussion

In 1992, van Eijk *et al.*, developed a PCR-RFLP method to type *BoLA-DRB3* alleles. At that moment, only 14 sequenced *BoLA-DRB3* alleles have been reported (Sigurdardottir *et al.*, 1991). Analysis of these sequences for restriction endonuclease cleavage sites resulted in the selection of *Rsa*I, *Hae*III and *BstY*I to study the polymorphism of exon 2 *DRB3* gene, since these three enzymes could distinguish all sequences reported by Sigurdardottir *et al.* (1991). However, more than one hundred sequences have been reported since 1992, which could be grouped in a total of 53 PCR-RFLP types alleles. Many of these groups include several sequences belonging to different subtypes and even distinct types.

The PCR-RFLP method through the analysis of 140 DNA sequences for *Rsa* I, *BstY*I, and *Hae* III restriction enzymes sites, showed that five restriction patterns could be identified by digestion with *BstY* I as a result of two polymorphic restriction sites and one deletion; nine types could be distinguished by *Hae* III as a consequence of five polymorphic restriction sites and one deletion; and twenty-five restriction patterns could be differentiated by *Rsa*I as a result of five polymorphic restriction sites and one deletion. Together, these enzymes could defined fiftythree PCR-RFLP types.

BstY I (or its isoschizomer *Xho* II, Udina *et al.*, 1998) is the less polymorphic of the three used enzymes yielding only five restriction patterns, one of which - the "b" pattern - was found in 33 (62%) out of the 53 PCR-RFLP defined alleles. The results obtained in the present paper showed that both *Msp*I and *Bst*YI enzymes exhibit five restriction patterns. However, using *Bst*YI, the number of defined PCR-RFLP alleles result quite minor than using *Msp*I. Furthermore, in cattle the *Msp*I seems to be less polymorphic than in horses (Díaz *et al.*, 2001).

The use of *Msp*I restriction enzyme with *Bst*YI, *Hae*III and *Rsa*I could be helpful to recognise some sequences in PCR-RFLP *DRB3*, improving the robustness of this typing method. In addition, the use of *Msp*I instead of *Bst*YI in the PCR-RFLP typing methods could offer other advantages. The *Msp*I patterns are easily identified than *Bst*YI ones (Figures 1 and 2). Some genotypes of unrelated heterozygous individuals could not be resolved because of overlapping fragment patterns between alleles. In those cases, double digestion is necessary to solve the problem. *Msp*I works well in the same reaction buffer and temperature than *Hae*III y *Rsa*I, thus simplifying double digestions. Finally, the costs may be reduced, since *Msp*I is ten-times less expensive than *Bst*Y I.

The method proposed by van Eijk *et al.*, (1992) has been extensively employed by several authors to characterise breeds and for population studies (*e.g.* Giovambattista *et al.*, 1996; Dietz *et al.*, 1997; Udina *et al.*, 1998; Maillard *et al.*, 1999; Gilliespie, 1999). In this context, the main disadvantage of including *Msp*I is that these animals will have to be typed again in order to allow comparison with new data. However, the proposed modifications in this paper could be useful to improve the original PCR-RFLP method designed by van Eijk *et al.*, (1992).

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