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## Two new variants of the bovine PAS-1 glycoprotein

P Sacchi, E Macchi, R Rasero, P Fiandra

### Abstract

Two new alleles (*A* and *E*) of the bovine *MUC* locus which encodes PAS-1 protein, a glycoprotein of the milk fat globule membrane, are reported. The *A* allele was found in Italian Brown while *E* was present in the Jersey and the Piedmont breeds.

**Keywords:** cattle, glycoprotein, milk fat globule membrane, polymorphism

The bovine PAS-1 is the major high molecular weight glycoprotein of the milk fat globule membrane (MFGM). Being a glycoprotein, it stains with periodic acid-Schiff's reagent or PAS; hence the name PAS-1. Patton and Patton (1990) first described the genetic polymorphism of bovine PAS-1: three codominant alleles controlling variants of relative molecular weight of about 180 000–200 000 were reported in Holstein–Friesian and subsequently (Patton & Muller 1992) in Ayrshire, Brown Swiss, Jersey and Guernsey breeds.

We describe the polymorphism of PAS-1 in five Italian cattle populations for which preliminary data have already been presented (Sacchi *et al.* 1994). A total of 275 individual milk samples were analysed from Italian Friesian, Italian Brown, Jersey, Angeln and Piedmont populations reared in North West Italy.

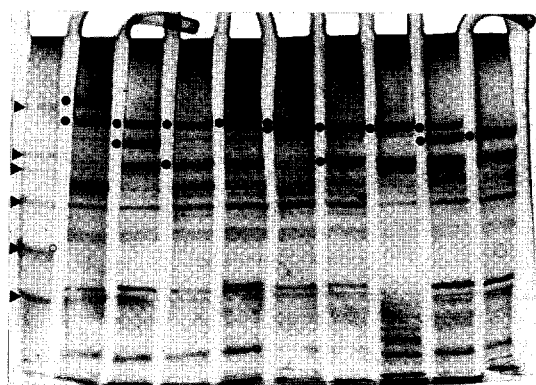
MFGM was centrifuged out of fresh milk and lipids were removed by repeated ethanol-ether extractions (Patton & Huston 1986). All samples were freeze-dried. Separation of polypeptide fractions was performed on 6% constant concentration SDS-polyacrylamide gel electrophoresis (PAGE) (Patton & Patton 1990; running time 15–18 h) and on precast gradient 4–15% mini-gels, *BIO-RAD* (running time 35–40 min), using whole or skimmed milk as well as purified freeze-dried membranes. The best results were obtained by gradient electrophoresis of purified membranes by which the routine analyses were performed.

PAS-1 does not stain with Coomassie blue, but it can be detected by PAS method and it stains more rapidly than other milk proteins with silver nitrate procedure (Silver Stain Kit, Sigma, St Louis, MO, USA), modified by adding 0.2% periodic acid for 1 h at 4°C after fixation, and 5 µg dithiothreitol/ml for 30 min before silver nitrate treatment.

We found five variants called A, B, C, D and E from anode towards cathode using gradient gels; their relative molecular weight was about 120 000, 140 000, 170 000, 180 000 and 220 000 respectively (Fig. 1).

A greater number of variants should exist because small differences in D mobility were observed amongst individuals, especially using 6% constant concentration SDS-PAGE. When separation was performed by this method, B, C and D showed the same relative mobility of the fractions obtained by Patton & Patton (1990), whereas A and E appeared to be new variants.

In the patterns for 25 dam–daughter pairs, involving variants B, C and D, each daughter had a variant in common with her mother. These data are in agreement with a mode of inheritance based on codominant alleles.



**Fig. 1.** Gradient 4–15% SDS-PAGE of PAS-1 in cattle (circles mark the PAS-1 fractions). From left: molecular weight references (triangles, from top to bottom, indicate: rabbit muscle myosin 205 000; *E. coli*  $\beta$ -galactosidase 116 000; rabbit muscle phosphorylase b 97 400; bovine albumin 66 000; egg albumin 45 000; bovine carbonic anhydrase 29 000), DE, BD, AD, D, CD, AC, C, BC, B.

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In accordance with the present nomenclature used for the human largest MFGM glycoprotein gene (Swallow *et al.* 1987; Audie *et al.* 1993), we propose for the bovine PAS-1 a locus designation *MUC* and suggest that the alleles are called *MUC<sup>A</sup>*, *MUC<sup>B</sup>*, *MUC<sup>C</sup>*, *MUC<sup>D</sup>* and *MUC<sup>E</sup>*. *MUC<sup>A</sup>* was only found in Italian Brown and *MUC<sup>E</sup>* was only found in Jersey and Piedmont. *MUC<sup>D</sup>* was the most common allele in all populations (Table 1).

Further investigations are needed to show the gene structure of bovine *MUC* and to compare it with the human gene.

**Table 1.** Allele frequencies

Population	Allele frequencies				
	A	B	C	D	E
Italian Friesian		0.282	0.260	0.458	
Italian Brown	0.036	0.143	0.166	0.655	
Angeln		0.211	0.167	0.622	
Jersey		0.268	0.062	0.634	0.036
Piedmont		0.255	0.109	0.618	0.018

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