IDENTIFICATION OF THE TWO COMMON ALLELES OF THE BOVINE $\alpha_s$-CASEIN LOCUS BY MEANS OF RFLPs.


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SUMMARY

The aim of the research was to find Restriction Fragment Length Polymorphisms (RFLPs) at the bovine $\alpha_s$-, $\alpha_2$-, and $\beta$-casein loci. Milk and DNA samples from 98 cows belonging to six breeds (Italian Friesian, Reggio, Modena, Podolian Type, Italian Brown, and Jersey) were considered. DNA samples were digested with the following enzymes: BamHI, BglII, EcoRI, EcoRV, HindIII, PstI, PvuII, and TaqI. Three endonucleases (TaqI, BglII, and EcoRI) evidence RFLPs at the $\alpha_s$-Cn locus. With the exception of few recombinants in the Reggio and Modena breeds, BglII and EcoRI polymorphic fragments correspond to B and C alleles of $\alpha_s$-casein. An RFLP was found at the $\alpha_2$-Cn locus with TaqI endonuclease and no RFLP was found at the $\beta$-Cn locus.

INTRODUCTION

It has been demonstrated that, in cattle, some casein genetic variants influence milk quality (for a review, see Grosclaude, 1988). In order to identify casein genotypes even in absence of gene product and, therefore, to make easier selection for favourable alleles, we began to search for RFLPs in the DNA regions containing casein genes in several species (cattle, sheep, goat, and Italian dairy water buffalo). Identification of the two common $x$-casein alleles at the DNA level has been independently described by Damiani et al. (1987), Rando et al. (1988), and Levéziel et al. (1988). Furthermore, some alleles of goat $\alpha_s$-casein can be also identified by means of RFLPs (Di Gregorio et al., 1989). In this paper we report results on RFLPs in the DNA regions containing $\alpha_s$-, $\alpha_2$-, and $\beta$-casein genes.

MATERIALS AND METHODS

Milk and DNA samples were obtained from 26 Italian Friesian, 34 Reggio, 20 Modena, 8 Podolian Type, 7 Italian Brown, and 3 Jersey cows. Milk was subjected to starch gel electrophoresis according to Aschaffenburg and Michalak (1968). DNA, digested with BamHI, BglII, EcoRI, EcoRV, HindIII, PstI, PvuII, and TaqI endonucleases, was subjected to Southern blot analysis as previously described (Masina et al., 1984) using plasmids C184, C411, and C468 (a gift from Dr. A.G. MacKinlay) containing bovine $\alpha_s$-Cn, $\alpha_2$-Cn, and $\beta$-Cn cDNAs, respectively, as probes.
RESULTS

\(\alpha_1\)-casein locus

Starch gel electrophoresis of milk samples showed that 68 cows were \(\alpha_1\)-Cn BB, 28 cows were \(\alpha_1\)-Cn BC, and 2 cows were \(\alpha_1\)-Cn CC. Fifteen selected DNA samples from cows with different \(\alpha_1\)-Cn genotypes were digested with BamHI, EcoRV, HindIII, PstI, and PvuII endonucleases. No RFLP was found, therefore, no further sample was examined with these endonuclease. BglII endonuclease shows allelic fragments of 3.4kb and 5.5kb. Other polymorphic fragments with a faint signal were not considered because of the difficulty of genotype attribution. EcoRI endonuclease shows a polymorphism with allelic fragments of 1.1kb and 0.9kb. BglII and EcoRI genotypes correspond perfectly in all the considered individuals (Table 1). An EcoRI 7.7kb fragment is invariant in all individuals, but two. In fact, in two \(\alpha_1\)-Cn BB Italian Brown cows another allelic fragment of 7.9kb is present at the heterozygous state. These two cows were also the only heterozygous carriers of a third rare BglII allele (genotype 3.4kb/3.8kb) (see Table 1). In addition, EcoRI endonuclease presents a RFLP with three alleles of 9.9kb, 5.7+4.2kb, and 5.5+4.2kb. Finally, TaqI endonuclease shows a polymorphism with three common alleles (7.2kb, 9.6kb, and 15.4kb) and a rare one (10.2kb), found at the heterozygous state only in one individual. In Fig. 1 the observed RFLPs are presented.

Informative genotypes (Table 1) show that the \(\alpha_1\)-Cn \(^B\) allele is in cis, with one exception, with the BglII 3.4kb or 3.8kb and the EcoRI 1.1kb fragments, whereas the \(\alpha_1\)-Cn \(^C\) allele is in cis, with three exceptions, with the BglII 5.5kb and the EcoRI 0.9kb fragments. The three latter exceptions

Figure 1 Autoradiograms of bovine DNA samples digested with EcoRI, BglII, and TaqI endonucleases and hybridized with the \(\alpha_1\)-Cn cDNA.
Table 1 Different genotypes at the protein and DNA level and their distribution in the six considered breeds.

<table>
<thead>
<tr>
<th>αs1-Cn</th>
<th>BglII</th>
<th>EcoRI</th>
<th>F</th>
<th>P</th>
<th>B</th>
<th>J</th>
<th>R</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB</td>
<td>3.4/3.4</td>
<td>1.1/1.1</td>
<td>24</td>
<td>5</td>
<td>*6</td>
<td>2</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>BB</td>
<td>3.4/5.5</td>
<td>1.1/0.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>BC</td>
<td>3.4/5.5</td>
<td>1.1/0.9</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>13</td>
<td>6</td>
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<tr>
<td>BC</td>
<td>3.4/3.4</td>
<td>1.1/1.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>CC</td>
<td>5.5/5.5</td>
<td>0.9/0.9</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Two cows are BglII 3.4kb/3.8kb and EcoRI 7.7kb/7.9kb (see text). F=Italian Friesian, P=Podolian Type, B=Italian Brown, J=Jersey, R=Reggio, M=Modena.

Belong to genetically related individuals of the Reggio breed. Furthermore, the αs1-Cn allele is in cis with the EcoRI 5.7 or 5.5+4.2kb fragments, whereas the αs1-Cn allele is in cis with the EcoRI 9.9kb fragment. Six recombinants C-EcoRI 5.7 or 5.5+4.2kb appear in the Reggio breed and one recombinant B-EcoRI 9.9kb appears in the Modena breed. Relationship between TaqI and αs1-Cn genotypes shows that the αs1-Cn allele is in cis with the 15.4kb or 7.2kb fragments, whereas the αs1-Cn allele is in cis with the 9.6kb fragment. In this case, several recombinants B-TaqI 9.6kb are present in the Reggio and Modena breeds.

In conclusion, the two most common haplotypes at the bovine αs1-Cn locus are the following:

- B-BglII 3.4kb-EcoRI 1.1kb-EcoRI 5.7 or 5.5+4.2kb-TaqI 15.4 or 7.2kb-
- C-BglII 5.5kb-EcoRI 0.9kb-EcoRI 9.9kb-TaqI 9.6kb-

Since the restriction map of the αs1-Cn gene is not known the relative localization of the polymorphic sites is impossible. However, BglII+EcoRI double digestions (not shown) of DNA samples homozygous for the two most common haplotypes show that EcoRI 1.1kb and 0.9kb allelic fragments are internal to the BglII 3.4kb and 5.5kb allelic fragments, respectively. Absence of recombinants between BglII and EcoRI polymorphic fragments is in agreement with this result.

αs2-casein locus

No individual differences were found at the protein level in all the considered milk samples. At the DNA level, only TaqI endonuclease shows a polymorphism with allelic fragments of 1.1kb and 1.8kb. The former is less frequent in all the considered breeds with a frequency around 0.05.
\( \beta \text{-casein locus} \)

Starch gel electrophoresis showed the following genotype distributions: 58 \( \beta \text{-Cn AA} \), 25 \( \beta \text{-Cn AB} \), 3 \( \beta \text{-Cn BB} \), 2 \( \beta \text{-Cn BC} \), and 10 \( \beta \text{-Cn AC} \). No DNA polymorphism was observed with the used endonucleases in 15 selected samples belonging to cows with different \( \beta \text{-Cn} \) genotypes. Restriction patterns obtained with \( \beta \text{-Cn} \) cDNA agree with the map presented by Gorodetsky et al., (1988).

**DISCUSSION**

Results presented in this paper demonstrate that by using \( \alpha_{s1} \text{-casein cDNA} \) as probe and BgIII or EcoRI endonucleases it is possible to identify, with few exceptions in the Reggio and Modena breeds, the bovine \( \alpha_{s1} \text{-casein genotypes} \) at the DNA level and, therefore, in males and non-lactating females. If we suppose identity by descent of the three recombinant C-BgIII 3.4kb-EcoRI 1.1kb observed haplotypes, the probability of wrong genotype attribution in Modena and Reggio breeds should be no more than 3-4%.

DNA polymorphism at the \( \alpha_{s2} \text{-Cn locus} \), which is monomorphic at the protein level in many European breeds (Grosclaude et al., 1978), could be useful to accomplish studies on linkage disequilibrium in order to locate this gene in a fine genetic map of the casein cluster.

In the DNA region containing the \( \beta \text{-Cn locus} \) no RFLP was found. However, since no recombinant among casein loci appears in all the considered informative dam-daughter pairs (see Grosclaude, 1979), it is possible to identify \( \beta \text{-Cn} \) genotypes in the progeny once linkage phase between a \( \beta \text{-Cn} \) allele and a DNA marker at any other casein locus is established in parents.

**REFERENCES**