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ALPHA-LACTALBUMIN GENOTYPES IDENTIFICATION IN ROMANIAN BLACK SPOTTED CATTLE BREED

IDENTIFICAREA GENOTIPURILOR ALFA-LACTALBUMINEI LA RASA BĂLȚATĂ CU NEGRU ROMÂNEASCĂ

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Alpha-lactalbumin (α-La) is a major milk protein essential for the biosynthesis of lactose at the level of mammary glands. α-La directly influences the quality and the volume of the milk since it is directly involved in the lactose synthesis (Ashwell et al., 1997). The PCR-RFLP test was performed to distinguish the different alleles in a population of Romanian Black Spotted cattle, a dairy breed. Genetic polymorphism was detected by digestion with the endonuclease MnlI, followed by electrophoresis in high resolution agarose gel stained with ethidium bromide. Sixty DNA samples from Romanian Black Spotted breed were analyzed for A and B variants. The PCR-RFLP test makes feasible the inclusion of α-La genotypes in breeding plans and cattle selection.

Key words: alpha-lactalbumin, genetic polymorphism, PCR, RFLP, cattle breed

Introduction

α-La is a constituent of lactose-synthetase, the enzyme responsible of the synthesis of lactose, in the final step where glucose is linked to galactose. The quantity of lactose released into the milk is a condition for its quality (Voelker Jr. et al., 1999). The primary sequence of α-La was first determined by Brew et al., 1970. The most common B variant consists of 123 aminoacids with a molecular weight of 14.175.

Lactose synthase synthesizes lactose which is released into the milk along with α-La. Because of its association with lactose production, α-La is thought to play a role in regulating milk volume. Bleck and Bremel (1993) discovered a variation in the control region of the α-La gene. The A variant of this gene is only found in Holstein cattle while other breeds studied all contained the B variant. Through milk production trait analysis, they showed that this α-La variant is associated with significant differences in milk production within Holsteins.

The objective of our work was to develop a fast and simple method to distinguish the allelic variants of α-La in Romanian Black Spotted breed and to evaluate the favourable genotypes for the production of milk quality.

Materials and Methods

DNA extraction: blood samples for DNA genotyping were supplied by sixty cows of the Romanian Black Spotted breed (ICDB Baloteşti farm). Genomic DNA isolation was done with Wizard Genomic DNA Extraction Kit (Promega) from fresh blood samples (300 µl).

PCR – RFLP test: for α-La genotyping we performed a simple polymerase chain reaction (GeneAmp® PCR System 9700) followed by enzymatic restriction. Genomic DNA was amplified for 45 cycles in a 25µl reaction containing: PCR buffer, $MgCl_2$, dNTPs, AmpliTaq DNA Polymerase and nuclease-free water, sense and antisense primers (Table 1).

Table 1

Primer sequences and annealing temperature

	Primer sequences	**Protocol**
α-La	for. 5'-CTCTTCCTGGATGTAAGGCTT-3' rev. 5'-AGCCTGGGTGGCATGGAATA-3'	95°C/30 sec; 58°C/30 sec; 72°C/1 min.

The first denaturation step was performed at 95ºC (10 min) and the last extension was 30 minutes (72°C).

PCR products were digested with restriction endonuclease (Table 2) at 37ºC for 3h. Restricted products were analyzed by electrophoresis in 2% high resolution agarose gel stained with ethidium bromide.

Table 2

Restriction enzymes used to digest the PCR amplicons and the restriction products

Length of PCR products bp	**Restriction enzymes**	**AA genotype** restriction fragments bp	**BB genotype** restriction fragments bp	**AB genotype** restriction fragments bp	**References**
α-La 166	*Mnl I*	78/52/36	114/52	114/78/52/36	Bleck and Bremel, 1993 Mao, 1994

PCR Amplification and Sequencing for α-La: the most widely used method to distinguish between AA, BB and AB genotypes of α-La gene is to cut the 166 bp PCR products (obtained using primers in Table 1) with *Mnl I* endonuclease at the recognition site $GAGG(N)_6\downarrow$. The lengths of the fragments we must obtain are 78/52/36 bp for AA, 114/52 bp for BB and 114/78/52/36 bp for AB. To confirm the α-La genotypes we decided to perform sequencing. The amplified fragments were purified with the Wizard PCR Preps DNA Purification System Kit (Promega)

and amplified for sequencing using the ABI Prism® BigDye Terminator Cycle Sequencing Ready Reaction and were run on ABI Prism 310 Genetic Analyzer. DNA Sequencing Analysis 5.1 Software (AppliedBiosytems) was used to process the sequences which were aligned with the Clustal X multiple alignment program and refined manually.

Results and Discussions

Identification of α-La genotype was performed by PCR amplification with specific primers designed for the sequences of interest. To confirm the lengths of PCR products of 166pb we performed electrophoresis in agarose gel stained with ethidium bromide (Figure 1).

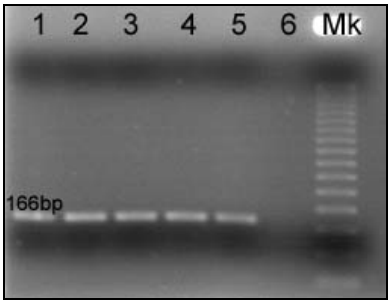

Figure 1. Electrophoresis pattern of amplified bovine genomic DNA with α-La specific primers separated in 2% agarose gel**.** *Lanes 1-5: fragments amplified for α-La locus. Lane 6: negative control. Lane 7: molecular size marker (50 bp DNA Step Ladder).*

Detection of the genetic polymorphism of the bovine α-La locus has been done by digestion of the PCR products with *Mnl I* restriction endonuclease (Bleck and Bremel, 1993; Mao, 1994). The enzymes cut the amplicons in three fragments of 78, 52, and 36 bp for the AA genotype and in two fragments of 114 and 52 bp for the BB genotype. Heterozygotes AB are a combination of the two alleles A and B (four fragments of 114, 78, 52 and 36 bp) (Figure 2).

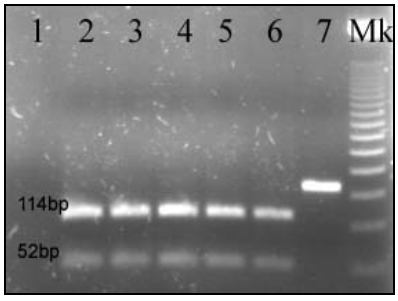

Figure 2. Electrophoresis pattern of amplified fragments after digestion with *Mnl I* enzyme**.** *Lane 1: negative control. Lanes 2-6: BB homozygous cattle, two fragments of 114 and 52bp. Lane 7, molecular size marker (50bp DNA Step Ladder).*

The milk-specific protein α-La is a key part of the lactose synthase complex in mammary epithelial cells. This enzyme complex is responsible for the production of lactose, the major osmole in milk and a major determinant of milk volume. Through the sequencing method mentioned above, sixty Romanian Black Spotted cattle were genotyped and analyzed for any potential effect on milk production traits, but only BB variant (114/52 bp) was detected.

Sequencing was performed in order to confirm the sequences of amplified fragments from the α-La gene but also to strengthen the diagnostic method taking into account that we found only BB genotype in the specimens analyzed by us. We identified and validated the presence of the restriction site for *MnlI* enzyme in the α-La gene (Fig. 3, 4).

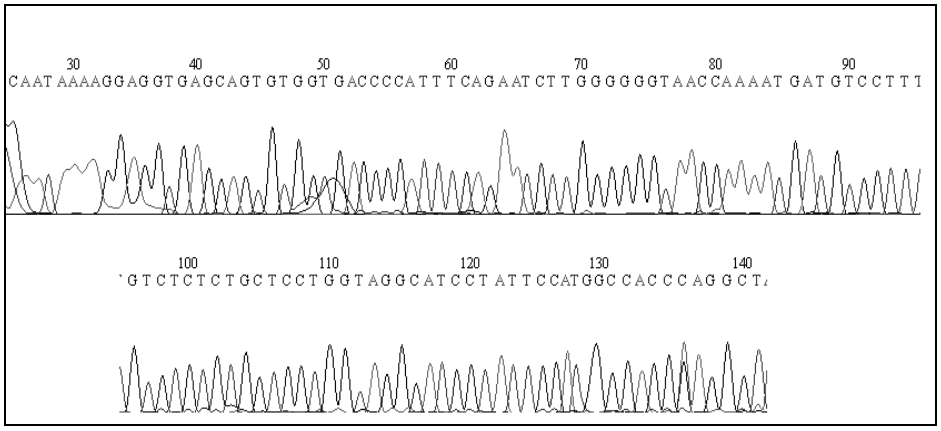

Figure 3. The sequence of the PCR products for a normal cow containing *Mnl I* restriction site**.**

In Figure 4, a region of a PCR fragment of the sequenced α-La gene is shown. Restriction site for *Mnl I* (left), normally used to detect different α-La genotypes was identified.

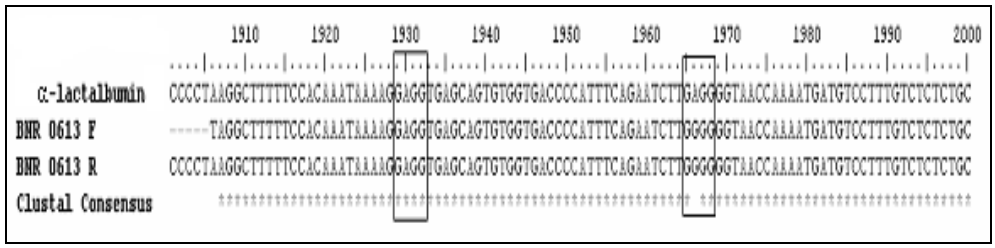

Figure 4. Clustal X alignment between a Gene Bank fragment from the α-La gene and our PCR products**.**

Conclusions

The results of RFLP analysis showed that of all the three genotypes (AA, BB and AB) we expected to find in the population studied for α-La gene, we detected only the BB genotype.

Detection methods based on PCR amplification and RFLP analysis are powerful tools to determine the presence of polymorphism of gene coding for α-La gene.

Using the PCR-RFLP technique, we established an easy, low-cost and efficient method that can be used to determine the genotype of dairy cattle.

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