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PCR-RFLP ANALYSIS OF GENETIC POLYMORPHISM OF THE LACTOFERRIN GENE IN TUNISIAN IMPORTED HOLSTEINS

B. Jemmali[1], M. Kamoun[1], M. Ksouri[1], B. Rekik[1] and S. Mhirsi[2]

[1]*Département Des Productions Animales, ESA Mateur, 7030 Tunisie*
[2] *Laboratoire de Génie Biologie INSAT Tunis*

*****Corresponding author: bornijemali@yahoo.fr

The rate of subclinical mastitis inferred from somatic cell scores is high in Tunisian dairy cattle. Other than management, susceptibility of cows to infections may be of great impact on the mammary health. Lactoferrin seems to influence resistance of dairy cows to mastitis infections. The objective of this study was to search for the genes coding for lactoferrin in imported Holstein cows managed in a large herd in the North of Tunisia. A total of 52 blood samples were collected. Genomic DNA was extracted and all individuals were genotyped for polymorphism of lactoferrin gene by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. Amplification of the lactoferrin gene fragment revealed an 1143 bp long product by electrophoresis following PCR. After restriction enzyme digestion with *HinfI*, two alleles were characterized by only one restriction fragment. All screened individuals were homozygous showing the same band pattern in all samples by the PCR-RFLP, implying the existence of only one genotype for the lactoferrin locus. This study may be generalized to cover the Tunisian Holstein population for possible identification of resistant animals to mastitis infection and that have polymorphic lactoferrin genes.

Keywords: Holstein cattle; lactoferrin gene; PCR-RFLP

Rekik et *al*. (2008) reported that somatic cell scores (SCS) in the Tunisian Holstein population were high in the first three lactations most likely due to high mastitis infection rates. These infections resulted in reduced milk and protein yields and long calving to first service and calving to conception intervals. The SCS has high genetic correlation with mastitis (0.60-0.80), and is one of the useful indirect measures of mastitis at present (Zhang *et al.*, 2007).

Although sizeable genetic gains for health traits were made using conventional selection methods which are money and mainly time-consuming, there is a growing attention towards improving health traits using genomic selection in dairy populations worldwide (e.g., Schaeffer, 2006; de Roos *et al.*, 2010). In many countries which are part of Interbull, genomic selection is developing rapidly. Routine genomic evaluations produce genomically enhanced breeding values (GEBV) of male calves which are only slightly less precise than conventional estimated breeding values (EBV) obtained after a formal progeny test. In such a context, several authors demonstrated that larger yearly genetic gains can be obtained by intensively using genomically evaluated young sires, without waiting for progeny tested results (Schaeffer, 2011).

In dairy cattle breeding the special attention is given to the candidate genes (alleles) and their association with production and health traits (Taylor et *al*., 2006; Zhou et *al*., 2006). Lactoferrin is an

iron-binding glycoprotein found in most exocrine secretions including tears, saliva and milk, and there are numerous reports of its antibacterial activity in vitro and in vivo (Nibbering et *al*., 2001; Wojdak et *al*., 2006). The lactoferrin gene can be used as a marker of somatic cell concentration in milk and, in consequence, as a marker of susceptibility/resistance to mastitis in dairy cows.

MATERIAL AND METHODS

Sample collection and DNA extraction

A total of 52 dairy cows were randomly selected from a private farm in the North of Tunisia. Blood samples were collected in vacutainer tubes containing ethylene diamine tetra-acetic acid (EDTA) (1 mg/mL). Genomic DNA was extracted using standard protocol (Easy-DNA™ Kit, Invitrogen) and stored at -20 °C until used in assay.

The concentration of DNA samples was estimated using UV-visible range spectrophotometer and diluted to 50 ng/µL before PCR amplification. All the DNA samples had 260/280 Optical density (OD) ratios in the range of 1.8 to 2, indicating high purity. DNA was also examined by loading samples on 0.8 % agarose gel and visualizing the band under UV light with a Gel Doc 1000 system (BioRad) after ethidium bromide staining.

The PCR was performed in a final volume of 50 µL containing 100 ng of template DNA, 50 pmole of each primer, 5µl of 10X PCR buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl), 1.5 mM MgCl2, 0.2 mM of dNTPs, and 1 U of *Taq* DNA polymerase, primers was performed as described by Zhao *et al.* (2008).

This solution was initially denatured at 94 °C for 5 min. followed by 39 cycles of denaturation (95 °C for 30 s), annealing (62 °C for 45 s), and elongation (72 °C for 1 min) and a final extension at 72 °C for 10 min. The PCR products were electrophoresed on 1.5% agarose gels in order to check the quality and specificity of DNA fragment amplification.

Restriction enzyme digestion

For PCR-RFLP analysis, the 1143 bp PCR products were digested with *HinfI* (BioLabs). Restriction fragments were separated by electrophoresis in a 2% agarose gel and their sizes were estimated using the molecular markers. The results were taken into account when the sum of all the restriction fragments for *HinfI* enzyme was in the range of 1143bp ± 100 (Fagundes and Dornelas, 2007). 20 µl of PCR products was digested for 4h at 37°C with 10 units of restriction enzyme. Digested products were separated by electrophoresis on a 1.5 % agarose gel and visualized with ethidium bromide under UV light with a Gel Doc 1000 system (BioRad) after ethidium bromide staining.

RESULTS AND DISCUSSION

Quality and quantity of extracted DNA from analyzed samples was tested by electrophoresis on agarose gel. DNA Electrophoresis showed a single band. DNA quantity was estimated using the molecular markers quantity for each band.

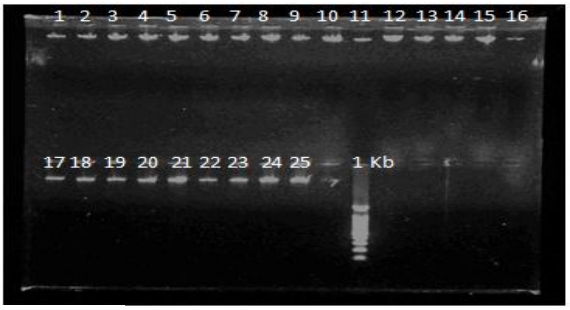

Figure 1. Quality of extracted DNA from analyzed samples. 1-25: cows DNA extracred samples 1kb: Molecular weight marker (Invitrogen DNA Ladder).

DNA samples were amplified and PCR product were shown on agarose gel. As expected, the size of PCR product of the lactoferrin gene was 1143 bp in lenght (Changhong *et al.*, 2009).

Results show the existence of the sequence encoding lactoferrin in all sampled cows. Identification of different genotypes for lactoferrin requires an enzymatic digestion. In our case, digestion was carried out by restriction enzyme *HinfI*. This endonuclease cuts at the restriction

site (5-G ↓ C-3 ANT, 3 -TNA C ↑ G-5) and subsequently allows the revelation of the restriction site if exists. Profile on agarose gel showed that all analyzed individuals were homozygous for the lactoferrin gene (Figure 3). All tested individuals showed a genotype A / A (a band size of 635 bp) (Figure3). The results found are similar to those found by Daly *et al.* (2006), Fagundes and Dornelas (2007), and O'Halloran *et al.* (2009).

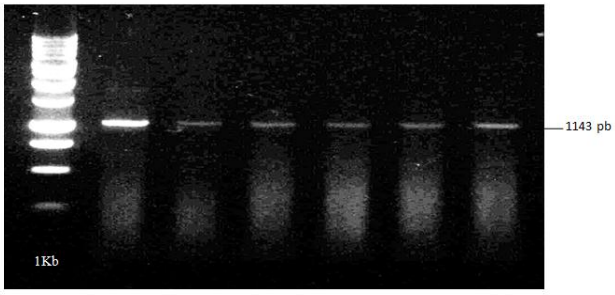

Figure 2. Size of PCR product of lactoferrin gene. 1kb: Molecular weight marker (Invitrogen DNA Ladder).

After digestion, only the A/A genotype was obviously detected. According to Wojdak *et al.* (2006), individuals that are homozygous for the A allele presented the lowest rates of somatic cells. Similar observations were reported by Changhong *et al.* (2009) who confirmed that cows with the A/A genotypes were resistant to mastitis infection.

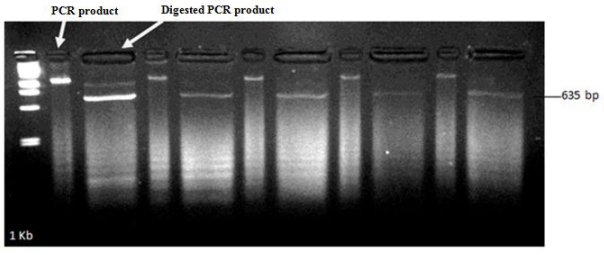

Figure 3. Gel electrophoresis of PCR product after digested with *HinfI* restriction enzyme for detection of lactoferrin gene polymorphism. 1 kb: Molecular weight marker (Invitrogen DNA Ladder).

CONCLUSION

In the present essay, imported cows were sampled in a private Tunisian farm to search for polymorphism in the lactoferrin gene. The results of PCR-RFLP showed the same band pattern in all samples, implying one genotype for the lactoferrin locus. Only the A/A genotype was found in sampled animals. This result can be used to design future studies to determine relations of lactoferrin alleles with resistance to mastitis in Tunisian Holsteins to collect data that may be used in genomic selection breeding animals.

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