ANALYSIS METHODS USED IN GENOMICS RESEARCH ON CATTLE BREEDS

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Abstract

The analysis of the cattle genome, mostly on genetic diversity and population structure, provides useful information for designing effective strategies to improve production, management and conservation of valuable genetic resources, especially of endangered breeds. Bovine DNA analysis involves a series of genetic analysis methods that result in data on the location of genes within the genome, information on certain molecular markers, SNP, etc. At the same time, the application of genomics techniques in the analysis of genetic material in cattle, in order to see the resistance to certain diseases, indicates the importance of the interdisciplinary dialogue between animal health and their genetics. Knowledge of the principles and methods of identifying genetic diversity and evolutionary processes a different species of animals contribute greatly to the implementation of programs for the conservation of valuable genetic resources.

This paper presents the most important methods used in the genomic analysis of cattle breeds, starting from DNA extraction, electrophoresis techniques as well as methods used in the analysis of the PCR reaction.

Key words: DNA, genomics techniques, cattle, genetic diversity

INTRODUCTION

Sequencing the bovine genome has dramatically increased the number of gene polymorphisms available. The combination of these new polymorphisms with variability in the quality of beef (e.g. tenderness, marbling) for different breeds in different rearing systems will be a future perspective. The development of high-performance DNA sequencing techniques and array technology analysis has increased the efficiency of bovine muscle physiology research with the ultimate aim of improving the quality of beef [5]. Significant advances in the genomics and mapping of the bovine genome have identified markers for some deleterious recessive genes, but the broader benefits of marker-assisted selection remain in the future [10]. Availability of genotyping assays has allowed a detailed assessment of the genetic diversity of cattle worldwide. Patterns of genetic differentiation, shared ancestry, and phylogenetic analysis may show the occurrence of gene flow, especially among populations from the same geographical area [8]. Knowledge of genetic diversity is essential for the management and sustainable use of genetic resources in livestock [11]. Indigenous cattle play an important role in the economic sustainability of the marginal and disadvantaged regions, and it is therefore important to preserve and develop their breeding [12]. Livestock breeding has shifted over the last decade to genomic selection. Due to genotype-by-environmental interactions, imperfect linkage imbalances and the existence of dominance and imprinting, purity and crossbred performances are not perfectly correlated. Further progress could be made by assuming realistic covariance structures between the genetic effects of the various breeding lines and by using larger marker panels and

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mixture distributions for the SNP effects [14]. Inbreeding control is a key concern in the management of local endangered breeds, which have often developed unique adaptation characteristics. Currently, genetic marker panels are available which allow the assessment of inbreeding and the occurrence of previous bottlenecks in a population. These panels also allow the identification of genes associated with specific production traits, where reliable phenotypic information is available. Amaral and his collaborators have been able to identify genetic markers that are significantly linked to longevity, reflecting the ability of cattle to remain productive under severe environmental conditions. The results indicate that genomic information can be used to control inbreeding and to implement genomic selection in cattle in order to improve adaptation and production characteristics [2]. Heat stress negatively affects the reproductive performance of milk cows. A study by Sigdel and his collaborators shows that the genetic basis underlying milk cow fertility under the conditions of heat stress. The findings of this study contribute to a better understanding of the genetics underlying the reproductive performance of dairy cattle under heat stress conditions and highlight novel genomic strategies to improve thermotolerance and fertility through marker-assisted breeding [13]. Knowing the structure and genetic diversity of the population is a vital requirement for the design and implementation of a breeding program [1].

MATERIALS AND METHODS

In order to achieve the assumed objectives of this study, there were consulted 14 scientific articles and a book, of different national and international databases. The most important aspects are presented in a different sections including: Molecular genetics techniques in the study on cattle breeds, with sections: a. DNA extraction techniques; b. Electrophoresis techniques; c. Amplification techniques of genes of interest by PCR (Chain Polymerization Reaction). All the articles reviewed in this paper are specialized, published in international journals with high impact factor and at the same time, are the latest research in the field of genetics.

RESULTS AND DISCUSSIONS

By means of the advancement of biotechnology, DNA-based methods are the most effective techniques for characterizing different animal species, as they are rapid and have higher stability under harsh conditions compared to protein-based methods [15]. Genomic data are the foundation for the improvement of breeds, while at the same time exploiting heritable traits and supporting conservation efforts for endangered local cattle breeds [9].

Molecular genetics techniques in the study on cattle breeds

a. DNA extraction techniques

In order to isolate DNA from blood samples, two methods can be applied, namely manual or automatic. Manual DNA extraction involves the use of a special extraction kit. Such a kit can be the Wizard Genomic DNA Purification which has in its composition four solutions: Cell Lysis Solution; Nucleic Lysis Solution; Protein Precipitation Solution and DNA Rehydration Solution. The following steps are taken in this process of extracting DNA from the blood: Cell lysis, Lysis of nuclei and precipitation of proteins, Precipitation and rehydration of DNA. For DNA extraction with this method, is used 300µl of blood, as biological material. The determination of the concentration and purity of the extracted DNA samples can be appreciated by the spectrophotometry technique with different models of spectrophotometers [6], [7]. The automatic method of extracting DNA from the blood provides very good results, both in terms of DNA purity and its quantity (table 1). This method differs from the other in that the blood collected must be frozen in advance. The technique is simple because it only requires the introduction of blood and consumables in the device, which performs DNA extraction, but the method is more expensive. In this case, too, there are three steps: Preparing the device and setting up the software, Dosing the reagents and finally, Adding the consumables [3], [4].

Table 1 The results on the amount of DNA extracted from bovine blood and the purity of DNA, following the application of three different extraction techniques [4]

DNA extraction technique	The amount of extracted DNA (ng/µl)	Purity of extracted DNA
Extraction of bovine blood DNA with Wizard ™ Genomic DNA Purification Kit	351.95±76.81	1.33±0.03
The rapid method of extraction of bovine blood DNA	209.79±13.65	1.46±0.02
The automated method of extracting bovine DNA from the blood.	50.54±15.16	1.77±0.03

In the statistical processing of data on DNA extraction by the three methods, significant differences were found between all the mean values, both between the quantities of DNA extracted and those of its purity level.

b. Electrophoresis techniques

Electrophoresis is a technique of separating molecules according to their molecular mass. Also, logarithmic dependence, migration distance by size, is well respected. Worldwide there are a large number of methods applied for the analysis of major milk protein polymorphisms. The most commonly used include: Capillary Electrophoresis; Polyacrylamide gel electrophoresis under native conditions (NATIVE-PAGE); Polyacrylamide gel electrophoresis under denaturing conditions (SDS-PAGE); Izoelectric Focusing Electrophoresis (IEF Technique); Agarose gel electrophoresis (EGA) [3], [4].

Capillary electrophoresis is a new technique that combines separation mechanisms electrophoresis and automation of chromatography. The extraordinary separation power, analytical speed and extreme sensitivity (a single molecule by laser-induced fluorescence detection) lead to intensive application of capillary electrophores by many of the world's best research laboratories [3], [4].

Electrophoresis (NATIVE-PAGE) is a method based on the principle of protein separation based on electrical charge, molecular mass, and spatial conformation. The technique applies both to the research of major milk protein polymorphisms and to the authenticity of dairy products [3], [4].

Electrophoresis (SDS-PAGE) is based on the principle of protein separation based on molecular weight. A discontinuous polyacrylamide gel having a supporting medium role and a specific substance, sodium dodecylsulfate (SDS) is used to denature the proteins. This technique can be applied to both protein separation and separation of DNA and RNA molecules [3], [4].

(IEF) electrophoresis is an electrophoretic method that separates proteins according to their isoelectric point (pI). Proteins are amphoteric molecules that can have positive, negative or zero electrical charge, depending on the pH in the environment. The electrical charge of a protein is given by the sum of the positive and negative electric charges, which characterize the component amino acids [3], [4].

Agarose gel electrophoresis (EGA) - to migrate the PCR products are used 3% agarose gel and a TBE electrolyte buffer. The agarose gel is previously stained with Midori Green or Ethidium Bromide. After solidification of the gel, PCR products are introduced into each well and the samples will migrate under the influence of an electric current for about 40 minutes. The gel image can be seen under UV light and the size of the amplicons can be estimated based on the distance traveled from the bands of a standard molecular weight DNA [6], [7].

c. Amplification techniques of genes of interest by PCR (Chain Polymerization Reaction)

The PCR technique or polymerase chain reaction, developed in the mid-1980s, revolutionized molecular genetics, making it possible to study and analyze genes by more accessible and very simple methods. This

technique was developed by Kary Mullis in 1983, starting from DNA replication characteristics, namely, with the DNA polymerase enzyme that uses a DNA strand as a template for the synthesis of a new complementary strand. This technique produces an enormous number of copies of DNA sequences (genes) without resorting to cloning.

Restriction Fragment Length Polymorphism (RFLP) technique is based on the hybridization properties that exist between two DNA fragments presenting a high degree of homology (RFLP-based hybridization) and the PCR technique - called PCR-RFLP, in which highlights the polymorphisms existing at the restriction enzyme sites.

AFLP technique - AFLP technique (Amplified Fragment Length Polymorphism) Molecular techniques that allow genetic fingerprints and polymorphisms to visualize differences in DNA samples are based on two principles: probing hybridization or amplification by the PCR reaction. The polymorphism based on the number of tandem rehearsals of satellite motifs is today most used in studying the genetic diversity of animal breeds and plant varieties [3], [4], [6], [7].

CONCLUSIONS

Given that currently the local cattle breeds are in a national genetic preservation program, with major risks in maintaining their genetic resources, one of the main objectives of this research is in the future to implement DNA sequencing techniques in the Genetics Laboratory to obtain data on the genetic diversity of this breeds, and subsequently to apply this information in possible conservation program. The use and testing of new molecular marker research techniques will help clarify aspects of the genetic diversity of local cattle breeds. At the same time, it is possible to establish associations between gene polymorphism and productive, reproductive or adaptation to environmental conditions. Also, the validation of some molecular analysis methods will allow the use of genetic information in national genetic preservation programs.

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