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USE OF PCR TECHNIQUES IN FOOD QUALITY CONTROL

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Abstract

The use of PCR based techniques in food quality control allows fast and sensitive detection of DNA. Few areas of its usage, the quality check of raw materials, identification of microbiological pathogens and identification of food source are explained below. Of these, results on investigation of the halothane locus in a pig breed 'Mangalica' are shown. These animals are resistant to stress, therefore they would not produce PSE meat.

Keywords: PCR, food analysis, PSE.

There are two major requirements for a modern analytical method in food quality control, they are accuracy and speed. Polymerase chain reaction (PCR) based assays not only meet these criteria but offer high sensitivity and specificity. PCR is an *in vitro* DNA amplification process, where a piece of target DNA is multiplied exponentially. The sequence of two oligonucleotide primers determines the amplified DNA fragment by specific hybridization. If an expression of a particular gene is of interest, RNA can also be detected by PCR after reverse transcription to DNA. Amplification of DNA does not need laborious sample preparation, it is fast and fully automated. PCR offers many applications both in basic and applied research, the number of scientific papers published monthly is very high. Therefore, anyone attempting to make a list on the use of PCR would simply fail or oversimplify the subject. Still, following I would like to explain the three major areas in the food quality control, where I use PCR.

Assurance of Supreme Quality Raw Materials

This title can be found mainly in plant or animal breeding programs, screening for disease resistant species or searching for quantitative trait loci (QTL). In pigs, for example, the pale, soft and exudative (PSE) meat is associated with a point mutation in the calcium release channel (CRC)

gene. Analysis of this mutation is therefore necessary to assure high quality meat products, and this may be carried out in a laboratory run by the food manufacturer and not only by the breeder. Our results in this field are summarised in the second part of this paper.

Microbiological Analysis

This is the most critical control point in food processing. Using traditional culturing methods, a reliable analysis requires long time (24–72 hours) and much work. The introduction of DNA based assays in this field leads to very specific and sensitive methods, where results can be obtained in 8 hours. As the specificity of PCR depends on the sequence of the oligonucleotide primers, it is possible to detect a particular species, sub-species or strains of microbes. If an arbitrary oligonucleotide primer, for instance $(GTG)_5$ is used in PCR, a random set of DNA fragments are generated (randomly amplified polymorphic DNA, RAPD). After agarose gel electrophoresis this results in a banding pattern, which is characteristic of the DNA source, in this case of the microorganism. Today there are some commercially available PCR kits for detection of *Salmonella* spp. or *Listeria monocytogenes*. PCR also allows studies on starter cultures during the fermentation process, which is of interest at our department.

Identification of Food Origin

On the market it is extremely important to analyse the origin and the purity of a food product. For such purposes immunochemical assays have been widely used. However, DNA is much more stable than proteins, its integrity is maintained at boiling temperatures. This permits analysis of cooked food. Moreover, the sensitivity of PCR allows the analyst to detect trace amounts of foreign material if it contained DNA (in other words foreign cells). We have developed a DNA isolation method from salami and we are investigating its DNA profile.

DNA techniques offer numerous applications in food quality control, but these methods are still to be standardised. This may be a very difficult task, because only very limited information is available about the DNA sequence of living organisms. I think for the next few years PCR will be used for quick analysis, and those samples in which a specific DNA is detected will be further analysed by traditional methods. These can be further replaced only after observing a strong correlation between the PCR signal and the expected origin of DNA.

Haptotyping Rare Swine Species at the Halothane Locus

Introduction

It is well known that the meat from stress susceptible pigs is pale, soft and exudative (PSE). No effort has been spared to find the gene(s) responsible for this phenomenon, and in 1990 it became clear that a single C to T mutation in the swine calcium release channel (CRC) gene is associated with the stress susceptible phenotype (FUJI et al., 1990). In the animal breeding programs pigs being homozygous for the mutation were eliminated (ARCHIBALD, 1991). However, these animals display some favourable traits such as back fat thickens and bigger litter size (MITCHELL and HEFFRON, 1982). Using PCR-RFLP method we studied the CRC genotype of a rare Hungarian breed 'Mangalica'. It has been introduced in Hungary in 1833 from Serbia. The animals weigh 60–65 kg at the age of one year and 200–300 kg when adult. This breed is almost extinct.

Material and Methods

Blood was collected from a family of three animals at the Research Stud of the University of Veterinary Medicine, Budapest. DNA isolation was carried out using a salting out method of Lahiri and Nurnberger (LAHIRI and NURNBERGER, 1991). In the DNA amplification reaction standard PCR conditions were used with primers described by VÖGELI et al., (1994):

5'-TCCAGTTTGCCACAGGTCCTACCA-3'
5'-ATTCACCGGAGTGGAGTCTCTGAG-3'

For genotyping, the wild type allele was cut with the restriction enzyme Hha I according to the manufacturer's recommendation (Sigma). 25 μl of the digested PCR product was loaded onto a 1% agarose gel and electrophoresis was carried out in 1xTBE for 2 hours at 80 V. The bands were visualized using ethidium-bromide staining and UV detection.

Results

In a preliminary study DNA from a German Landrace pig was used to optimise PCR conditions. Next, from three 'Mangalica' pigs DNA was successfully isolated. Amplification of part of the CRC gene resulted in a PCR fragment of the expected size. Hha I enzyme digestion and subsequent gel electrophoresis revealed that all animals were homozygous for the wild-type allele (*Fig. 1*).

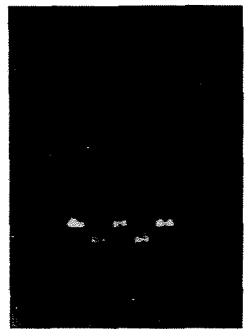

Fig. 1. Analysis of the amplified DNA fragments from three animals using 1% agarose gel electrophoresis. Lanes 1,3,5: after *Hha I* digestion (495 bp). Lanes: 2,4,6 directly after PCR (659 bp). Lane 7; DNA size marker ($\lambda/BstEII$)

Discussion

Popular pig breeds (i.e. Pietran) display a high risk of stress susceptibility. This results in a major economical loss due to their PSE meat. Elimination of the susceptible genotype would lead to loss of favourable traits too. In our work all the investigated 'Mangalica' pigs carried the wild-type allele at the CRC locus. We will further characterize this locus using microsarellite genetic markers. This will shed light on the evolution of the stress susceptible phenotype and probably justify the breeding value of the 'Mangalica'.

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