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Detection of pork in admixed meat and meat products by species-specific PCR technique

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ABSTRACT

The objective of this study was to detect the adulteration of pork in meat and meat products employing PCR assay. A PCR assay was successfully optimized for amplification of 263 bp DNA fragment extracted from pork using designed species-specific primer pairs based on mitochondrial 16S rRNA gene. The optimized PCR assay was subsequently validated for its specificity with DNA extracted from cattle, buffalo, sheep, goat, pig and chicken meat. The primer pair was found to be specific for pork and no cross reaction was observed with other species used in this study. Subsequently, optimized PCR assay was evaluated for its efficiency to amplify the DNA extracted from heat treated meat and meat emulsion. No adverse effect of heat treatments was found on PCR amplification of pork DNA. Thus, the developed primer pairs were found to be specific for pig.

Key words: Pork, meat admixture, heat treated, speciation, PCR

Advances in DNA technology have lead to the development of rapid assay for identification of meat species. Initially, the DNA hybridization was used for this purpose (Chikuni *et al.* 1990, Ebbenhoj and Thomsen 1991), but recently this has been replaced by PCR techniques. Mitochondrial DNA was preferred due to the presence of higher copies per cell, which increases the chances of accurate results even in heat treated processed meat and meat products (Greenwood and Paboo 1999, Bellagamba *et al.* 2001).

The techniques currently used for meat species identification include PCR amplification of primers based on conserved mitochondrial or nuclear DNA, followed by sequencing (Girish *et al.* 2004) or restriction enzyme analysis (Pfeiffer *et al.* 2004, Girish *et al.* 2005, Aida *et al.* 2007). However, species-specific PCR assay was found to be rapid and cost effective for identification of meat species due to specific detection of target sequence, even in heat treated processed meat and meat products without the need of further sequencing or digestion of the PCR products with restriction enzymes (Rodriguez *et al.* 2004) and successfully used for identification of various species of meat (Ilhak and Arslan 2007, Martin *et al.* 2007,Mane *et al.* 2007, Frezza *et al.* 2008).

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This study evaluated a method for porcine species identification in heat treated meat and meat emulsion using designed species-specific PCR based on mitochondrial DNA. The objective was to develop a technique that could be useful for rapid and authentic detection of undesirable meat species in meat and meat products.

MATERIALS AND METHODS

DNA extraction: The DNA was extracted from meat samples of cattle (ox), buffalo, sheep, goat, pig and chicken collected from local slaughterhouses and experimental abattoir of the Institute using blood and tissue kit as per the instructions given by manufacturer. The same kit was also used for extraction of DNA from heat treated meat and meat emulsion.

The DNA was also extracted from blood samples collected from different species. The blood was collected in sterile 15 ml polypropylene tube containing 0.5 ml of 0.5 M ethylene diamine tetra acetate (EDTA) solution, which acts as an anticoagulant. The collected blood samples were also preserved at –20°C till DNA isolation. The DNA samples of certain breeds of different species were also collected from different labs of this institute.

Primer design: Species-specific primer for the detection of pork and chicken DNA was designed based on mitochondrial 16S rRNA gene, following the alignments of available gene sequences from GenBank database using primer designing soft-ware. The primer pair designed was

synthesized from Metabion International, Germany. The details of pig specific primer pair used in the present investigation are given below:

Forward: 5' GCC TGG TGA TAG CTG GTT GTC 3'

Reverse: 5' TGT TTG AGG ATC GGG TTA TGG CC 3'

PCR Amplification of specific fragments: The reaction mixture was prepared in a 500µl in a total volume of 50µl containing 5µl of 10X PCR buffer, 15 mM $MgCl_2$, 200µM each of dNTP, 1–2 Units of *Taq* DNA polymerase (Qiagen, USA), 20 pmol each of forward and reverse primer, 1µl of DNA template (20–30 ng) and remaining nuclease free water.

The PCR conditions programmed on master cycler gradient thermocycler were as follows: initial denaturation at 94°C for 2 min followed by 30–35 cycles of denaturation at 94°C for 0.5 min, annealing at 56.5°C for 0.5 min and extension at 72°C for 1 min. Then final extension was done at 72°C for 5 min. The PCR product was kept at –20°C for further use.

Analysis of PCR Amplified DNA Fragments: The submarine horizontal agarose gel electrophoresis was used for analysis of PCR products using two percent agarose in gel. For that 0.4 g of agarose (Ambion, USA) was put in 20 ml of 1X TBE solution and heated to completely dissolve the agarose. Then 1µl (5%) ethidium bromide solution was added as gel visualizing agent and mixed thoroughly. The electrophoresis was done for 90 min at 80V. The PCR product was finally analyzed using UV transilluminator and documented by Gel documentation system. The ready to use 100 bp was used for in the present work as a molecular marker.

Evaluation of PCR Efficiency in admixed meat and meat products: The sensitivity of the assay was tested in admixed minced meat and meat emulsion (at 10%, 5% and 1%) taking due care during sampling.

Preparation of Meat Emulsion and Heat Treatments: Meat emulsion was prepared with the help of bowl chopper. The pre-weighed quantity of minced meat was blended with 1.7, 0.4, 2% of common salt, sodium tripolyphosphate, sugar respectively and 150 ppm sodium nitrite was added and chopping was done for about 1–2 min. Refined vegetable oil (4%) was slowly incorporated during chopping. Condiments paste, spice mixture and maida were also added at 4, 1.5 and 3% respectively and chopping was continued till desired consistency of emulsion was achieved. The condiments paste was prepared from onion and garlic in a ratio of 4:1, while readymade spice mix of reputed brand was procured from the market.

In the present investigation meat and meat emulsion were heat treated at different temperatures to evaluate applicability of optimized PCR assay in meat and meat products. The heat treatments given to meat and meat emulsions were: steam cooking at 100°C for 45 min and autoclaving at 121°C (15 psi) for 15–20 min.

RESULTS AND DISCUSSION

In the present study PCR assay was developed for rapid and specific identification of pork species in meat and meat products to protect the consumer from fraudulent practices of meat adulteration. The PCR assay was successfully optimized for amplification of 263 bp DNA fragment from pig meat using species-specific primer pairs designed based on mitochondrial 16S rRNA gene (Fig. 1). The primer pair amplified the DNA fragments from pork without any non-specific amplification. The species-specific PCR assay was earlier successfully applied for identification of meat species by various workers (Zhang *et al.* 1999, Mane *et al*. 2007, Mane *et al*. 2009, Ilhak and Arslan 2007, Martin *et al.* 2007, Frezza *et al.* 2008). The species-specific PCR assay was found to be rapid and cost effective and specifically detects the targeted sequence of the species, without the need of further sequencing or digestion of the PCR products with restriction enzymes (Rodriguez *et al.* 2004).

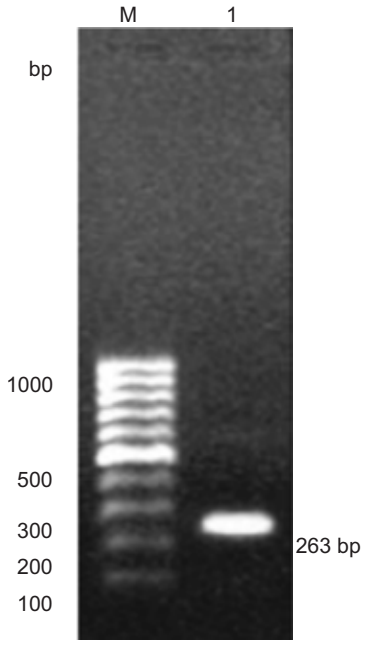

Fig. 1. PCR amplification of mitochondrial 16S rRNA gene of pig extracted from meat.

Lane M: 100 bp Ladder; Lane 1: PCR amplified fragment of pig DNA

The specificity of the developed PCR assay was cross validated with DNA extracted from cattle, buffalo, sheep, goat, pig and chicken meat. For that DNA was extracted from meat tissues of the species under investigation and subsequently PCR amplification was tested under optimized conditions. After repeated testing in the cross validation, it was found that single DNA fragments of 263 bp size was amplified only in pig species DNA without any cross reaction and spurious amplification with other meat species used in this study (Fig 2). For specific detection of meat species in

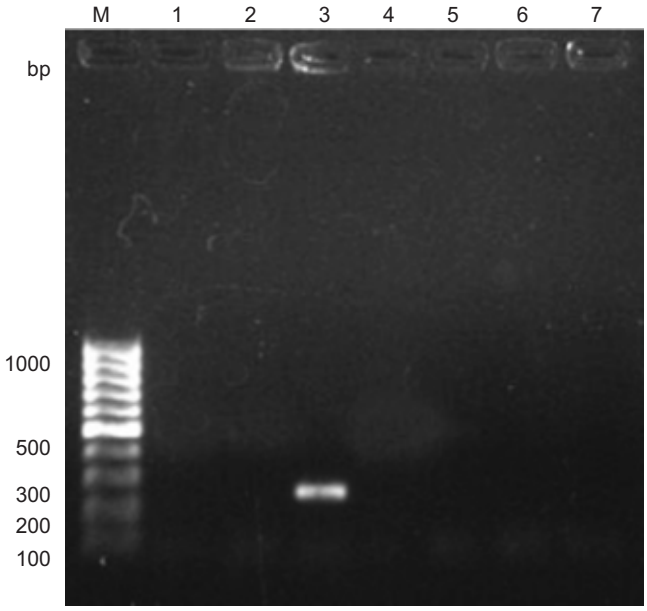

Fig. 2. PCR amplification pattern of mitochondrial 16S rRNA gene with different meat species.

Lane M: 100 bp Ladder; Lane 1: Cattle; Lane 2: Buffalo; Lane 3: Pig; Lane 4: Sheep; Lane 5: Goat; Lane 6: Chicken; Lane 7: Negative Control

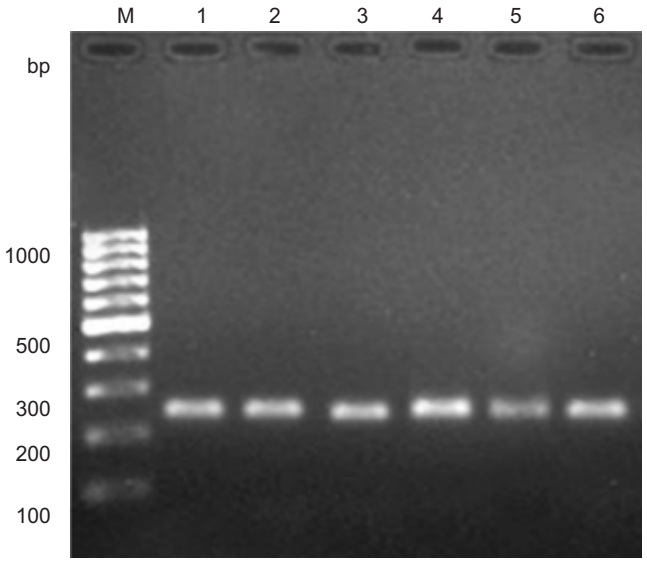

Fig. 3. PCR amplification pattern of mitochondrial 16S rRNA gene in admixed heat treated meat and meat emulsion (at 1% level of adulteration).

Lane M: 100 bp Ladder; Lane 1: Raw Meat; Lane 2: Raw Meat Emulsion; Lane 3: Cooked Meat; Lane 4: Cooked Meat Emulsion; Lane 5: Autoclaved Meat; Lane 6: Autoclaved Meat Emulsion

presence of other meat species DNA is highly valuable for regulating the meat and meat products and protect the consumer from meat adulteration (Hopwood *et al.* 1999).

The developed PCR assay was then evaluated for its efficiency of amplification from the DNA extracted from heat treated meat and meat emulsion. In the experiments, the PCR assay was successfully applied for the DNA extracted from heat treated, admixed meat and meat emulsion, even from autoclaved meat and meat emulsion (120°C/15psi/20 min) without any adverse effect (Fig. 3). In the earlier work, the PCR assay was successfully applied for identification of meat species in heat treated meat and meat products under various processing conditions (Kesmen *et al.* 2007, Mane *et al.* 2007) without any major adverse remarks. However, the amplification pattern was weak and inconsistent in pan fried meat products (Arslan *et al.* 2006). Also no effect of processing conditions and ingredient used for emulsion preparation was observed on PCR amplification. The detection limits was found to be 1% (wt/wt) in meat and meat emulsion. The whole procedure takes hardly 4–5 hrs after DNA extraction to produce the results. The species-specific PCR assay was found to be highly sensitive for specific detection of pork. The technique is rapid and cost effective valuable tool for regulating meat and meat products authenticity. The developed pig specific PCR assay could be useful tool for detection of pork in meat and meat products.

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