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## Rapid Detection of Pork in Processed Food Using Polymerase Chain Reaction Amplification Technology: A Preliminary Report

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**Abstract:** Pork consumption is prohibited in some religions. Therefore, religious people are adamant about importing processed food, which may contain or has been contaminated with pork or swine-derived products. In Sudan, no reliable assays exist for detecting the presence of pork in processed food. Currently, regulatory officials rely on a paper trail for this verification. To address the void in scientific regulatory monitoring, a means of a reliable, rapid, sensitive and specific method for detection of pork in processed food is urgently needed. The swine mitochondrial cytochrome-b (mtcyt-b) gene was used as a target DNA for PCR amplification. Using a pair of primers (PSL1 and PSR4), the mtcyt-b PCR resulted in amplification of a 525 base pair (bp) PCR product. The sensitivity of this mtcyt-b PCR was found to be 100 fg of DNA (equivalent to 1000 copies) as determined by DNA concentration and number of copies of mtcyt-b DNA, extracted from whole blood sample obtained from pigs. The mtcyt-b PCR assay provides a simple, rapid and reliable method for detection and identification of fresh, marinated or cooked pork in processed food produced for human consumption. In addition, this PCR assay should support future policies regarding import regulations for food industry.

**Key words:** PCR, swine-derived products, nucleic acid amplification

### INTRODUCTION

In food industry, quality control and consumer satisfaction require that the origin of materials used in processed food be labeled on the products. Therefore, in some African countries, regulatory officials require labeling of the product for this verification. Fresh pork is a protein in nature, which could be detected by immunologic assays. However, it is rather difficult to identify cooked, marinated or dried pork by immunological assays due to protein denaturing. Therefore, nucleic acid hybridization and sequencing have been successfully applied for animal species identification in human food and animal feed<sup>[1-3]</sup>.

Application of the Polymerase Chain Reaction (PCR) and its adaptation for food analysis have proliferated because of the simplicity, specificity and sensitivity of this technique for the examination of food ingredients<sup>[2]</sup>. These new approaches are alternatives to immunological and DNA hybridization methods. The objective of the present study was to develop a rapid, sensitive and

specific assay for detection of pork or swine-derived products in processed food using nucleic acid amplification technology, commonly known as Polymerase Chain Reaction (PCR).

### MATERIALS AND METHODS

**Collection of blood samples:** Blood samples were collected in clean sterile vacutainers, containing Ethylene Diamine Tetraacetic Acid (EDTA), from different animal species including pigs, cattle, sheep, goat, camel, deer, horse, donkey, chicken and fish. The blood samples were used for extraction of total genomic and mitochondrial cytochrome-b (mtcyt-b) DNA. The extracted DNA was used as the target DNA for PCR amplification.

**Extraction of DNA from blood samples:** Whole blood was used for extraction of mtcyt-b DNA using a commercially available QIAamp blood kit (QIAGEN Inc. Chatsworth, CA, USA) according to the manufacturer's instructions. Briefly, 200 mL of whole

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blood, 20  $\mu\text{L}$  of proteinase K stock solution and 200  $\mu\text{L}$  of lysing buffer were pipetted into 1.5 mL epindorf tube and the mixture was incubated at 37°C for 30 min and then at 70°C for 10 min. Two hundred microliter of absolute ethanol was added to the sample and the mixture was mixed by vortexing. The mixture was transferred to the QIAamp spin column and placed in a clean 2 mL collection tube and centrifuged at 8000 rpm for 1 min. The QIAamp spin column was washed twice using 500  $\mu\text{L}$  of washing buffers and spin for 1 min. The QIAamp spin column was then placed in a clean 1.5 mL epindorf tube and the DNA was eluted with 200  $\mu\text{L}$  double distilled water preheated at 70°C. Maximum DNA yield was obtained by spinning at 12,000 rpm for 1 min. The DNA concentration was determined by spectrophotometer at 260-wave length. Five microliters of the suspended nucleic acid was used as a target DNA in the PCR amplification.

**Extraction of DNA from processed pork:** Extraction of DNA from cooked, smoked and marinated pork was performed basically as described for the whole blood extraction procedure. The marinated pork was further treated by freezing and thawing and finally incubated at 70°C for 10 min to lyse the content of the food sample. The insoluble component of the food sample was discarded by spinning at 8,000 RPM at room temperature, for 1 min. Two hundred microliter of the supernatant was used for extraction of mtcyt-b DNA using the QIAamp blood kit as previously described<sup>[3]</sup>.

**Selection of the primers for PCR amplification:** For mtcyt-b DNA amplification step, oligonucleotide Primers were selected from the published sequences of the swine mitochondrial Cytochrome-b gene and used in these PCR assays<sup>[6]</sup>. Primers (PSL1 and PSR4) were selected for the synthesis of swine-specific PCR product. PSL1 included bases 63-84 of the positive sense strand of the swine mitochondrial mcyt-b gene 5: CCC AGC CCC CTC AAA CAT CTCA. PSR4 included bases 566-588 of the complementary strand 5: ATG TAC GGC TGC GAG GGC GGT AA. The mtcyt-b PCR using primer PSL1&PSR4 would result in a 525 bp product.

All primers were synthesized on a DNA synthesizer (Milligen/Bioscience, a division of Millipore/Burlington, MA) and purified using oligo-pak oligonucleotide purification columns (Glen Research Corporation, Sterling, VA.) as per manufacturer's instructions.

**Polymerase chain reaction:** A stock buffered solution containing 250  $\mu\text{L}$  10X PCR buffer, 100  $\mu\text{L}$  of  $\text{MgCl}_2$ , 12.5  $\mu\text{L}$  of each dATP, dTTP, dGTP and dCTP was prepared in 1.5 mL eppendorf tube. The primers were used

at a concentration of 20  $\mu\text{M}$  and double distilled was added to bring the volume of the stock buffer solution to 1.5 mL. Two microliter of the primers, 5.0  $\mu\text{L}$  of the target DNA and 42  $\mu\text{L}$  of the stock solution were added into 0.5 mL PCR tubes and mixed by vortexing. 1.0  $\mu\text{L}$  of Taq DNA polymerase (Perkin Elmer) at a concentration of 5.0 units was used. All PCR amplification reactions were carried out in a final volume of 50  $\mu\text{L}$ . The thermal cycling profiles were as follows: A at 95°C, followed by 40 cycles of 94°C for 1 min, 57°C for 30 sec and 72°C for 45 sec and a final incubation at 72°C for 10 min. Thermal profiles were performed on a Techne PHC-2 thermal cycler (Techne, Princeton, NJ.). Following amplification, 15 microliters from each PCR reaction containing amplified product were loaded onto gels of 1.0% SeaKem agarose (FMC Bioproduct, Rockland ME) and electrophoresed. The gels were stained with ethidium bromide and the swine-specific PCR products were easily identified following visualization under UV light.

## RESULTS AND DISCUSSION

The results of this study indicated that the mtcyt-b PCR-based assay affords sensitive and specific detection of mtcyt-b DNA in swine blood and pork samples used in this study. The pork -specific 525-bp PCR product was visualized onto an ethidium bromide-stained agarose gel from  $\geq 100$  fg mtcyt-b DNA of swine blood (Fig. 1A). Using 1 pg of pork DNA target, the 525 bp specific PCR products was detected in whole blood from a variety of different swine-derived samples including, fresh pork, marinated and cooked pork (Fig. 2). The amount of 1.0 ng DNA from other animal species including cattle, sheep, goat, camel, deer, horse, donkey, chicken and fish failed to demonstrate the swine-specific 525 bp PCR product (Fig. 3).

Breeding of pigs and consumption of pork by non-muslims is not uncommon in Islamic countries. It is, therefore, becoming a necessity to develop a molecular diagnostic technique for verification of food free of pork or swine-derived products in such countries. Differentiation of sheep and goat meat was shown by comparison of their nucleotide sequences through direct sequencing techniques and digestion of PCR product with restriction endonuclease<sup>[7]</sup>.

Analysis of mitochondrial DNA (mtDNA) with restriction enzymes was proposed to identify mtDNA genotypes among North American cervid: moose, caribou, elk, White-tailed deer, and mule deer. Comparison of nucleotide sequences of the complete mtDNA or isolated genes for example, the gene coding for small ribosomal

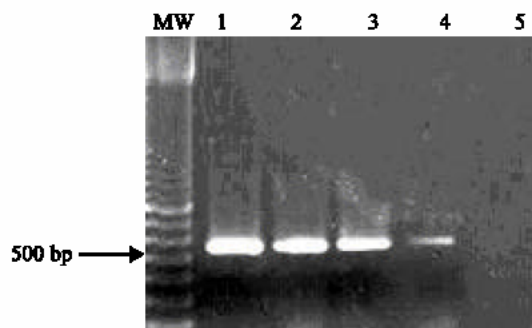


Fig. 1: Sensitivity of the polymerase chain reaction for specific identification of swine mtcyt-b DNA. The 525 bp amplification product was detected from 100 fg (equivalent to 1000 copies of mtcyt-b DNA) extracted from pig blood. Lane M: molecular weight marker (100 bp ladder); Lane 1-5: 100, 1.0 pg, 100, 10 and 1 fg of pig mtcyt-b DNA, respectively

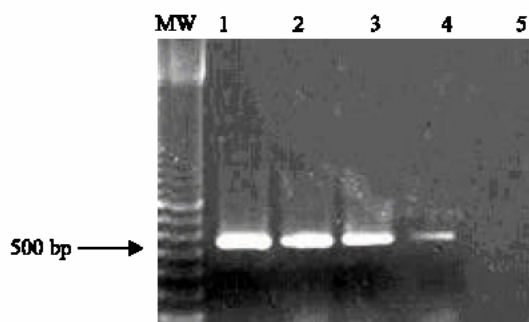


Fig. 2: Detection of the specific 525-bp swine PCR products from pork samples. Lane MW: molecular weight marker; Lane 1-2: 1.0 pg of mtcyt-b DNA extracted from smoked pork; Lane 3-4: mtcyt-b DNA extracted from cooked pork. Lane 5: 1.0 pg of mtcyt-b DNA extracted from sheep (negative control)

RNA (12S and 16S rRNA) and Cytochrome-b (cyt-b) was studied. Amplified genes were used for the study of intraspecific and interspecific variation of animals, including fish, birds and mammals<sup>[8]</sup>. Primers that amplify the whole mtcyt-b gene of approximately 1140 bp were described. Partial amplifications from a wide variety of hoofed mammals for direct nucleotide sequencing of these mtcyt-b segments and comparison of sequences to estimate molecular phylogenetic and mtDNA relationships among species, were also studied using well characterized primers<sup>[9]</sup>.

Analysis of the PCR fragment with endonuclease enzymes to detect Restriction Fragment Length Polymorphisms (RFLPs) was also described<sup>[10,11]</sup> This analysis is useful to differentiate species instead of

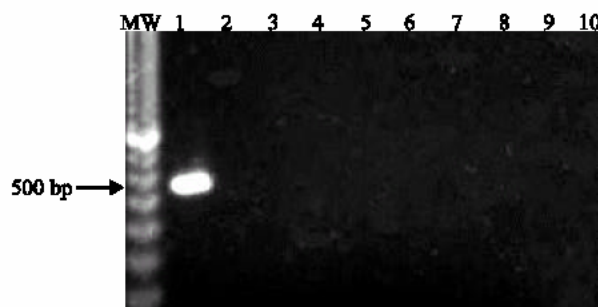


Fig. 3: Specificity of the polymerase chain reaction for specific identification of swine mtcyt-b DNA. The 525-bp amplification product was detected from 1.0 pg of mtcyt-b DNA extracted from pig blood but not from blood of ruminant species including cattle, sheep, goat and deer mtcyt-b DNA of Lane M: molecular weight marker; Lane 1: 1.0 pg of pig mtcyt-b DNA; Lane 2-10: 1.0 ng of mtcyt-b DNA including cattle, sheep, goat, camel, deer, horse, donkey, chicken and fish, respectively

sequencing the common fragment. Application of PCR-RFLP technique was described for differentiation of meat species particularly food animals<sup>[12]</sup>. This technique demonstrated the significant advantage of the coamplification of multicopy mtDNA from various species in comparison to species-specific single-copy gene PCR assay for analysis of a heated and marinated meat mixture. Recently, we reported on a nested PCR assay for specific identification of sheep meat<sup>[13]</sup> and for detection and differentiation of sheep and goat meat<sup>[9]</sup>. Similar studies were described for specific identification of beef or bovine-derived products in commercial animal feed concentrates<sup>[5]</sup>. The described PCR detection assay detected the mtcyt-b DNA from whole blood as well as other tissues such as fat (tallow) and gelatin as the mtcyt-b DNA is present in all tissues of pigs<sup>[1]</sup>. As this assay proved to be a successful procedure for rapid detection of pork or swine-derived products, the next phase will be to carry out field trials and interlaboratory studies to assess their use outside of the research laboratory for the purpose of quality control and consumer's preference or satisfaction. Further studies are currently under way to increase the sensitivity of this PCR assay using nested amplification and to compare it with conventional methods currently used for detection and identification of pork or associated swine-derived products.

The scientific data presented in this study suggested that the swine mtcyt-b PCR protocol, described in this study, could be used as a simple method for rapid detection and identification of pork or swine derived-

derived products in processed food or food ingredients. In addition, the mtcyt-b PCR provides scientific information that could provide a means for verification of the swine protein status in specified food.

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