

Porcine DNA Detection in Finished Meat Products Using Different Mitochondrial DNA (*mt*-DNA) on Polymerase Chain reaction

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Abstract

This study was conducted to detect the presence of porcine DNA in meat products in the market using different mitochondrial (*mt*) DNA on conventional polymerase chain reaction (PCR). Porcine DNA detection in meat products was tested due to some issues associated with the adulteration of food products in Malaysia. This is an important issue especially for Halal authentication which is required for some religious practices such as in Islam and Hinduism. Many techniques have been developed for determining the Halal status of food products. In this paper, Polymerase Chain Reaction method was used to detect the presence of porcine DNA in meat products. Positive and negative controls were always present for each batch of extraction. DNA of raw pork meat was used as a positive control while nucleus free water is used as negative control. DNA of meat products was amplified by using species-specific primer namely *mtATP6* with band size of 83-bp and Pork1 and Pork2 with band size of 531-base pair (bp) mitochondrial (*mt*) DNA D-loop primer to detect pork species. The present study demonstrated that none of the samples were contaminated with porcine residuals but selected samples with pork meat were positive. The species-specific PCR amplification yielded excellent results for identification of pork derivatives in food products and it is a potentially reliable and suitable technique in routine food analysis for Halal certification.

Keywords: Porcine DNA; Detection; Meat products; Mitochondrial DNA; PCR

Introduction

Diversity is one of many very desirable characteristics of processed muscle foods. Processed meats consist of sausages, cured and smoked, non-comminuted meats (ham and bacon), restructured products, and canned products. Processed meats can be successfully manufactured from beef, pork, lamb, venison, chicken, turkey, and seafood [1].

The major authenticity concerns for Muslim consumer in meat and meat products include pork substitution, undeclared blood plasma, using prohibited ingredients, pork intestine casings and non-halal method of slaughter [2]. But in most countries, food manufactures choose to use porcine derivatives because they are cheap and readily available [3].

Pork and its derivative are *Haram* (unlawful or prohibited) to be consumed by muslims. Pork is a typical meat in Malaysian market while, wild boar (*Sus scrofa*) or *babi hutan* is found in the Malaysian rain forest [4]. According to Nakyinsige [2] porcine derivatives used in the meat processing industry include; pork fat (lard), mechanically recovered meats (MRM), porcine gelatine and porcine blood plasma.

Problems related to adulteration of meat species in ground and comminute products have been a widespread problem in some retail markets, while meat species identification is a major global concern [5]. In order to protect Muslim consumers from fraud and adulteration several analytical approaches have been made to identify animal species in food products [6].

PCR identification of species using mitochondrial DNA (*mt*-DNA) gives a series of advantages due to its present in thousands of copies per cell [7]. DNA molecule being the targeted compound for species identification compared to protein due to their high stability and present in most biological tissues.

Protecting Muslim consumer from adulterated food product with non Halal ingredient is a must. The aim of this study was to detect the

porcine DNA and species identification using mitochondrial DNA for Halal authentication.

Materials and Methods

Sample for analysis

A total of 31 (n=31) samples randomly selected from supermarket in Selangor and Kuala Lumpur area which are finished meat product; and two of them made from pork meat; pork sausages and breakfast meatball.

DNA extraction

The DNA extraction for each samples were extracted using Qiagen Blood and Tissue kit. Sample weighing about 100-150 mg of ground finished product were placed into 2.0 ml sterile micro centrifuges tubes. 180 μ l ATL buffer and 20 μ l Proteinase K was added into the micro centrifuges tubes with sample and vortex. The tube was incubated overnight (16-18 hours) at 56°C for cell lyses. After the incubation process complete, the micro centrifuges tube was vortex for 15 seconds to mix thoroughly. Then 200 μ l AL buffer was added and vortex again. After that, the micro centrifuges tube was incubated at 70°C for 10 minutes. After 10 minutes, the sample in tube was vortex and undergo centrifuge at 8000 rpm for 1 minute. 200 μ l ethanol (100%) was added and mix thoroughly by vortex. All the mixed was transferred into spin

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column and centrifuged at 8000rpm for 1 minute. Flow through was discarded. 500 µl AW1 was added to spin column and centrifuges for 1 minute at 8000 rpm. Collection tube was changed and flow through was discarded. Then, 500 µl AW2 was added and centrifuges at maximum speed (13000 rpm) for 5 minutes. The flow through was discarded and centrifuged again at maximum speed for just 1 minute to dry the membrane. After that, the spin column was inserted into micro centrifuges tube and ready for elution step. 100 µl of AE buffer was added on top of membrane and incubated at room temperature for 1 minute. Then, centrifuge at 8000 rpm for 1 minute. The DNA was collected in the micro centrifuges tube. The DNA was stored at -20°C prior to use as DNA template in PCR amplification.

PCR amplification

83-bp primers: PCR amplification was performed in 20 µl reaction volume containing 10 µl ready to use Atlas Taq 2x PCR mix, 1 µl of each forward and reverse primers, 4 µl of 50 ng/µl DNA template and H₂O PCR grade up to 20 µl. The reaction was performed in Eppendorf thermal cycler with the following cycling condition: after an initial heat activation at 95°C for 9 min, 45 cycles were programmed as follows: 92°C for 30 s, 55°C 30 s, 72°C 30 s and final extension at 72°C for 5 minutes. The PCR products were separated by electrophoresis through 2.5% agarose gel with pre-stain red safe.

531-bp primer: Mitochondrial (*mt*) DNA D-loop region fragments namely Pork1 and Pork2 were amplified by PCR. Amplification of DNA were carried out in a final volume of 50 µl in tubes containing 100ng/µl DNA template, 2X PCR Master Mix (BioAtlas), 1 µl of each 100mM primers and nuclease free water (NFW) mark up to volume. Initial denaturation perform for 3 min at 92°C; followed by 30 cycles of amplification at 92°C for 20 s (denaturation), 58°C for 20 s (hybridization) and 72°C for 30 s (elongation) were carried out using thermal cycler (Eppendorf).

Results and Discussion

The production and consumption of poultry products have been on the increase globally (Lasekan et al. 2013) [8]. Recently, issues related to adulteration of food products arose in Malaysia. So identifying the species of meat in the finished meat products is the main target to fulfil Halal requirement and Islamic laws.

Mitochondrial DNA sequence is highly conserved in different species of animals [9]. These present studies describe a conventional PCR method for detection of porcine materials in meat product using different sizes of amplicon of mitochondrion (*mt*) DNA. This method previously described by Montiel-sosa [7], was used for identification of species-specific in pork meat and fat in meat which targeted D- loop region of *mt*-DNA. Yoshida founded *mtATP6* primers with amplicon length 83-bp able to detect the porcine DNA in feedstuff [10].

Result demonstrated on agarose gel shows that the amplification of PCR was done successfully when the band of desired amplicon size was appeared on the gel image. Negative control and positive control always present in every batch of amplification process to determine the ability of the primer used.

Analysis was started with the samples with positive porcine DNA result which are pork sausages and breakfast meatball (pork meat). The result was used to compare with other finished meat product that bought from the markets. Based on the Figure 1 and Figure 2 all pork samples shows the band with 83-bp and 531-bp complimentary with positive control which is the DNA obtain from fresh pork meat.

Based on the figure above, both *mt*-DNA primers are appears to

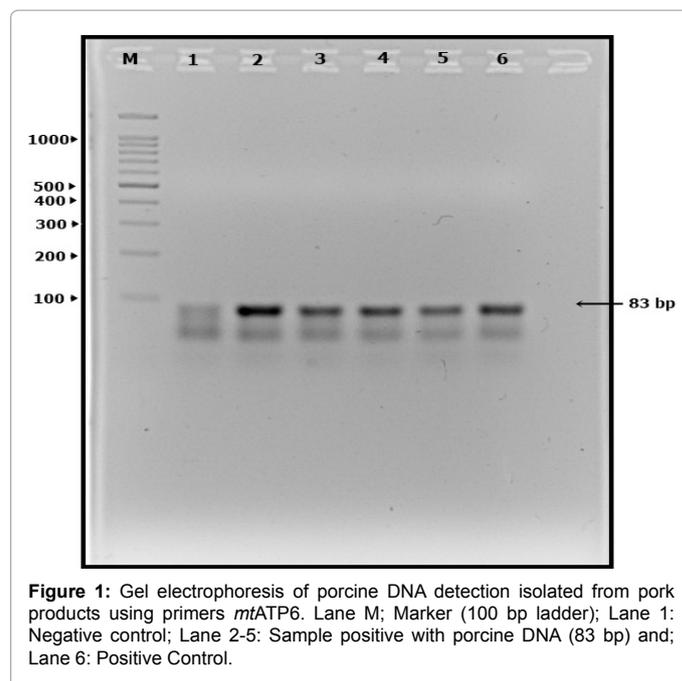


Figure 1: Gel electrophoresis of porcine DNA detection isolated from pork products using primers *mtATP6*. Lane M; Marker (100 bp ladder); Lane 1: Negative control; Lane 2-5: Sample positive with porcine DNA (83 bp) and; Lane 6: Positive Control.

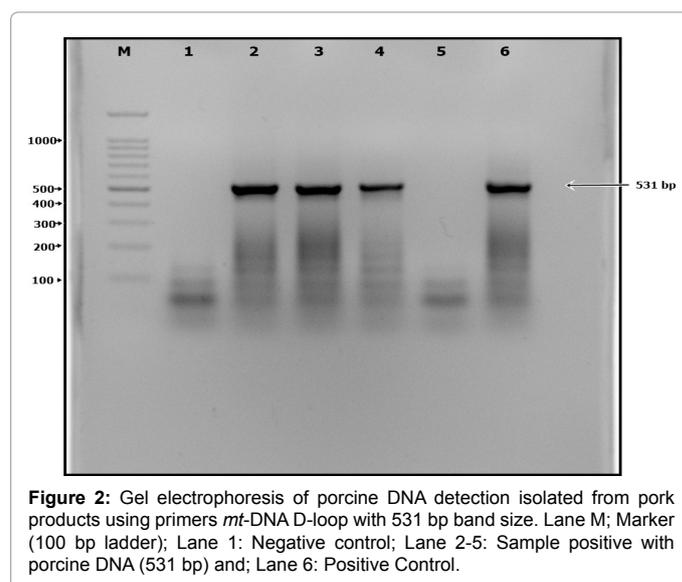


Figure 2: Gel electrophoresis of porcine DNA detection isolated from pork products using primers *mt*-DNA D-loop with 531 bp band size. Lane M; Marker (100 bp ladder); Lane 1: Negative control; Lane 2-5: Sample positive with porcine DNA (531 bp) and; Lane 6: Positive Control.

be highly species-specific that able to detect the presence of porcine DNA in processed food. Studies by Yoshida [10] shown that *mtATP6* clearly detected damage or fragmented porcine DNA in feed effectively. Thus, the amplification process was expended to various types of meat products. Table 1 below shows the results for all samples tested.

Based on Table 1 all processed food, meat based and seafood product are free from contamination, addition or substitution with porcine derivatives except for pork products. The *mt*-DNA primer gives a series of advantages due to its present in thousands of copies per cell.

In this study, different sizes of primers length, 83-bp and 531-bp was used but results obtain shows both primers are highly specific for pork *mt*-DNA. Food products which contain fish, seafood, beef and chicken based does not amplified at 83-bp and 531-bp due to highly species-specific primers.

Sample	Porcine Mitochondria DNA (<i>mt</i> DNA)	
	83-bp	531-bp
1. Ladder (1 Kbp)	Valid	Valid
2. Negative control	Valid	Valid
3. Fish fillet A	Not detected	Not detected
4. Fish fillet B	Not detected	Not detected
5. Fish fillet C	Not detected	Not detected
6. Chicken nugget G	Not detected	Not detected
7. Beef sausages G	Not detected	Not detected
8. Chicken ball G	Not detected	Not detected
9. Chicken lyoner	Not detected	Not detected
10. Fish cake	Not detected	Not detected
11. Chicken frankfurter	NA	Not detected
12. Chicken jumbo	NA	Not detected
13. Beef jumbo	NA	Not detected
14. Streaky beef	NA	Not detected
15. Chicken burger	NA	Not detected
16. Chicken burger R	NA	Not detected
17. Chicken frankfurter F	NA	Not detected
18. Crab stick	NA	Not detected
19. Lamb sausages	NA	Not detected
20. Minced meat	NA	Not detected
21. Chicken nugget P	NA	Not detected
22. Fish sate A	Not detected	Not detected
23. Fish sate B	Not detected	Not detected
24. Squid sate A	Not detected	Not detected
25. Squid sate B	Not detected	Not detected
26. Squid sate C	Not detected	Not detected
27. Fish snack	Not detected	Not detected
28. Fish sandwich	Not detected	Not detected
29. Fish ball A	Not detected	Not detected
30. Fish ball B	Not detected	Not detected
31. Fish ball C	Not detected	Not detected
32. Pork sausages	Detected	Detected
33. Breakfast Meatball	Detected	Detected
34. Positive control (Porcine DNA)	Valid	Valid

*NA-not available

Table 1: Shows the results of Porcine DNA in food products using *mt* DNA primers.

Conclusion

In conclusion, processed food products with damage or fragmented DNA also can be used for DNA detection using specific primers. Both mitochondria oligonucleotide primers from Monteil and Yoshida et al. [7,10] are designed for detection of pork and their derivatives. This method is reliable for porcine detection in meat product and can be used to detect fraud and to ensure that foods comply with religious regulations.

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