

## Halal authentication of raw meats using PCR amplification of mitochondrial DNA

<sup>1,2\*</sup>Sahilah, A.M., <sup>1</sup>Norhayati, Y., <sup>1</sup>Norrakiah, A. S., <sup>1</sup>Aminah, A. and <sup>1</sup>Wan Aida, W. M.

<sup>1</sup> School of Chemical Sciences and Food Technology, Faculty of Science and Technology Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

<sup>2</sup> Institute of West Asian Studies (IKRAB), Universiti Kebangsaan Malaysia

**Abstract:** Chicken (*Gallus gallus*), cattle (*Bos taurus*), goat (*Capra hircus*), pig (*Sus scrofa domestica*) and wild boar (*Sus scrofa linneus*) raw meats were examined using PCR amplification of mitochondrial DNA. Three oligoprimers were used to amplified mitochondria DNA (mtDNA) of *cytochrome b* (two types of primers) and mitochondrial 12s rRNA (mt-12s rDNA) (one type of primer) gene for vertebrate-specific. The amplification PCR products of 359 bp and 531 bp were successfully amplified from the *cyt b* gene of pig and wild boar meats using two type of mtDNA. None of the band was observed for chicken, cattle and goat. While, the amplification product of all meats using mt-12S rDNA gene were successfully produced a single band with molecular size of 456 bp, which were as expected due to all animals were vertebrate-specific. Thus, this primer could not be used to detect the pig DNA in raw meat samples. In the present work, the PCR amplification of mtDNA of *cytochrome b* has been shown as a suitable tool for rapid detection of pig DNA in foods.

**Keywords:** Halal authentication, PCR amplification, and mitochondrial DNA (mtDNA)

### Introduction

Halal is come from Arabic word for permissible which refers to religious acceptable or lawful. In Islam, animal can be categorized into two groups, Halal animals and non-Halal (Haram) animals. Halal animals can be divided into two groups: Halal animal with proper slaughtered through Syariah Law such as cow, goat, sheep, chicken, duck, rabbit and the others which have been allowed in Islam; On the other hand, if all animal mentioned which have not been slaughtered according to Islamic Law, they and their derivatives would still be considered as non-Halal or Haram, and Halal animal without slaughtered such as fish and crustacean. Whereas, pig and its inheritance or any other derived food, dog, rat, and the others which forbidden in Islam are non-Halal or Haram animals.

Adulterations of pig meat in food or processed food are possible due to substitution of high quality meat to cheaper materials (Al- Jowder *et al.*, 1997). The potential use of pig meats are possible as replacement of beef, chicken and goat meat, due to its cheap price. Besides the tight enforcement from local authority such as Department of Islamic Development Malaysia (JAKIM) for Halal certification applications which complied with Halal standards and integrity of Halal, scientific evidence against fraud is vital in supporting the Halal authentication.

The used mitochondria DNA (mtDNA) approaches for the identification of pig species has been reported by several authors due to the mtDNA genes are present in thousands of copies per cell, the large variability of mt-DNA allows identification of precise pig DNA (Monteil-Sosa *et al.*, 2000; Lenstra *et al.*, 2001). In this work, we examined 5 raw meats namely chicken (*Gallus gallus*), cattle (*Bos taurus*), goat (*Capra hircus*), pig (*Sus scrofa domestica*) and wild boar (*Sus scrofa linneus*) with 3 different oligoprimers of mtDNA tool for halal authentication. We included the wild boar (babi hutan) meat which was obtained from Malaysian native (Orang asli) to look at the capability of mtDNA primers in the detection of pig and its related species.

### Materials and Methods

#### Sample preparation

A total of 5 (n=5) raw meats namely chicken (*Gallus gallus*), cattle (*Bos taurus*), goat (*Capra hircus*), pig (*Sus scrofa domestica*) were purchased from retail market in area of Selangor. While, fresh wild boar meat (*Sus scrofa linneus*) was frozen and obtained from Perkampungan Orang Asli, Temerloh, Pahang. All samples were stored in ice container while transporting and kept in refrigerator before used.

\*Corresponding author.

Email: [sahilah@ukm.my](mailto:sahilah@ukm.my), [sahilah.am@gmail.com](mailto:sahilah.am@gmail.com)  
Tel: 03 8921 5444; Fax: 03 8921 5410

### PCR amplification

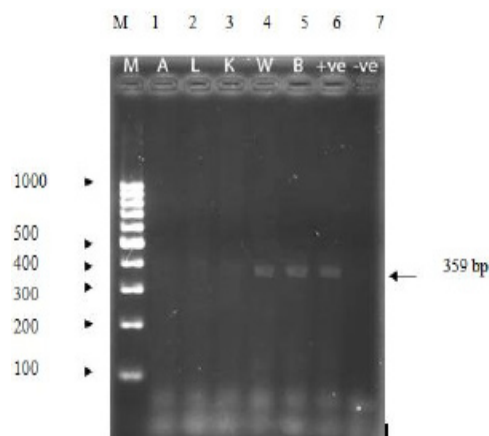
A total of 3 pair primers were utilized in each PCR reaction. Two pairs of mitochondria *cyt b* primers used in this work were described by Lenstra *et al.* (2001) (CYT b1 5'-CCA TCC AAC ATC TCA GCA TGA TGA AA-3' and CYT b2 5'-GCC CCT CAG AAT GAT ATT TGT CCT CA-3' and Monteil-Sosa *et al.* (2000) (Pig F 5'-AAC CCT ATG TAC GTC GTG CAT-3' (15592) and Pig R 5'-ACC ATT GAC TGA ATA GCA CCT-3' (16124)). While, the mitochondrial 12s rRNA (mt-12s rDNA) (12SL 5'-AAA CTG GGA TTA GAT ACC CCA CTA and 12SH 5'-GAG GGT GAC GGG CGG TGT GT-3') gene using universal, vertebrate-specific primers as described by Pandey *et al.* (2007). The primers were supplied from First Base Laboratories (Selangor, Malaysia). Amplification of the mitochondria *cyt b* gene was performed in a final volume of 50  $\mu$ l. Each reaction mixture contained 50  $\mu$ l volume containing 10.0  $\mu$ l of 10X PCR buffer (100 mM of Tris-HCl, 500 mM KCl and 0.1% TritonTMX-100), 4  $\mu$ l of 25 mM MgCl<sub>2</sub>, 0.5  $\mu$ l of 10 mM dNTPs (Promega, Madison, USA), 1.5  $\mu$ l of 5  $\mu$ M each primer (Forward and reverse), 0.5  $\mu$ l of 2.0 units of Taq DNA polymerase (Promega, Madison, USA), 29.0  $\mu$ l of nuclease free water (NFW) and 3  $\mu$ l of 100 ng DNA template. A negative-DNA control was performed by adding 3  $\mu$ l of NFW, a positive control was performed by adding 3  $\mu$ l of the DNA sample. PCR was carried out in Eppendorf thermal-cycler (Eppendorf, Germany) with a temperature program consisting of the initial denaturation at 94°C for 2 min to complete denature the DNA template, followed by 35 cycles of denaturation at 94°C for 15 seconds, annealing for 1 min at 42°C, polymerization at 72°C for 1 min and final elongation at 72°C for 2 min. Negative controls (water) were included in each PCR amplification, in order to verify the PCR efficiency and to detect contamination. The amplification products were analyzed by electrophoresis using 1.0% (w/v) agarose gel in 1X TAE buffer (40 mM Tris-OH, 20mM acetic acid and 1mM of EDTA; pH 7.6) at 90 V for 40 minutes and stained by ethidium bromide. A 100 bp DNA ladder (Vivantis, Malaysia) was used as size reference. The gels were visualized using UV transilluminator (AlphaImager™ Gel Documentation).

### Results and Discussion

Halal authentication is refer to any products which contained pig or its derivatives especially in foods. The used of PCR techniques to amplify the mtDNA of *cyt b* in identifying pig DNA in foods has been reported by several authors (Monteil-

Sosa *et al.*, 2000; Aida *et al.*, 2005; Kesmen *et al.*, 2007; Chandrika *et al.*, 2009). A detection based on mtDNA is popular due to its different specificity expressed in the species or genera through the study of mtDNA. There are approximately 104 copies of mtDNA available per cell compared to only one copy of genomic DNA. Thus it is more efficient to detect species-specific DNA using mtDNA than genomic DNA (Cheng *et al.*, 2003).

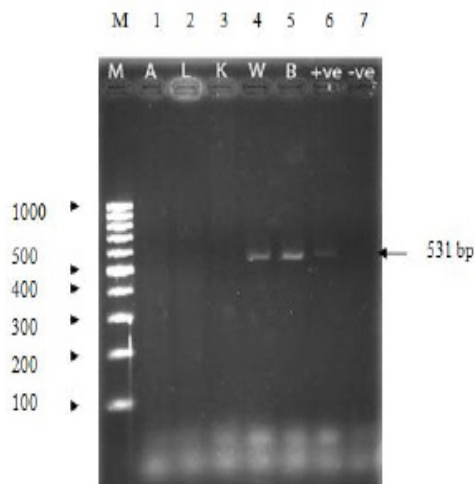
In the presence study, the PCR amplification analysis was conducted on chicken, cattle, goat, pig and wild boar meat using 3 type of mitochondria oligonucleotide primers. Figure 1 shows the PCR amplification products of all meats by mt DNA oligonucleotide primers of *cyt b* as described by Lenstra *et al.* (2001). Using these primers the amplification products of both, pig and wild boar meat produced a single band, respectively with molecular size of 359 bp. While, no band observed on chicken, cattle, goat meat and negative control (Lane C) in each experiment.



**Figure 1.** Specific PCR amplification of raw meats by *cytochrome b* primers of mitochondria DNA (Lenstra *et al.*, 2001). Lane M, 100 bp DNA ladder (molecular weight in base pair, bp); Lane 1, Chicken; Lane 2, cattle; Lane 3, goat; Lane 4, wild boar; and Lane 5, pig; Lane 6-7, positive control and negative control.

It has been reported, using primers as described by Lenstra *et al.* (2001), a fragment of 359 bp would appear for pig, chicken, cattle, goat, turkey, water buffalo, sheep, horse and man. However, our findings were in contrast with Lenstra *et al.* (2001), where no amplified PCR fragments were observed for chicken, cattle and goat in repeated experiments (Figure 1).

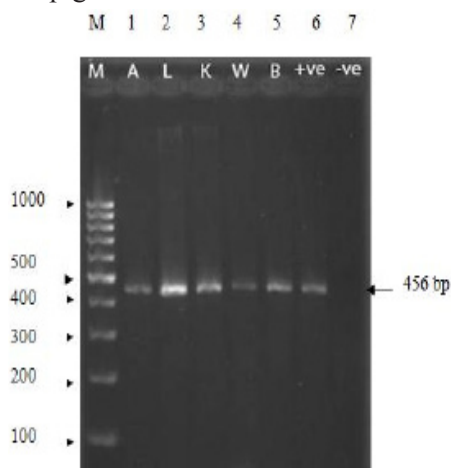
The species-specific mt *cyt b* primers using primers as described by Monteil-Sosa *et al.* (2000), produced a single band with molecular size of 531 bp on pig and wild boar meat as shown in Figure 2. Our finding were in agreement with Monteil-Sosa *et al.* (2000) whose reported that the oligoprimers used were highly specific for pig mtDNA and the amplified PCR product would a fragment with its molecular weight of 531 bp in size. The primers allow



**Figure 2.** Specific PCR amplification of raw meats by *cytochrome b* primers of mitochondria DNA (Montiel *et al.*, 2000). Lane M, 100 bp DNA ladder (molecular weight in base pair, bp); Lane 1, Chicken; Lane 2, cattle; Lane 3, goat; Lane 4, wild boar; and Lane 5, pig; Lane 6-7, positive control and negative control.

an easy detection of pig DNA without conducted any restriction fragment length polymorphisms (RFLP) analysis.

As shown in Figure 3, the amplification product of all meat using mt-12S rDNA gene (Pandey *et al.*, 2007), produced a single band with molecular size of 456 bp. Since all meat samples were vertebrate animal, as expected all meat produced a single band of 456 bp. However, this primer is not useful in the detection of pig DNA.



**Figure 3.** Specific PCR amplification of raw meats by 12S rRNA primers of mitochondria DNA (Pandey *et al.*, 2007). Lane M, 100 bp DNA ladder (molecular weight in base pair, bp); Lane 1, Chicken; Lane 2, cattle; Lane 3, goat; Lane 4, wild boar; and Lane 5, pig; Lane 6-7, positive control and negative control.

## Conclusion

In conclusion, this study suggests an analytical approaches for identification of pig and wild boar DNA based on mtDNA analysis for Halal integrity. The technique is also useful to trace pig meat adulteration which allows the rapid detection of pig DNA in the meat products.

## Acknowledgements

This study was supported by Fundamental Research Grant Scheme (UKM-ST-06-FRGS0010-2008), Ministry of Higher Education (MOHE), Malaysia.

## References

- Aida, A. A. Che Man, Y. B., Wong, C. M. V. L., Raha, A. R. and Son, R. 2005. Analysis of raw meats and fats of pigs using polymerase chain reaction for Halal authentication. *Meat Science* 69: 47-52.
- Al-Jowder, O., Kemsley, E. K. and Wilson 1997. Mid-infrared spectroscopy and authenticity in selected meats: feasibility study. *Food Chemistry* 59: 195-201.
- Chandrika, M., Zainon, M. N., Maimunah, M., Lesley, M. B., Jinap, S. and Son, R. 2009. Meat species identification and Halal authentication analysis using mitochondrial DNA. *Meat Science* 83:57-61.
- Cheng, Y. H., Wen, C. H., Ding, S. T., Kao, C. C. and Kuo, T. Y. 2003. Detecting meat and bone meal in ruminant's feeds by species-specific PCR. *Journal of Animal Feed Science* 12: 851-860.
- Kesmen, Z., Yetim, H. and Sahin, F. 2007. PCR assay for the identification of animal species in cooked sausages. *Meat Science* 77 (4): 649-653.
- Lenstra, J. A., Buntjer, J. B. and Janssen, F. W. 2001. On the origin of meat-DNA techniques for species identification in meat products. *Journal Veterinary Science Tomorrow* 2:1.
- Monteil-Sosa J. F., Ruiz-Pesini, E., Montoya, J., Roncales, P., Lopez-Perez, M. J. and Perez-Martos, A. 2000. Direct and highly species-specific detection of pig meat and fat in meat products by PCR amplification of mitochondrial DNA. *Journal of Agriculture and Food Chemistry* 48: 2829-2832.
- Pandey, P. K., Dhotre, D. P., Dharme, M. S., Khadse, A. N., Hiremath, U. I., Chaudhari, R. D., Patole, M. S. and Shouche, Y. S. 2007. Evaluation of mitochondrial 12S rRNA gene in the identification of *Pantera pardus fusca* (Meyer, 1794) from field-collected scat samples in Western Ghats, Maharashtra, India. *Current Science* 92: 1129-1133.