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Extraction and detection of animal deoxyribonucleic acid (DNA) species on lipsticks using Polymerase Chain Reaction (PCR) assay

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Abstract : Extraction and detection of animal DNA species was conducted by polymerase chain reaction (PCR) assay using universal mitochondria DNA (MtDNA) primers for vertebrate animal (CYT*b*) and species-specific for porcine (SIMp) genes. Prior to DNA extraction, triplicate lipsticks of each samples were pretreated with water and phosphate buffered saline (pH 7.0, 0.1 M) and incubated at 56 °C for 3 hours, before DNA was extracted using commercial Epicentre MasterPure™ Complete DNA Purification Kit. DNA extraction using this kit demonstrated a good DNA recovery. The extracted DNA was then tested using PCR assay and produced amplicons of 359 (CYT*b*) and 398 bp (SIMp) by gel electrophoresis, respectively. DNA extraction method in the present study demonstrated a good quality of DNA recovery and useful for animal species identification using PCR assay.
Keywords: DNA extraction, Lipsticks, Animal species identification, Polymerase chain reaction (PCR), porcine detection.

Introduction

Lipsticks are popular and regularly use among women across entire world. However, the lipstick may content forbidden ingredients from animal source which are not allowed due to religious, health and vegetarian issue. Halal (permissible) quality of cosmetic is required to abide in Muslims daily live and any forbidden animal source such as porcine is not allowable. It is also influence the other communities such as Jews, allergic toward porcine ingredient and vegetarian[1,2]. Lipstick normally contain wax, oils and fats, antioxidants, emollients, coloring and gelatin[3]. Major component of lipstick is wax and its give the structure of the lipstick. Other ingredient such oils and fats is to give a shiny finish to the lips. While, possible reasons emollient and gelatin are added in the lipstick are to make the consumers feel soft when it applies on the lips.

DNA-based techniques of polymerase chain reaction (PCR) techniques are well known as a specific, reproducible, sensitive, rapid processing time and low costs[4]. The DNA stability has offered PCR

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techniques using DNA amplification of specific target gene is feasible. Mitochondria DNA (mtDNA) is the most interested target gene because its DNA is stable and resistant under conditions associated with high temperature, pressure and chemical treatments (Madesis *et al*. 2009). The most mtDNA targeted gene is *cyt* b, the other primer targets used in conventional PCR for detecting porcine DNA include tRNA-ATP8, D-loop, 12S rRNA, 16s DNA, ATP8 and ATP6[5, 6, 7, 8, 9]. However, the ingredients as such in lipstick may influent the recovery of good quality DNA. The major problem in lipstick DNA extraction may come from oils and fats content. Thus, DNA extraction technique is crucial prior PCR analysis.

In the present study, we report a protocol to extract the DNA from lipstick and evaluate it quality using PCR analysis. This study is the step of preventing misconduct of lipstick products for consumer's choice. The PCR analysis is conducting using two pairs of primer that are universal for animal and porcine primers.

Materials and Methods

Lipstick samples

New and unopened lipsticks of ten (n=10) different brands samples were purchased from supermarkets at Kuala Lumpur in June 2016 and namely as lipsticks A to J. The lipsticks were shaved and extraction was carried out in triplicates.

DNA extraction

The shaved lipsticks were pretreated by adding 50 µL of water and 550 µL of Phosphate Buffered Saline (Sigma, USA) (pH 7.0, 0.1 M) and incubated at 56 °C for 3 hours, before transferring the supernatant to a new 1.5 mL centrifuge tube. The genomic DNA was then extracted using the Epicentre Masterpure^{tm} Complete DNA Purification Kit and the amount of Proteinase K and RNase A were increased if necessary. The purity and concentration of the DNA extracted was determined using the Alpha Imager Gel Documentation (DKSH, Germany).

Oligonucleotide primers

The oligonucleotide primers used in this study were universal mtDNA primers of CYTb[10] and species-specific of porcine DNA primers of SIMp [10]. The primers sequence of universal vertebrate using in this study were: *Cyt*b F: 5'-CCA TCC AAC ATC TCA GCA TGA AA-3' and *Cyt* b R: 5'-GCC CCT CAG AAT GAT ATT TGT CCT CA-3'; and SIMp F: 5' -GAC CTC CCA GCT CCA TCA AAC ATC TCA TCT TGA TGA- 3' and SIMp R: 5' -GCT GAT AGT AGA TTT GTG ATG ACC GTA-3'. Both amplicons produced were 359 and 398 bp band, respectively. All of the mtDNA primers were synthesized and supplied by First Base Laboratories Sdn. Bhd. (Selangor, MY).

Polymerase Chain Reaction (PCR) assay

The extracted DNA was then tested using PCR assay using universal mtDNA primers of CYT*b* and species-specific of porcine DNA primers of SIMp. For CYT*b* primers, the assay was performed in a 25 µL containing 1 µL of 50-100 ng DNA, 12.5 µL of the universal PCR Mastermix (Promega, USA), 8.5 µL of sterile distilled water and 1 µL pM each of the forward and reverse primers (Helix Biotech, MY). Amplification was performed in thermocycler (BioRad) with a temperature program consisting of the initial denaturation at 95°C for 2 minutes followed by 35 cycles of denaturation at 95 °C for 30 seconds and annealing at 55ºC for 30 seconds and extension at 72ºC for 40 seconds. The amplicons were analyzed by electrophoresis in a 1.5% (w/v) agarose 1X TBE (0.1 M Tris, 0.1 M boric acid, 0.2 mM EDTA) at 120 Volts for 60 minutes. Gels were stained with ethidium bromide. The amplified fragments were visualized with Alpha Imager (DKSH, Germany).

The PCR assay for species-specific porcine primers was performed in a 25 µL reaction assay contains 1 µL of 50-100 ng DNA, 12.5 µL of the universal PCR Mastermix (Promega), 8.5 µL of sterile distilled water and 1 µL pM each of the forward and reverse primers (Helix Biotech, MY). Amplification was performed in a BioRad Thermal-cycler with a temperature program similar as mentioned above for primers CTY*b*. The amplicons were analyzed by electrophoresis as mentioned above as well as gel visualization of amplified

fragments. Both, agarose gel for primers CTYB and SIMp, the 100 bp ladder (Promega, USA) was used as a DNA size marker, respectively.

Results and Discussion

Ten (n=10) lipsticks samples were tested for the presence of animal DNA species namely as lipsticks A to J. Prior of animal DNA species detection, Limit of detection of species-specific primer SIMp for porcine DNA was examined and the detection limit was as low as 0.01 ng. This concentration was expected as reported by [10] as rapid methods for the identification of meat species and meat products by PCR assay (Figure 1).

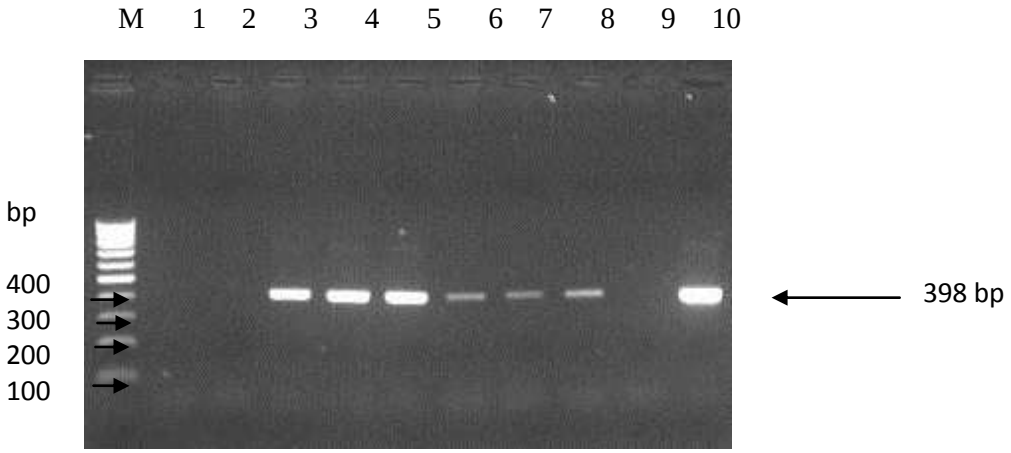

Figure 1: Limit of detection (LOD) for primers SIMp on 1.5% (w/v) agarose gel. Lane M: 100 bp ladder; Lane 1: Negative control; Lane 2: Water; Lane 3: 20 ng; Lane 4: 10 ng; Lane 5: 5 ng; Lane 6: 1 ng; Lane 7: 0.1 ng; Lane 8: 0.01 ng ; Lane 9: 0.001 ng; Lane 10: Positive control (Porcine DNA).

Of 10 lipstick samples, 3 were positive (Lipstick C, G and J) towards universal mtDNA primers of CYT*b* [10]. As indicated in Figure 2, lipstick C, G and J showed 359 bp bands for CYT*b* primers. Since the primers were universal for any vertebrate animal, the clear band were also shown for porcine and mutton meat DNA (Figure 2).

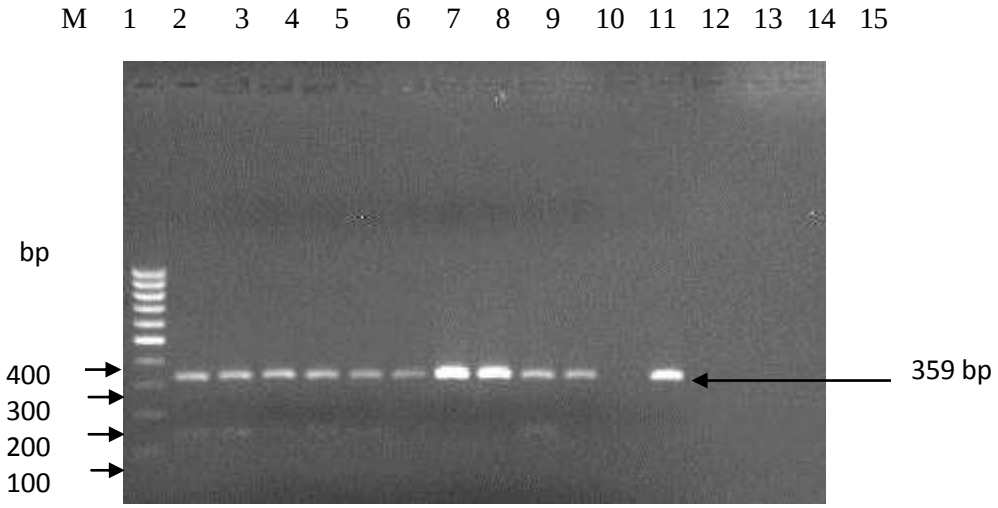

Figure 2 : A ns of animal DNA isolated from lipsticks using CTY*b* primers. Lane M: 100-bp ladder; Lane 1-3: Lipstick C; Lane 4-6: Lipstick G; Lane 7-9: Lipstick J; Lane 10: mutton; Lane 11: Negative control; Lane 12: porcine (positive control), Lane 13: water

Lipstick C, G and J were then tested for porcine DNA, as shown in Figure 3 the said lipstick samples were also positive toward porcine DNA using species-specific SIMp primers. The faded bands indicated the original concentration of DNA in PCR assay may be low in concentration, thus low intensity of the bands produced on the agarose gel analysis. Although the DNA was low it has sufficient information to allow

identification [2, 8]. While, lipstick samples A, B, D, E, F, H and I were negatives either toward universal mtDNA (CYTb) or porcine DNA using SIMp primers.

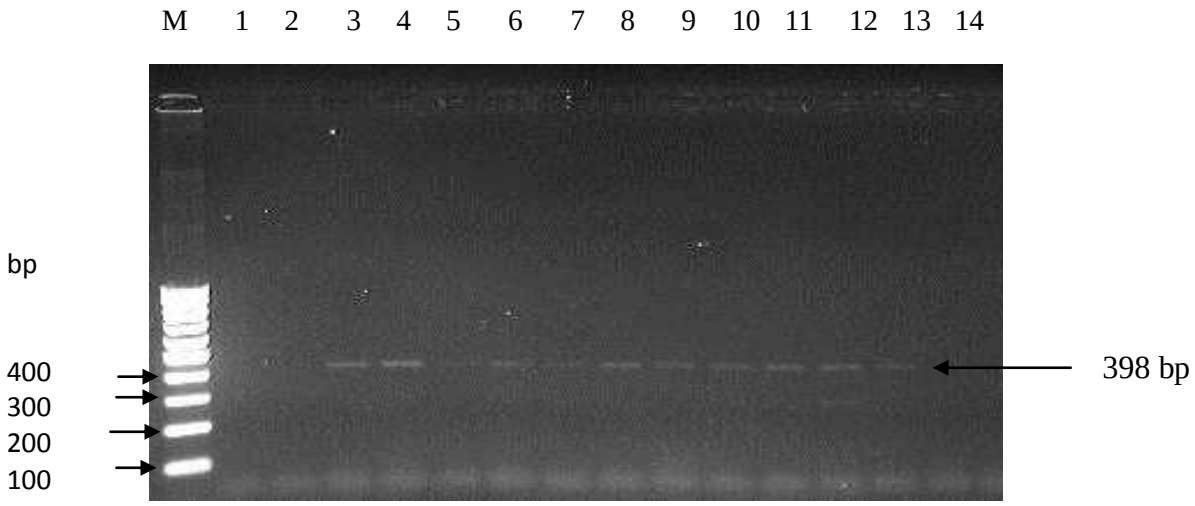

Figure 3: Amplicons of animal DNA isolated from lipsticks using primers SIMp. Lane M:100-bp ladder; Lane 1: Negative control; Lane 2: Water; Lane 3-5: Lipstick C; Lane 6-8: Lipstick G; Lane 9-11; Lipstick J; Lane 12: Positive control (porcine); Lane 13: 0.01 ng of porcine DNA; Lane 14: mutton

Conclusion

In conclusion, the DNA extraction method used in the present study demonstrated a good DNA quality leading to the removal of inhibitors, thus useful for animal DNA species identification in lipstick samples.

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