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ARTICLE

Identification of Pork Adulteration in Processed Meat Products Using the Developed Mitochondrial DNA-Based Primers

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Abstract

The identification of pork in commercially processed meats is one of the most crucial issues in the food industry because of religious food ethics, medical purposes, and intentional adulteration to decrease production cost. This study therefore aimed to develop a method for the detection of pork adulteration in meat products using primers specific for pig mitochondrial DNA. Mitochondrial DNA sequences for pig, cattle, chicken, and sheep were obtained from GenBank and aligned. The 294-bp mitochondrial DNA D-loop region was selected as the pig target DNA sequence and appropriate primers were designed using the MUSCLE program. To evaluate primer sensitivity, pork-beef-chicken mixtures were prepared as follows: i) 0% pork-50% beef-50% chicken, ii) 1% pork-49.5% beef-49.5% chicken, iii) 2% pork-49% beef-49% chicken, iv) 5% pork-47.5% beef-47.5% chicken, v) 10% pork-45% beef-45% chicken, and vi) 100% pork-0% beef-0% chicken. In addition, a total of 35 commercially packaged products, including patties, nuggets, meatballs, and sausages containing processed chicken, beef, or a mixture of various meats, were purchased from commercial markets. The primers developed in our study were able to detect as little as 1% pork in the heat treated pork-beef-chicken mixtures. Of the 35 processed products, three samples were pork positive despite being labeled as beef or chicken only or as a beef-chicken mix. These results indicate that the developed primers could be used to detect pork adulteration in various processed meat products for application in safeguarding religious food ethics, detecting allergens, and preventing food adulteration.

Keywords food authentication, PCR, processed meat products

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Introduction

Detecting the adulteration of processed meat with unwanted food ingredients is one of the most important food quality-related issues. Food ingredient authentication is related to human health since ingredients may include allergenic or toxic substances. Furthermore, certain groups of people will not eat specific meats because of their religious food ethics and preferences (Ortea *et al*., 2012). Hsieh *et al*. (1997) reported that multispecies adulteration were found in commercial meat products. In addition, products labeled beef only are often intentionally adulterated with pork owing to the economic advantage that pork provides. This is found to be especially in the countries having expensive beef such as Korea, Japan, China, and so on (Singh and Neelam, 2011; Soares *et al*., 2013).

Food labeling regulations in many countries require that the meat species used in processed meat products must be declared for consumers because of religious food ethics, medical purposes, and personal food preferences (Doosti *et al*., 2014). However, processed meat products are still mislabeled for meat species, especially pork, which is either intentional or accidental (Tanabe *et al*., 2007). An accurate detection method for pork therefore needs to be developed for the prevention of food adulteration.

Existing detection methods for the identification of pork in processed meat products rely on protein or DNA analysis. Protein-based analytical methods include immunological assays (Anguita *et al*., 1996; Chen and Hsieh, 2000), chromatography (Chou *et al*., 2007), and peptide examination (Aristoy and Toldrá, 2004). However, protein-based analytical methods may not be appropriate for processed meat products since proteins can be denatured during processing. On the other hand, DNA-based analyses such as the polymerase chain reaction (PCR), real time PCR, PCR-restricted fragment length polymorphism (PCR-RFLP), and species-specific PCR are used more frequently in identifying fraudulent meat products (Man *et al*., 2007; Murugaiah *et al*., 2009; Soares *et al*., 2010), since these methods can detect small amounts of DNA and amplify specific target regions (Saiki *et al*., 1988). DNA-based analyses also have numerous advantages including simplicity, rapidity, sensibility, and specificity (Lockley and Bardsley, 2000).

The objective of this study was to design primers for the PCR amplification of a specific region of pig mitochondrial DNA and subsequently use these primers to identify mislabeled processed meat products.

Materials and Methods

Identification of pork-specific mitochondrial DNA sequences for primer design

Pig, cattle, chicken, and sheep mitochondrial DNA sequences were obtained from GenBank (database accession no. AF034253, V00654, AY235570, and AF010406, respectively) and aligned using the MUSCLE multiple sequence alignment program (http://www.ebi.ac.uk/Tools/msa/muscle/). The regions most specific and unique to pork were identified among the aligned sequences using BLAST, and primers were subsequently designed. Sequences of the final selected primers were as follows: Pork-Forward (F) (5'- GGT TCT TAC TTC AGG ACC ATC-3'), and Pork-Reverse (R) (5'- GTG TAC GCA CGT GTA TGT AC-3') (Table 1).

Processed meat sample preparation

To evaluate primer sensitivity, ground meat samples were purchased from butcher's shop, and processed meat samples were prepared by mixing ground pork (boston butt), ground beef (shank), and ground chicken (breast) as follows: i) 0% pork-50% beef-50% chicken, ii) 1% pork-49.5% beef-49.5% chicken, iii) 2% pork-49% beef-49% chicken, iv) 5% pork-47.5% beef-47.5% chicken, v) 10% pork-45% beef-45% chicken, and vi) 100% pork-0% beef-0% chicken. All mixtures were kneaded by hands, subsequently placed into Eppendorf tube, and cooked in a water bath at 70°C for 3 min. The cooked meat was minced with a knife, and 200 mg was used for DNA extraction.

Monitoring pork adulteration

A total of 35 packaged meat products, such as 14 patties, 8 nuggets, 8 meatballs, and 5 sausages were purchased from a commercial vendor in Itaewon, Korea. Most products were frozen and some products were refrigerated. The product labels indicated that purchase products were manufactured with only chicken, beef, or mixture of these two meats. All products were stored at -20°C until used in the experiments.

DNA extraction

Genomic DNA was extracted from 200 mg of each of the processed meat products using the PowerPrep^{TM} DNA Extraction from Food and Feed Kit (Kogenbiotech Co., Ltd., Korea). Six hundred microliters of lysis buffer A and 40 μL of lysis buffer B were added to 200 mg of each of the processed meat products, followed by incubation at 65°C for 1 h. Thereafter, 400 μL chloroform was added and centrifuged at 12,000 rpm for 10 min. Two hundred microliters of the supernatant was mixed with 200 μL binding buffer and 200 μL isopropanol, and the mixture was transferred into the DNA binding column. The DNA

Table 1. Primer sequences specific for the pig mitochondrial DNA D-loop

Primer	Sequence (5' → 3')	Length of base pairs (bp)	Length of PCR product (bp)
Pork-F	GGT TCT TAC TTC AGG ACC ATC	21	294
Pork-R	GTG TAC GCA CGT GTA TGT AC	20	

was washed twice with 75% ethanol and finally the DNA was eluted by 100 μL distilled water.

PCR assay

The PCR amplification was performed in a 50 μL reaction volume using 25 μL *Taq* PCR Master Mix Kit (Qiagen, Germany), 2 μL of each primer, 2 μL extracted DNA, and 19 μL of distilled water. The amplification profile was an initial denaturation step at 95°C for 5 min, followed by 40 cycles at 95°C for 20 s, 55°C for 20 s, and 72°C for 30 s. In order to confirm amplification of the target sequence, the PCR product was electrophoresed on a 2% agarose gel in 1×TAE buffer (Biosesang, Korea) at 100 V for 20 min. A 100-bp DNA Ladder (Dynebio Inc., Korea) was used as the size marker and images were captured in a UV-transilluminator (TF-20M, Vilber Lourmat, France).

Results and Discussion

Food adulteration has been an issue for many years in processed meat products. In particular, pork is often mixed in other meat products such as beef, because it is cheaper. Thus, various pork detection methods have been developed. Among these methods, species-specific oligo Vilber Lourmat nucleotide primers have been used to detect pork in processed meat products. This study targeted the 294-bp long pig mitochondrial DNA D-loop region since mitochondrial DNA is highly conserved in many animal species, is stable from heating, and can be used to detect pork fat (Montiel-Sosa *et al*., 2000). This suggests that the primers designed in our study could be used to detect pork in heat-treated products.

There are two methods to detect pork adulteration in processed meat products, namely protein and DNA analysis. DNA-based methods are used more frequently than protein-based methods to detect adulterated food, because of the limitations of protein-based methods such as protein denaturation following heating (Fajardo *et al*., 2010). Meat mixtures were prepared with the inclusion of pork at a concentration of 0%, 1%, 2%, 5%, 10%, and 100%, and subsequently heated to determine primer compatibility and detection limits. Study results indicate the developed primers could detect as little as 1% pork in the heat treated meat products (Fig. 1).

To monitor pork adulteration in commercial processed meats with the primers developed in our study, 35 processed meat products were purchased including patties, nuggets, meatballs, and sausages labeled as 0% pork. Of the 35 products, three (8.6%) were pork-positive (Table 2). The positive samples were one meatball and two sausage products. The meatball was labeled as beef only, and the sausages were labeled as beef and chicken, and chicken only. Murugaiah *et al*. (2009) reported that adulteration occurs predominantly in comminute or ground meat products. In our study, all pork-positive samples were from ground meat products.

Most ground meat is accidentally adulterated with unwanted meat species during the process of grinding. It is, thus, essential that grinders are thoroughly cleaned when changing meat species, alternatively different meat grinders should be used for different meat species. However, pork is also intentionally added to beef products because of production costs (Doosti *et al*., 2014). In many countries, labels declaring the specific meat species used are

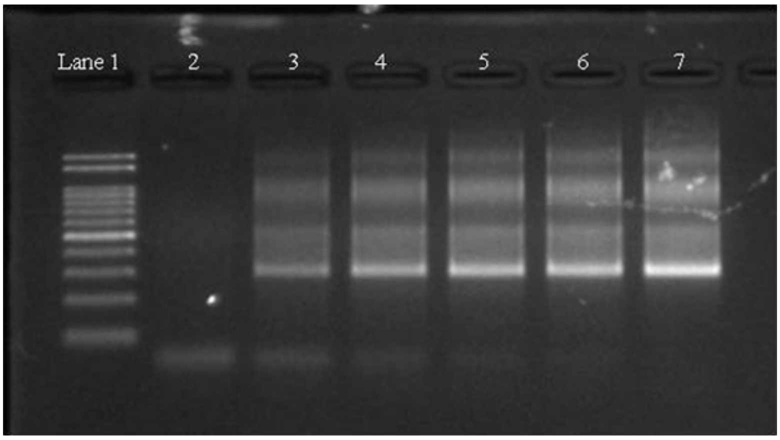

Fig. 1. Agarose gel electrophoresis of PCR products amplified from heated meat; Lane 1: Marker, Lane 2: 0% pork, Lane 3: 1% pork, Lane 4: 2% pork, Lane 5: 5% pork, Lane 6: 10% pork, Lane 7: 100% pork.

Table 2. Detection of pork adulteration in commercial processed meat products by PCR

Sample no.	Product category	Labeling	Company	Presence of pork
1	Patties	Beef	A	-
2	Patties	Chicken	B	-
3	Patties	Chicken	C	-
4	Patties	Vegetables	D	-
5	Patties	Chicken	E	-
6	Patties	Beef	E	-
7	Patties	Chicken	F	-
8	Patties	Chicken	G	-
9	Patties	Chicken	H	-
10	Patties	Beef	H	-
11	Patties	Vegetables	I	-
12	Patties	Beef	J	-
13	Patties	Beef	K	-
14	Patties	Beef	L	-
15	Nuggets	Chicken	M	-
16	Nuggets	Chicken	N	-
17	Nuggets	Chicken	N	-
18	Nuggets	Chicken	O	-
19	Nuggets	Chicken	B	-
20	Nuggets	Chicken	P	-
21	Nuggets	Chicken	Q	-
22	Nuggets	Chicken	R	-
23	Meatball	Beef	S	-
24	Meatball	Beef	T	-
25	Meatball	Chicken	F	-
26	Meatball	Chicken	F	-
27	Meatball	Chicken	F	-
28	Meatball	Beef	J	-
29	Meatball	Beef	U	+
30	Meatball	Beef	V	-
31	Sausage	Beef+ Chicken	W	+
32	Sausage	Fish	X	-
33	Sausage	Fish	X	-
34	Sausage	Beef	Y	+
35	Sausage	Beef	Z	-

mandatory because of food allergens, religious food ethics, and prevention of food fraud (Ayaz *et al*., 2006; Gendel, 2012; Soares *et al*., 2013); however, as shown in Table 2, adulteration still occurs in commercial products. It is therefore important that an accurate and simple method to detect pork adulteration in processed meat products is developed.

In conclusion, the primers developed in our study could be used to detect the presence of pork adulteration in various processed meat products, even when heat treated. In addition, pork adulterated commercial meat products were identified, and thus, government agencies should consider monitoring mislabeling of meat products that include pork.

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