DETECTION OF PIG CYTOCHROME B IN BEEF SAUSAGE FOUND IN TRADITIONAL MARKETS AROUND JAKARTA

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Abstract

Processed fast food products are currently in great demand by the people of Indonesia, especially with basic ingredients such as beef, chicken or other meats. Sausage is a fast food processed food that is at risk of mixing other food ingredients that can not be known by consumers. The halal of food products is considered very important by Muslim community and is taken into consideration in consuming food products. The food category in Indonesia still often mixes or falsifies halal food with prohibited ingredients, one of which is processed products such as sausages. Contamination of processed food products based on sausages was found to contain pig contamination circulating in the one of market in Indonesia by testing as many as 5 out of 6 sausage samples were detected positively contaminated with pig which resembled a Polymerase Chain Reaction (PCR) product sequencing results of 95% with *Sus scrofa* species. The purpose of this study is to detect pig contamination in processed food products such as sausages sold in traditional markets and modern markets and the benefits of this research to detect *cytochrome b* pig genes in sausages and provide information to the public about the halal status of sausages on the market. The research method uses the PCR Technique which is able to be used as a tool to detect contamination of other meats with using a pig specific primer. Sausage samples was obtained from around Jakarta. DNA of pigs and bovine was extracted as positive and negative controls, as well as testing all of sausage samples. The results showed that from all of sausage samples amplified by PCR there were no pig *cytochrome b* genes. This finding informed that the sausages collected from market around Jakarta were negative to pig components.

Keyword: Halal, Sausage, Cytochrome b

1. INTRODUCTION

Food products contaminated with prohibited ingredients were still often occur in halal food one of which is sausage. Food product contaminants found in the one market Indonesia with test samples are sausages, meatballs, shredded meat, satay, beef jerky, and nuggets. Food product samples from 109 samples that tested positive for processed food contained pig (Gusti, 2014). The same thing also found contamination of processed beef based sausage products circulating in the market Indonesia. 5 out of 6 samples of beef sausages were detected positively contaminated with pig which has a similarity of 95% PCR product sequencing results with Sus scrofa species (Priyanka, 2017)

Various techniques to identify the types of meat used in processed food products continue to be developed in order to avoid having food mixed with other meat ingredients (Priyanka, 2017). The purpose of this study was to detect pig in processed food products such as beef sausages sold in markets around around Jakarta (Tradisional markets). While the benefits of this research are to detect the *cytochrome b* pig gene in beef sausages and provide information to the public about the halal status of beef sausages on the market.

2. MATERIALS AND METHODS

2.1. Sample Preparation

The research was implemented at Molecular Laboratory of Al Azhar University Indonesia. The research was conducted from February 2019 to December 2019. The sausage sampling areas in Table 1 from traditional market in around Jakarta. Sausage samples that have been collected are then separated from the plastic casing on the outside of the sausage and cut into small pieces inside a petri dish. Total sampling obtained as many as 12 samples.

Table 1. List of sausage sample

Market	Sample Code
Market 1	P1S1
	P1S2
	P1S3
	P1S4
Market 2	P2S1
	P2S2
Market 3	P3S1
	P3S2
	P3S3
	P3S4
Market 4	P4S1
	P4S2

2.2. Tools And Materials

The tools used tube eppendorf 2 ml, microtube PCR, micro pipettes [PIPETMAN] (10 µl, 200 µl, 1000 µl µl), Tip (10 µl, 200 µl, 1000 µl µl), vortex, centrifugation, waterbath shaker, refrigerator 4°C, Freezer 20°C, Laminar air flow, electrophoresis, PCR, UV, autoclave, Dneasy mericon food, QIAquick Spin Coum Kit, hot plate, Analytical scales, gloves, masks, scalpels, mortars, petri dishes, marker pen.

The materials used sausage samples with 12 brands, fresh beef, fresh meat pig, *cytochrome b* specific primers, 70% alcohol, liquid nitrogen, buffer lysis (Tris-HCL, EDTA, NaCl), SDS 20%, NaCl 6M, isopropanol, PCI solution, CI solution, 70% ethanol, parafilm paper, proteinase K (Bioline), food buffer lysis, chloroform, TAE buffer, PB buffer, AW2 buffer, EB buffer, myTaqTM HS Red Mix, loading dye, DNA ladder, Visafe Red gel stain, agarose. The primary used is a pig-specific porcine DNA primer that amplifies *cytochrome b* fragments in pigs in the order of primary bases (Table 1) (Tanabe, et al., 2014)

Table 2. Base sequence and Primary

		Sequence
Pig (Porcine DNA)	Forward Primary	5'-CTTGCAAATCCTAACAGGCCTG-3'
	Reverse Primary	5'-CGTTTGCATGTAGATAGCGAATAAC-3'

2.3. Step Research

2.3.1. DNA Extraction From Pig and Bovine

Pig raw meat were cut into 100 mg sterile in Laminar air flow. The sample is taken in the middle, the sample is frozen with liquid nitrogen and crushed until smooth, the sample is put into 2 ml microtube. A buffer lysis solution (Tris-Hcl, EDTA, NaCl) was added as much as 400 µl, 20% SDS as much as 40 µl and proteinase K as much as 20 µl. Samples were incubated at 65°C (1 hour). Samples were added 6M NaCl as much as 300 µl and vortexed in 30 seconds. Subsequently the sample was centrifuged at 10,000 xg (30 minutes). The supernatant is removed and transferred to a new tube, then an isopropanol solution is added and incubated in -20°C (1 hour). Samples were centrifuged 10,000 xg (20 minutes). Pellet DNA is taken or discarded with isopropanol solution and 70% ethanol is added as much as 300 µl, then ethanol is discarded and the tube is air dried. The final step was added ddh2o for 50 µl.

2.3.2. DNA Extraction of Sausage Samples using the QIAGEN Kit

Sausage samples are cut and taken in the middle. The sample weighed 200 mg. The sample is frozen with liquid nitrogen and crushed until smooth. Samples were put into a microtube, and 1 ml of food buffer lysis was added and 2.5 K proteinase K was added. Samples on Vortex (15 seconds). The samples were incubated in a 60 ° C waterbath (30 minutes), then placed at a

room temperature of 15-25°C. Samples were centrifuged 2,500 xg (5 minutes). 500 µl of chloroform was put into a new microtube and 700 µl of the supernatant was put into a microtube containing chloroform, then the sample was vortexed (15 seconds) and centrifuged 14,000 xg (15 minutes). 1 ml of PB buffer is inserted into a new microtube and added the phases of the previous vortex and centrifugation results, then the vortex. The supernatant mixture and PB buffer were put into the QIAquick Spin Colum (Kit) 600 ml, then centrifuged 17,000 xg (1 minute). The solution at the bottom of the tube is discarded and refilled with the remaining 600 µl of the supernatant mixture and PB buffer, then centrifuged 17,000 xg (1 minute), then the bottom solution is thrown back. The AW2 buffer was put into 500 µl into the QIAquick Spin Colum (Kit) and centrifuged 17,000 xg (1 minute), then the solution was discarded. The tube is re-centrifuged (1 minute) 17,000 xg. The final step was added EB buffer to the 100 µl QIAquick Spin Colum (Kit) and centrifuged at 17,000 xg (1 minute).

2.3.3. DNA Quality Check

Gel Agarose 1% make by mixing 0.25 grams of agarose and 25 ml TAE 1x, then the mixture is heated and homogenized with a magnetic stirer. After it was cooled ± 5 minutes added Visafe Red Gel Stain (2 µl). The gel is poured into a balanced electrophoresis mold plate that has been set in a comb, then the gel is left to harden for ± 20 minutes. The gel was placed on an electrophoresis device and soaked in TAE buffer 1x. A 1 µl loading dye was placed on parafilm paper and DNA solution. 4 µl sausage samples were then homogenized, the mixture was put into a well. The electrophoresis device is connected to a voltage source of 100 volts, 400 mv, and a time of 30 minutes. The final step will be seen gel doc in the UV.

2.3.4. Visualization of Amplification Results

The results of the amplification are then visually analyzed with GelDoc in UV. Samples tested positive for contamination of pigs if the results of visualization of sausage samples were formed or visible bands / bands that appeared and in accordance with positive control.

3. RESULTS AND DISCUSSION

3.1. DNA Extraction Pig and Bovine

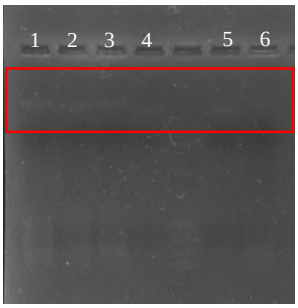

Figure 1. Dna extraction results;
1-4; Positive controls (pig), 5-6; Negative controls (bovine)

Figure 1 showed the results of a pig and bovine DNA extraction which indicating the presence of a DNA band. DNA genome quality resulted better than bovine genome DNA. The pig genome DNA tape is slightly thicker though a little smear, whereas the DNA tape of the bovine genome is thinner. Smears on extraction results can be assumed as fragmented DNA due to mechanical treatment processes, so DNA fragments with smaller molecular weights move faster away from wells (Fibriana, Widianti, Retnoningsih, & Susanti, 2012).

3.2. PCR Optimization Pig Cytochrome B

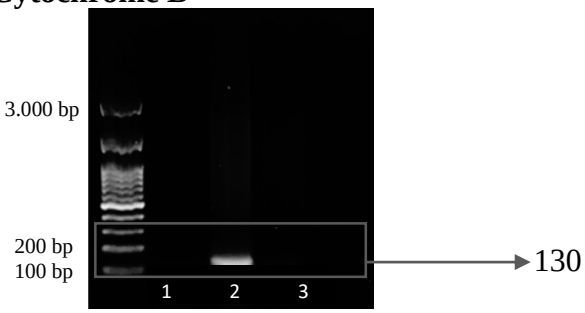

Figure 2. Optimization PCR Temperature;
1; without DNA templates (Control), 2; positive control (pig), 3; negative control (bovine)

Figure 2 showed that there is a DNA band with size of 130 bp (according to the target gene). At the time of the PCR reaction, the selection of annealing temperature was optimal because of the presence of DNA bands in the positive control or primers used specifically for pig *cytochrome b* primers. PCR temperature optimization is done first to get the right PCR conditions so that specific PCR products are produced (Ludyasari, 2014).

PCR amplification was made with a total volume per tube of 13.5 µl consisting of 20 µl DNA templates; 0.5 µl primary forward; 0.5 µl reverse primer; MyTaq 12.5 µl. Whereas for PCR temperature optimization are pre-denaturation temperature of 95°C (3 minutes), denaturation temperature of 95°C (10 seconds), annealing temperature of 60°C (15 seconds), and extension temperature of 72°C (30 seconds) with an amount cycle of 35. Each PCR tube is labeled according to the code. The master mix protocol and PCR cycle are in accordance with the protocol of myTaqTM HS Red Mix. The results of PCR amplification were visualized using electrophoresis and seen with GelDoc.

3.3. DNA Extraction and Amplification PCR Sausage Samples

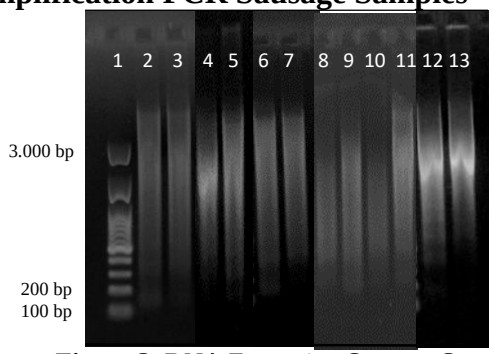

Figure 3. DNA Extraction Sausage Sample;
1; Leadder, 2-5; Sample (P1S1,P1S2,P1S3,P1S4), 6-7; Sampel (P2S1,P2S2),
8-12; Sample (P3S1, P3S2, P3S3, P3S4), 13-14; Sample (P4S1,P4S2)

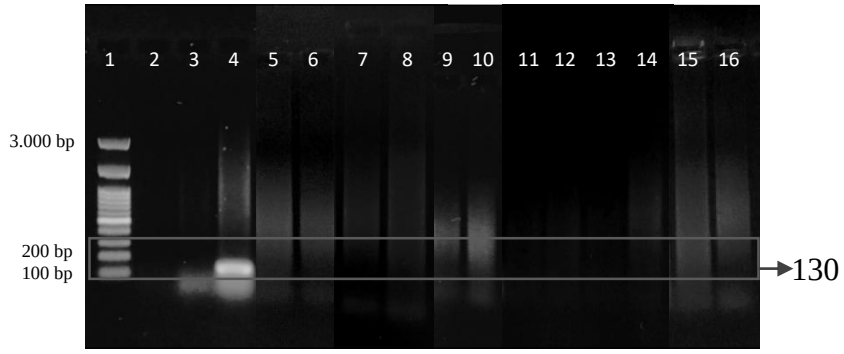

Figure 4. Amplification PCR Sausage Sample;
1; Leadder, 2; without DNA templates, 3; negative control (bovine), 4; positive control (pig),

5-8; Sample (P1S1,P1S2,P1S3,P1S4), 9-10; Sampel (P2S1,P2S2),
11-14; Sample (P3S1, P3S2, P3S3, P3S4), 15-16; Sample (P4S1,P4S2)

Figure 3 DNA extraction showed that the results DNA smears caused by DNA extraction from processed food products cannot be fragmented because the processed sausage products have gone through the cooking and heating process and the addition of ingredients other than meat. This situation caused the results of DNA extraction from processed food products are very difficult to get well fragmented DNA. Smears are assumed to be fragmented DNA because of the mechanical and physical processes in meat products such as heating and treatment processes which can reduce the quality of the resulting DNA, so that fragments of DNA with smaller molecular weight moves faster away from wells (Fibriana, Widianti, Retnoningsih, & Susanti, 2012). Whereas figure 4 in the visualization of PCR products in accordance with the desired target gene, which is a specific band with a 130 bp amplicon size. Every sausage sample showed there are no DNA band. The results beef sausage product tested did not contain pig and was found to be negatively contaminated with pig.

4. CONCLUSION AND RECOMMENDATIONS

Based on research revealed that there was no pig components in beef sausages collected from 4 traditional markets around Jakarta. The results reflected that the beef sausage product tested did not contain pig and was found to be negatively contaminated with pig. Based on research, it's expected that in future research the total sample used will be extend, food products taken from supermarkets and tested using various PCR methods such as real time PCR, RAPD, etc.

5. ACKNOWLEDGEMENT

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