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The Identification of Pork Contamination on Beef by Polymerase Chain Reaction (PCR)

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ABSTRACT

This study aim was to determine the ability of the Polymerase Chain Reaction (PCR) method for identification of pork contamination. The research sample consisted of beef as a negative control, pork which was diluted and mixed with beef as research material to be examined for pork contamination. The validation test method in this study was by testing the sensitivity in serials of dilution of pork (1:10^1: 1:10^3; 1: 10^5; 1: 10^7). PCR amplification result showed that this method was able to detect pig DNA contamination in 1: 10^5 pork dilution. It can be concluded that PCR has the ability to detect pork contamination on beef quite sensitive and accurate.

Keywords: Pork contamination, PCR, sensitivity, pig DNA

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INTRODUCTION

The case of counterfeiting beef with pork is very detrimental to the community, especially Muslims. Muslims are forbidden by their religion to consume pork (1). There are several ways that have been done by researchers to detect the presence of pig elements in food. PCR (Polymerase Chain Reaction) is one of the most frequently used ways to detect the element of pigs in food. The element of pigs that can be detected by PCR is pig DNA derived from pig cells that are still presence on food processing (2, 3).

PCR technology uses cytochrome B primers which have high sensitivity to detect pig DNA. Therefore, the approach with the PCR method using cytochrome B primers is proposed as an analytical method in the supervision of processed meat products. Swine cytochrome B gene is a gene that is often used in the identification of pig DNA in food products in the PCR technique. One of them was in Yuni Erwanto's research that uses cytochrome B to identify pork contamination in meatballs that circulate in the local market, in Jogjakarta (4). Validation of an analytical method is an important factor because only the analytical method has proven its validity, so the measurement results can be justified and used as a basis for subsequent calculations. Validation of the analysis method of pork contamination in meat products using PCR focuses more on testing the validity of specifications and precision (5).

Based on the description above, this study was conducted to determine the effectiveness of
method of identification of pig DNA using PCR techniques using cytochrome B primers.

It is hoped that the PCR technique used in this study can be applied in several ways cases of detection of pig elements in commercial products in the community.

MATERIALS AND METHODS

Sample Preparation

Fresh pork, and beef were bought from the market in the Surabaya region. Both meat was put
separate with different tools to avoid crossing contamination. After packing the meat is stored
at temperatures of -20°C until it is used for research. On this research was beef individually or mixed with pork with a dilution of pork of 10^{-1}, 10^{-3}, 10^{-5} and 10^{-7} mixed homogeneously. After DNA isolation was analyzed by PCR using primers specific and PCR with universal primer mitochondrial genes cytochrome B (6).

DNA Isolation

Meat formulation with various concentrations of pork performed DNA isolation. Method extraction according to the DNA Kit High isolation protocol Pure PCR Template (Roche, Mannheim, Germany). Sample as much as 50-100 mg inserted into the tube capacity 1.5 ml. Extraction begins by adding 200-300 µl tissue buffer and 40 µl proteinase K, and incubated at 55° C for 16 hours. A total of 200 µl binding buffers were added, then incubated 70 ° C for 10 minutes. Total of 100 µl isopropanol is added and mixed well. High Filter Tube is prepared in a collection tube. Sample put in the filter tube. All tubes were inserted into the table top centrifuge and centrifuge at 8000 x g for 1 minute. The solution was removed from the collection tube, 500 µl buffer removal inhibitors added to the High Filter Tube, then centrifugate at 8000 x g for 1 minute. High Tube filter was taken and the solution was removed. A total of 500 µl wash buffer added to the High Filter Tube, centrifugation at 8000 x g for 1 minute. High Filter Tube taken and dispose of the solution. A total of 500 µl wash buffer was added on the High Filter Tube, then centrifuged at 8000 x g for 1 minute. After removing the solution, then centrifuged at maximum speed at 13,000 x g for 10 seconds. Collection tube is discarded, then High The filter

tube is put into 1.5 ml microcentrifuge new tube. A total of 200 µl prewarmed elution buffer added, then centrifuged at 8000 x g for 1 minute. The DNA solution was stored at -20° C to further analysis (6).

DNA amplification using the PCR method

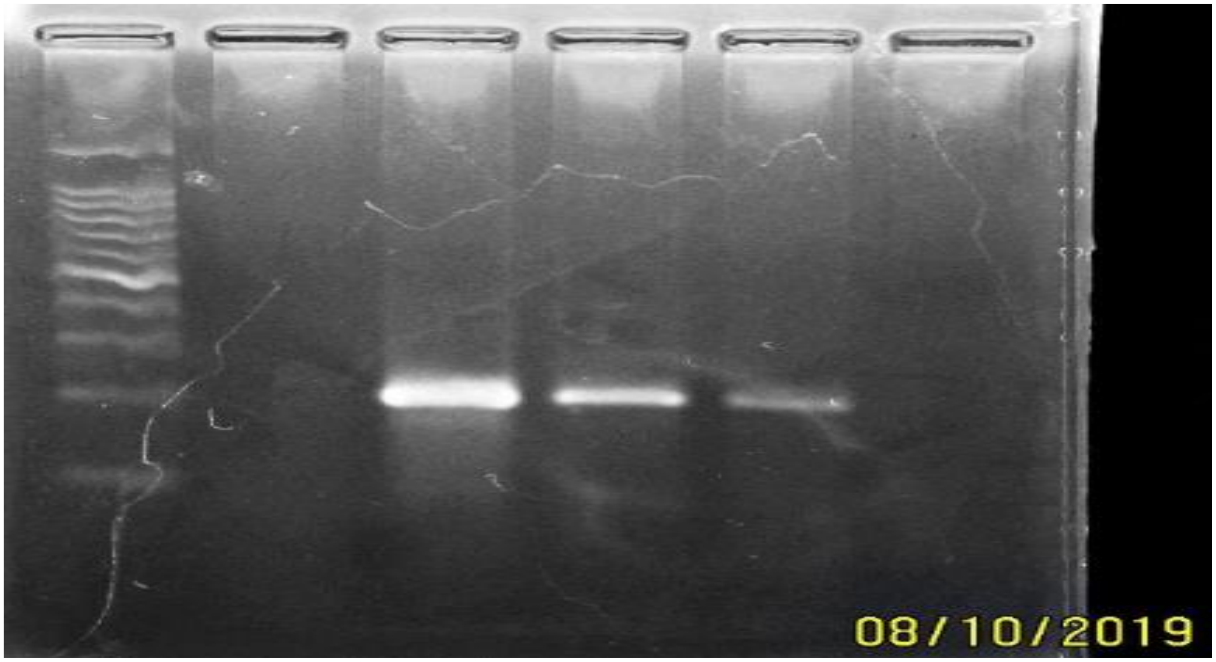
The pig primary sequences used for PCR amplification are in the base sequence 5'-GCC TAA ATC TCC CCT CAA TGG TA-3 'and 5'-ATG AAA GAG AAT AGA TTT TCG-3 ' (7). Prepare a 12.5 PCR mix solution (MgCl2, dNTPs, Taq DNA Polymerase, loading), 7.5 DNA samples, 2.5 forward and reverse primary sequences and nuclease-free water to adjust the volume, then homogenized (8, 9, 10).

The initial denaturation conditions are at a temperature of 94^{o}C for 4 minutes followed by 35 cycles of denaturation at a temperature of 94^{o}C for 30 seconds, primary attachment at 56^{o}C for 40 seconds, and the initial DNA extension at 72^{o}C for 30 seconds. The final extension was carried out at 72^{o}C for 5 minutes (7).

Electrophoresis of PCR products

PCR amplification products are analyzed using 2% agarose gel in 0.5 x TBE dye, and stained with ethidium bromide. Marker is also put inside agarose gel used to determine DNA size from PCR product, electrophoresis is run for 40 minutes with constant voltage 100 v. The result of electrophoresis is observed under ultraviolet light. The results obtained is a pattern of DNA bands (DNA bands) that shows the differences thickness and clarity of DNA bands. The 100 bp DNA ladder is used as a marker (11, 12). The PCR product of pig DNA is 212 bp (7).

RESULTS AND DISCUSSION



M 1 2 3 4 5

Figure 1. Visualized PCR product results on the electrophorogram.

M: marker; lane1: beef meat (negative control); lane2: beef meat + diluted pork 10^{-1}; lane3: beef meat + diluted pork 10^{-3}; lane4: beef meat + diluted pork 10^{-5}; lane5: beef meat + diluted pork 10^{-7}

PCR is the method chosen in this study because of its high efficiency and sensitivity in detecting it accurately, even though the purity value obtained is below the specified range (13, 14). The other components needed besides DNA template in this activity, namely primer. In this study, the primer used is a cytochrome B primers, which specifically produces only one band of a certain size and only amplifies pig DNA. The use of primer spesific for pig is intended to determine whether or not the presence of pig DNA contaminants in the meat product being tested (15, 16). This study did not use duplex PCR, due to the use of mitochondrial DNA cytochrome B gene only from pig DNA (7).

In sensitivity testing used one isolate of pork DNA which was diluted to 1: 10^{1}, 1: 10^{3}, 1: 10^{5} and 1: 10^{7}. Electrophorogram results (Figure 1) can be seen in the lane 1 to 5. From the four series of dilution produces a variety of thickness of bands. In lane 1, there is no band which is an indicator of beef being not contaminated with pork. However, the results of the band which are most clearly seen in lane 2 are 1: 10^{1} and it has not produced a band at 1: 10^{7} dilution (lane 5). The band can not be seen clearly, probably because the concentration produced by the dilution is too low to be used in the PCR process, so it is not able to amplify. Therefore, in lane 5 from figure 1, it was found that PCR examination with cytochrome B pig cannot detect pork with dilution 1: 10^{7}. This detection limit value can be obtained if the solution has good homogeneity. The concentration of template DNA required for PCR activities ranged from 10-100µg / µL, so that the total DNA concentration obtained in this method was sufficient to be used in the PCR process. By looking at Figure 1 it can be concluded that lane 5 has DNA concentration of less than 10-100µg / µL. For this reason, the concentration uniformity is carried out with DNA dilution according to the optimal concentration in PCR amplification. The optimal concentration in PCR amplification at 30 cycles to produce a thick band is a concentration of 50 µg / µL (17). All DNA isolates that have passed the quantitative analysis testing process and meet the existing requirements. Furthermore, it can be used in PCR-based molecular analysis. PCR is the method chosen in this study because of its high efficiency and sensitivity in detecting accurately (18, 19), although the detection of

pork contamination is faster using Fourier Transform Infrared Spectrophotometry (FTIR) spectroscopy (18).

The PCR process begins with a denaturation step carried out at 94˚C for 30 seconds, in this step, the DNA double helix breaks down into two strands of a single DNA template, the primary attachment (anneling) at 56˚C. Based on the results of the optimization of the anneling temperature, it was found that the optimum anneling temperature of the cytochrome B primer was 56˚C using the PCR method. At this temperature, it can specifically identify pig DNA targets and the DNA of cows. Therefore, in this study, an anneling temperature of 56˚C was used for 30 seconds. Anneling is an important parameter for successful PCR product results (20, 21). The next stage, namely the extension stage, takes place at a temperature of 72˚C, at this stage the polymeration process occurs to form new DNA strands.

PCR was performed using pig cytochrome B primers. This primer is very often used to identify various types of commercial products in various countries. Cytochrome B used as a primer because this is a mitochondrial gene that is protected by a membrane, derived from the maternal, and always in the cell (22). This study uses cytochrome B primers which have been used by Tanabe on research in 2007, to detect the presence of pork, chicken, goat, sheep and horses in various foods. Therefore, it is considered this primer is very specific for detecting pig DNA (23).

CONCLUSION

From the result of this study, it was found that the optimization of PCR techniques using Cytochrome B should preferably use annealing temperature of 56˚C. This PCR technique has pork detection limit is 1: 10^5 dilution consentration. The technique used here has quite a high sensitivity and specificity.

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