AUTHENTICATION OF RAW CHICKEN MEAT FROM PORK CONTAMINATION USING GENE *CYT-B* WITH DUPLEX-POLYMERASE CHAIN REACTION ANALYSIS

AUTENTIKASI DAGING AYAM SEGAR DARI KONTAMINASI DAGING BABI MENGGUNAKAN GEN CYT-B DENGAN ANALISIS DUPLEX- POLYMERASE CHAIN REACTION

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ABSTRACT

Halal is one of important aspects in consumer protection. Meat and processed meat products are food that should be controlled strictly because those are prone to be adulterated by pork contamination. Therefore, it is necessary to provide detection technique which is accurate, fast and cheap. The objective of this research was to identify the presence of impurities of pork meat on raw chicken meat using gene *Cyt-b* with *duplex*-PCR analysis. This research used six samples of raw chicken meat and raw pork. Raw chicken meat was bought from supermarkets in the city of Surakarta and raw pork was obtained from pig slaughterhouse. The percentage of raw pork contamination on raw chicken meat was designed as much as 1, 5, 10, and 25%, respectively. The DNA genome was isolated according to DNA isolation protocol from Genomic DNA Mini Kit. In addition, duplex-PCR was performed based on protocol of KAPA2G Fast Multiplex PCR kit. The data was descriptively analyzed by directly looking the DNA bands on the gel documentation apparatus. The result showed that specific DNA bands for chicken and pig were completely appeared on 1.5% of agarose gels. Duplex-PCR detect contamination of pork on raw meat of chicken at all contamination levels. This research proved that the duplex-PCR detect the contamination of pork until the level of 1%.

(Keywords: Authentication, Chicken meat, *Cytochrome b*, *Duplex*-PCR, Pork contamination)

INTISARI

Halal merupakan aspek penting dalam perlindungan konsumen. Salah satu pangan yang harus dikontrol secara ketat adalah daging dan produk hasil olahannya karena rawan terjadi pemalsuan sehingga diperlukan teknik deteksi yang sensitif, akurat, cepat, dan murah. Tujuan penelitian ini adalah untuk mengidentifikasi adanya cemaran daging babi pada daging ayam segar menggunakan gen cyt-b dengan analisis duplex-PCR. Penelitian ini menggunakan enam sampel daging segar yang terdiri dari daging ayam segar dan daging babi segar. Daging ayam segar diperoleh dari supermarket di Kota Surakarta dan daging babi segar diperoleh dari Rumah Potong Babi (RPB). Rancangan penelitian berdasarkan persentase kontaminasi daging babi segar pada daging ayam segar dengan level kontaminasi sebesar 1, 5, 10, dan 25%. Isolasi DNA dilakukan mengikuti protokol dari Genomic DNA Mini Kit dan duplex-PCR dilakukan sesuai petunjuk dari KAPA2G Fast Multiplex PCR kit. Analisis data menggunakan deskriptif kualitatif dengan menganalisis hasil visualisasi pada gel documentation. Hasil duplex-PCR pada agarose gel 1,5% mampu mendeteksi cemaran daging babi pada daging ayam segar di semua level kontaminasi. Penelitian ini membuktikan bahwa duplex-PCR mampu mendeteksi kontaminasi daging babi sampai level 1%.

(Kata kunci: Autentikasi, Cytochrome b, Daging ayam, Duplex-PCR, Kontaminasi daging babi)

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Introduction

The authentication of meat is a sensitive and important issue concerning food products, especially in Moslem countries (Erwanto *et al.*, 2014). It is closely related to food safety, i.e. the issues of pork contamination and its derivatives on food product (Murugaiah *et al.*, 2009; Koppel *et al.*, 2011). Pork contamination causes controversial reactions in society because pork is clearly prohibited for the Islamic community, many people are highly hypersensitive to pork, and there are vegetarian life styles (Rahmawati *et al.*, 2015). In Indonesia, the pork contamination could be occurred during meat processing. Grinding process is a common activity to produce meat products, and it is susceptible to pork contamination in raw meat. Erwanto *et al.* (2014) investigated consumers in Yogyakarta obtained ground meat from the traditional market which is grinded using the traditional equipment that also may be used for pork. Therefore, an effective method should be developed to specifically and selectively identify the pork on raw meat from other species.

Recently, authenticity testing and analytical techniques have immeasurably improved and now it can draw on a wide variety of techniques and methods, each method is appropriate and specific to deal with a particular problem (Ballin, 2010). Methods based on DNA identification have been applied to meat specification such as polymerase chain reaction (Aida *et al.*, 2005; Sahilah *et al.*, 2011), Duplex-PCR (Soares *et al.*, 2010), Multiplex-PCR (Koppel *et al.*, 2011), PCR-RFLP (Erwanto *et a*l, 2014) and real time PCR (Dooley *et al.*, 2004; Kesmen *et al.,* 2009; Ali *et al.*, 2012) were methods to detect the contamination in pork which have been developed.

Methods used to analyze contaminants in pork processed food products namely Polymerase chain reaction (PCR) is one of molecular biology approaches used to authenticate and to detect species origin in meat by amplifying spesies specific sequence in the genome such as fragment of mt-DNA *Cyt-b* gene (Girish *et al.,* 2005). Contaminants analyze which is used was mitochondrial DNA (mt-DNA) is done based on the existence of a sequence-specific DNA fragment of species on meat (Aida *et al.*, 2005; Ling-Sun, 2003).

Cytochrome b (*Cyt* b) is one of gen in mitochondria DNA and it was often used to identify species from raw material in marine product (Sotelo *et al.*, 2001). The PCR analysis of species-specific mitochondrial DNA sequences is the most common method currently used for identification of meat species in food and seems to be the best as a 'routine' test, because it is easy, rapid and allows the discrimination of several species at the same time (Bottero and Dalmasso, 2011), and it only requires a small sample size (Che Man *et al.*, 2007; Nakyinsige *et al.*, 2012). The use of mitochondrial DNA in PCR analysis can also enhance sensitivity because each cell has about a thousand mitochondria and each mitochondria has ten copies of DNA (Jain, 2003). Therefore, the objective of this research was to identify the presence of impurities of pork meat on raw chicken meat using *Cyt-b* gene by duplex-PCR analysis.

Materials and Methods

Sample collection

Pork and chicken meat samples were obtained from commercial sources. Both of samples was labeled to ensure that all samples were perfectly separated, and then samples were stored in the refrigerator until used for further analysis (Ni'mah *et al.*, 2016).

The grinding process of chicken meat and pork was done separately. The meat samples were cut into small size 1x1 cm, and then the meats were ground using meat grinders. Moreover, the percentage of raw pork contamination on raw chicken meat was designed as much as 1, 5, 10, and 25%, respectively. The meat samples were weighed and manually mixed until homogeneous for DNA extraction analysis.

DNA extraction

The isolation of DNA was conducted according to the Protocol of the Genomic DNA Mini Kit for animal tissues (Geneaid Biotech Ltd., Taiwan). Firstly, 30 mg of meat tissue was placed into 1.5 ml tube and mashed by micropestle, and then 200 µl of GT buffer was added into the tube and homogenized by using a grinder. Furthermore, 20 µl of proteinase K was added to the tube, homogenized back and incubated at 60°C for 30 minutes. After that, 200 µl of GBT buffer was added into tube, and it was homogenized and incubated at 60ºC for 20 minutes.

Supernatant was moved into a new 1.5 ml tube and added 200 μl of ethanol absolute and it was homogenized. Furthermore, the sample was moved to GD column which is placed on top of 2 ml collection tube, and it was centrifuged at 14,000-16,000 rpm for 2 minutes and then the 2 ml collection tube was replaced with the new one. In addition, 400 μl of Buffer W1 was added into the sample, and it was back centrifuged at 14,000-16,000 rpm for 1.5 minutes. The rest of the mixture was dumped, and GD column was inserted back into tube 2 ml collection. Furthermore, 600 μl of wash buffer was added to the GD column and centrifuged for 3 minutes at 14,000-16,000 rpm. GD column was put into a new 1.5 ml tube and added 100 μl of elution buffer heated at 60°C, and it was centrifuged at 14,000-16,000 rpm during 1.5 minutes. Finally, the DNA was ready to use for subsequent analysis. The DNA concentration was determined by observing the intensity of DNA bands on 1% of agarose gels.

Simplex- and Duplex-PCR

The PCR was performed with a total volume of 25 μl consisting of 10.5 μl aquabidest; 0.5 μl of each primer (10 M); 12.5 μl 2X Fast KAPA2G Multiplex Mix (Kapa Biosystems, Inc., United States); and 1 μl of DNA template. The PCR was carried out to amplify fragments of DNA mitochondrial *Cyt*-b gene, and the primer used in this study was previously published by Matsunaga *et al.* (1999) which is presented in Table 1. PCR program was performed using the GeneAmp® PCR System 9700 (Singapore) as follows: initial temperature of denaturation at 95°C for 3 minutes, followed by 30 cycles of denaturation at 95°C for 15 seconds, annealing temperature at 60°C for 30 seconds and extension at 72°C for 30 seconds and also final extension at 72°C for 1 minute. The results of duplex-PCR were visualized in 1.5% agarose gel using gel documentation (Vilber Lourmat Infinity 1100126M, France).

Results and Discussion

DNA extraction

As many as 6 raw meat samples were designed and used for the isolation of DNA in this study. The results showed that the DNA of the genome was successfully extracted from raw meat in all levels contamination of pork on raw chicken meat (Figure 1).

The DNA genome extracted in this study was low concentration indicated by smear DNA bands on the 1% agarose gel (Figure 1). The low DNA concentration yielded in this study may due to the meat samples have been cooked before isolation process. Previous study reported that genomic DNA can still be extracted from cooked meat such as sausage, cutlet and leftover meals (Matsunaga *et al.,* 1999; Nakyinsige *et al.*, 2012; Zhang, 2013). Basic principle of DNA extraction is by breaking up the cells from tissues until the DNA genome was completely separated from other cell components like protein, DNA and RNA (Faatih, 2009).

Simplex- and Duplex-PCR

This research conducted both simplex- and duplex-PCR methods. Simplex-PCR was applied to optimize PCR conditions while duplex-PCR was performed to detect contamination of pork in chicken meat raw. The existence of pork contamination in the meat mixtures was indicated by a 398 bp of DNA band. On the other hand, a species of chicken was shown by the existence of 227 bp of DNA band.

Table 1. Marker, primer pairs, PCR product size, and annealing temperature for each species used in this study

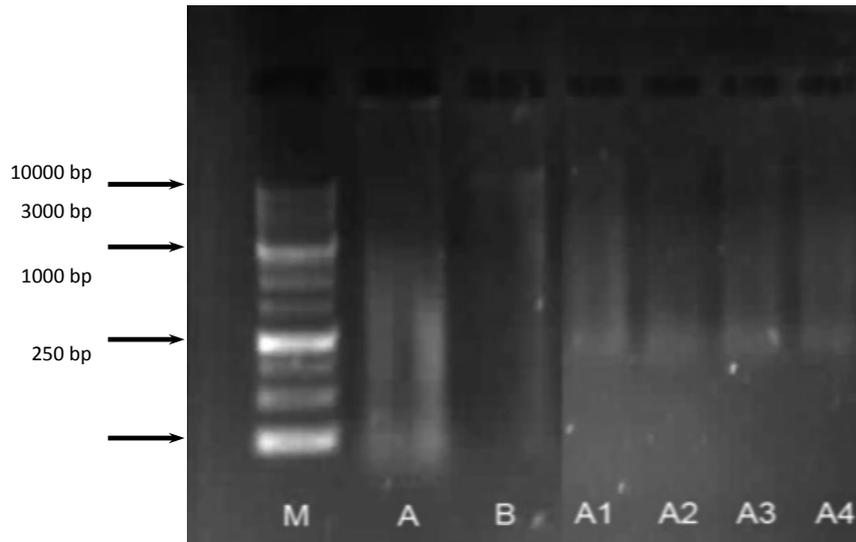| Species	Marker	Primer pairs	PCR product size	Annealing temperature
Chicken (*Gallus gallus*)	mt-Cyt b	F: 5'-GACCTCCCAGCTCCATCAA-ACATCTCATCTTGATGAAA-3' R: 5'-AAGATACAGATGAAGAAG-AATGAGGCG-3'	227 bp	60°C
Porcine (*Sus scrofa*)	mt-Cyt b	F: 5'-GACCTCCCAGCTCCATCAA-ACATCTCATCTTGATGAAA-3' R: 5'-GCTGATAGTAGATTTGTGA-TGACCGTA-3'	398 bp	60°C

Source: Matsunaga *et al.* (1999).

Figure 1. Visualization of DNA isolation on 1% agarose gel. Marker Ladder (M), 100% of chicken meat (A), 100% of pork (B), A1 to A4 were chicken meat sample contaminated by pork: 75%: 25% (A1), 90%: 10% (A2), 95%: 5% (A3) and 99%: 1% (A4).
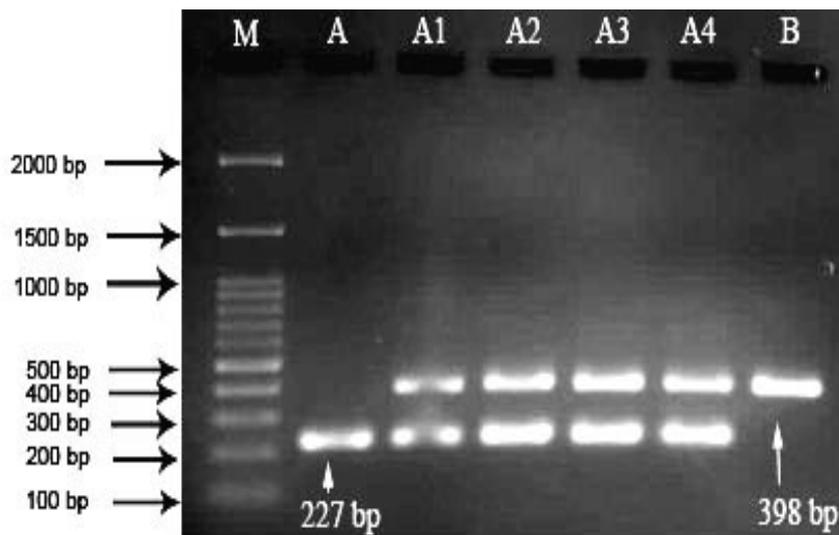

Figure 2. Visualization of duplex-PCR on 1.5% agarose gels. Marker Ladder (M), 100% chicken (A), 100% of pork (B), A1 to A4 were chicken meat sample contaminated by pork: 75%: 25% (A1), 90%: 10% (A2), 95%: 5% (A3) and 99%: 1% (A4).

This research was conducted to detect contamination of pork in chicken raw meat up to the level of 1%. Previous study reported that both raw and cooked meats could be detected by using multiplex-PCR from mt-DNA Cyt-b gene up to level of 8% (Matsunaga *et al.*, 1999; Ni'mah *et al.*, 2016). Multiplex-PCR was also used successfully as one way to authenticate a good product of meat raw or processed products, and it could be used by a company as the reference of quality control to produce food (Nakyinsige *et al.*, 2012; Zhang, 2013).

Conclusion

Pork contamination in raw chicken meat could be detected by Duplex-PCR of mt-DNA Cyt b gene. The sensitivity of authentication method was able to detect pork contamination in raw chicken meat until 1%.

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