The 1st International Conference on

HALAL-THOYYIB INDUSTRY & RESEARCH

"Halal industry and its challenge toward global safety and prosperity"

PROCEEDING

ISSN: 2355-4029

FEBRUARY 19- 20,2014

WIDYALOKA CONVENTION HALL
BRAWIJAYA UNIVERSITY, INDONESIA

Detection of Porcine DNA Residue by Polymerase Chain Reaction on Food Processing Equipments after Ritual Purification

Rosy Hutami [1)], Joko Hermanianto[2)], and Nancy D. Yuliana [2)]
[1)] *Graduate School of Food Science, Bogor Agricultural University, Indonesia*
[2)] *Department of Food Science and Technology, Bogor Agricultural University, Indonesia*

***Abstract*—A commercially available conventional PCR, based on a multi-copy target cytochrome b (*cyt b*) using porcine specific primers, has been validated for the Halal/Kosher authentication of porcine detection. Detection of porcine DNA residue on najis affected equipments after ritual purification *(sertu)* is required to provide initial data of syariah purification implementation in industrial equipments. In this study, the probability of conventional PCR method in observing porcine DNA residue on affected equipments after ritual purificated was 1:3. It means 75% of the sample analysis was disobservable using coventional PCR analysis method. The porcine DNA band was once found at 398 bp. The concentration of DNA extraction on the affected equipments varied from 2.4-24.2 ng/μl. The method were used Phire Direct PCR Dillution Buffer combining with Phusion High-Fidelity PCR kit.**

***Keywords*—porcine, PCR, DNA.**

I. Introduction

HALAL food is defined as food that does not contain and contact with any prohibited *(haram)* materials or substances to be consumed by Muslims and its processing step does not break the Islamic law [1]. As long as the increasing of consumers awareness in halal products importance, the willingness of manufacturers to produce halal products is increase too. For the manufactures, ensuring halal food status, it is not only enough just by using the guaranteed halal raw materials, but also have to ensure whole the food production chain; including raw materials halal status utilization, manufacturing process, pure equipments utilization, until released product.

Porcine and its derivatives is prohibited to be consumed according to the Islamic law and the materials itself is chategorized as *heavy najis.* In case, there were manufactures who want to stand as halal food industries may have processed heavy *najis* such as porcine and its derivatives, so their process equipments have contact with *najis* and become *mutanajjis* (unpure thing because of direct exposure of *najis*). In other hand, the food industries wish their affected equipments can be reused to produce halal product. So they expect there is any cleaning procedure that can purify their affected equipments. Because, it should need a high cost if they replace the equipments by the new ones. The goodwill of the industries to move from non-halal food production base to halal food production base is a worthy thing to be supported by all of the stakeholders. Scholars, scientists, and technologists have to support food industries to implement the halal food productions system.

Reusing the affected equipments as the pure ones need a ritual purification according to Islamic law. Ritual purification needs to be done on areas which have direct contact with heavy Najis such as porcine and its derivatives and dog saliva. Ritual purification of the affected equipments is by one washing of earth water mixture and six washings of pure water according to Syafi'i school of thought [2].

In the implementation, earth can not always be used to purify anykind of equipments because sometimes it will affect and break the equipments and the products [3]. Based on clean in place (CIP) method for food industries, cleaning agents that is commonly used for food processing equipments (especially for the stainless steel based) are acid solution (such as HCl and HNO_3), alkali solution (such as NaOH), and detergents [4],[5]. Those materials were also used for cleaning affected processing equipments when ritual purification *(sertu)* can't be implemented. But, there is no performance data of those cleaning agents in cleaning porcine and its derivatives residue on the affected equipments.

Porcine detection at molecular level has been diversely used nowadays especially DNA-based methods [6]. The use of DNA-based methods provides different levels of identifcation: (i) individual traceability to ensure food safety; and (ii) traceability of individuals to their source breed or species to detect possible labelling adulteration [7]. The process of multiplication of DNA sequences by polymerase chain reaction technique (PCR) is another alternative in the determination of the existence of fraud or other species contamination in a product [8], [9].This method uses a universal DNA marker or a specific marker which is only found in a species of animal [10].

Cyt b gene is a gene that is often used to identify the species of the animal and the source of the animal product because it has eternal part at the level of species[11]. Fragment specific for pigs obtained at 398 bp [12].

The aim of this research is to detect porcine DNA residue on the affected equipments after ritual purification *(sertu)* as initial data of syariah purification implementation in industrial equipments model.

II. MATERIALS AND METHODS

2.1 DNA Sample Preparation

Twenty (20) grams of pork was exposed to 500 ml stainless steel mug up to half of the mug. The mug was washed once with earth and water mixture and six times with pure water. The volume of water used is 400 ml for each wash. The earth was mixed with water until the form as like as mud, then the mixture was rubbed inside the mug and 400 ml water was added into the mug. Each washing step was shook with orbital shaker at room temperature for 5 minutes at 120 revolution per minute (rpm). Then the re-pure equipment was swabbed. The swabs were dipped in distilled water and the water were analyzed as the sampel.

2.2 Specific Primer

Specific primers used for amplification of DNA fragments pig followed the method of Matsunaga et al. [13]. Forward
primer used is 5'-GAC CTC CCA GCT CCA TCA AAC ATC TCA TCT TGA TGA AA-3'. While a reverse primer specific for pig is 5'-GCT GAT AGT AGA TTT GTG ATG ACC GTA-3' from the cytochrome b (*cyt b*) as target gen.

2.3 DNA Extraction

The process of DNA extraction used Phire Direct PCR dillution buffer. 500 µl of sampel was added with 20 µl dillution buffer. The quantitative measurement of DNA purity and its concentration was done by using a spectrophotometer to ensure successful DNA extraction, while the DNA test results in a qualitative extraction is done by electrophoresis on 1% agarose gel run at 100 V for 40 min.

2.4 PCR Amplification

Amplification of specific DNA fragments was made by PCR (polymerase chain reaction) method. Reaction components using Thermo Scientific Phusion High-Fidelity PCR Kit, as much as 15 µL, consisted of 0.2 µL forward primer, 0.2 µL reverse primer, 3 µL 5x Phusion HF buffer, 1 µL $MgCl_2$, 0.3 µL dNTPs, 0.004 units Taq DNA polymearse, 0.4 µL DMSO, and 8.7 µL H_2O.The process of amplification was run on a GeneAmp ® PCR System 9700 (Applied Biosystems ™). The reaction conditions included the pre-denaturation step for 30 s at 98°C, denaturation step for 5 min at 98°C, annealing step for 30 s at 60°C, extension step for 5 min at 72°C, and the last extension step for 5 min at 72°C.

2.5 The Interpretation Results Visualization and Amplification

Visualization of amplification performed on agarose gel 1,5% (v/w) was stained with EtBr (ethidium bromide) above transiluminator UV irradiation. Specific DNA fragment of pig were analyzed by standard DNA size marker (100 bp).

III. RESULT AND DISCUSSION

3. 1 Total DNA Quality

The purity of the DNA solution can be counted by comparing A260 nm to A280 nm. Limit of purity commonly used in molecular analysis on the ratio of A260/A280 is 1.8-2.0 [14]. The DNA sampel purity was not good enough, because the ratio of A260/A280 is 1.45-2.53. Although there were no negative values obtained. The concentration of DNA extraction varied from 2.4-24.2 ng/µl. It was low to medium concentration, because the limit of concentration commonly used is not less than 50 ng/µl.

3.2 Amplification of Specific Fragment of DNA Cyt b

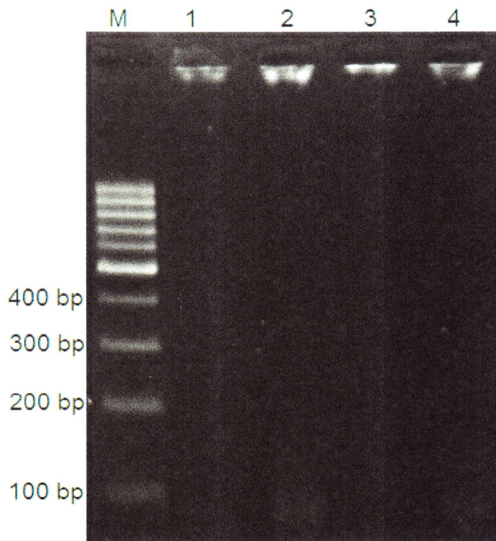

Fig. 1 Amplification of specific DNA fragments of *cyt b* on porcine affected equipments after ritual purification. M: marker 100 bp, 1-2 : blank porcine DNA sample for 1st repeatation, 3-4 : blank porcine DNA sample for 2nd repeatation.

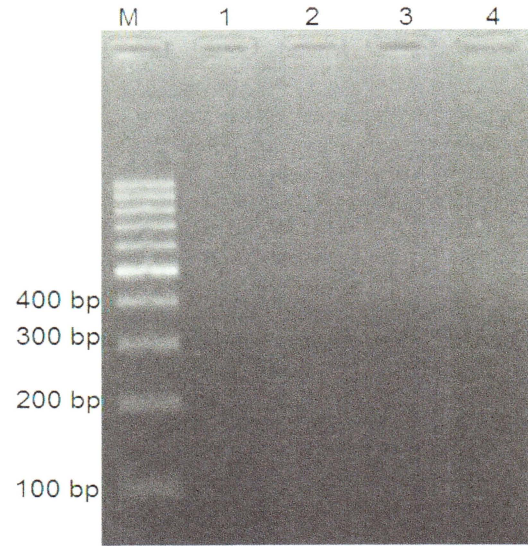

Fig. 3 Amplification of specific DNA fragments of *cyt b* on porcine affected equipments after ritual purification. M: marker 100 bp, 1-2 : blank porcine DNA sample extraction, 3-4 : blank porcine DNA sample for 1st repeatation.

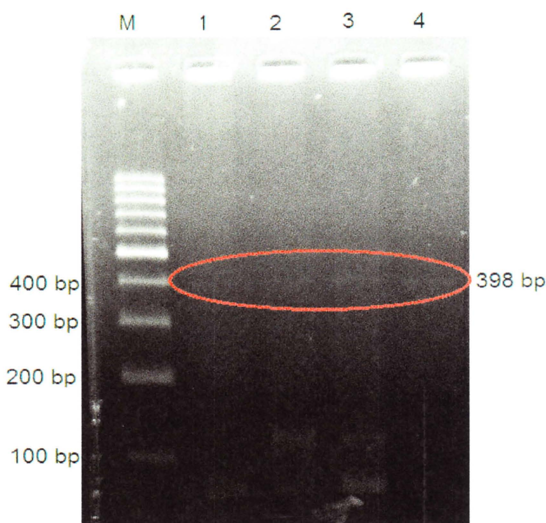

Fig. 1 Amplification of specific DNA fragments of *cyt b* on porcine affected equipments after ritual purification. M: marker 100 bp, 1-2 : blank porcine DNA sample for 1st repeatation, 3-4 : blank porcine DNA sample for 2nd repeatation.

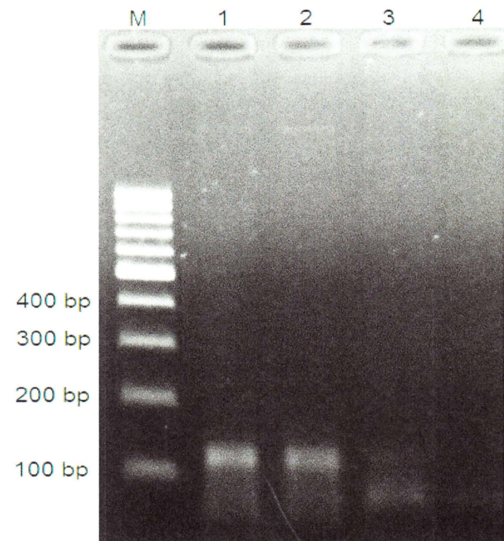

Fig. 4 Amplification of specific DNA fragments of *cyt b* on porcine affected equipments after ritual purification. M: marker 100 bp, 1-2 : blank porcine DNA sample for 1st repeatation, 3-4 : blank porcine DNA sample for 2nd repeatation

As shown in Fig. 1-4, the conventional PCR method proposed in this study allowed us only to detect porcine DNA residue on the affected equipments qualitatievely. The amplification of each DNA sample was unclearly observed in a range between 2.4-24.2 ng/µl concentration of DNA extraction. The probability of the observable residue DNA in this study is 1:3. It means 75% of the sample analysis was disobservable using coventional PCR analysis method.

IV. CONCLUSION

Ritual purification technique left 2.4-24.2 ng/μl concentration of DNA extraction on the affected equipments. In this study, the probability conventional PCR method in observing porcine DNA residue on affected equipments after ritual purificated was 1:3. It needs to improve the extraction and amplification technique to get the best data.

V. PLANS

After collecting data of ritual purification performance in removing residue DNA, researchers will analyse the performance of others cleaning agents, such as NaOH, HNO_3, HCl, and detergents. Also, researchers will analyse other residues like fatty acid residue and protein residue by proper methods.

VI. ACKNOWLEDGMENT

The author acknowledge Djuanda University as the sponsor of author's attendance in International Conference Halal Thoyyib Industry and Research.

VII. REFERENCES

[1] [MoRA RI] Ministry of Religious Affairs of the Republic of Indonesia. 2001. Decree of the Minister of Religious Affairs of the Republic of Indonesia on the Guidelines and Procedures for Examination and Determination of Halal Food. Jakarta (ID) : DEPAG RI.

[2] *Five School of Thought Fiqh : Ja'fari, Hanafi, Maliki, Syafi'i, Hambali.* Lentera Pub. Masykur AB, Muhammad A, Al-Kaff I; (Ed) Abudan F, Shahab U, ID, 2000.

[3] [MUI] Majelis Ulama Indonesia. 2011. FatwaMajelis Ulama Indonesia Nomor 9 Tahun 2011 tentang Pensucian Alat Produksi yang Terkena Najis *Mutawassitah* (Najis Sedang) dengan Selain Air, ID, 2011.

[4] [FAO] Food and Agricultural Organization, Cleaning and sanitation in meat plants. Available : http ://www.fao.org/docrep/010/ai407e/ ai407e26.htm, 2013.

[5] S. Niamsuwan, P. Kittisupakorn, IM Mujtaba, "Minimization of water and chemical usage in the cleaning in place process of a milk pasteurization plant". *Songklanakarin J. Sci. Technol*, vol. 33, pp : 431-440.

[6] S. Tanabe, M. Hase, T. Yano, M. Sato, T. Fujimura, H.A. Kiyama, "A real time quantitative PCR detection method for pork, chicken, beef, mutton, and Horseflesh in foods," *Biosci. Biotechnol. Biochem,* vol 71, Dec. 2007, pp. 3131-3135.

[7] C. M. Davit, D. Marchi, M. Cassandro, "Genetic traceability of livestock products : A review," *Meat Sci. J,* vol 77, Oct. 2007, pp :437-449.

[8] C. L. Zhang, M. R. Flowler, N. W. Scott, G. Lawson, A. Slater, "A taqman real-time PCR system for the identification and quantify rication of bovine DNA in meats, milks, and cheeses." *Food Ctrl. J,* vol 18, Oct. 2007. pp : 1149-1158.

[9] S. Ghovvati, M.R. Nassiri, S. Z. Mirhoseini, A. H. Moussavi, A. Javadmanesh, "Fraud identification in industrial meat products by multyplex PCR assay," *Food Ctrl. J, vol 20, Sep 2008. pp : 696-699.*

[10] H. Nuraini, A. Primasari, E. Andreas, C. Sumantri, "The use of cytochrome b gene as a specific marker of the rat meat (Rattus norvegicus) on meat and meat products," *Medpet J. IPB,* Apr 2012. pp : 15-20.

[11] R. Widayanti, "Study of cytochrome b gen and D-Loop region as genetic markers in Tarsius sp." (Dissertation), IPB, ID. 2006.

[12] I. Obrovska, I. Steinhauserova, M. Nebola, "The application of the PCR method to the identification of meat species", *Folia Vet,* vol 46, pp :113-118.

[13] T. K. Matsunaga, R. Chikuni, S. Tanabe, K. Muroya, J. Shibata, Yamamda, Y. Shinmura, "A quick and simple method for the identification of meat species and meat products by PCR assay," *J Meat Sci,* vol. 51, 1999, pp: 143-148.

[14] *Molecular Cloning a Laboratory,* CSH Laboratory Press, Sambrook J, Fritsch EF, Maniatis T, US, 1989 .