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Isolation, Amplification and Characterization of Foodborne Pathogen Disease Bacteria Gene for Rapid Kit Test Development

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Abstract. There is a lot of public concern over food safety. Food-safety cases recently, including many food poisoning cases in both the developed and developing countries, considered to be the national security threats which involved police investigation. Quick and accurate detection methods are needed to handle the food poisoning cases with a big number of sufferers at the same time. Therefore, the research is aimed to develop a specific, sensitive, and rapid result molecular detection tool for foodborne pathogens bacteria. We, thus, propose genome level approach with Polymerase Chain Reaction. The research has successfully produced a specific primer to perform amplification to fim-C S. typhi, E. coli, and pef. Salmonella typhimurium genes. The electrophoresis result shows that amplification products are 95 base pairs, 121 base pairs, and 139 base pairs, and all three genes are in accordance with the size of the in silico to third genes bacteria. In conclusion, the research has been successfully designed a specific detection tool to three foodborne pathogen bacteria genes. Further stages test and the uses of Real-time PCR in the detection are still in the trial process for better detection method.

INTRODUCTION

Food security, especially food poisoning, becomes very important study and center of increasing concern. The global incidence of the disease because of food poisoning is very difficult to estimate. It was reported in 2011 in the United States that almost every one of the six populations (about 48 thousand people) [1] suffered pain, 128 thousand treated in the hospital, and 3000 people were transparently reported dead. In Indonesia during 2010-2014, reaching 1218 cases of food poisoning cases, in the first three months of 2016 Food and Drug Regulatory Agency (BPOM) Indonesia recorded 35 cases with 1378 casualties and 26 fatalities [2]. In dealing with case of food poisoning outbreak which is also taken as extraordinary occurrence requires not only doctors and medical assistants but also police investigators in a short time. Therefore, it is necessary to provide fast and accurate detection method.

Food poisoning is known to be caused by microorganisms contaminated such as bacteria, viruses, fungi, and parasites that have the ability to infect humans through food and beverages [3–4]. Analysis of the literature shows there are 31 bacteria often found in cases of food poisoning bacteria include Escherichia coli, Salmonella enterica species, Vibrio species, Shigella species, Clostridium perfringens, and Toxoplasma gondii [5]. In previous studies Salmonella UNJ team has succeeded in developing a method of detection of Salmonella typhi with a technique Polymerase Chain Reaction (PCR). We have also demonstrated that the primer pairs FW-int2-Rev-1a fim new Gen-C Salmonella typhi has a high specificity and sensitivity in detecting Salmonella typhi bacteria from pure cultures, nor the blood of typhoid fever patients [6].

In addition to developing methods of detection by PCR of Salmonella typhi, Salmonella UNJ team has also managed to express recombinant proteins fim-C-S. typhi, which has been potential as a vaccine or detection through
serological approach [7]. Fim-C (fimbrial-C) gene is a virulence factor that plays a role in the attachment and colonization (adherence and colonization) of Salmonella typhi bacteria. The gene is also known to function and encode a potential protein located in the surface area. Based on the presence of this potential protein, so that it can be targeted in designing a detection tool in genomic and proteomic level [8]. Besides fim-C Salmonella typhi and Escherichia coli gene, this study also developed pef Salmonella typhimurium gene as a detection tool. pef gene serves as a gene that mediates binding to the intestinal epithelial cells, and facilitates the accumulation of fluid within the luminal space [9].

This research aims to develop a detection method that is fast, accurate, and specific to the Salmonella species bacteria and other bacteria that cause food poisoning by using Polymerase Chain Reaction (PCR). This method has the advantage to detect the bacteria at the same time in the reaction mixture, differentiates types of bacteria quickly and accurately, and requires a short time compared with other methods.

MATERIALS AND METHOD


RESULTS AND DISCUSSION

The isolation of genomic and gene amplification of fim-C Salmonella typhi, Escherichia coli and pef of Salmonella typhimurium have been successfully carried out. The result of isolation and amplification of genome is shown in Fig. 1a and Fig. 1b. Figure 1a shows the results of electrophoresis genome of Salmonella typhi bacteria, Salmonella typhimurium, Shigella dysenteriae and Escherichia coli from a pure culture. The success of the isolation marks with the appearance of DNA bands on characterization results by electrophoresis 1.5% agarose gel. DNA band of all bacteria is located at a higher position than the first line of the marker DNA or larger than 1.5 kilo base (kb). The appearance of DNA bands shows that these results correspond to the results from the search to DNA bacteria from several genomes in the silico database. The analysis showed the genomic DNA of Salmonella typhi bacteria of 4.81 mega base pairs (mb), Salmonella typhimurium of 4.86 mb, Escherichia coli of 4.8 mb, and Shigella dysenteriae of 4.34 mb [12–14].

Figure 1b shows the results of genome amplification of the Salmonella typhi bacteria with primer pairs fim-C: S. typhi-Fam produces DNA band sized 95 base pairs, Salmonella typhimurium with the primer pair pef-S: tpm-Fam produces DNA band in size 139 base pairs, and Escherichia coli with primer pairs fim-C-EC-Fam produces amplicon in size 121 base pairs. The amplification process also uses a positive control from previous research that is primer pairs FW-in2-Rev-1a fim-C-new Salmonella typhi which produces a band 0.2 kilo base (kb) or 200 base pairs (bp). This indicates that the method of Polymerase Chain Reaction using primer's results from the design can detect bacteria Salmonella typhi, Salmonella typhimurium and Escherichia coli in samples of genomic DNA from a pure culture.

As previously stated by some researchers that the use of PCR method has advantages in specificity, sensitivity and efficiency in detecting Salmonella species [15–17], so that the development of detection methods in this study is also expected to improve the quality to human life, especially the people of Indonesia. The results from this study become basis in the development of the multiplex-PCR system, and Real-Time PCR is expected to provide better results than conventional PCR methods [18–19].
FIGURE 1. The Characterization of the bacterial genome and the amplification results of sample bacteria. Figure 1 (a): (1) DNA Ladder 1 Kb (Thermo Scientific), (2) The genome of the Salmonella typhimurium bacteria, (3) The genome of the Shigella dysenteriae bacteria, and (5) The genome of the Escherichia coli bacteria. The processes were characterized by a 1.5% agarose gel with ethidium bromide. Figure 1 (b): (1) DNA Ladder 100 bp (Thermo Scientific), (2) DNA band from S. typhi as Positive Control (3) Amplicon DNA sized 95 bp (fimCF-fimCR of Salmonella typhi), (4) Amplicon DNA sized 139 bp (pefF-pefR of S. typhimurium), and (5) Amplicon DNA sized 121 bp (fimCF-fimCR-C of Escherichia coli). The processes were characterized by a 2% agarose gel with ethidium bromide. The Sighting results were conducted with UV light with a wavelength of 260 nanometers.

CONCLUSIONS

This research has successfully developed a detection method that is rapid, specific and accurate through PCR method using a primer pair fim-C for the Salmonella typhi and E. coli bacteria, as well as pef primers for the Salmonella typhimurium bacteria. Amplicon produced from pure cultures amplification sized consecutive 95 base pair for the fim-C Salmonella typhi genes, 121 base pair for Escherichia coli fim-C gene and the size of 139 base pair for the pef Salmonella typhimurium gene. These results can be used as basis detection to the other bacteria that cause food poisoning in a short time, fast, accurate and specific.

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