Detection of Pathogenic Bacteria in Shrimp Paste through an Enrichment Stage Using Nutrient Broth Medium

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Abstract. Terasi products are one of the home industry products in Indonesia. Shrimp paste is produced from fermented small shrimp (called rebon) and small fish (called teri). As a food product marketed to the public, shrimp paste also has the potential to be contaminated with pathogens, both during production and after being sold. One of the pathogens that is highlighted in shrimp paste products comes from bacteria. In fact, the Indonesian Food and Drug Supervisory Agency (BPOM) emphasizes that specific pathogenic bacteria that you should be wary of can contaminate shrimp paste products. The sample is a packaged shrimp paste product that is ready to be marketed in Lombok Island. The enrichment stage was carried out using Nutrient Broth (NB) medium for 0 to 24 hours. The mixture was inoculated on Baird-Parker Agar (BPA) medium for detection of gram-positive pathogenic bacteria and Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS Agar) medium for detection of gram-negative pathogenic bacteria. The effect of the enrichment stage using NB medium resulted in a high increase in cell numbers. The highest increase occurred at 18 hours of enrichment, both in BPA and TCBS Agar medium. The enrichment method can be used as a preparation step for advanced applications such as molecular detection, cell activity assays, and culture collections.

Keywords: detection, enrichment, pathogenic bacteria, shrimp paste

1 Introduction

The presence of pathogens in food products causes a large number of diseases in humans. Diseases that arise as a result of pathogenic contamination of food are known as foodborne diseases, while the pathogenic biological agents that cause them are called foodborne pathogens. Various cases of foodborne diseases in an area are suspected to be caused by the consumption of food contaminated with pathogens in the form of bacteria, fungi, viruses, parasites, protozoa, worms, and algae [1][2][3]. At least more than 200 cases of foodborne diseases have been identified with various symptoms and

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causative pathogens. The most common symptoms found are nausea, vomiting, pain in the stomach area and digestive tract, diarrhea, headaches, fever, and various other disorders, even death [4].

Food contaminated with pathogens is unsafe to consume and poses a health threat worldwide. Prevention efforts have been made, including cooking, separating cooked and raw food, cold/frozen storage, and maintaining the cleanliness of food tools and ingredients [5]. Standardization of production processes is also carried out with food safety regulations and control from food regulatory bodies. Pathogen detection has also been used, both in the quality control stage and after the product is marketed. Pathogen detection is key to prevention and identification of problems related to health and food safety [6][7].

Shrimp paste is a product of processed fish in dry and fermented form. Various typical Indonesian dishes cannot be separated from shrimp paste as one of the seasoning components. Terasi is generally made from small shrimp (rebon) and small fish (teri). Shrimp paste also requires special handling to avoid pathogen contamination. Most biological hazards in shrimp paste products come from bacteria. There are 8 (eight) types of pathogenic bacteria that must be controlled in shrimp paste products, namely *Salmonella spp., Clostridium perfringens, Clostridium botulinum, Listeria monocytogenes, Campylobacter jejuni, Staphylococcus aureus, Vibrio cholerae,* and *Bacillus cereus* [2].

One detection method that is often used is the culture method. Using the culture method is considered to require more time, at least in a matter of days. The culture method provides advantages because the test is sensitive, inexpensive, and can be easily used in the laboratory. In addition, the use of culture methods for food samples can provide qualitative or quantitative information regarding the number and types of microorganisms living in food samples [8]. However, sometimes there are conditions where the detection results for pathogenic bacteria are very poor or even undetectable. This is mostly due to the presence of pathogenic bacterial cells in the samples being very low or in non-viable conditions [9]. One step that can be attempted before the definitive culture identification stage is enrichment. The use of enrichment media allows the growth and repair of pathogenic bacterial cells in the sample. This initial growth is very necessary to ensure that detection results are not mistaken. Thus, it is hoped that pathogenic bacteria in shrimp paste will grow better at the enrichment stage and can be specifically detected when cultured. Therefore, this research aims to develop a preparation stage for a method for detecting pathogenic bacterial cultures in shrimp paste through a reliable enrichment stage.

2 Materials and methods

2.1 Population and Sample

The population in this research is shrimp paste products produced by home industry in Lombok Island. The sample is a packaged shrimp paste product that is ready to be marketed. The samples of shrimp paste products were taken from the shrimp paste producer in Lombok Island. The collection of shrimp paste products for test samples was carried out using a random sampling technique.

2.2 Collection and Preparation of Shrimp Shrimp Product Samples

Sample collection and preparation of shrimp paste products was carried out using the Indonesian quality standard of SNI 2326:2010 concerning Methods for Sampling Fishery Products. This standard is a requirement stipulated in the SNI Document 2716:2016 concerning Shrimp Paste.

2.3 Enrichment Stages

A total of 10 grams of sample was added to 100 mL of Nutrient Broth (NB) medium, then homogenized. The mixture was inoculated in a shaker-incubator at 37°C with a rotation speed of 150 rpm for 0, 6, 12, 18, and 24 hours. A total of 0.5 mL of the mixture was inoculated on Baird-Parker Agar (BPA) medium for detection of gram-positive pathogenic bacteria and Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS Agar) medium for detection of gram-negative pathogenic bacteria in 2 times. The inoculation results in TCBS and BPA medium were incubated at 37°C for 24 hours. Negative controls were grown in a selection medium without a mixture of samples containing bacterial cells.

3 Results and discussion

3.1 Enrichment of gram-positive pathogenic bacteria

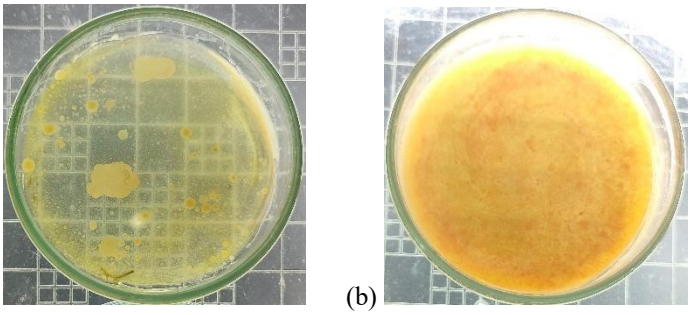
The effects of the enrichment stages of gram-positive pathogenic bacteria using NB medium can be seen in Table 1. The results of enrichment using NB medium were shown in the growth of gram-positive pathogenic bacteria on the BPA medium. The results were obtained by the growth of cultures on the selection medium. Slight growth was found on all plates in the 0-hour enrichment treatment. This was shown by only a few colonies growing on the BPA medium (Figure 1a). The longer the enrichment, the more colonies will be able to grow on the BPA medium. In the 18 and 24-hour enrichment treatment, colony growth was very dense so almost all of the plates were covered with pathogenic bacteria (Figure 1b).

Table 1. Enrichment results using NB medium on gram-positive pathogenic bacteria

Sample	Enrichment time (hours)				
	0	6	12	18	24
C1	+	++	++	+++	+++++
	+	++	++	+++	+++++
N1	+	++	++	+++	+++++
	+	++	++	+++	+++++
P1	+	++	++	+++	+++++
	+	++	++	+++	+++++
Q2	+	++	++	+++	+++++
	+	++	++	+++	+++++

Control BPA	-	-	-	-	-

The (+) sign indicates growth; the more (+) signs indicate higher qualitative growth.



(a) (b)

Figure 2. Growth of gram-negative pathogenic bacteria on BPA medium of sample C1; (a) 0-hour of enrichment; (b) 18-hour of enrichment

3.2 Enrichment of gram-negative pathogenic bacteria

The effects of the enrichment stages of gram-negative pathogenic bacteria using NB medium can be seen in Table 2. The results obtained were more or less the same as on the BPA medium, the growth of culture occurred on the selection medium. Slight growth was still found on all plates in the 0-hour enrichment treatment (Figure 2a). In contrast to the enrichment treatment for gram-positive pathogenic bacteria, the enrichment of gram-negative pathogenic bacteria grown on TCBS Agar medium showed a very dense increase in the 18-hour of enrichment and almost covered the entire medium plate (Figure 2b).

Table 2. Enrichment results using NB medium on gram-negative pathogenic bacteria

Sample	Enrichment time (hours)				
	0	6	12	18	24
C1	+	+++	++++	+++++	+++++
	+	+++	++++	+++++	+++++
N1	+	++	+++	+++++	+++++
	+	++	+++	+++++	+++++
P1	+	++	+++	+++++	+++++
	+	++	+++	+++++	+++++
Q2	+	+	++	++++	+++++
	+	+	++	++++	+++++
Control TCBS	-	-	-	-	-

The (+) sign indicates growth; the more (+) signs indicate higher qualitative growth.

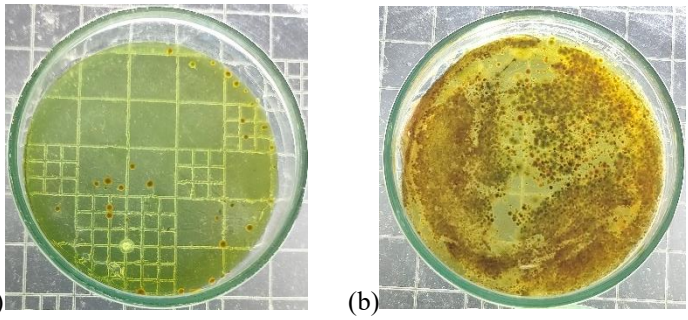


Figure 2. Growth of gram-negative pathogenic bacteria on TCBS Agar medium of sample C1; (a) 0-hour of enrichment; (b) 18-hour of enrichment

3.3 The need for an enrichment stage in pathogen detection

The enrichment stage in detecting pathogenic bacterial cultures in food samples is very important to carry out. Foodborne pathogens are contaminating agents that often do not show signs of damage to food. The existence of which is very difficult to detect by direct observation means that pathogen detection systems need to be designed to be very sensitive. However, sometimes food pathogenic bacteria are in very low numbers so enrichment treatment is needed to increase the numbers so they are easy to detect [10]. However, sometimes there is a bias that needs to be remeasured by the enrichment stage in pathogen detection. The enrichment stage has great potential to change the taxonomic profile of environmental samples other than the targeted organisms. One technique that can be used is to use metagenomics on the diversity of pathogens in the sample. Comparison of these data can provide more validity to existing pathogen detection results [11]. Therefore, alternative methods can be sought to improve pathogen detection using culture methods. Regarding specificity, PCR-based method such as multiplex PCR and qPCR methods can also be used to detect pathogens [12][13][14][15]. Basic PCR methods was known to have been used in research on the detection of Salmonella spp. on shrimp paste with a satisfactory success rate [16][17]. However, PCR detection only targets one type of pathogenic bacteria, while the security threat from other pathogenic bacteria can only be identified by carrying out PCR using another set of DNA primers. Thus, the speed of processing time, practicality, and cost savings for detecting pathogenic bacteria in shrimp paste can be improved compared to just using conventional PCR [18][19][20][21][22][23]. However, this still requires optimization to sharpen the validity of the detection results. Apart from this, an inexpensive and practical enrichment stage must also be developed in order to increase the expected pathogen detection results.

4 Conclusions

The effect of the enrichment stage using NB medium resulted in a high increase in cell numbers. The highest increase occurred at 18 hours of enrichment as indicated by the high number of colonies on the BPA medium for the detection of gram-positive pathogenic bacteria and TCBS Agar medium for detection of gram-negative pathogenic

bacteria. The high enrichment results can help ensure that the pathogenic bacterial cells in the sample are viable cells. This enrichment method can be used as a preparation step for advanced applications such as molecular detection, cell activity assays, and culture collections.

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