Identification of Bacteriocin Gene in *Lactobacillus acidophilus*

Siska Telly Pratiwi[1(✉)] and Panca Andana[2]

[1] Department of Microbiology, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia
siska.telly@lecture.unjani.ac.id
[2] Medicine Study Program, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia

Abstract. Lactic acid bacteria have the ability to produce antimicrobial substances such as bacteriocins. They are of great interest to research due to some of the lengthy annals of safe use and the generally considered safe (GRAS) and presumed safety (QPS) status. Bacteriocins are small, hydrophobic, cationic peptides with bacteriostatic or bactericidal activity in various microbes other than the producing strain. The production of bacteriocin depends on its strain and culture optimization. Genes encoding bacteriocin are located in chromosomes or plasmids. This study was carried out to detect the bacteriocin gene in *Lactobacillus acidophilus* FNCC 0051 and ATCC 4356. Non-lactic acid bacteria (*Bifidobacterium bifidum*), nuclease-free water, and distilled water are used as the negative control. The DNA extraction was carried out following Agilent Technologies® DNA Extraction Kit with some modifications. Primers used in this study are specific gene primers for acidocin LF221A, and the PCR cycling condition was done based on it. The result of using PCR showed that *Lactobacillus acidophilus* FNCC 0051 and ATCC 4356 have acidocin LF221A gene. It proved by electrophoresis analysis that the amplicon showed bands with a size less than 100 bp. The conclusion is that *Lactobacillus acidophilus* in this study contains gene encoding bacteriocin. Vice versa, non-lactic acid bacteria such as *Bifidobacterium bifidum* does not carry the bacteriocins gene.

Keywords: Acidocin · Bacteriocin · DNA · Gene · Lactobacillus acidophilus

1 Introduction

Lactic acid bacteria (LAB) are rod or coccus-shaped Gram-positive bacteria, non-motile, producing lactic acid as the result of their carbohydrate fermentation [1]. Besides lactic acid, they produce bacteriocins and organic acids that have antimicrobial activity [2]. A*erococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* are considered the primary LAB genera [3]. LAB have been used in the last decades for preservation techniques such as fermentation, pharmacological product, and even new

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antibiotics development. The antimicrobial substances they produce are responsible for it [4].

Bacteriocins are in great interest among other antimicrobial substances because of their heat and pH stability, broad-spectrum, and low toxicity [5]. Additionally, it is considered Generally Recognize as Safe (GRAS) by the United States Food and Drug Administration (US-FDA) and Qualified Presumption of Safety (QPS) by the European Food Safety Authority (EFSA) [6]. Bacteriocins are ribosomal synthesized, small, hydrophobic, cationic peptides encoded by a chromosome or plasmid gene [7]. There are many kinds of bacteriocins classified by their amino acid sequences, mechanism of action, and secretion mechanism [8]. Moreover, their production depends on their strain and culture optimization [9].

Lactobacillus acidophilus (*L. acidophilus*) is one of the primary genera of LAB that has proven to have the ability to produce bacteriocins. It mainly produces class II bacteriocins (acidocin, acidophilicin, lactacin) that have low molecular weight, heat-stable, and form pore in target bacteria [10]. Studies show that *L. acidophilus* strain American Type Culture Collection (ATCC) 4356 grown in US and Food and Nutrition Culture Collection (FNCC) 0051 grown in Indonesia produce bacteriocin that has an inhibitory effect on other bacteria [11, 12]. Unfortunately, no studies have shown the bacteriocin gene in the aforementioned strains. Thus, this study aimed to detect the bacteriocin gene in *L. acidophilus* ATCC 4356 and FNCC 0051.

2 Materials and Methods

This experimental-laboratory study focused on detecting bacteriocin gene in two strains of *L. acidophilus* that will describe further. The detection used Polymerase Chain Reaction (PCR) with specific primer. This study has three negative controls: non-LAB strain, distilled water, and Nuclease-Free Water (NFW). The study was conducted in the Microbiology Laboratory and Biochemical-biomolecular Laboratory of Jenderal Achmad Yani University and the Eijkman Molecular Biology Research Center.

2.1 Bacterial Strains and Culture Optimization

L. acidophilus FNCC 0051 and ATCC 4356 were the strains used for DNA identification. And *Bifidobacterium bifidum* (*Bif. bifidum*) as the negative control. They obtained from Gadjah Mada University (UGM) in Indonesia and ATCC. Cultures were grown in de Man Rogosa Sharpe Agar (MRSA) at 37 °C for 24 h with 7.5% CO_2 [12]. Each colony morphology was identified by observing its size, form, margin, color, and elevation. Meanwhile, microscopically its identified by Gram stain.

In preparation for DNA extraction, colonies are suspended at 0.5 McFarland concentration. It is equivalent to an absorbance value of 0.250 Au in a 450 nm spectrometer. 5 ml of 0.5 McFarland suspension of each strain were used for DNA extraction.

2.2 DNA Extraction

The extraction was based on the protocol of Agilent Technologies® DNA Extraction Kit with some modifications. DNA that had been extracted was washed with pure ethanol

by centrifugation, dried with Kimwipes®, and stored in sterile distilled water. Distilled water used for incubating DNA was saved as the negative control. So it will show if there is any contamination.

2.3 Primer Preparation

As follows are primer pairs used in this study. It is a specific primer for acidocin LF221A: forward primer 5′-AGAGTTTGATCCTGGCTCAG-3′ and reverse primer 5′-GGTTACCTTGTTACGACTT-3 [13]. Majhenič et al. [14] said that the gene encoding acidocin LF221A has a size of 69 base pairs (bp). The Freeze-dried primer was centrifuged first at 8000 rpm at 10 °C for 10 min to bring all the primer down to the bottom of the tube. After that, it was diluted with sterile distilled water according to product protocol and diluted more to make the working solution. The working solutions and stocks were stored in the freezer and defrosted gradually before use.

2.4 PCR Setting and Electrophoresis

In one PCR reaction, there are 50 μl of the mixture. It consists of 25 μl MyTaq™ HS Red 2x, 2 μl forward primer, 2 μl reverse primer, 20 μl NFW, and 1 μl DNA template. The thermal cycler is programmed with an initial denaturation at 95 °C for 1 min. Followed by 35 cycles containing denaturation at 95 °C for 30 s, annealing at 46.3 °C for 30 s, and extension at 72 °C for 1 min. After that, the program continued by a final extension at 72 °C for 5 min and held at 4 °C for 10 min. Five samples were subjected to PCR reaction: *L. acidophilus* FNCC 0051, *L. acidophilus* ATCC 4356, *Bif. bifidum*, distilled water used in DNA extraction and primer dilution, and NFW used in PCR.

Amplicons were analyzed by gel electrophoresis compared with a 1 kb DNA ladder. The DNA ladder was placed in the first lane of the electrophoresis. The amplicons from the samples mentioned above are third to seventh lanes in a row.

3 Results and Discussion

On macroscopic observation, the colonies of *L. acidophilus* were white with flat edges and protruding from the surface. On the other hand, *Bif. bifidum* showed colonies with milky white flat edges and convex. The colony description as per the characteristics of *L. acidophilus* and *Bif. bifidum* [15, 16]. Each bacteria that have grown is Gram-positive bacteria. Additionally, *Bif. bifidum* has a slightly shorter body than *L. acidophilus*, as shown in Fig. 1. It signifies that the bacteria used in this study were as desired species.

The PCR reaction that was carried out is amplified or increased the amount of target DNA in the PCR mixture. The amplification process can occur if the DNA template in the mixture contains the target DNA [17]. The amplified target DNA can be visualized by gel electrophoresis. In principle, the DNA fragments in the PCR results will move on the media and stop at one place according to their size. The PCR reaction causes DNA fragments to have the same size in large numbers to occupy the same place in the electrophoresis media. The gathering place for these fragments will form a band that

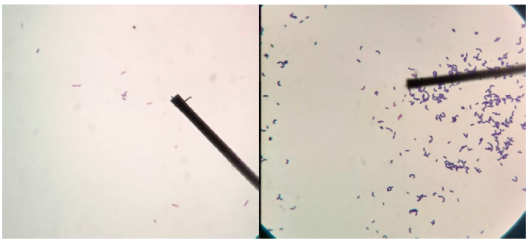

Fig. 1. Microscopic observation of *L. acidophilus* and *Bif. bifidum.*

Fig. 2. Electrophoresis analysis result.

can be seen with the naked eye [18]. Therefore, the target DNA amplified through PCR reactions can be confirmed by electrophoresis to see the size of the DNA.

In this study, the electrophoresis results show as shown in Fig. 2. The first column is DNA extracted from *L. acidophilus* FNCC 0051. The second column is DNA extracted from *L. acidophilus* ATCC 4356, and the third column is DNA extracted from *Bif. bifidum*. The fourth and fifth columns are distilled water, and NFW is a negative control. The last three columns did not show any bands, so the PCR results did not contain large amounts of DNA fragments of the same size. In contrast, the first and second columns show a band less than 100 bp.

It is known that the size of the DNA encoding acidocin LF221A is 69 bp [14]. The statement as per the results shown in electrophoresis. So, *L. acidophilus* FNCC 0051 and *L. acidophilus* ATCC 4356 showed the gene encoding the bacteriocin acidocin LF221A. However, the band shown by *L. acidophilus* ATCC 4356 was thinner than that of *L. acidophilus* FNCC 0051. It means that *L. acidophilus* FNCC 0051 can produce acidocin LF221A better. Even so, various things can cause the thin band in the second column.

Contamination is the most common reason that causes amplification errors in PCR reactions. This contamination can occur during the DNA extraction process or in the reagents used in PCR reactions. In addition, the annealing temperature (Tm) has an important role in the PCR reaction. The selection of Tm that is too low will cause the primer to bind nonspecifically to the DNA template. On the other hand, a Tm that is too

high makes it difficult for the primer to bind to the DNA template, so the resulting PCR product is not perfect [19]. Therefore, this bacteriocin gene detection can be considered relevant because the resulting band corresponds to the size of the target DNA and no contaminants were found in the negative control.

4 Conclusion

This study proves that *L. acidophilus* FNCC 0051 and *L. acidophilus* ATCC 4356 are lactic acid bacteria with bacteriocin genes. They contain acidocin LF221A gene. Meanwhile, non-lactic acid bacteria such as *Bif. bifidum* does not have acidocin LF221A gene.

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