Plantaricin characteristics for teat dipping in milking cow treatment

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Abstract. Fresh milk is perishable food, especially due to the microbial activity in it. Milk that contains lots of water and nutrients is indeed suitable for growth. Milking process allows the milk contamination from bacteria and impurities. Bacteriocin produced by lactic acid bacteria (LAB) has been known to display antimicrobial properties against some Gram-positive and negative bacteria. Therefore, bacteriocins can be used as biopreservative on both raw and processed foods. *Lactobacillus plantarum* is a bacteriocin-producing lactic acid bacterium known as plantaricin. Plantaricin IIA-1A5 has been previously isolated from Indonesian BAL of *L. plantarum* IIA-1A5. Because of this reason in this research, the characteristics are highlighted plantaricin IIA-1A5 in fresh cow's milk as antibacterial in the milking process. Lactoscan is used to analyze fat, Solid Non Fat (SNF), and protein in fresh milk. The results showed that the lactoscan results and microbiological characteristics of fresh milk have met SNI 3141.1 2011.

1. Introduction

Fresh cow's milk is high in nutrients and water and has neutral acidity. Milk is a food that contains complete nutrients such as protein, fat, vitamins and minerals. The content of nutrients, water and the optimum pH value makes milk a good medium for the growth of various types of microorganisms. This causes milk to become perishable food and has the potential to cause health problems for consumers [1]. Milk contains complete and high nutritional value. Fresh milk contains 87.5% water, 5% lactose, 3.5% protein, and 3%-4% fat [2]. The high nutritional content in milk provides the potential as an excellent growth medium for bacteria, both pathogenic bacteria and spoilage bacteria [3]. Bacterial contamination is able to develop rapidly so that the milk becomes damaged and is not suitable for consumption.

Fresh milk is perishable food, especially due to the microbial activity in it. Milk that contains lots of water and nutrients is indeed suitable for growth. The usual handling of fresh milk to extend its shelf life is cooling. At low temperature (refrigerator temperature), metabolism of bacteria will be disturbed so that the ability to reproduce and destroy milk is very limited [4].

The microbiological quality of milk is an important parameter in determining the safety of milk and its processed products. One of the bacteria that need to be tested to determine milk safety is Total Plate Count and *Escherichia coli*. The presence of these bacteria in food products such as milk and their processed products can cause food poisoning due to the toxins produced [5]. The presence of *E. coli* in fresh milk generally comes from milking, and unhygienic milk processing. These bacteria can make milk unsafe for consumption [6].
Bacteriocin produced by lactic acid bacteria (LAB) has been known to display antimicrobial properties against some Gram-positive and -negative bacteria [7]. The use of bacteriocin in food preservation has been shown to reduce the contamination of pathogenic bacteria and prolong shelf-life of products [8]. *Lactobacillus plantarum* is a bacteriocin-producing lactic acid bacterium known as plantaricin. Plantaricin IIA-1A5 has been previously isolated from Indonesian BAL of *L. plantarum* IIA-1A5[9]. This plantaricin has been shown to inhibit the growth of *E. coli*, *Salmonella* and *S. aureus* [10].

The quality of dairy products is strongly influenced by the quality of raw materials. Based on the potential for milk development at KUNAK which is the focus of milk development in Bogor and the importance of information on the raw material content of fresh milk in product development, it is necessary to conduct an assessment of the microbiological and physicochemical characteristics. In this research, the characteristics are highlighted plantaricin IIA-1A5 in fresh cow's milk as antibacterial in the milking process.

2. Method

2.1. Whey making

According to Fatmarani et al (208), fresh cow's milk pasteurized at a temperature of 75 °C for 15 minutes, then the milk cooled down to a temperature of 37 °C. The rennet was then inoculated into pasteurized milk with a concentration of 0.02 g L⁻¹ of milk. The milk coagulates after 30-60 minutes during the inoculation process and forms a curd. Curd used to make cheese, and the by-product liquid from this process is called whey. Whey was used as a medium for growing *Lactobacillus Plantarum* IIA-1A5 [11].

2.2. Production and purification of plantaricin IIA-1A5

According to Arief et al (2015), the production of plantaricin IIA-1A5 from *L. plantarum* IIA-1A5 culture was performed [9]. Secreted plantaricin IIA-1A5 was then purified from the medium by ammonium sulfate precipitation and cation exchange chromatography [12].

2.3. Dialysis

Dialysis was carried out using a dialysis membrane with a diameter of 3.5 µm and immersed in a phosphate buffer (KH₂PO₄ and K₂HPO₄) with a concentration of 20 mM and a pH of 6.8 for 24 hours at 4 °C. The phosphate buffer then replaced 4 times every 6 hours [8].

2.4. Lowry method protein level analysis

Determination of protein content as follows: 1 mL of protein suspension was put into a test tube and added 1 mL of Na₂CO₃ 2% (w / v) in 0.1 N NaOH, 1% Na + K + tartrate (w / v), and 0.5% CuSO₄ (w / v) with a ratio of 10: 0.5: 0.5. A total of 3 mL of Folin-ciocalteu was added to the test tube. After incubation for 10 minutes, the absorbance was read at A 650 nm. For the standard curve, BSA with a concentration of 0 is used; 0.1; 0.2; 0.3; 0.4, and 0.5 mg mL⁻¹ [13].

2.5. Antibacterial activity of plantaricin IIA-1A5

Briefly, 10⁶ cfu mL⁻¹ stock of *E. coli* (as a model pathogenic bacteria) was spread on to Muller Hinton Agar (MHA/Difco) media. Fifty microliters of plantaricin IIA-1A5 was poured into 6 mm-diameters well on agar. The plate was then incubated at 37 °C for 24 h. The area of inhibition was calculated from the diameters of the inhibition zones. These experiments were carried out in triplicate [8].

The research was conducted at a dairy farm in the Kawasan Usaha Peternakan (KUNAK) of Cibungbulang, Bogor from Juli to October 2020, and in the Integrated Laboratory, Animal Product Technology Division, Department of Animal Production and Technology, Faculty of Animal Science, IPB University. This research trial used cow’s teat from dairy farms in Kunak, Cibungbulang, Bogor. Furthermore, microbiological tests and physicochemical tests were carried out.
The study was conducted using a randomized block design (RBD) with 3 replications. The treatment design consisted of control, plantaricin, and povidone iodine. If different results are obtained, continue with the Tukey test.

3. Result and discussion

Table 1 shows that the plantaricin obtained from 22 liters of whey cheese yields as much as 89 mL. Plantaricin 89 mL produced a protein yield of 3.2 and a protein yield mL$^{-1}$ was produced as much as 3.03. The purification process of plantaricin is carried out in stages. After ammonium sulfate precipitation and cleaned using dialysis, then followed by the Lowry Protein Assay Method.

**Table 1.** Protein yield obtained from crude plantaricin according to Lowry Protein Assay Method.

| Plantaricin obtained (mL) | Protein yield (mL$^{-1}$) | Protein yield (89 mL) |
|--------------------------|---------------------------|-----------------------|
| 89                       | 3.03                      | 3.2                   |

Based on the research results of [14], that Plantaricin IIA-1A5 has broad-spectrum antimicrobial capabilities against Gram-positive and Gram-negative pathogenic bacteria. Based on [15], the pattern of action of bacteriocins in general is to kill bacteria through the formation of pores in the cell membrane which causes the cells to leak and eventually die. Based on [16], optimal antimicrobial activity of 90% -100% from plantaricin PASM1 from *Lactobacillus plantarum* A-1 shown in the pH range 5.5 to pH 7.

**Table 2** shows that cattle B produced the highest fat content (3.2%) and solid non-fat (SNF) (7.81%) than cattle A and C. Cattle C produced the highest protein content (2.8%) compared to cattle A and B. These results are in accordance with SNI 3141.1: 2011 that the minimum fat content (3.0%), minimum SNF (7.8%), and minimum protein content (2.8%). The results obtained for these three types of cattle are almost the same, because these cows come from the same pen with the same feed management, workers, and treatment.

**Table 2.** Mean of physicochemical characteristics fresh milk.

| Content | Type of cattle (%) |
|---------|-------------------|
|         | A     | B     | C     |
| Fat     | 3.03  | 3.2   | 3.06  |
| SNF     | 7.78  | 7.81  | 7.79  |
| Protein | 2.77  | 2.78  | 2.8   |

Table 3 shows that fresh milk from cattle A, B, and C for the number of Total Plate Count and *E. coli* bacteria are still in the normal range. Milk produced from healthy livestock and hygienic milking practices normally contains $10^6$ cfu mL$^{-1}$ milk SNI [16]. The amount of these biological elements changes depending on production conditions such as animal health and sanitation as well hygiene during milking, handling, transportation and milk products. These microorganisms have an effect on changes in the quality and contamination of milk. Temperature control is very important to prevent changes in milk quality associated with bacterial growth. Traditional milking practices can also stimulate the growth of contaminant microbes. One of the bacteria included in coliform is *Escherichia coli*. *E. coli* belongs to the *Enterobacteriaceae* family and includes Gram-negative bacteria, rod-shaped with a length of 2.0 to 6.0 micrometers, *E. coli* is present in single or paired forms, motile or non-motile. The minimum water activity ($a_w$) that allows *E. coli* growth is between 0.95 and 0.96 [17].

**Table 3.** Mean of microbiological characteristics fresh milk.

| Content | Type of Cattle (cfu mL$^{-1}$) |
|---------|-------------------------------|
|         | A     | B     | C     |
| TPC     | 2.5x10$^5$ | 2.8x10$^5$ | 1.87x10$^5$ |
| *E. coli* | <1.0x10$^1$ | <1.0x10$^1$ | <1.0x10$^1$ |
4. Conclusion
Plantaricin obtained from 22 liters of cheese whey is 89 mL. Lactoscan results and microbiological characteristics of fresh milk have met SNI 3141.1 2011.

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