Chapter 8

Prevention of *Staphylococcus aureus* Contamination on Animal Products Using Indonesian Natural Products

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Additional information is available at the end of the chapter

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Abstract

Foodborne transmission of pathogenic microorganisms has been recognized as an important hazard. One of foodborne pathogens that was well known for 30 years, that associated with animals, have presented as illness-causing agents in humans, is *Staphylococcus aureus*. *S. aureus* is a bacterium that produces enterotoxin, causing poisoning to humans. These bacteria are found in foods that contain high protein such as sausage, eggs, meat, beef, poultry products, and milk products. *S. aureus* is a Gram-positive bacterium that is an indicator of contamination from the worker and tools. *S. aureus* contamination on raw animal products such as eggs, raw beef, and poultry products also milks in Indonesia has been reported by many researchers. Indonesia is a tropical country that has high humidity, heavy rain, and two seasons (dry and wet) that contribute to *S. aureus* contamination especially in animal products. Furthermore, poor postmortem handling on animal products also causes the contamination. Preventive methods are needed for food processing and food storage especially for animal products in Indonesia. This chapter in this book explains the contamination of *S. aureus* in animal products in Indonesia and the preventive methods used in Indonesia to reduce the contamination. Plant extracts, herbs, spices, bacteriocins, and lactic acid bacteria have been widely used in food processing in Indonesia that proved as biopreservatives for animal products.

Keywords: prevention, *Staphylococcus aureus*, animal products, Indonesia

1. Introduction

Foodborne transmission of pathogenic microorganisms has been recognized as an important hazard. The predominant foodborne pathogens that were known 30 years ago are *Salmonella, Clostridium botulinum, Clostridium perfringens, and Staphylococcus aureus* and have been joined by a widening array of pathogens of bacterial, viral, and parasitic origin. Those

pathogens that were only seen associated with animals have been presented as illness-causing agents in humans [1]. *S. aureus* is a Gram-positive bacterium that is an indicator of contamination from the workers and tools. *S. aureus* is a normal flora on the skin and in the respiratory organs in humans, and it is generally found in 20–50% of healthy population [2]. *S. aureus* contamination in food could also occur after the food has been cooked. In relation to cases of food poisoning, *S. aureus* enterotoxin intoxication on consumers occurs through the establishment of contamination on food consumed. This enterotoxin is resistant to heat (heat stable), acid-resistant, and resistant to the effects of proteolytic enzymes such as pepsin and trypsin.

S aureus contamination is found in animal products that are marketed in Indonesia, such as eggs, chickens, and raw beef and also other raw meat products. Poor handling and improper storage methods cause the contamination of *S. aureus,* which survive on kitchen utensils and unwashed hands.

Some *S. aureus* contamination that is in animal products marketed in Indonesia is as follows:

a. Chicken eggs

Egg contamination can be derived from the environment. *S. aureus* would stick to the eggshell and subsequently on holding it penetrates into the egg through the pores in the eggshell.

b. Chicken meats

Population of *S. aureus* contamination on chicken breast meats is 2.71 ± 0.02 log CFU/g up to 3.25 ± 0.28 log CFU/g in Java Island, Indonesia (research result). *S. aureus* contamination on breast chicken meat can be derived from the contents of the digestive tract during slaughtering process in the poultry abattoir. Contamination on the carcass also occurs from the air or feces that contaminates skin and carcass [3]. External factors that influence the contamination of *S. aureus* are pH value and a_w (water activity) on breast chicken meat. a_w that is optimum in food for growth factor of *S. aureus* is 0.8–1.0.

c. Beef

Contamination of *S. aureus* on fresh beef at traditional market in West Java, Indonesia, has been investigated. The population of *S. aureus* was approximately 2.48 log CFU/g [4] and increased continuously every hour in the room temperature of storage.

2. Antimicrobials agents against *Staphylococcus aureus*

2.1. Spices, herbs and plant extract as antimicrobials against *Staphylococcus aureus*

The compounds found in herbs and found beneficial as traditional medicine can also be used as antibacterials and natural preservatives. The use of antibacterial synthetic or synthetic preservatives in foods such as the addition of formaldehyde or borax (borax) if taken

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continuously will cause a disease. The existence of the above phenomenon encourages people to find the best solution for health. An alternative solution is to replace synthetic antibacterial agents with natural antibacterial agents. Preventive methods have been used through the application of indigenous Indonesian herbs, plant extracts, and spices. The antimicrobial activities of plant extracts used for seasoning in foods have been recognized. The most common plant secondary metabolites that have antimicrobial activities occur in the following groups: alkaloids, anthraquinones, coumarins, essential oils (terpenoid and phenylpropanoids), flavonoids, steroids, and triterpenoids [5]. Some of them have antimicrobial activities.

2.1.1. Curcuma domestica val

Turmeric (*Curcuma domestica* val) is one of the plants that is used for traditional medicine by our ancestors long ago. Turmeric has great potential in the pharmacological activity that is, anti-inflammatory, anti-immunodeficiency, anti-virus (bird flu virus), anti-bacterial, and anti-fungal [6]. The antibacterial properties in turmeric are caused by the chemical content of its main and essential oil curcuminoid.

2.1.2. Ginger

Ginger can grow in the lowlands of the mountainous regions with an altitude of 0–1500 m above sea level. It has been used in food for seasoning in Indonesia. Meat cooked with ginger can have longer storage duration than without ginger. Ginger contains gingerol bioactive compound, which is a major component that can be converted into shogaol or zingerone shogaol formed from gingerol during the heating process [7].

2.1.3. Garlic

Raw garlic can be minced, pressed, sautéed, pickled, boiled, and juiced. Garlic sulphur compound(s) is(are) the primary bioactive agent(s). The major thiosulfanates, allicin, account for approximately half of the total of thiosulfanates from the *Allium sativum* genus [8]. Allicin was described as colorless oil, extremely pungent for the principal odor and taste of garlic. It was reported that allicin in concentrations of 1: 85,000 in broth was bactericidal to a wide variety of Gram-negative and Gram-positive organisms. A 5% garlic extract concentration has a germicidal effect on *S. aureus* [9]. Garlic extract used for seasoning in Indonesian food has strong antibacterial activities against *S. aureus* (**Figure 1**).

2.1.4. Clove oil

Clove oil has a potential as a preservative for food products and is known as Generally Recognized As Safe (GRAS) as a food ingredient. In addition, various studies have shown that clove oil has antimicrobial properties against *Salmonella* sp., *Listeria monocytogenes, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli,* and *S. aureus*. An amount of 0.25/100 ml of clove oil could inhibit *S. aureus*. The application of clove oil in processed meat products showed that at a concentration of 1 ml/l, it reduces the bacterial population significantly ($P < 0.05$), as much as 0.88 log CFU/g.

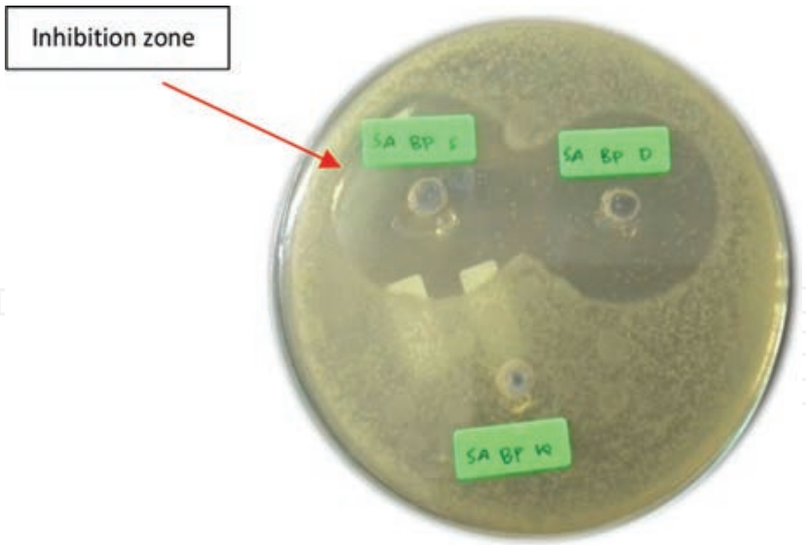

Figure 1. Inhibition zone of antimicrobial activities of garlic extract against *S. aureus*.

2.1.5. Roselle flower

An essential ingredient contained in rosella flower petals is the pigment anthocyanin that forms flavonoids and acts as antioxidants. Anthocyanin that causes the red color of this plant contains delfinidin-3-siloglukosida, delfinidin-3-glucoside, and sianidin-3-siloglukosida, while flavonoids contain gossypetin and mucilage (rhamnogalacturonan, arabinogalactan, and arabinan). *Hibiscus sabdariffa* Linn (Roselle flower) also contains phenol compounds that can be chemically defined by the presence of the aromatic ring carrying one (phenol) or more (polyphenols) substitution of hydroxyls [10]. The working of phenol in killing microorganisms is by cell protein denaturation. Phenol derivatives interact with bacterial cells through adsorption process involving hydrogen bond. At low levels, protein complex forms phenol by weak bonds and immediately occurs as decomposition, followed by phenol penetration into cells, causing precipitation and protein denaturation. Roselle flower extracts were proven for their antibacterial activities against *S. aureus* (**Figure 2**) and are used in yoghurt products in Indonesia as flavoring and preservatives.

2.1.6. Red dragon fruit extract

Flavonoids, phenols, hydroquinones, and saponins are the phytochemical compounds found in red dragon fruit peel extract. Steroids and triterpenoids compounds are also found in the red dragon fruit peel. The phytochemical substances of red dragon fruit extract have antibacterial activity that reacts with the bacterial cell wall proteins.

Red dragon fruit peels were extracted by modification maceration. Dragon fruits were cleaned and peeled manually before being cut into small sizes (2 mm). Red dragon fruit peels were dried at 50°C with an oven and ground to a powder. Peel powder was added with a solvent (1:50) for 60 min and filtered. The solution was evaporated at a vacuum evaporator temperature of 60°C. The extract was stored at −20°C and continued to be used in the

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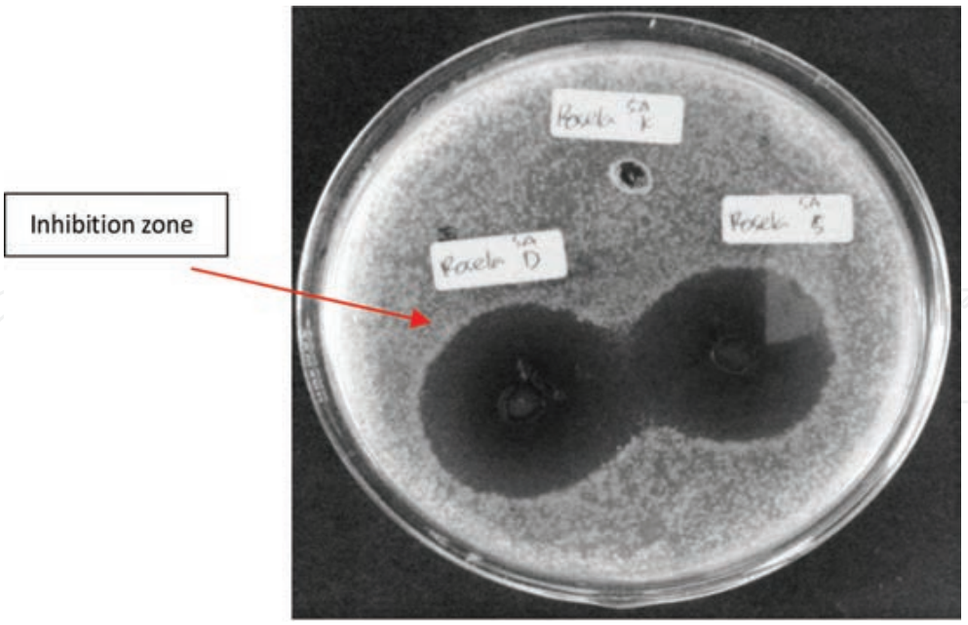

Figure 2. Inhibition zone of antimicrobial activities of Roselle flower extract against *S. aureus*.

antimicrobial analysis. Analysis of antimicrobial activity was performed by the well diffusion method. Bacterial culture was inoculated in NaCl 0.85% to obtain the bacteria concentration of 10^8 CFU mL^{-1}. The dilution of the bacterial culture was done to obtain a culture concentration of 10^6 CFU mL^{-1}. The other culture was grown in Mueller-Hinton Agar medium (Difco™, USA) and provided with holes as well with a predetermined diameter. Extracts were inserted into the well and covered with filter paper. Grail was stored in a refrigerator for 2–3 h, followed by incubation at 37°C for 24 and 48 h. The antimicrobial activity was characterized by the formation of clear zones around wells and measured for its diameter (mm). The inhibition zone produced by red dragon fruit peel extracts showed strong antibacterial activity (**Figure 3**).

Gram-positive bacterium, *S. aureus* ATCC 25923, was more sensitive to the antibacterial activity of red dragon fruit peel extract. The Gram-positive bacteria are more susceptible to antibacterial activity due to the absence of a lipoprotein wall that is capable of preventing antimicrobial compounds. Red dragon fruit peel extract due to its antibacterial compounds such as phenolic compounds could inhibit the growth of bacteria [13]. Application of red dragon fruit extract on beef sausages showed that *S. aureus* was not detected during 20 days of cold storage.

2.1.7. Teak leaf extract

Teak leaf extracts have a composition of flavonoids, alkaloids, tannins, anthraquinones, and naphthoquinones as antimicrobial substances that inhibit the growth of bacteria [11]. Addition of teak leaf extracts effectively inhibited *S. aureus* in the sausages. The 0.5 and 1% concentrations of teak leaf extracts addition on sausage formula in the processing could effectively inhibit *S. aureus* [12]. The method of teak leaf extraction is as follows: The extraction of teak

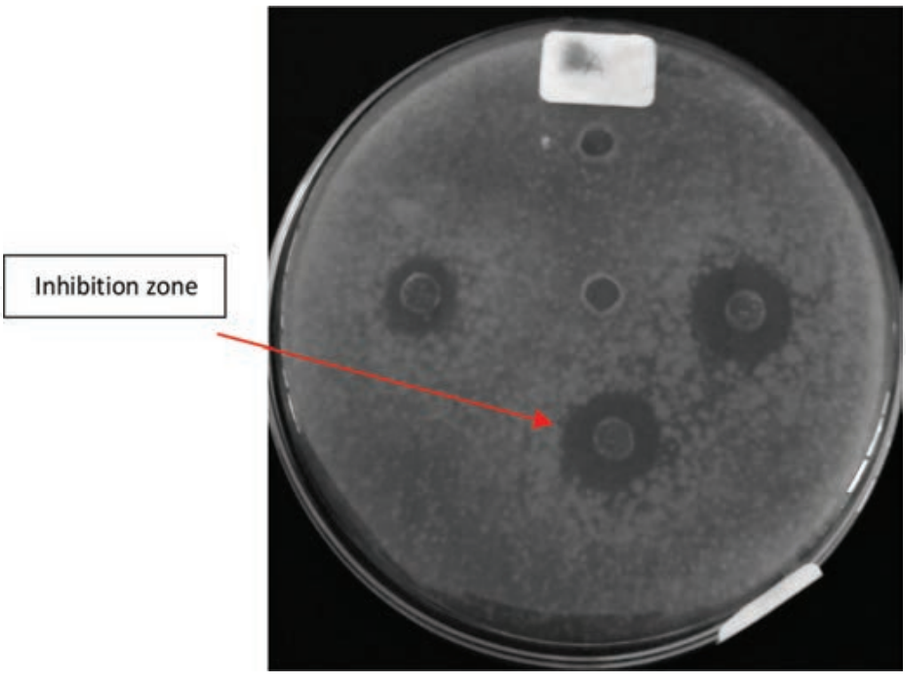

Figure 3. Inhibition zone of antimicrobial activities of red dragon fruit extract against *S. aureus*.

leaf was performed using ethanol extraction. Fresh teak leaf was oven-dried at 60°C for 24 h, chopped, and blended. Two hundred milliliters of 96% ethanol was then added into 20 g of teak leaf powder (10:1 ratio) and was boiled using waterbath at 70°C for 2 h. The mixture was centrifuged at 6000 rpm for 15 min. Ethanol was then removed by air-dry evaporation. The inhibition zone of antimicrobial activities was performed using diffusion methods [12]. The result of the antibacterial activities of teak leaf extract against *S. aureus* is shown in **Figure 4**.

2.2. Bacteriocins as antimicrobials and their application as meat product biopreservatives

Bacteriocins produced by Indonesian lactic acid bacteria *Lactobacillus plantarum* IIA-1A5 was purified and characterized. Plantaricin IIA-1A5 has been previously isolated from Indonesian lactic acid bacteria of *L. plantarum* IIA-1A5. This plantaricin has been shown

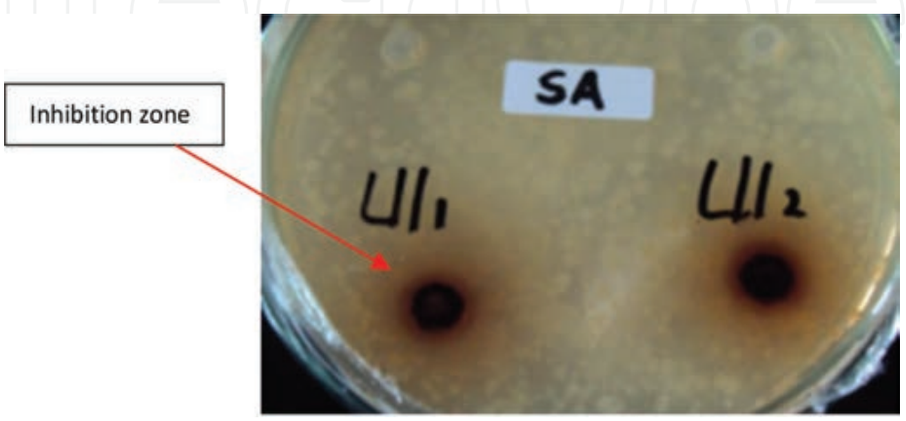

Figure 4. Inhibition zone of antimicrobial activities of teak leaf extract against *S. aureus*.

to inhibit the growth of *S. aureus* [13], making it a promising preservative substance to replace the use of chemical preservatives. Plantaricin could be digested by trypsin enzyme. It was heat stable at 80°C for 30 min and 121°C at 15 min, also active in a broad pH range of 4.0–9.0. Plantaricin IIA-1A5 could inhibit the growth of pathogenic bacteria, such as *E. coli, Salmonella Typhimurium, Bacillus cereus,* and *S. aureus.* Plantaricin IIA-1A5 showed good characteristics as an antimicrobial [14]. Plantaricin IIA-1A5 employs bactericidal activity since it disrupts the cell membrane and promotes the release of ions, proteinaceous, and genetic materials [13]. The cell wall of Gram positive has a thicker peptidoglycan layer, which is dominantly composed of lipoteichoic acid (LTA). The LTA is the target recognition of bacteriocin, facilitating the absorption of bacteriocin in the cell wall of Gram-positive bacteria [15].

2.2.1. Genes involved in the production of plantaricin

The genes responsible for bacteriocin production in *L. plantarum* IIA-1A5 are at least organized in two different operons: *plnABCD* and *plnEFI* [13]. The genes have been sequenced. PlnB (representative of operon plnABCD) amino acid sequence is derived from translation of partial DNA sequences using the software APE plasmid editor (http://biologylabs.utah.edu/jorgensen/wayned/ape/). Regardless of its open-frame reading, partial sequence of plnB is shown in **Figure 5**.

To identify what kind of protein is encoded by plnB gene, we performed protein BLAST. BLAST results showed that the DNA sequence has 100% similarity with the histidine kinase genes for plantaricin on *L. plantarum* (**Figure 6**). The histidine kinase has been reported as one of the genes responsible for bacteriocin production. It is located in the locus responsible for plantaricin production in some of the plantarum strain. Histidine kinase is a quorum sensor to monitor the cell density of a bacterial population. At a certain concentration threshold, histidine kinase will be activated through a certain mechanism and induced with the production of bacteriocin [13].

Sequencing of plnEF gene and translation plnEF gene to amino acid has been conducted. PlnEF amino acid sequences were also obtained from the translation of DNA sequences using the software ApE plasmid editor (http://biologylabs.utah.edu/jorgensen/wayned/ape/). PlnB translation of DNA sequences to amino acid sequences is presented in **Figure 7**.

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  1 TTC AGA GCA AGC CTA AAT GAC GGT AGT ATT GCA AGT GTT CAA CAT TTG AAG AAT GAG ATA  60
  1 Phe Arg Ala Ser Leu Asn Asp Gly Ser Ile Ala Ser Val Gln His Leu Lys Asn Glu Ile  20

 61 TTG CGC GGG TTA GTT GTA CAG AAG TTT TTT TAT GCG AAA CAG TGT GGG GTT AAG TTG ACG  120
 21 Leu Arg Gly Leu Val Val Gln Lys Phe Phe Tyr Ala Lys Gln Cys Gly Val Lys Leu Thr  40

121 ATT GAA ATA GCT AAC ACT GAC TTT ATT CTC AGT CAT GGT GTT ACA GTG  168
 41 Ile Glu Ile Ala Asn Thr Asp Phe Ile Leu Ser His Gly Val Thr Val  56
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Figure 5. Amino acid sequences translation of plnB derived from its DNA sequences. The number on the left and right side shows the numbering sequence of the DNA sequence (top row) and amino acids (the second row). Bases in DNA are written in capital letters, while the amino acids are written in the format of three letters.

Figure 6. Multiple amino acid sequence alignment PlnB (35555) with homologous proteins. Each homologous proteins used in the alignment presented in the access code in the database. The red sequence shows the location of homology in the alignment.

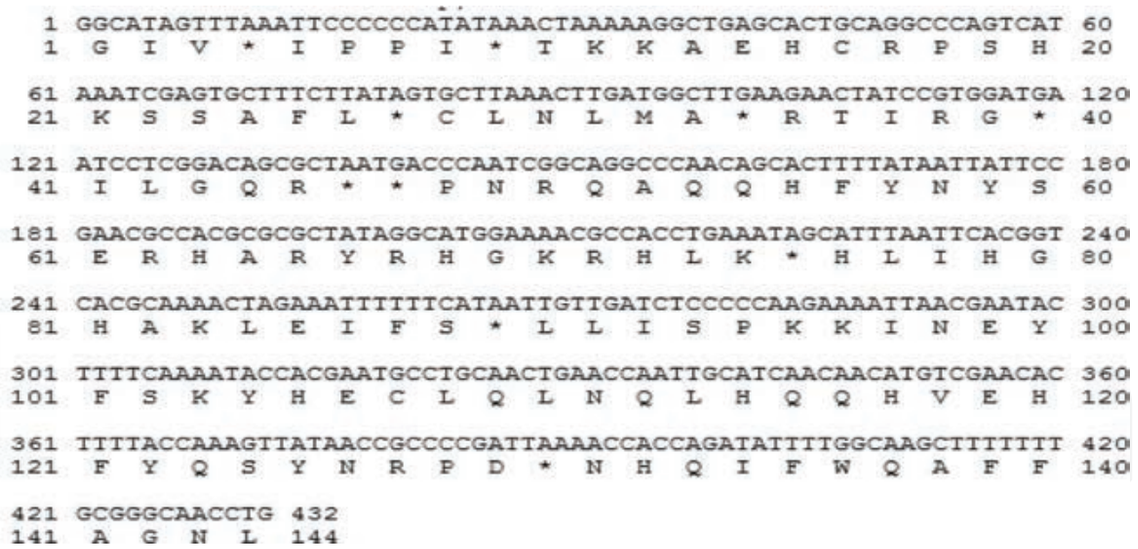

Figure 7. PlnE translation of DNA sequences into amino acids. The number on the left and right side shows the numbering sequence of the DNA sequence (top row) and amino acids (the second row). Bases in DNA are written in capital letters, while the amino acids are written in one letter.

BLAST results showed that the DNA sequence of plasmid Ape editors has a high homology with the pln locus from several strains of plantaricin from *L. plantarum*. This means plnE correct encoding plantaricin [13]. Alignment results either in whole or in part show that the homology of plnE is more than 90% with various strains of the plantaricin (**Figure 8**).

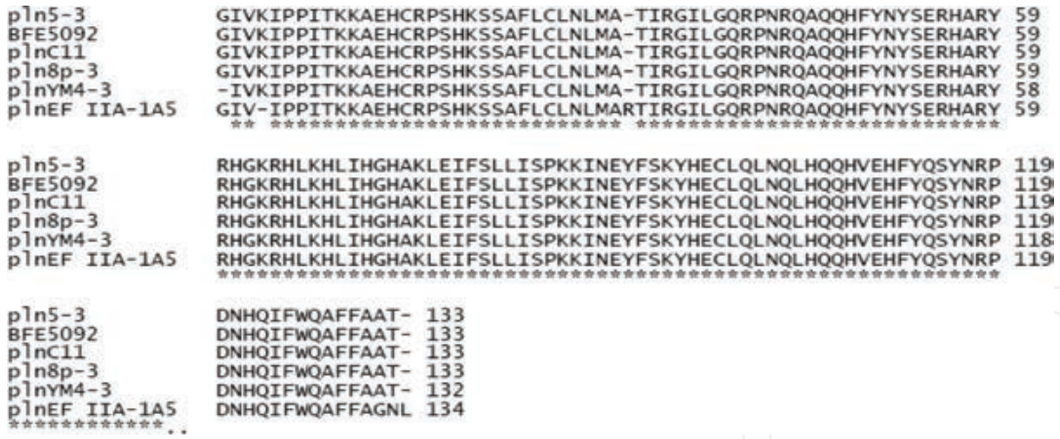

Figure 8. Multiple sequence alignment of amino acid sequencing of plantaricin EF.

2.2.2. Application of plantaricin IIA-1A5 as a biopreservative

Plantaricin IIA-1A5 could be used as biopreservatives for raw beef after slaughtering from abattoir. The initial contamination of *S. aureus* on raw beef is 2 log CFU/g. The addition of 0.2% plantaricin IIA-1A5 by spraying it onto raw beef surface could enhance the safety of beef from *S. aureus* contamination. Population of *S. aureus* on beef with 0.2% plantaricin is lower than maximum standard allowed by Indonesian standard of fresh beef (2 log CFU/g). In control (without plantaricin addition), population of *S. aureus* increased continuously every hour (3 log CFU/g). Plantaricin IIA-1A5 is able to extend the shelf life of meat stored at room temperature, according to physicochemical and microbiology quality [4].

Another application of plantaricin is as a biopreservative in meat products. *S. aureus* has been observed in meatballs without preservatives after 5 h of storage at room temperature. The 0.3% plantaricin IIA-1A5 addition displayed inhibition of *S. aureus* to be as strong as 0.3% nitrite. Until 20 h storage at room temperature, meatballs with nitrite or plantaricin IIA-1A5 were considerably safe to be consumed, which is a proven and promising potential use of plantaricin as a nitrite replacer for meatballs preservative [16].

3. Bacteriocin produced by *S. aureus*

S. aureus produced bacteriocins and bacteriocin-like substances that were correlated with the presence of a plasmid usually involved in type B exfoliative toxin production. The bacteriocinogenic plasmids carried by the *S. aureus* strains are identified as plasmids larger than 40 kb that code for a high-M bacteriocin and that do not confer immunity [17]. *S. aureus* was isolated from bovine mastitis cases in 56 different Brazilian dairy herds and has been successfully investigated to produce antimicrobial substance (AMS). The bacteriocins may possess potential practical applications since they were able to inhibit important pathogens such as *B. cereus* and *L. monocytogenes* isolated from nosocomial infections [18] and show a potential application in food preservation [19]; meanwhile, the pathogenicity of *S. aureus* should be discussed

for safety of bacteriocin. The antimicrobial activity of the bacteriocin produced by *S. aureus* is detected to be resistant to heat treatment at 65°C; however, treatment at 80°C completely abolished its antimicrobial properties [19].

Although *S. aureus* also produced bacteriocin, it could not kill and inhibit the cell itself because of immunity system. Bacteriocin-producing bacteria protect themselves from similar bacteriocin by immunity proteins. When these proteins are expressed in sensitive cells, they strongly protect against externally added similar bacteriocin. The immune system can work synergistically to protect the producing cells from their own bacteriocin [17]. Plasmid carried by the *S. aureus* strains confers immunity identified as small plasmids (8.0–10.4 kb), which code for bacteriocins or bacteriocin-like substances with a low M [18].

4. Conclusion

To prevent *S. aureus* contamination, many antimicrobial substances originated from Indonesia and are widely used for food processing and as preservatives. Herbs, plant extracts, spices, and indigenous bacteriocin isolated from Indonesian lactic acid bacteria also prove to be effective antimicrobial agents for animal products. Many different types of mode of action and antimicrobial mechanisms could be synergic as animal product preservatives in Indonesia.

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