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Research Article

Application of Antimicrobial Agents Produced by *Lactobacillus plantarum* IIA-1A5 as Natural Preservative on Beef during Room Temperature Storage

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Abstract: *Lactobacillus plantarum* IIA-1A5 is indigenous lactic acid bacteria isolated from Indonesian beef. *Lactobacillus plantarum* IIA-1A5 was reported could produce bacteriocin, called plantaricin IIA-1A5. The aims of this research was to analyze application of plantaricin IIA-1A5 as a natural preservative on beef. Based on antagonistic test, plantaricin IIA-1A5 had good moderate antimicrobial activity against pathogenic bacteria isolated from human's feces that cause diarrhea such as *Salmonella* 38, *Enteropathogenic Escherichia coli* K11 and *Shigell*a A33. Application of plantaricin IIA-1A5 was effective as a natural preservative on beef stored at room temperature by inhibiting the growth of *Escherichia coli* and *Staphylococcus aureus*. Plantaricin IIA-1A5 could kill all of the *Escherichia coli* after 5 h storage. Plantaricin IIA-1A5 could reduce the population of *Staphylococcus aureus* in beef during room temperature storage. Interestingly, plantaricin produced by *Lactobacillus plantarum* IIA-1A5 was effective against gram negative and positive bacteria. According to physichochemichal and microbiology quality, plantaricin IIA-1A5 was recommended as biopreservative agents for beef.

Keywords: Bacteriocin, beef, *Lactobacillus plantarum*, natural preservative

INTRODUCTION

Utilization of Lactic Acid Bacteria (LAB) in food ingredients as natural biopreservative agents have been much studied and one of them is bacteriocin. Bacteriocin is a peptide compound produced by lactic acid bacteria, which have antimicrobial activity. The bacteria which are derived from LAB have the potential to be applied to food because they are not toxic and do not produce toxins, so called food-grade microrganisms, known as Generally Recognized As Safe (GRAS), that is, microorganisms that expose no risk to health and some types of the bacteria are even useful for health. *Lactobacillus plantarum* is one of the lactic acid bacteria producing bacteriocin known as plantaricin (Diep *et al.* 1996; Holo *et al*., 2001; Maldonado *et al*., 2003).

Arief *et al*. (2013) succeeded in isolating several strains of *L. plantarum* referred to as *L. plantarum* 1A5, 1B1, 2B2 and 2C12. The four of strains have the inhibition capability of pathogenic bacteria groups. *L. plantarum* IIA-1A5 is a Indonesian indigenus strain of *L. plantarum* isolated from Indonesian beef, as identified by using 16S rRNA gene sequencing methods (Arief 2011; Arief *et al*., 2015). *L. plantarum* IIA-1A5 has been found to produce bacteriocin with some specific characteristics, such as ability to with stand in pasteurization temperature (80°C for 30 min) and sterilization temperature (121°C for 15 min), remaining active in a pH range of 4 to 9, can be degraded by protease enzyme and being proven able to inhibit the growth of pathogenic bacteria like *Escherichia coli*, *Salmonella typhimurium*, *Bacillus cereus* and *Staphylococcus aureus* (Arief *et al*., 2013).

The research focused on the application of pure plantaricin as natural preservative on beef which is expected to replace the use of chemicals as food preservatives. The use of chemicals as food preservatives are very risky to human health, especially when the users do not follow the terms of the security commission, they tend to be toxic to the body tissues. Plantaricin application will be a breakthrough for the development of biotechnology in food preservation because it is safe to be consumed, active in a low concentration and has a good shelf life.

MATERIALS AND METHODS

Plantaricin purification aimed to obtain pure plantaricin, which consisted of several stages, among others, a stage of partial purification using ammonium sulfate, dialysis and purification using cation exchange chromatography as practiced by Arief *et al*. (2013), but

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there was a modification, where in Arief *et al*. (2013) cell free supernatant evaporation was not done. In this research, evaporation was conducted in cell-free supernatant until the volume reached half of the initial volume.

Antimicrobial activity of Plantaricin IIA-1A5: Antimicrobial activity of pure plantaricin against pathogenic bacteria such as *Salmonella* 38, EPEC K11 and *Shigella* A33, which were tested using a well diffusion method (Savadogo *et al*., 2006). Pathogenic bacteria were refreshed 2 times and incubated for 24 hours at 37°C. The culture of pathogenic bacteria was inoculated in 0.85% NaCl so that its concentration became 10^8 cfu/mL (compared with standard solution of Mc.Farland). The culture was as much as 1 mL and diluted with 9 mL of 0.85% NaCl so that its concentration became 10^7 cfu/mL. The same dilution was repeated in order to obtain bacterial concentration of 10^6 cfu/mL. 1 mL of pathogenic bacteria culture was pipetted on a petri dish and added by 20 mL of medium MHA (*Muller Hinton agar*, Oxoid). Once the agar hardened, a well was made using a cork borer with a diameter of 5 mm. Next, pure plantaricin IIA-1A5 was pipetted into the well as much as 50 µL and then stored in a refrigerator at 7°C for 3 h. After that the plate was incubated at 37°C for 24 h. Finally, antimicrobial activity was shown by the formation of a clear zone around the well.

Application of bacteriocin plantaricin IIA-1A5 to Beef: Seventy five grams of *top side* beef was placed in two sterile plastic (control and 0.2% plantaricin). For plantaricin treatment, 0.2% plantaricin was diluted 100x with aquabidest. Then, plantaricin is evenly sprayed to all parts of the beef and wait 30 min until plantaricin has seeped into the beef. Next, the beef is divided in four sterile plastic to be analyzed at 0, 5, 10 and 15 h at room temperature.

Microbiological quality analysis of beef: Briefly, 25 g of beef was suspended in 225 mL of *Buffer pepton water* (BPW) (w/v). Microbiological analysis was carried out by a pour plate method using media of *Baird Parker Agar* (BPA, Merck) to test *Staphylococcus aereus* bacteria, *Xylose Lysine Desoxycholate Agar* (XLDA) to test *Salmonella* bacteria and of *Eosyn Methylene Blue Agar* (EMBA, Merck) test for *E. coli*. Pathogenic bacteria were taken from the dilutions of 10^1,10^2 and 10^3. The samples were incubated for 24 hours at 37°C (AOAC 2005).

Physicochemical quality analysis of beef: pH values were measured using a pH meter (Hanna Instruments, USA). Water activity was measured with a_w meter SAL-T and Sensor-Check SC Number 75 with manufacturer's protocols.

Data analysis: The research data was collected and analyzed using the Analysis of Variance (ANOVA) based on a completely randomized design (2×3 factorial) with two treatment (0.2% plantaricin and control) with 3 replications. If there is a significant difference between the treatments, it will be followed by Tukey's test (Steel and Torrie, 1995).

RESULTS AND DISCUSSION

Antimicrobial activity of Plantaricin IIA-1A5: Enteropatogenic *Escherichia coli* (EPEC K11) was the bacteria isolated from the feces of children with diarrhea (Budiarti, 1997). EPEC is one of the six virotypes of *E. coli* that can cause acute diarrhea. The second pathogenic bacteria most frequently detected in patients with diarrhea are *Shigella* (Eppy, 2009). *Salmonella* is a type of bacteria that often contaminate foods of animal origin such as meat, milk and other dairy products which can result in an outbreak of salmonellosis. Salmonellosis outbreak could cause about 3 million deaths each year in developing countries (Zein *et al*., 2004).

Plantaricin produced by *Lactobacillus plantarum* IIA-15A could inhibit the growth of *Salmonella* 38, EPEC K11 and *Shigell*a A33 (Table 1). Antimicrobial activities of plantaricin IIA-1A5 againts those pathogenic bacteria was a good moderate level based on criteria from Ismail *et al*. (2013). Plantaricin IIA-1A5 against EPEC K11 was better than antibiotics, because EPEC K11 bacteria are resistant to tetracycline and ampicillin (Budiarti and Mubarik, 2007). Raw beef and chicken are the foods that often cause diarrhea. Therefore, plantaricin IIA-1A5 can be recommended as a natural preservative to reduce the risk of diarrheal disease in Indonesia.

In general, plantaricin IIA-1A5 produced by gram positive bacteria could inhibit gram negative bacteria, called an unusual case (Todorov *et al*., 2007). Boziaris and Adams (1999) reported that gram negative organisms had protective outer membranes blocking bacteriocins work. Some *Lactobacillus plantarum* strains, that produced bacteriocins that could inhibit gram negative bacteria, had been reported. *Lactobacillus plantarum* ST202Ch and *Lactobacillus* ST216Ch produced bacteriosin which had antimicrobial activity against gram positive and negative bacteria (Todorov *et al*., 2005). *Lactobacilllus plantarum* isolated from two Nigerian fermented foods, ogi and Tufu, inhibited more than 40% of strains of gram negative bacteria from catfish (Ogunshe and Olakbode, 2009). Inhibitory activity of *Lactobacillu*s depended on the producers and the pathogenic bacteria strains (Smetankova *et al*., 2014).

Aplication of plantaricin IIA-1A5 on beef: The values of pH and water activity (a_w) are the most

Table 1: Diameter of inhibition zone: (mm) of plantaricin IIA-1A5 against pathogenic bacteria

Percentage of protein	Concentration of protein (µg/mL)	EPEC K11	*Salmonella* 38	*Shigella* A33
0%	0	0	0	0
0.2%	0.153	7.21±0.23	7.32±0.23	7.14±0.26
1%	0.765	7.24±0.21	7.42±0.34	7.25±0.43
10%	7.653	7.37±0.25	7.58±0.88	9.10±0.02

High antimicrobial activity: inhibition zone >10 mm in diameter; Moderate antimicrobial activity: inhibition zone 5-10 mm in diameter; Low antimicrobial activity: inhibition zone <5 mm; No antimicrobial activity: no inhibition zone

Table 2: Physicochemical quality of beef storaged at room temperature storage

		Storage time (hour)			
Physicochemical quality	Treatment	0	5	10	15
Water activity (a_w)	Plantaricin 0.2%	0.90±001	0.90±0.02	0.90±0.01	0.90±0.02
	Control	0.89±0.03	0.89±0.01	0.88±0.01	0.89±0.02
pH	Plantaricin 0.2%	5.44±0.08	5.43±0.05	5.44±0.07	5.43±0.04
	Control	5.38±0.08	5.40±0.07	5.42±0.01	5.44±0.04

Table 3: Microbiological quality of beef at room temperature storage

		Storage time (hour)			
Bacteria	Treatment	0	5	10	15
E. coli (log cfu/gr)	Plantaricin 0.2%	$1.49±0.2^{c}$	0^{c}	0^{c}	0^{c}
	Control	$1.98±0.40^{a}$	$2.27±0.35^{ab}$	$2.42±0.36^{ab}$	$2.83±0.49^{ab}$
S. aureus (log cfu/gr)	Plantaricin 0.2%	$2.02±0.23^{b}$	$2.00±0.26^{b}$	$1.98±0.02^{c}$	$1.73±0.13^{c}$
	Control	$2.49±0.11^{a}$	$2.69±0.11^{a}$	$2.72±0.23^{a}$	$2.92±0.30^{a}$
Salmonella	Plantaricin 0.2%	Negative	Negative	Negative	Negative
	Control	Negative	Negative	Negative	Negative

Different subcript for each bacteria is significant difference ($p<0.05$)

important physicochemical parameters of food quality. The pH value could affect meat color, tenderness and quality of food (Jelenikova *et al*., 2008). Table 2 shows that at the beginning of spraying plantaricin IIA-1A5 as a natural preservative on beef could increase the water activity of meat, because the sprayed plantaricin was first diluted in aquabidest. After 5 h storage, a_w value of beef tended to decrease. Arief *et al*. (2012) stated that the use of preservative plantaricin in meatballs tended to decrease the water activity. Actually, a decrease in a_w was an appropriate condition to inhibit the growth of bacteria in food. The decrease in the water activity in food was done by adding solids, ionic, hydrophilic colloids, freezing and drying.

The pH values obtained in the study were good, ranging from 5.3 to 5.4. According to Puolanne *et al*. (2001), the average value of the final meat pH ranged from 5.4 and 6.0, depending on the glycolytic potential at the slaughter time. The difference in pH values in beef with 0.2% plantaricin and control (without plantaricin) was caused by the water activity which was slightly higher in beef with 0.2% plantaricin compared with control. After 5 hours storage, the pH value of the beef with 0.2% plantaricin tended to be more stable according to the study Arief *et al*. (2012). This is in contrast to the pH value of the meat without plantaricin which tended to increase during storage time. The increase in the pH value reflected the degree of spoilage to the beef through the process of protein degradation by the production of free amino acids, which led to the formation of alkaline compounds such as NH_3 and amines (Vásquez *et al*., 2009).

Total microbial or *Total Plate Count* (TPC), *Staphylococcus aureus*, *Escherichia coli* are indicator of contamination, because this bacteria are naturally present in meat and if the amount exceeds the normal range will cause foodborne disease. *Escherichia coli* is a bacteria that is used as an indicator of sanitation. In the beginning, *E. coli* contamination in beef with 0.2% plantaricin addition met appropiate standards by Indonesian standars that is a maximum of 1 log cfu/g (Table 3). Plantaricin IIA-1A5 was effective in inhibiting the growth of *E. coli*, there is no colony of *E. coli* grew at plate after 5 hours storage. This is different to the control (without plantaricin addition), *E. coli* increased every hours. The consumption of this bacteria in certain limit may promote diarrhea or other foodborne diseases. Bacteriocin can distrupt the cell of bacteria, causing dead of bacteria (Hata *et al*., 2010). Kalalou *et al.* (2010) reported that the bacterial cell suspension of *L. plantarum* on camel meat stored at a temperature of 10ºC could reduce *E. coli* viability by 2 log cfu/g.

Staphylococcus aureus is a gram positive bacteria indicator of contamination from the worker and tools. After 5 h storage, bacteria *Staphylococcus aureus* tended to decrease. Population of *Staphylococcus aureus* on beef with 0.2% plantaricin is lower than maximum standart allowed by Indonesian standard of fresh beef (2 log cfu/g). In control (without plantaricin addition), population of *Staphylococcus aureus* increased continuously every hour (3 log cfu/g). Based on Table 3, it was known that the beef did not contain *Salmonella*. This suggested that the beef was not

contaminated by the infected cattle dung. Salmonellosis affecting cattle could contaminate the surrounding food. *Salmonella* is a harmful pathogenic bacterium. Based on microbiology quality, plantaricin IIA-1A5 was effective to inhibit gram positive and negative bacteria. This is in contrast with nissin that is only capable against gram positive bacteria (McAuliffe *et al*., 1999).

CONCLUSION

Plantaricin IIA-1A5 has a good moderate antimicrobial activity against pathogenic bacteria cause diarhea. Plantaricin IIA-1A5 is effective in inhibiting gram negative and positive pathogenic bacteria normally present on beef so that it can be recommended as a natural preservative for meat. Plantaricin IIA-1A5 is able to extend the self life of meat stored at room temperature, according to physichochemichal and microbiology quality.

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