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Purification of Bacteriocin from *Lactobacillus plantarum* IIA-1A5 Grown in Various Whey Cheese Media Under Freeze Dried Condition

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ABSTRACT

Lactobacillus plantarum **IIA-1A5 is a lactic acid bacteria (LAB) that has been reported to have capacity to produce bacteriocin, usually called plantaricin. This bacteriocin is usually produced in commercial synthetic media. However, the media is expensive, thus finding a novel source which is less expensive and abundant is necessary. The present work was aimed to compare the use of gouda and mozarella cheese whey as growth media of** *L. plantarum* **IIA-1A5 producing plantaricin, as well as to evaluate the effectiveness of freeze dried plantaricin as an antimicrobial agent. The results showed that gouda and mozarella cheese whey were applicable for growth media of** *L. plantarum* **IIA-1A5 and production of plantaricin. The plantaricin produced from the whey showed a size of about 9.6 kDa. Freeze dried plantaricin was shown to be relatively stable in the second week of storage, but there was a slight decrease in protein concentration during storage, indicating that the protein was partially denatured and precipitated. However, the freeze dried plantaricin showed inhibitory activities against** *S. aureus* **ATCC 25923,** *B. cereus* **ATCC 21332,** *S. thpymurium* **ATCC 14028,** *E. coli* **ATCC 25922, and** *P. aeruginosa* **ATCC 27853 during storage at room temperature.**

Keywords: cheese whey, growth media, bacteriocin, freeze dried, plantaricin

INTRODUCTION

Lactic acid bacteria (LAB) constitute a general term of bacteria which ferment lactose primarily to lactic acid. These bacteria have been used in food production and recognized as health-enhancing bacteria. LAB offer important roles in the fermentation industry of yoghurt, cheese, butter, yakult, and sour milk. Additionally, they are also capable of extending the food shelf life since the bacteria can produce antibacterial compounds such as lactic acid, hydrogen peroxide, diacetyl, and bacteriocin. Bacteriocin is a type of protein and has been known to display antimicrobial activities and non-toxic compounds. The need for new strains of LAB that carry probiotic characteristics for probiotic food development is increasing. One of them is *Lactobacillus plantarum* IIA-1A5 isolated from Indonesian local beef (Arief *et al*., 2015b). Plantaricin IIA-1A5 from *L. plantarum* IIA-1A5 showed a remarkable antibacterial activity against *Staphylococcus aureus* and that to be bactericidal (Arief *et al*., 2015a). The use of bacterial bacteriocin may reduce the use of chemicals as food preservatives.

In order to produce bacteriocin, the bacteria require appropriate growth medium to grow and produce bacteriocin. In addition, other factors affecting the production of bacteriocin are the type of bacteria, fermentation condition, and the presence of nutrition. Further, large scale production of bacteriocin is hampered by the high production cost, mainly, due to the growth medium. The media commonly used to produce bacteriocin from LAB is a commercial synthetic medium such as deMann Rogosa Sharp Broth (MRSB). Nevertheless, MRSB is regarded as expensive media, thus other potential source of media need to be investigated. The use of cheese whey as growth media is expected to replace commercial synthetic media.

As a by-product of cheese industry, whey is considered as a less valuable and problematic waste. However, whey still contains several important nutritional components such as lactose, nitrogen (protein, peptides, and amino acids). The presence of protein may provide a great source of nutrition for bacteria (Anwar *et al*., 2012). Production of bacteriocin and storage process can decrease the survival of bacteria, leading to reduction of its bio-preservative properties. The storage of fresh bacteriocin in long period may decrease its activity, thus the most proper technique for preservation of bacteriocin needs to be determined. Freeze drying is commonly used technique to preserve bacteriocin. Fu & Etzel (1995) reported that freeze drying was known to have a desirable effects on storability of bacteriocin. Freeze drying is a common preservation technique used

to preserve the culture of lactic acid bacteria. The study aimed to investigate the use of gouda and mozarella cheese whey as growth media of *L. plantarum* IIA-1A5 for plantaricin production, and to test the antimicrobial activity of plantaricin preserved by freeze drying.

MATERIALS AND METHODS

Materials

Gouda cheese whey was obtained from PT Bukit Baros Cempaka, Sukabumi, meanwhile mozzarella cheese whey was obtained from PT Waluya Wijaya Farm Sentul, Bogor. Gouda and mozzarella cheese wheys were taken in fresh and cold conditions, then they were immediately treated to avoid damage.

Physical Characteristics of Cheese Whey

pH value. To measure pH, whey solution (100 mL) was dipped by electrode of pH meter. The pH value was displayed in the device (AOAC, 2005).

Viscosity. Viscometer (brookfield digital viscometer model DV-E, USA) was used to observe whey viscosity (expressed as cP). Spindle (number 5) was set at a speed of 50 rpm, and then submerged in 250 mL of whey. The viscosity was displayed in the reading scale.

Water Activity *(a_w).* Water activity of whey was determined using a_w meter (Novasina ms^1 Set-aw), operated according to standard procedure of company. Prior to use, the calibration was performed using $BaCl_2.2H_2O$ solution (kept for 3 minutes until reaching 0.9 on the reading scale).

Specific gravity. A total of 250 mL whey were analyzed for specific gravity using lactodensimeter (Funke gerber model 6610, Germany). The test was carried out according to standard procedures of Gerber.

Total lactic acid bacteria (LAB). Total LAB was determined using pour plate method with BPW dilution medium (9 mL each dilution). The whey was diluted 10^{-1} to 10^{-8}. Diluted whey (1 mL) at dilution of 10^{-6}, 10^{-7}, 10^{-8} was transferred into sterile petri dish, and MRSA (about 15 mL) was added. The petri dish was shaken to make bacteria spread evenly, left to solidify agar, and incubated at 37 °C for 24-48 h in reverse. Each dilution was made triplicates, and each replication was duplicated. Total LAB formed was then calculated using the standard plate count (SPC) method (BAM, 2001).

Chemical Characteristics of Cheese Whey

Chemical composition of whey (fat, solid non fat, density, lactose, solids, protein) was performed using lactoscan tool. Whey (25 mL) was set in cuvet and analyzed by lactoscan. The results of analysis were then displayed in the instrument after 10 min.

Culture Enrichment of *L. plantarum* IIA-1A5

L. plantarum IIA-1A5 (1 mL) was grown on cheese whey (9 mL) medium that had been sterilized at 115 °C for 3 min and incubated for 24 h at 37 °C. The process is repeated as much as 3x a good culture (Waluyo, 2008).

Bacteriocin Production

Culture of cheese whey media were multiplied (8000 mL), with a ratio of 1 (culture): 9 (cheese whey media). Then centrifuged (Himac CR21G, Japan) (20000x g for 20 min, 4 °C). The cell free supernatant obtained from the culture was then filtrated with 0.20 µm, and neutralization of pH with the addition of NaOH 1N to 6.8. The supernatant was evaporated using Heidolph VV micro evaporator until reducing the volume to half of the previous volume with supernatant saturation for 48 h and then ammonium sulphate was added to reach 80% concentration. The precipitate of this saturation process was then separated by centrifugation (20000x g for 20 min, 4 °C). The crude plantaricin deposits obtained were dialyzed with a 20 µm dialysis membrane and immersed in phosphate buffer (KH_2PO_4 and K_2HPO_4) with concentration of 20 mM and pH of 6.8 for 24 h. The phosphate buffer was replaced every 4 h. All the steps were carried out at 4 °C (Arief *et al*., 2013). Then chromatography was purified using sepharose HiTrap FF (GE Healthcare).

Purification of Cation Exchange Chromatography

Prior to being used, the HiTrap FF volume column of 5 mL was washed with aquabidest three times the volume of the column and then equilibrated with 20 mM phosphate buffer of pH 6.0, each four times the volume of the column. The dialysis sample was fed into the column manually with the syringe and flowed through solution coming out of the collected column. Samples in the column were then added with an elution solution containing NaCl at various concentrations of 0 M, 0.05 M, 0.1 M, 0.15 M, 0.2 M, 0.25 M (NaCl 0.5 M in LE1, LE2, LE3, LE4, LE5, and LE6) . The volume of the elution solution was 1 times the volume of the column and the elution results were accommodated in one tube and set as one fraction. All these stages were done at 4 °C. The presence of proteins eluted at these fractions was detected by the UV-VIS spectrophotometer at 280 nm wavelength.

Electrophoresis SDS PAGE

Electrophoresis was performed to determine the molecular weight and confirm the purity, the selected fraction was collected and analyzed with sodium dodecil sulfuric acid - polyacrylamide gel electrophoresis (SDS-PAGE) 15% using electrophoresis set mini protein gel (Bio-Rad, Hercules, CA, USA) (Kim & Ahn, 2000). Samples given 1 : 2 buffer samples were further vortexed and heated 95 °C for 3 s. Polyacrylamide gel consisted of 2 stages of stacking and resolving. Polyacrylamide gels between stacking and resolving

stages were only distinguished by the percentage of gel. Stacking gel used 15% of gel, while resolving gel only used 4% gel as a barrier to enter the sample to the well. The sample used was 10 μL and 10 μL of protein marker (Bio-Rad) was used at one well as a standard calculation. Protein marker used was 140 kDa (low protein). Running electrophoresis is carried out with a constant current of 40 mA with a voltage of 125 Volts for 2.5 h or until all the sample were falled above the bottom of the gel. Gel staining was done with Coomassie Brilliant Blue R250 (Sigma, St.Louis, MO, USA).

Preservation with Freeze Drying

Preservation of bacteriocin plantaricin IIA-1A5 using the freeze drying method was carried out with temperature of -40 °C for 72 h with freeze drying tool. Freeze dried plantaricin product was stored in a closed schoot bottle. During the storage process, bacteriocin plantaricin was stored in room temperature for one month.

Evaluation of Antimicrobial Activity

Antimicrobial activity of bacteriocin plantaricin was examined/measured by using modification method of Dhiman *et al*. (2011), to determine the antimicrobial activity of the plantaricin by pouring 0.1 mL of pathogenic bacteria into 20 ml muller hinton agar (MHA) media that was formed in order in the petri dish. Then the paper discs (Oxoid, United Kingdom) was immersed in a bacteriocins plantaricin IIA-1A5 using different eppendorfs as much as 100 mL of different medias. The submerged paper discs were placed on MHA media that had been planted with pathogenic bacteria. Pathogenic bacteria used was *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella thpymurium* ATCC 14028, and *Bacillus cereus* ATCC 21332. The petri dish that had been added paper disc was closed using filter paper, and then incubated at 37 °C for 24-48 h. The results of the antimicrobial activity of bacteriocins plantaricin IIA-1A5 was determined by the formation of a clear zone around the paper discs and the diameter of a clear zone was measured using calipers (Mitutoyo).

Protein Analysis

Protein content of bacteriocin plantaricin IIA-1A5 was measured according to Lowry *et al*. (1951) as follows: Fifty microliters of samples of bacteriocin plantaricin IIA-1A5 was taken and poured into the tube. Then aquades were added as much as 4 mL. Subsequently reagent solution was added. Reagent solution consisted of 2 kinds. Reagent 1 contained 0.1 N NaOH and 2% Na_2CO_3. Reagent 2 contained 0.5% $CuSO_4$ in 1% Na-K Tartrate. Ten milliliters of reagent 2 was taken to be added to reagent 1. Furthermore, after adding aquades, 5.5 mL of reagent solution was added and awaited 10 min at room temperature. As much as 0.5 mL of folin ciocalteau reagent was added and the mixture was allowed to turn the color from blues to blackish. After the blue black color was changed to blackish, the absorbance was read at a wavelength of 650 nm. For the standard curve, bovine serum albumin (BSA) was used with the concentrations of 0; 0.1; 0.2; 0.3; 0.4; 0.5 mg mL^{-1}.

Statistical Analysis

Physical and chemical characteristics of cheese whey were statistically evaluated using T-Test. Antimicrobial activity test data and protein content during storage were analyzed using factorial randomized design followed with advanced tests using Duncan (Steel & Torie, 1991). Data processing was done by using computer software program of Microsoft Excel 2010 and SPSS for Windows version 21.

RESULTS

Physical and chemical properties of cheese whey included pH value, viscosity, water activity, specific gravity, total LAB of cheese whey, total LAB of fermented whey, fat, solid non fat, specific gravity, lactose, solids, and protein (Table 1). Gouda cheese whey (WG) had pH of 4.97, which was significantly lower than mozzarella cheese whey (WM) of 5.40. Similarly, water activity of WG was significantly lower than that of WM ($P<0.05$). The higher pH was also associated with high viscosity. Total LAB between WG and WM had a significant difference ($P<0.05$), which was caused by the different addition of bacteria during the cheese making process. The cheese whey sample was obtained from different cheese making companies. Weight value of WG was significantly lower than that of WM. Total LAB of fermented whey between WG and WM showed no significant difference ($P>0.05$). Fat content of WG was significantly higher than that of WM. The presence of fat in whey was derived from the milk fat globules which were included in the curd filtration process after the coagulation. Solid non fat value of WG was significantly lower than that of WM. Whey used in this study had high lactose levels ranging from 3.68% to 4.51%. Solids of WG was significantly lower than that of WM. Protein in cheese whey ranged from 2.45 to 2.89%.

Based on SDS-PAGE experiment, plantaricin IIA-1A5 of WG and WM was not significant difference, namely 9.69 kDa and 9.63 kDa, respectively (Figure 1). This suggests that the plantaricin was classified as IIa (<10 kDa). The gel resolving formulation used to determine the molecular weight of the plantaricin IIA-1A5 was 15% concentration. In the case of antimicrobial activity, plantaricin showed a growth resistance against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 as observed in the decrease of OD_{600} value with and without the addition of plantaricin IIA-1A5. The addition of plantaricin IIA-1A5 was performed at 1 h. Growth inhibition of *S. aureus* and *E. coli* was depicted in Figure 2a and Figure 2b, respectively. Low OD_{600} values indicate a decrease in absorbance value, which means a decrease in activity of bacteria after 10 h of incubation.

Table 1. Characteristics of physical and chemical composition of cheese whey

Variables	Sample type	
	WG	WM
pH	4.97±0.39	5.40±0.09
Water activity	0.79±0.02[a]	0.86±0.01[b]
Viscosity (cP)	0.30±0.00[a]	0.47±0.06[b]
Specific gravity (kg m^{-3})	1.02±0.00	1.02±0.00
Total LAB whey (log cfu mL^{-1})	7.62±0.08[a]	6.89±0.27[b]
Total LAB fermented whey (log cfu mL^{-1})	8.47±0.28	8.27±0.21
Fat (%) bb	2.12±0.59	1.53±0.49
Solid non fat (%) bb	6.65±0.71	7.86±0.95
Density (%) bb	24.40±2.30	29.56±3.35
Lactose (%) bb	3.68±0.39	4.51±0.58
Solids (%) bb	0.55±0.06	0.65±0.08
Protein (%) bb	2.45±0.26	2.89±0.35

Note: Means in the same row with different superscripts differ significantly (P<0.05); WG: whey cheese gouda; WM: whey cheese mozarella; LAB: lactic acid bacteria.

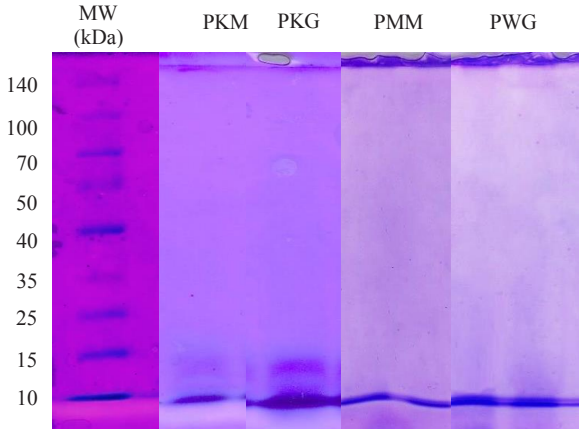

Figure 1. Profile of SDS PAGE plantaricin IIA-1A5; PKM: profile rude plantaricin IIA-1A5 media mozarella, PKG: profile rude plantaricin IIA-1A5 media gouda, PMM: profile pure plantaricin IIA-1A5 media mozarella, PWG: profile pure plantaricin IIA-1A5 media gouda.

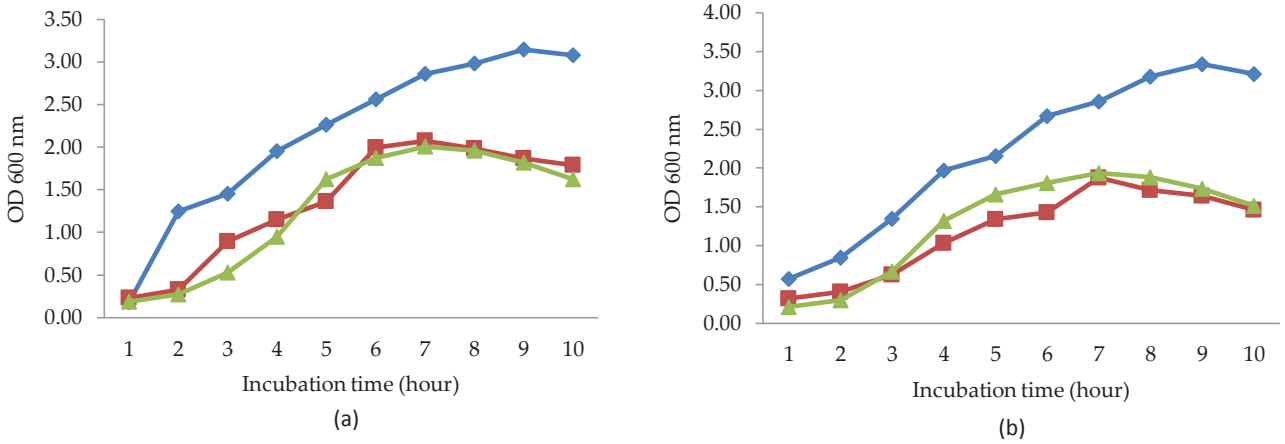

Figure 2. Activity mode (OD) curve of bacteriocin plantaricin against *S. aureus* ATCC 25923 (a) and *Eschericia coli* ATCC 25922 (b) for 10 hours; TBP: without bacteriocin plantaricin (-•-), PWM: plantaricin media whey mozzarella (-■-), PWG: plantaricin media whey gouda (-▲-).

Table 2 exhibits the inhibitory zone of plantaricin, which indicates antimicrobial activity against pathogenic bacteria. The inhibitory zone of plantaricin IIA-1A5 of WG and WM showed a significant effect (P<0.05) on all bacteria tested. Although the inhibitory effect of plantaricin whey mozarella (PWM) and plantaricin whey gouda (PWG) against all observed bacteria differed, but it did not differ between the two bacteriocins plantaricin IIA-1A5. Antimicrobial activity of PWM and PWG showed a clear zone of 7.53-13.90 mm in diameter. Decrease in the inhibitory zone occurred in the second week, which might be linked with the decreased protein concentration (Table 2) present in plantaricin stored at room temperature. The protein content of plantaricin IIA-1A5 before the freeze drying treatment for WM and WG was 29.88 mg mL^{-1} and 32.57 mg mL^{-1}, respectively. Decrease in protein levels during storage of plantaricin IIA-1A5 started in the second week (Table 2).

DISCUSSION

We found that WG had lower pH than WM, but both were still classified as acid whey. Similarly, Spreer (1998) reported that sweet whey was obtained from coagulation method using enzyme, while acid whey was prepared from coagulation method using acid. A fairly obvious difference between sweet whey and acid whey is indicated by their pH. Sweet whey has a higher pH value ranging from 6.20-6.40, while acid whey has a more acidic pH ranging from 4.60-5.00. WG and WM were classified as an acid whey. Solid non fat values between WG and WM as a standard when compared with standard BPOM (2016) is not less than 30% for acid whey. The presence of lactose in whey is necessary especially since whey is used as a bacterial growth medium. Lactose is a carbohydrate source for lactic acid bacteria that will convert lactose to lactic acid during the fermen-

Table 2. Inhibitory zone (mm) and protein (mg mL^{-1}) content of freeze-dried plantaricin IIA-1A5

Sample type	Type of bacteria	Storage (weeks)					Average
		0	1	2	3	4	
Inhibitory zone							
PWM	SA	12.12±1.79	11.71±0.37	10.91±0.61	10.46±0.40	7.76±0.15	10.59±1.75
PWG		12.41±0.79	11.73±1.08	11.36±0.87	11.13±0.97	7.53±0.76	10.83±1.93
Average		12.27±1.24^{a}	11.72±0.72^{ab}	11.13±0.72^{ab}	10.80±0.76^{b}	7.64±0.50^{c}	
PWM	BC	12.20±1.87	10.77±1.66	10.76±0.50	9.69±0.66	8.39±0.93	10.36±1.69
PWG		12.11±0.62	11.91±0.83	11.55±1.57	9.99±1.49	7.95±0.62	10.70±1.87
Average		12.16±1.25^{a}	11.34±1.33^{a}	11.15±1.13^{a}	9.84±1.04^{b}	8.17±0.75^{c}	
PWM	ST	12.41±2.72	11.40±0.26	10.61±0.73	10.29±0.22	8.11±0.28	10.56±1.83
PWG		12.05±0.97	11.63±0.97	10.85±0.76	10.54±0.40	8.22±1.06	10.66±1.56
Average		12.23±1.83^{a}	11.51±0.65^{ab}	10.73±0.68^{b}	10.41±0.32^{b}	8.17±0.69^{c}	
PWM	EC	12.67±0.07	11.21±0.67	10.97±1.09	10.24±1.73	7.78±0.43	10.57±1.86
PWG		13.31±1.68	12.48±1.99	11.24±0.87	10.67±1.68	7.77±0.53	11.09±2.32
Average		12.99±1.12^{a}	11.84±1.50^{ab}	11.11±0.89^{b}	10.45±1.54^{b}	7.77±0.43^{c}	
PWM	PS	13.90±1.14	11.76±0.88	10.46±0.49	9.91±1.61	7.81±0.38	10.77±2.25
PWG		13.54±1.15	12.15±0.34	10.34±0.55	9.62±1.86	7.95±0.44	10.72±2.20
Average		13.72±1.05^{a}	11.95±0.63^{b}	10.40±0.47^{c}	9.76±1.56^{c}	7.88±0.38^{d}	
Protein							
PWM		31.05±3.92	30.75±3.95	22.17±0.94	21.49±1.63	21.24±1.82	25.34±5.25^{a}
PWG		34.90±1.60	34.18±3.18	28.80±0.87	27.08±0.43	25.47±1.68	30.08±4.21^{b}
Average		32.98±3.40^{a}	32.47±3.71^{a}	25.49±3.72^{b}	24.29±3.24^{b}	23.35±2.79^{b}	

Note: Means in the same row with different superscripts differ significantly (P<0.05); PWM: plantaricin media whey mozarella and PWG: plantaricin media whey gouda; SA: *Staphylococcus aureus*, BC: *Bacillus cereus*, ST: *Salmonella thpymurium*, EC: *Escherichia col*i, PS: *Pseudomonas aeruginosa*.

tation process, so that high levels of lactose in whey are required to ensure adequate food availability for the lactic acid bacteria used.

The SDS-PAGE electrophoresis used has similar resolving concentrations with Karthikeyan & Santosh (2009) to determine the molecular weight of *L.acidophillus* bacteriocin using a resolving gel with a concentration of 15%. The molecular weight of the plantaricin IIA-1A5 prepared using WG and WM was higher in comparison with previous results such as plantaricin IIA-1A5 of 6.41 kDa (Arief *et al*., 2015b) and *L. plantarum* FGC12 of 4.1 kDa (Xinran *et al*., 2017). Although they are different in molecular weight, those plantaricins are regarded as a group IIa and relatively stable against heat (Zacharof & Lovitt, 2012). Other bacteriocin produced by *L. plantarum* included plantaricin 163 with a molecular weight of 3.5 kDa (Hu *et al*., 2013), plantaricin LR14 alpha with a molecular weight of 3.0 kDa (Tiwari & Srivastava, 2008), and plantaricin K25 with a molecular weight of 1.7 kDa (Wen *et al*., 2016) . The molecular weight of the bacteriocin produced by *L. plantarum* IIA-1A5 is different from the previously reported bacteriocin. Therefore, it can be concluded that the bacteriocin produced from *L. plantarum* IIA-1A5 is a new bacteriocin. Class I and II are the major classes of bacteriocins that have potential for use in commercial applications. Different *Lactobacillus plantarum* strains remarkably influenced plantaricin characteristics and protein concentrations produced in the calculation of electrophoresis SDS-PAGE (Kia *et al*., 2016).

Higher OD_{600} values showed no decreased rate, but there was an increase in absorbance value, indicating increased number of bacterial cells after 10 h of incubation. Decreased value of OD_{600} per hour incubation time showed a minimal concentration that could inhibit the growth of *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. The bacteriocin is lactic acid bacteria that can generally break down glucose to produce lactic acid. This causes the pH of the media to be low (<4.5) so it can inhibit the growth of pathogenic bacteria (Nurhajati *et al*., 2012b). Hafsan (2014) reported that inhibition of bacteriocin against pathogenic bacteria was due to changes in the membrane gradient potential and the release of intracellular molecules as well as the entry of extracellular substances. Additionally, Nurhajati *et al*. (2012a) stated that bacteriocin is synthesized during the exponential growth phase. The incubation time that extends after the stationary phase can cause the bacteriocin activity to decline because proteases are released from the cell as the cell enters the phase of death. OD_{600} observation results from bacteriocin plantaricin IIA-1A5 showed no difference in bacterial inhibition activity of both bacteriocin media. These findings are in accordance with Pratiwi (2008), reported that the presence of clear zone demonstrated that antibiotics effectively inhibited bacterial growth. However, current literature suggests that cell membrane permeability plays a very important role on the mode of action of bacteriocins (Ge *et al*., 2016). In this study, plantaricin IIA-1A5 demonstrated the ability to increase the permeability of cell membranes, leading to pore formation and eventual cell death. Similar modes of action were also seen in Enterocin FH99, active against *Listeria monocytogenes,* and bacteriocin BacC1,

active against *S. aureus* (Goh & Philip, 2015; Kaur *et al*., 2013).

Antimicrobial activity of plantaricin IIA-1A5 is indicated by the formation of clear zone around disc paper. This zone may differ in its size due to several factors. The results showed that inhibitory zone varied with a difference in the type of indicator pathogenic bacteria. This result was supported by Arief *et al*. (2013), finding that antimicrobial properties of the plantaricin IIA-1A5 against Gram positive and Gram-negative bacteria closely related to bacterial strains. In a previous study, *L. plantarum* IIA-1A5 grown in commercial MRSB media for production of plantaricin had good antimicrobial activity against *E.coli* ATCC 25922, *S. thypimurium* ATCC 14028, *S. aureus* ATCC 25923 ranged from 6.86-12.38 mm (Arief *et al*., 2015b). Decrease in inhibitory zone is affected by storage of bacteriocin plantaricin IIA-1A5 at room temperature. Todorov *et al*. (2015) suggested that low inhibitory activity in the experimental media might result from the reduced antimicrobial activity of bacteriocin due to the roles of organic acids. The other factors are characteristics of different indicator bacteria. The indicator bacteria included Gram positive and Gram negative bacteria, and their sensitivities against antimicrobials were not similar. Gram-positive bacteria are more sensitive, whereas Gram-negative bacteria are more resistant.

Maltodextrin encapsulation protects the protein during freeze dry process and makes it dry quickly. The statement is consistent with the that of Bae & Lee (2008) that physical properties, such as wettability and density, were greatly improved by increasing the maltodextrin ratio in the wall systems, which may affect encapsulation efficiency and flavor release during production and storage. However, there is no further study about how the types and contents of maltodextrin affect the encapsulation properties. Therefore, a better understanding of the roles of maltodextrin in encapsulation systems is very important to improve product development and modification of existing process and products. Decrease in protein levels of the plantaricin IIA-1A5 during storage begins in the second week. This was in accordance with Millette *et al*. (2007) finding that the storage temperature may affect the antibacterial ability of the bacteriocin extract powder, as does the nisin powder. Temperature is capable of affecting the rate of change in a product quality associated with the occurrence of chemical or biochemical reactions in the product. The higher environmental temperature positively related to the chemical or biochemical reactions which triggered the more rapid product damage.

CONCLUSION

Gouda and mozzarella cheese whey were applicable for growth media of *L. plantarum* IIA-1A5 that enabled to produce plantaricin IIA-1A5. It could still show a good stability after preserved by freeze drying treatment, although its protein content and antimicrobial activity seemed to decrease at the second week of storage. Freeze-dried plantaricin IIA-1A5 prepared from WG and WM inhibited growth of Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 21332) and Gram-negative bacteria (*Salmonella thpymurium* ATCC 14028, *Eschericia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853) during storage at room temperature.

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