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The growth and production of antimicrobial compounds from *Lactobacillus plantarum* IIA-1A5 on cheese whey medium

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ABSTRAK

Lactobacillus plantarum IIA-1A5 yang diisolasi dari daging sapi di Indonesia, menghasilkan bakteriosin yang disebut plantarisin IIA-1A5, menunjukkan aktivitas penghambatan pada bakteri patogen Gram positif dan negatif. Namun, produksi dari bakteriosin ini untuk aplikasi dan studi lebih lanjut membutuhkan biaya yang mahal karena penggunaan media bakteri. Maka, penelitian ini bertujuan untuk menganalisis kelayakan whey keju sebagai media pertumbuhan untuk menghasilkan bakteriosin. Kurva pertumbuhan *Lactobacillus plantarum* IIA-1A5 dalam menghasilkan senyawa antimikroba terjadi pada fase logaritmik pada waktu inkubasi jam ke-28 dan 32. Purifikasi plantarisin IIA-1A5 menghasilkan peptida dengan berat molekul 9.59 KDa yang terdiri dari whey dan whey+ (20g/L sukrosa, 12.5 g/L tryptone and 7.5 g/L ekstrak ragi), dan berada pada kelas IIa bakteriosin (<10 kDa). Konsentrasi protein plantarisin IIA-1A5 dengan perlakuan whey+ (sukrosa, tryptone, and ekstrak ragi) dan whey masing-masing adalah 1883.17 mg/ml dan lebih rendah dari 325.58 mg/ml. Berdasarkan uji aktivitas antimikroba menggunakan metode kertas cakram, plantarisin IIA-1A5 menunjukkan aktivitas antimikroba terhadap *Staphylococcus aureus* ATCC 25923; perlakuan whey dan whey+ masing-masing menghasilkan 38,02 IU/dL dan 321 IU/dL. Efektivitas sifat antimikroba plantarisin IIA-1A5 dalam media whey terbukti melalui hasil penelitian ini. Hal ini juga menunjukkan bahwa whey berpotensi digunakan sebagai media pertumbuhan untuk produksi bakteriosin.

Kata Kunci: *Lactobacillus plantarum, antimikroba, whey keju, plantarisin*

ABSTRACT

Plantaricin IIA-1A5 is a bacteriocin produced by *Lactobacillus plantarum* IIA-1A5 which is isolated from Indonesian beef, and it inhibits activity of Gram negative and positive pathogenic bacteria. However, preparation of the antibacterial agent for further applications or studies is costly due to the usage of a bacterial medium. Therefore, this study was aimed to investigate the feasibility of cheese whey as a growth medium for production of the bacteriocin. The growth curve of *Lactobacillus plantarum* IIA-1A5 in producing antimicrobial compounds was found to occur in the logarithmic phase with an incubation time of 28 and 32 hours. Purification of plantaricin IIA-1A5 produced peptides with a molecular weight of 9.59 kDa consisting of whey and whey+ (20g/L sucrose, 12.5 g/L tryptone and 7.5 g/L yeast extract); thus, the peptide was grouped as class IIa (<10 kDa) bacteriocin. The protein concentration of plantaricin IIA-1A5 with whey+ (sucrose, tryptone, and yeast extract) treatment and

whey treatment was 1883.17 mg/ml and lower than 325.58 mg/ml, respectively. Based on the antimicrobial activity test using a paper disc method, plantaricin IIA-1A5 demonstrated antimicrobial activity against *Staphylococcus aureus* ATCC 25923; the whey and whey+ treatment yielded 38.02 IU/dL and 321 IU/dL, respectively, while antimicrobial activity against *Escherichia coli* ATCC 25922 using whey and whey+ treatment yielded 44.85 IU/dL and 172.08 IU/dL, respectively. The effectiveness of the antimicrobial properties of plantaricin IIA-1A5 in the whey medium is proven through the results of this study. In short, the whey is appropriate growth medium for bacteriocin production.

Keywords: Lactobacillus plantarum, antimicrobial, whey cheese, plantaricin

INTRODUCTION

Milk is mostly consumed not only in fresh (raw) form, but also in its processed form, which includes cheese. In regard to cheese, the processing of this product is accompanied with the release of huge amount of a by-product known as whey. The by-product amount is usually even higher than that of the main product. For instance, 8-9 L id whey will be obtained during production of 1 kg of cheese from 10 L of milk. Whey or serum proteins are soluble milk proteins representing about 20% of total milk proteins. The β-lactoglobulin, α-lactalbumin, proteose peptone, serum albumin, immunoglobulins in order of abundance form the bovine whey proteins and whey from by product of cheese industry (Kilara and Vaghela, 2018). Despite being considered as a by-product, they contains remarkable nutritional components, which are not only good for infants but also for microorganism growth.

Lactobacillus strains have been known as safe microbes for consumption regarding their presence in human diets and their essential roles in gut health. In terms of *Lactobacillus plantarum,* the microbe is regarded as Gram-positive, short-rod and micro-aerophilic bacteria. Besides classified as non-spore forming bacteria, *Lactobacillus plantarum* is non-respiring bacteria belonging to hetero-fermentative group of lactobacilli, and is widely applied in various food industries as a starter culture and preservative (Arasu *et al*., 2013). *Lactobacillus plantarum* IIA-1A5 was originally isolated from Indonesian beef, the PO (Peranakan Ongole) breed, (Arief *et al.,* 2015[a]) and was proven capable of producing plantaricin IIA-1A5 (Arief *et al*., 2013). Regarding antimicrobial effects of plantaricin specifically produced by *L. plantarum*, it was reported to more actively inhibit Gram-negative pathogenic bacteria (Gong *et al*., 2010; Abo-Amer, 2007; Kia *et al*., 2015). Therefore, the plantaricin IIA-1A5 can be a promising antimicrobial bacteriocin (Arief *et al*., 2013).

The bacteriocins produced by lactic acid bacteria (LAB) have been the center of attention as they are generally regarded as safe (GRAS) and have potential application as natural preservatives in the food industry (Sheoran and Tiwari, 2019). The high cost of the LAB culture medium, which is the bacteriocin, has opened to doors to various methods as an alternative, substitute or reduction such as the use of synthetic media via the addition of natural components. Former study successfully isolated plantaricin production using probiotic *L. plantarum* CRA52 in whey permeate (Sharma *et al*., 2021); isolated plantaricin IIA-1A5 from *Lactobacillus plantarum* IIA-1A5 (Arief *et al*., 2015[a]). However, its application as an antimicrobial agent in Indonesia is still limited. Further researches are needed to uncover the suitability of plantaricin IIA-1A5 for growth medium and natural media. This presnt work aimed to discover the feasibility of cheese whey as a growth medium for the production of bacteriocin, it is envisaged that this study will lend fundamental insights in order to leverage the innate nutritional potential of raw products such as whey and it is envisaged that have potential application as natural preservatives in the food industry

MATERIALS AND METHODS

Preparation of Bacteria *Lactobacillus plantarum* IIA-1A5

Refreshment of *Lactobacillus plantarum* IIA-1A5 was carried out to reactivate the bacterial cultures in MRSB (de Man Regosa Sharpe Broth) media (Oxoid, England). The bacterial reactivation was conducted by growing the sample (1 mL) on MRSB media (9 mL), followed with incubation at 37°C for 24 hours; the process was repeated 3 times (Arief *et al.,* 2015[a]).

Growth Analysis of *Lactobacillus plantarum* IIA-1A5 on Various Media

The growth analysis of *Lactobacillus plantarum* IIA-1A5 for establishing their growth curve followed previous from Daneysa *et al.* (2015) with modification. The bacterium was grown in three different media, including 100% MRS *broth,* 100% cheese whey media, and cheese whey supplemented with 20 g/L sucrose, 12.5 g/L tryptone, and 7.5 g/L yeast extract. LAB in the samples was quantified according to pour plate method in Buffer Peptone Water – BPW (Oxoid, England). For all samples, 1 mL of diluted sample in BPW was inoculated and mixed with MRS (de Man Regosa Sharpe) agar, and incubated at 37°C for 48 hours.

Measurement of pH and Total Titratable Acidity

A calibrated pH meter (Schoot Instruments) (S1 Analytics GmbH, Tokyo, Jepang) was used to check pH of sample and pH 7 standards prior to the measurement. The percentage of lactic acid was determined by titration method. 10 mL of the sample was poured into an Erlenmeyer flask followed by the addition of 1% drops phenolphthalein indicator. The sample was then titrated with 0.1 N of NaOH every 4 hours until a light pink color formed (AOAC, 2005).

Plantaricin IIA-1A5 Purification

Plantaricin IIA-1A5 was purified using a modified method from Arief *et al.* (2015[b]). Briefly, culture of *Lactobacillus plantarum* IIA-1A5 was performed in whey and whey+ for 20 hours at 37°C in absence of shaking. The cells were discarded by centrifugation at 10000 rpm, 4°C for 20 min), subsequently sterile-filtered through 0.2 µm cut-off membrane to collect cell-free supernatant. The bacteriocin was obtained after purification using ammonium sulfate ($(NH_4)_2SO_4$) precipitation and cation-exchange chromatography. The addition of ammonium sulfate was set to reach 90% saturation at 4°C with constant stirring. Furthermore, the mixture was stirred for 2 hours at 4°C. Subsequently, protein precipitate was centrifuged at 20000 rpm for 30 min at 4°C to collect pellet which was then resuspended in 20 mm Sodium Phosphate Buffer (SPB) (Merck, Germany) at pH of 6. Sample was desalted via 2.00 kDa cut-off dialysis membrane against SPB before the cation-exchange chromatography.

Cation-exchange chromatography analysis was operated at flow rate of 1 mL/min and performed using a SP sepharose fast flow cation-exchange column (GE Healthcare, Pittsburgh, PA, USA) equilibrated with SPB. To detect protein present in the eluted fractions, UV-VIS spectrophotometer (Agilent, Germany) was applied at 280 nm wavelength.

SDS-PAGE Electrophoresis

Molecular size and purity of the fractions were determined using SDS-PAGE experiment (Bio-Rad, Hercules, CA, USA) (Laemmli, 1970; Arief *et al.,* 2015[b]; Gallagher, 2006). The fractions were loaded to a 15% polyacrylamide gel, while the gel was then stained with Coomassie Brilliant Blue R-250 (Sigma, St.Louis, MO, USA). The amount of purified protein by plantaricin IIA-1A5 was quantified using Lowry protocols (Lowry *et al.,* 1951; Goldring, 2012)

Antibacterial Properties of Plantaricin IIA-1A5

Procedure for evaluating antibacterial activity referred to Dhiman *et al.* (2011). Briefly, a total of 0.1 µl bacteria suspension was poured into 20 mL-Muller Hinton Agar medium in 90 mm-petri plates. Different concentrations of plantaricin were prepared and introduced to 6 mm-paper discs (Whatman Oxoid, United Kingdom). After planted to the agar, incubation was carried out at 37°C for 24 h for *S.aureus* ATCC 25922 and *E.coli* ATCC 25923. After completed, antimicrobial activity of plantaricin was evaluated according to clear zone formed.

Measurement of Amino Acid Composition

Amino acid profile was determined using High-Performance Liquid Chromatography (HPLC) procedure. Sample preparation and extraction followed protocols prescribed by Arief *et al.* (2015[b]). Principle of the amino acid profiling in plantaricin IIA-1A5 was based on hydrolysis of the peptides to form free amino acids under 110°C in 6 N of HCl for 24 hours. The free amino acids were then quantified using liquid chromatography instrument (Agilent 1200 series HPLC system, Palo Alto, CA, USA). The amino acids were separated by using a ZORBAX SB RRHT C18 column (921×50 mm) with particle size of 1.8 µm (Agilent Technologies). Buffer A and B were used, i.e. 0.5 mM tridecafluoroheptanoic acid in HPLC-grade water and 100% acetonitrile, respectively. HPLC

condition was set as follows: initial flow rate of 2.4 ml/min, gradient separation as recommendation by Agilent Technologies maintained at 95°C and 550 bars. Diode-array detector SL was used to detect chromatograph at 80 Hz data rate.

Statistical Analysis

The experimental data were tabulated and statistically evaluated Analysis of Variance (ANOVA) based on a completely randomized design. Significant difference between means were compared using Tukey test (Gaspersz, 1991).

RESULTS AND DISCUSSION

The Growth Curve

Figure 1 depicts bacterial growth of *L. plantarum* IIA-1A5, indicating that the bacteria experienced logarithmic phase during 28 and 32 hours. Statistical analysis indicates that the growth medium has no effect on the total LAB (Lactic Acid Bacteria) ($P>0.05$). This is interesting to note because it means that MRSA can be replaced with the whey medium or with other additional supplements (yeast extract, sucrose, and tryptone). Based on the assumption of Todar (2009), it is possible that the medium component might affect the growth rate of the bacteria. This is proven by the fact that the whey components yielded similar effects to that of LAB growth. Whey protein hydrolysate area bundantly available renewable resources and utilization of these economically viable feedstocks as production medium compared with the conventional medium (Reddy Tadi *et al.*, 2017). Martinez *et al.* (2013) stated that the production of bacteriocin occurs during the phase of late lag or early stationer phase.

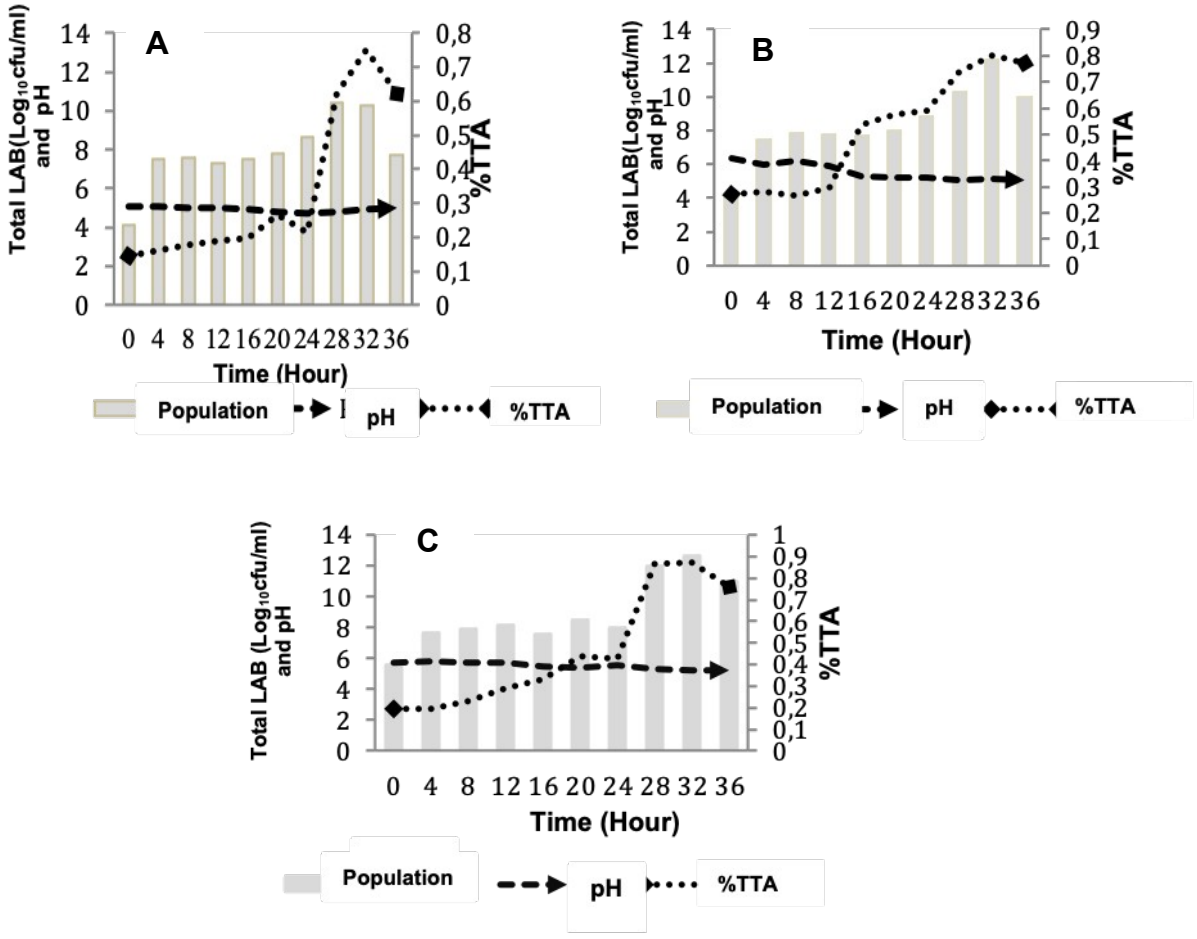

Figure 1. The growth curve *of Lactobacillus plantarum* IIA-1A5 in whey (A), whey+ (sucrose, tryptone, yeast extract) (B) and MRSB (C) medium.

The results revealed that incubation time significantly affected population of LAB ($P<0.05$). This phenomenon is acceptable since the growth curve deals with the cell division process, which is highly associated with time. The growth curve also indicates that the bacteria have a long adaptation phase for each treatment, because the growth media that were used were different from the previous growth media (MRSB and Whey). This is also accompanied by a stationer phase after 28 hours. Our experimental results are in accordance with the results of Kleerebezem and Quadri (2001), which may show high adaptability of the bacteria in various conditions. The productivity and yield of microbial metabolite is mainly influenced by factors, namely, medium constituents, physical parameters (temperature and pH), and genetic makeup of producing strain (Ip and Chen, 2005). Production of *Lactobacillus plantarum* SLG1 in MRS medium was maximized after 24 hours (the stationary phase of growth) (Pei *et al*., 2018).

Furthermore, the type of media and incubation period significantly affected the final pH of both media ($P<0.05$). Meanwhile, the effect of incubation period on pH was found to be acceptable, for which the significant effect of media implies that the biochemical activities of LAB closely relates to presence of lactic acid. Meanwhile, changes in pH level obviously resulted from organic acid produced by *L. plantarum* IIA-1A5. Sidhu *et al.* (2020) The pH is also a chief factor that affects the antibacterial activity of bacteriocins. This is similar to the results of (Reddy Tadi *et al.,* 2017) Interestingly, complete sugar consumption was observed in the presence of CaCO3, which proved that pH change influences lactic acid production and substrate consumption.

In addition, the percentage of total titratable acidity was also significantly affected by the media and incubation period ($P<0.05$). An increase was observed for the lactic acid content derived from the accumulation of LAB acids. The formation of acids is influenced by the addition of sucrose. This is similar to the results of (Faridah *et al.*, 2017) in that antibacterial activity, in the form of bacteriocin, was produced by *L. fermentum* strain A323L at an optimum level after being fermented for 24-28 hours with growth media. whey protein hydrolysate was selected as an elite nitrogen source for lactic acid fermentation nitrogen significant impact on lactic acid productionowing to its low cost and abundant availability (Reddy Tadi *et al.*, 2017)

Plantaricin Purification

Many reports have shown that the four-step purification method can at least be used to purify plantaricin. This study used a two-step protocol: purification of plantaricin IIA-1A5 through precipitation of ammonium sulfate followed by cation-exchange column chromatography. First, plantaricin IIA-1A5 was saturated with ammonium sulfate from cell-free culture supernatant up to 90%, in which the total precipitated amount was 30 g (from 1 liter of culture whey). Fractions from ammonium precipitation in the plantaricin IIA-1A5 was then loaded into ion exchange column chromatography. In this case, plantaricin formed a strong binding with cationic resin at pH 6.8. Figure 2 describes plantaricin IIA-1A5 elution profile produced from *Lactobacillus plantarum* IIA-1A5 growth in whey+ (sucrose, tryptone, yeast extract) and whey medium observed using UV-Vis spectrophotometer at 280 nm. Fraction number with higher peaks were chosen as plantaricin for activity characterization. After purified using SP sepharose fast-flow, the amount of plantaricin IIA-1A5 reached 74.26 mg/L, being considerably higher than previous works. Hata *et al.* (2010) purified plantaricin ASM1 using SP-Sepharose fast-flow binding and yielded 17.5 mg/mL. In addition, Arief *et al*. (2015[b]) reported quantity of plantaricin IIA-1A5 reaching up to 4.5 mg/L after purified using SP Sepharose fast-flow binding with media consisting of MRS broth supplemented with 3% yeast extract.

According to SDS-PAGE electrophoresis, molecular weight of plantaricin IIA-1A5 developed from both studied media was 9.59 kDa (Figure 3), which belonged to class IIa (<10 kDa). The size was slightly higher than that of previous work (Arief *et al.* 2015[b]), which might be due to the different composition of the medium used in the production of plantaricin IIA-1A5. Vaz et al. (2011) stated that larger molecular weight of plantaricin IIA-1A5 from the whey medium presumably related to the different medium composition compared to that of the control medium.

However, this value is higher than the plantaricin that was produced by *L. plantarum* PKLP5, which was around 5.1 kDa weight (Sidhu and Nehra, 2020). Plantaricin IIA-1A5 is part of class IIa (<10 kDa). Class II comprises a

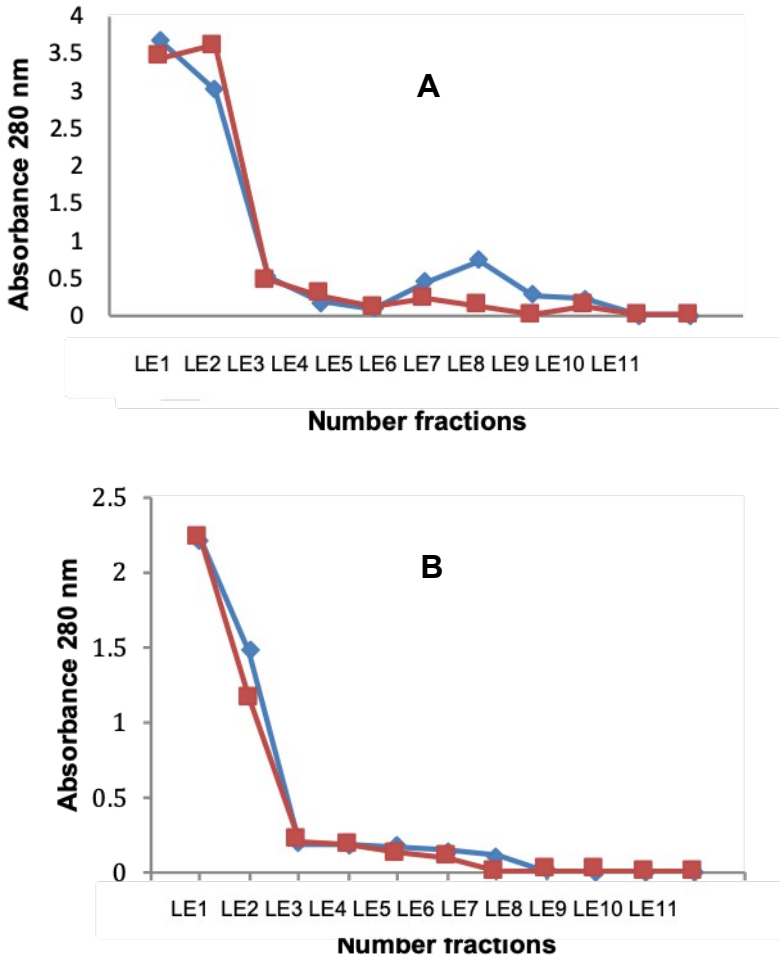

Figure 2. Plantaricin IIA-1A5 elution profile produced from *Lactobacillus plantarum* IIA-1A5 growth in (A) whey+ (sucrose, tryptone, yeast extract) and (B) whey medium observed using UV-Vis spectrophotometer at 280 nm.

thermostable peptide (<10 kDa made from 37-48 amino acids). In this case, Barbosa *et al.* (2016) divided the classification, Class I bacteriocins are small (<5 kDa) and thermostable peptides with residues of lanthionine and methyl lanthionine (thioether amino acids), and class II are small (≤10 kDa).

The concentration of plantaricin generated by *Lactobacillus. plantarum* IIA-1A5, in the treatment of whey+ with a total protein, was 1883.17 mg/mL, and the total protein in whey was 325.58 mg/ml. Total protein of plantaricin with media whey + was higher than media whey because in media whey + was added by sucrose, tryptone and yeast extract that increased the total protein of plantaricin produced. Tryptone and yeast extract were protein substrate. The concentration of plantaricin ASMI protein was 17500 mg/mL (Hata *et al.,* 2010), while plantaricin LR14 was in the range of 59.21 mg/mL (Tiwari and Srivastava, 2008; Arief *et al.,* 2015[b]). These results proved that the different *Lactobacillus plantarum* strains affected the characteristics of the plantaricin produced (Arief *et al.,* 2015[b]).

Antimicrobial Activity of plantaricin

Table 1 shows that plantaricin IIA-IA5 produced from whey or whey + media displayed remarkable antimicrobial activity towards *S. aureus* ATC 25923 and *E. coli* ATCC 25922. Plantaricin IIA-1A5 harvested from whey medium showed antibacterial activity against *Staphylococcus aureus* ATC 25923 and *E. coli* ATCC 25922, i.e. 38.02 IU/dL and 44.85 IU/dL, respectively. Meanwhile, plantaricin IIA-1A5 produced from whey+ medium inhibited *S. aureus* ATC 25923 and *E. coli* ATCC 25922 with

Table 1. Antimicrobial Activites of plantaricin IIA-1A5

Media	*E. coli* ATCC 25922 (IU/dL)	*S. aureus* ATCC 25923
Whey	44.85±1.12[a]	38.02±1.25[a]
Whey+	172.02±1.68[b]	321.10±0.67[b]
Ampicilin	53125±5622	53125±5621
Penicilin	67665±615.18	67665±615.18
Gentamicin	82093±1074	82093±1074
Chloramphenicol	28670±9262	37490±6729
Novobiocin	29184±16960	38157±29088

Different superscripts in the same column show significant difference (P<0.05)

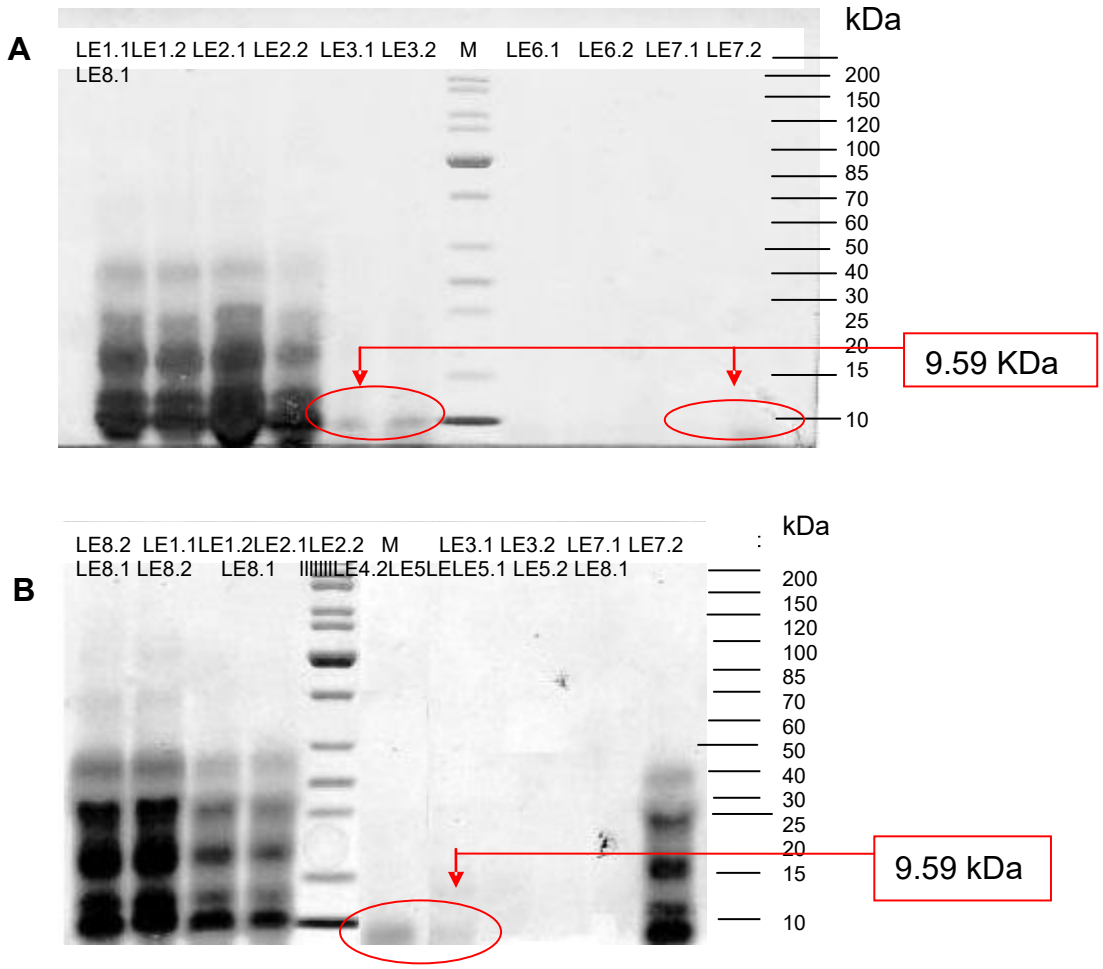

Figure 3. SDS-PAGE of the plantaricin IIA-1A5 purification produced from *Lactobacillus plantarum* IIA-1A5 growth in (A) whey+ (sucrose, tryptone, yeast extract) and (B) whey medium

activity of 321.10 IU/dL and 172.10 IU/dL, respectively.

The bacterial inhibition by the plantaricin was also reported by Kia *et al*. (2016), in which plantaricin IIA-1A5 showed remarkable inhibition against *E. coli* ATCC 25922 with an inhibition zone diameter of approximately 10 mm. Similarly, Pei *et al.,* (2018) reported antimicrobial activity of Plantaricin SLG1 had inhibitory activities towards both Gram positive bacteria and Gram negative bacteria, it was also able to inhibit the growth of some fungi. For control of such pathogens, use of bacteriocins is of great interest as they are generally recognized as safe natural biopreservatives (Silva *et al*. 2018). Plantaricin LD4 have been tested against pathogenic bacteria such as *S. aureus, Salmonella typhi, Vibrio sp., Pseudomonas aeruginosa* and *Escherichia coli* (Kumar *et al.*, 2016).

It is clear that plantaricin IIA-1A5 from both whey and whey+ media exhibited antimicrobial activity. Nevertheless, the activity was much lower compared with commercially available antibiotics (ampicilin, penicillin, gentamicin, chloramphenicol, and novobiocin). The strength of antimicrobial activities of *E. coli* ATCC 25922 using whey was only 0.08% compared to ampicilin, 0.06% compared to penicillin, 0.05% compared to gentamicin, 0.16% compared to chlorampenicol, and 0.18% compared to novobiocin. Meanwhile, whey+ showed only 0.32% antimicrobial strength compared to ampicilin, 0.25% compared to penicillin, 0.20% compared to gentamicin, 0.63% compared to chlorampenicol, and 0.71% compared to novobiocin. The strength of antimicrobial activities for *S. aureus* ATCC 25923 in whey was only 0.07% compared to ampicilin, 0.05% compared to penicillin, 0.04% compared to gentamicin, 0.10% compared to chlorampenicol, and 0.10% compared to novobiocin. Furthermore, whey+ showed only 0.31% strength compared to ampicilin, 0.26% compared to penicillin, 0.21% compared to gentamicin, 0.55% compared to chlorampenicol, and 0.43% compared to novobiocin. This discrepancy is might be due to concentration issue. Different nature of plantaricin IIA-1A5 (peptide) and commercial antibiotics (nonpeptide) might also account for the strength of their antimicrobial activity. In addition, organic solvents used in commercial antibiotic might also contributed to the activity of commercial antibiotic. Nevertheless, the result showed the promising of plantaricin IIA-1A5 to be further developed as an antibiotic replacer.

Interestingly, Table 1 shows that antimicrobial activity of plantaricin IIA-1A5 from whey+ was significantly greater ($P<0.05$) than that of from whey. The antibacterial activity of plantaricin IIA-1A5 from whey for *E. coli* ATCC 25922 was 4-5-fold lower than that of from whey+. Meanwhile, the activity of plantaricin IIA-1A5 from whey for *S.aureus* ATCC 25923 was 10-fold higher than that of from whey+. Considering SDS-Page results, the plantaricin produced from both media were apparently different and affected by the media. This might account for the differences in their activity. In addition, whey+ might contain some functional peptide exhibiting more antimicrobial activity that might contributed to the plantaricin IIA-1A5 activity. Similarly, During the translation of plantaricin, the peptide, or its amino acids, might fused to plantaricin IIA-1A5 hence increasing its antimicrobial activity. This might also explained the differences in the plantaricin IIA-1A5 size with the addition of whey. Bacteriocins are ribosomally-synthesized antimicrobial proteins or peptides produced by bacteria, which kill or inhibit bacterial strains closely related to producer (Silva *et al.*, 2018). Whey protein extensively used as an antimicrobial in edible film and as protective material to improve the shelf life of food and the importance of whey protein as a source of new-generation functional ingredients in the food industry (Kumar *et al*.,2018)

Amino Acid Compositions

Amino acid is an organic component that consists of amino and carboxyl. The composition of amino acid can be used to determine quality and property of the protein. In term of quality, protein with all of the essential amino acid that required in the human body was considered as high quality of protein and vice versa. Meanwhile, in term of property, amino acid composition of the protein modulates structure of protein which further dictated the overall properties of protein, including its function, solubility, polarity and others. Whey proteins were traditionally separated from caseins via isoelectric precipitation of the caseins by adjusting the pH of milk to 4.6 the whey proteins remained in the soluble phase at this pH, producing a whey stream termed acid whey (O' Mahony and Fox, 2013).

Around 95% of the milk protein component is synthesized from amino acid and another 5% of

the amino acid is obtained from blood cells. The components that are absorbed by the blood cells are albumin serum and immunogblobin. Whey proteins represent about 20% of the total protein in bovine milk and are mostly made up of β-lactoglobulin (β-Lg), α-lactalbumin (α-La), bovine serum albumin (BSA), immunoglobulins (Igs), glycomacropeptide (GMP, if the whey is from cheese manufacture), and proteose peptone (Sharma, 2018). the whey fraction, which contains a diverse profile with more than a hundred proteins(Liao *et al.,* 2017)

Table 2 presents composition of amino acid of whey+, which is considerably higher over amino acid in whey media. The result clearly showed that plantaricin IIA-1A5 from whey+ medium contained higher Asp, Lys and Trp than that from whey medium. Furthermore, the greater Pro presenting in amino acid profile of plantaricin IIA-1A5 from whey+ medium significantly reveals that Pro contribute to the secondary protein structure (Johnson, 1999)**.** Whey produced via enzymaticcoagulation of milk is termed sweet whey and contains glycomacropeptide (Hazlett *et al.*, 2018)**.** From these points, the results of amino acid composition clearly indicated that plantaricin IIA-1A5 from both media are different in their structures and possibly the function (antimicrobial activity). This might explained the differences of plantaricin IIA-1A5 obtained from current study with previous report (Arief et al., 2015[a]) obtained solely from MRSB media.

Table 2. Composition of Amino Acids in Plantaricin IIA-1A5

Amino acid	Whey+ (pmol/ul)	Whey (pmol/ul)
Polar		
Aspartic Acid$^{(Ne)}$	1433.7±177.68^{a}	154.78±71.65^{b}
Glutamic Acid$^{(NE)}$	87.10±5.04	72.28±2.25
Asparagine$^{(Ne)}$	653.42±95.71	615.1±42.05
Serine$^{(NE)}$	214.5±16.99^{a}	38.22±2.33^{b}
Cystine$^{(ES)}$	18.69±0.51	ND
Glutamin$^{(NE)}$	27.457±12.24^{b}	191.92±0.00^{a}
Threonine$^{(ES)}$	2762±49.92	ND
Argininine$^{(ES)}$	43.80±0.02	54.40±1,31
Tyrosine$^{(NS)}$	42.74±1.74^{b}	12096±0.00^{a}
Lysine$^{(ES)}$	1118.2±1497.85	68.05±9.88
Histidine$^{(ES)}$	58.65±9.60	ND
Non Polar		
Glycine$^{(NE)}$	25.95±0.32	167.3±23.01
Alanine$^{(NE)}$	125.9±120.7	310.7±49.12
Trytophan$^{(ES)}$	544.5±0.00^{a}	44.82±0.00^{b}
Valine$^{(ES)}$	13.16±1.69^{b}	57.16±0.00^{a}
Methionine$^{(ES)}$	59.64±0.65	59.85±21.98
Phenylalaninen$^{(ES)}$	16.28±0.44	15.89±3.11
Isoleucine$^{(ES)}$	49.166±0.00^{a}	13.24±0.33^{b}
Leucine$^{(ES)}$	4.644±0.00	0.0889±0.00
Prolin$^{(NE)}$	82316±240.67	61.69±0.00

Different superscript letter in the same column are significantly different (P<0.05), NE (Non essential), ES (essential). ND = Not Determined

The differences in the amino acid composition of plantaricin IIA-1A5 from whey and whey+ media might be due to the presence of tryptone and yeast extract. This means that supplementation with tryptone and yeast extract is a promising way to produce plantaricin by *Lactobacillus plantarum* IIA-1A5. The increase in plantaricin production rate is consistent with the increase in protein compound (amino acids). This result is acceptable since tryptone and yeast extract are essentially proteins composed of amino acid. The essential amino acid of tryptophan, threonine, lysine, and histidine showed the highest concentration compared to the non-essential amino acid. Similarly, Edwards *et al.* (2009) reported the appreciable performance of whey as source of fortification due to its essential amino acid content such as lysine, tryptophan, isoleucine, leucine threonine, and valine. However, Table 2 shows that cysteine and threonine were not detectable. This is not due to the absence of these amino acids; rather it might be due to very low levels of these amino acids. Similar finding was published by Atrih *et al.* (2001), reporting that plantaricin C19 contained a huge quantity of glycine and hydrophobic amino acids (majorly alanine and valine), while tryptophan as well as cysteine could not be determined. On whey proteins besides being a source of essential and branched chain amino acids, β-Lg is an important source of biologically active peptides that are inactive within the native sequence of the protein, but can be released by in vivo or in vitro enzymatic hydrolysis (Sharma, 2018)

The polar amino acid was higher in amount than the non-polar amino acid and hence might improve the final solubility. The composition of amino acids is one of the factors that affect the functional properties of a protein (Table 2). This is similar to the results of Sinz and Schwab (2012), in which *L. plantarum* is richer in detectable dipeptides, in which peptide hydrolase showed more intense activity in the cells. The accumulation of amino acids from dipeptide was internally higher than free amino acids; thus, it confirms essential contribution of dipeptides as nitrogen sources for microorganism (Saguir *et al*., 2008).

CONCLUSION

Our result clearly whey is promising to be further developed, used as a growth medium for the production of bacteriocin from L. plantarum IIA-1A5. We also believe that this strategy is feasible for growth and production bacteriocin from other LAB strains replacing a costly medium and have potential application as natural preservatives in the food industry.

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