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Characterization of plantaricin IIA-1A5 biosynthesized by *Lactobacillus plantarum* IIA-1A5 in corn steep liquor based medium

Tuti Rostianti Maulani[1*], Betty Sri Laksmi Jenie[2], Irma Isnafia Arief[3] and Sukarno Sukarno[2]

[1]Department of Food Technology, Math'laul Anwar University, Pandeglang 42273, Banten, Indonesia.
[2]Department of Food Science and Technology, Bogor Agricultural University, Darmaga Bogor 16680, West Java, Indonesia.
[3]Department of Animal Production and Technology, Faculty of Animal Science, Bogor Agricultural University, Darmaga, Bogor 16680, Indonesia.
Email: tutirostianti@gmail.com

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ABSTRACT

Aims: To characterize the plantaricin IIA-1A5 crude extract that biosynthesized by *Lactobacillus plantarum* IIA-1A5 using corn steep liquor (CSL) based medium.
Methodology and results: *Lactobacillus plantarum* IIA-1A5 was grown in several media containing different components including corn steep liquor (CSL), molasses and MRS (de Man Rogosa Sharpe) as control medium for 24 h at 37 °C. Antibacterial activities of the cell-free supernatant were expressed as diameter of inhibition zones observed by paper disc method**.** The results showed that CSL medium produced cell-free supernatant of *L. plantarum* IIA-1A5 with significantly higher antibacterial activity againts *Staphylococcus aureus* ATCC 25923 (9.81 mm), *Lactobacillus monocytogenes* ATCC 7644 (9.61 mm), *Bacillus cereus* (8.97 mm) and *Escherichia coli* ATCC 25922 (9.23 mm) were not significantly different compared to control MRS broth media (9.59 mm). CSL medium added only with 3% yeast extract and Tween 80 produced supernatant which showed similar antibacterial activity either to 10% molasses or control medium (Medium K and B). The CSL medium was considered more efficient and low cost, therefore this medium was selected for production and characterization of plantaricin IIA-1A5 crude extract. Further characterization performed by SDS PAGE analysis showed that crude plantaricin had molecular weight of approximately 9.9 kDa, higher than that produced in control medium (8.0 kDa). However, both plantaricins were categorized under the same class for small bacteriocin (class II). This study also revealed the plantaricin IIA-1A5 produced in CSL medium was stable to heat and pH and not significantly different compared to control MRS broth media. The antibacterial activity of plantaricin IIA-1A5 crude extract against *S. aureus* ATCC 25923 (10.09 mm) was not significantly different with 1000 ppm sodium benzoate (9.70 mm) and 300 ppm sodium nitrite (9.82 mm).
Conclusion, significance and impact of study: The CSL medium produced cell-free supernatant of *L. plantarum* IIA-1A5 had significant antibacterial activity characterization againts *S. aureus* ATCC 25923, *L. monocytogenes* ATCC 7644, *B. cereus* and *E. coli* ATCC 25922. Comparison of the inhibition activity of plantaricin IIA-1A5 crude extract against pathogen with synthetic preservatives indicated that plantaricin IIA-1A5 crude extract have the potency to replace synthetic preservatives. CSL based medium is potential to be used for low-cost plantaricin IIA-1A5 production.

Keywords: Bacteriocin, corn steep liquor, *L. plantarum* IIA-1A5, plantaricin IIA-1A5

INTRODUCTION

Bacteriocins are ribosomal synthesized peptides exhibiting antibacterial activity mainly against bacteria closely related to the produced microorganism. The spectrum activity of the bacteriocins can be narrow or can be relatively broad to inhibit a range of target microorganism (Mantovani *et al*., 2011). Bacteriocin is considered as the among most promising preservatives in the food industry. Earlier in 1969, World Health Organization (WHO) announced that nisin, produced by *Lactococcus lactis* subsp. *lactis* was a kind of food preservative and Food and Drug Administration (FDA) declared that nisin was generally recognized as safe food preservatives in 1983 (Henning *et al*., 1986). The bacteriocins from lactic acid bacteria (LAB) have attracted significant attention because of their potential to be used as non-toxic and safe additive for food preservation (Henderson *et al*., 1992).

Several strains of *Lactobacillus plantarum* have been

*Corresponding author

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reported to produce bacteriocins, usually called plantaricin. Many plantaricins have small molecular mass (<10 kDa) and were heat and pH stable (Diep *et al*., 2009; Hata *et al*., 2010; Todorov *et al*., 2011; Arief *et al*., 2013; Arief *et al*., 2015). It has been demonstrated that *L. plantarum* IIA-1A5 which is an indigenous strain that isolated from Indonesian beef was shown to produce bacteriocin, named plantaricin IIA-1A5 (Arief *et al*., 2013). It has been shown to has antimicrobial activity against pathogenic bacteria including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella typhimurium*. The purified plantaricin IIA-1A5 had been reported could be digested by trypsin and was stable at 80 °C for 30 min and 121 °C for 15 min and a wide range of pH (Arief *et al*., 2013).

Bacteriocin usually produced in MRS broth which was relatively expensive for industrial scale production (Lue *et al*., 2003). Studies on lowering the production cost for bacteriocins have been reported (Trinetta *et al*., 2008; Juárez Tomás *et al*., 2010). The use of low-cost media such as agro-industrial byproduct was considered more economical than MRS broth and provide the advantage in reducing the environmental pollution (Bali *et al*., 2014). Corn steep liquor (CSL) and molasses might be potential candidates as cheap nitrogen and carbon source, respectively, for bacteriocin production. CSL is a byproduct of the corn starch industry which contained nitrogenous components, protein and amino acids and high trace elements (Choi *et al*., 2013). Molasses are waste product from sugar cane industry that are rich in carbon source. Utilization of CSL or molasses for bacteriocin production had been reported previously by several researchers (Coetzee *et al*., 2007; Todorov, 2008; Mahrous *et al*., 2013; Bali *et al*., 2014). Optimization study on culture conditions for bacteriocin production by *L. acidophilus* CH1, *L. acidophilus* M2 and *L. pentosus* CH2 found that CSL was the best medium for bacteriocin production which demonstrated high antimicrobial activity against *B. subtilis* (Mahrous *et al*., 2013). Bali *et al.* (2014) produced bacteriocin by *Enterococcus faecium* BS13 using waste products such as whey supplemented with 4% CSL and polysorbate 80 at 40 °C and pH 6.5, which displayed maximum bacteriocin activity against *Lactobacillus brevis* MTCC 1750.

Agro-based byproducts in Indonesia such as CSL and molasses are relatively abundant but have not been optimally utilized yet. Therefore, the objectives of this study were to determine the potential of CSL as substrates for plantaricin IIA-1A5 production and characterize the obtained plantaricin IIA-1A5 crude extract which biosynthesized by *L. plantarum*.

MATERIALS AND METHODS

Bacterial cultures and argo-based byproducts

Lactobacillus plantarum IIA-1A5 was obtained from Microbiology Laboratory of the Department of Animal Production and Technology Bogor Agricultural University, Indonesia. *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *B. cereus* and *L. monocytogenes* ATCC 7644 were used as indicator microorganism. All indicator microorganisms were culture for 24 h in brain-heart infusion (BHI) media. On the other hand, CSL and molasses were obtained from corn starch industry (Cilegon Banten) and sugar cane industry (Subang, Indonesia), respectively.

Effect of incubation period on plantaricin IIA-1A5 production

Ten percent (v/v) of overnight culture of *L. plantarum* IIA-1A5 (10^8 CFU/mL) was inoculated into 100 mL MRS broth supplemented by 3% yeast extract and 1.0 g/L tween 80 and incubated at 37 °C for 36 h (Arief *et al*., 2013). Samples were drawn at 4 h-intervals during incubation and measured for pH and antibacterial activity. Antibacterial activities were expressed as zone of inhibition using paper disc method (5 mm diameter paper dish, Oxoid). This indicated by the presence of a clear zone around the paper dish, created by the neutralized cell-free supernatant pH 6.8. The optimum incubation time to produce plantaricin IIA-1A5 was selected based on the greatest growth inhibition zone produced by the CFS against *S. aureus* ATCC 25923.

Effect of different medium compositions on plantaricin IIA-1A5 production

CSL and molasses were used as replacement for nitrogen and carbohydrate component in MRS broth, respectively. Nitrogen content (by using Kjeldahl Method) in CSL was 19.7 g/L and glucose content (by using HPLC) in molasses was 66.8 g/L. Based on the nitrogen and glucose content of CSL and molasses, respectively, various media were prepared as follows: (K) MRS broth, (A) CSL, (B) 10% molasses, (C) CSL plus 10% molasses, (D) CSL, 2.3 g/L peptone, 20.0 g/L glucose and minerals, (E) 10% molasses, 10.0 g/L peptone, 8.0 g/L meat extract and minerals, (F) CSL plus 10% molasses, 10.0 g/L peptone and minerals. All media were further supplemented with 3% yeast extract and 1.0 mL/L tween 80. The minerals that added to several groups (medium D, E and F) consisted of 2.0 g/L di-potassium hydrogen phosphate, 1.0 ml/L tween 80, 2.0 g/L ammonium citrate, 5.0 g/L sodium acetate, 0.2 g/L magnesium sulfate and 0.05 g/L manganese sulfate. The pH of all media was adjusted to 6.2 before autoclaving.

A 10% (v/v) of 24 h old culture of *L. plantarum* IIA-1A5 was added into various media (as stated above medium A-F, K) and incubate for 24 h. After 24 h incubation, cells were harvested by centrifugation (12,000 x *g*, 15 min, 4 °C), and the cell free supernatant (CFS) was evaporated at 60 °C for 1 h and adjusted to pH 6.8 followed by filter sterilization using 0.22 µm filter. The best and effectivenes medium was selected based on the highest bacteriocin activity against *S. aureus* ATCC 25923 demonstrated by the CFS.

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Crude extract preparation

Cell-free supernatant (CFS) of *L. plantarum* IIA-1A5 grown in the selected media was added with gradient concentrations of ammonium sulfate (20%, 40%, 60%, 80% and 90%), stirred gently for 2 h at 4 °C and then centrifuged (12,000 x *g*, 15 min, 4 °C). The supernatant was dialyzed with 2.0 kDa cut-off membrane to obtain plantaricin crude extract. The plantaricin crude extract was further characterized and tested for antibacterial activity using paper disc method (Arief *et al*., 2013).

Antibacterial activity of plantaricin IIA-1A5

Fifty microliters of plantaricin IIA-1A5 was dropped and evenly distributed on 5 mm diameter paper disc (Oxoid Millipore) and placed on the plates containing targeted microorganism. The plates were then incubated at 37 °C for 24 h and antibacterial activity against *S. aureus* ATCC 25923, *L. monocytogenes* ATCC 7644, *B. cereus* and *E. coli* ATCC 25922 was measured as the diameter of inhibition zone including the diameter of paper disc (5 mm).

Stability of plantaricin IIA-1A5 crude extract to pH and heat

The stability of plantaricin IIA-1A5 crude extract to pH and temperature was examined according to Arief *et al*. (2013). Briefly, samples in different tubes containing 3.0 mL of plantaricin IIA-1A5 were adjusted to different pH (2.0; 4,0 and 6.0) with 1 N HCl or 1 N NaOH and incubated at room temperature (25 °C) for 1 h. After incubation, the samples were readjusted to pH 6.8. The effect of temperature on plantaricin IIA-1A5 was performed by heating 1.0 mL plantaricin IIA-1A5 in microcentrifuge tube at 80 °C for 30 min, 100 °C for 15 min and 121 °C for 15 min. After that, plantaricin IIA-1A5 antibacterial activity was determined against *S. aureus* ATCC 25923, *L. monocytogenes* ATCC 7644, *E. coli* 25922 and *B. cereus*. Antibacterial activity was expressed as the diameter of clear zone developed by plantaricin IIA-1A5 against indicator bacteria.

Determination the molecular weight of plantaricin IIA-1A5 crude extract

Electrophoresis was carried out by adding the plantaricin IIA-1A5 crude extract solution 10 µL and low molecular weight of 2.5 to 45 kDa marker (Spectra Multicolor Low Range Protein Ladder) into the SDS PAGE well. Electrophoresis was carried out for approximately 2 h with a constant voltage of 70 V, 28 mA. Migration was observed with bromophenol blue dye, with 4% (v/v) stacking gel using 15% acrylamide separator gel, followed by staining with 0.1% silver nitrate (silver staining) for 20 min at 4 °C. The gel was photographed to be documented and measured the distance to which protein bands of the sample and marker were measured. The SDS PAGE results analyzed the migration distance of the bands formed on the separator gel (Laemmli, 1970).

Mode of action of plantaricin IIA-1A5 crude extract

The mode of action of plantaricin IIA-1A5 crude extract was observed on the growth of two indicator bacteria *S. aureus* ATCC 25923 and *L. monocytogenes* ATCC 7644. A total of 200 mL of trypticase soy broth (TSB) was inoculated with 1% (v/v) 24h-old culture *L. monocytogenes* ATCC 7644 and *S. aureus* ATCC 25923 (in different tube) and incubated for 3 h at 37 °C. As much as 20 mL plantaricin IIA-1A5 crude extract was added to the culture and the growth of both indicator bacteria was measured by spectrophotometer (OD_{600nm}) hourly over a 10 h period. Samples without addition of plantaricin IIA-1A5 was used as control (Todorov *et al*., 2012).

Comparison of the antibacterial activity of plantaricin IIA-1A5 to sodium benzoate and sodium nitrate

This test was performed according to Arief *et al*. (2012). The inhibitory activity of plantaricin IIA-1A5 crude extract was compared to sodium benzoate (1000 ppm) and sodium nitrite (300 ppm) against *L. monocytogenes* ATCC 7644 and *S. aureus* ATCC 25923 by paper disc method as described above.

Statistical analysis

The data values were presented as mean of standard of deviation of triplicate measurements. Data were subjected to one way ANOVA (analysis of variance) and continued by Duncan test to identify difference in the assay. Differences was considered as statistically significant if $p<0.05$.

RESULTS AND DISCUSSION

Effect of incubation time on plantaricin IIA-1A5 antibacterial activity

The optimum time for plantaricin IIA-1A5 antibacterial activity was determined based on the inhibition zone against *S. aureus* ATCC 25923 (Arief *et al*., 2013). The results showed that plantaricin IIA-1A5 activity increased from 6.49 mm after 1 h of growth to 9.79 mm after 24 h (Table 1) and decreased to 8.44 mm after 36 h. Thus, the optimum time of plantaricin IIA-1A5 production by *L. plantarum IIA*-1A5 was observed at 24 h cultivation (Table 1). Todorov *et al*. (2011) also found 24 h incubation as the optimum production by *L. plantarum* ST31 and *L. plantarum* ST16Pa, respectively. The pH of the medium decreased from 6.0 to 4.13 (Table 1). Similar decrease of bacteriocin activity after 24 h have also been observed during bacteriocin ST16Pa production (Todorov *et al*., 2011) and bacteriocin produced by *E. faecium* ST311LD (Todorov and Dicks, 2005). This loss of bacteriocin activity might be due to proteolytic degradation, protein aggregation or instability of proteins at this low pH

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Table 1: Effect of incubation period on antibacterial activity of cell-free supernatant (CFS) of *L. plantarum* IIA-1A5 against *S. aureus* ATCC 25923.

Incubation time (h)	Inhibition zone (mm)*	pH
1	6.49 ± 0.16[a]	6.0 ± 0.04[a]
4	6.55 ± 0.41[a]	5.52 ± 0.01[a]
8	7.01 ± 0.16[a]	5.28 ± 0.01[a]
12	7.09 ± 0.34[a]	4.54 ± 0.02[a]
16	8.78 ± 0.10[b]	4.31 ± 0.02[c]
20	8.83 ± 0.47[c]	4.3 ± 0.02[c]
24	9.79 ± 0.07[c]	4.26 ± 0.01[c]
28	9.25 ± 0.06[b]	4.17 ± 0.04[b]
32	8.57 ± 0.07[b]	4.16 ± 0.04[b]
36	8.44 ± 0.74[b]	4.13 ± 0.03[b]

*Mean inhibition zone diameters, including the diameter of the paper disc (5 mm). Different superscript following value on the same line indicated statistically significant differences (p<0.05).

(Parente *et al*., 1994; De Vuyst *et al*., 1996; Aasen *et al*., 2000).

Effect of different medium composition on plantaricin IIA-1A5 antibacterial activity

The effect of different medium composition on plantaricin IIA-1A5 activity against *S. aureus* ATCC 25923 was shown in Table 2. Based on Table 2, it shows the plantaricin IIA-1A5 activity produced from CSL or 10% molasses was comparable to control medium (MRS broth). The utilization of CSL or molasses with additional nutrients in production media (Medium D and E) remarkably increased the plantaricin IIA-1A5 activity compared with MRS broth. Medium D and E produced the highest inhibition zone i.e. 11.36 mm and 10.46 mm, respectively, against *S. aureus* ATCC 25923. Inhibition zone created by control plantaricin IIA-1A5 was significantly lower (9.59 mm) than that produced in medium D and E above. Other medium compositions (A, B and F) produced plantaricin IIA-1A5 with activities against *S. aureus* ATCC 25923 were not significantly different compared with control. The medium composition C did not improve the plantaricin IIA-1A5 activity and demonstrates lower inhibition zone (7.92 mm) against *S. aureus* ATCC 25923. In this result, medium D has highest antibacterial activity but medium D used additional complex medium (CSL, pepton, D(+) glucose, mineral) which can increase the production cost. On the other hand, medium A produced a fairly high antibacterial activity significant to MRSB medium. Therefore, medium A (CSL) was selected for crude extract preparation and characterization.

The activities of plantaricin IIA-1A5 crude extract produced in selected CSL medium (medium A) showed inhibition zone of the antibacterial activity against *S. aureus* ATCC 25923 (9.81 mm), *L. monocytogenes* ATCC 7644 (9.61 mm), *B. cereus* (8.97 mm) and *E. coli* ATCC 25922 (9.23 mm) were not significantly different compared to MRSB medium (Table 3).

Table 2: Effect of medium components on antibacterial activity of plantaricin IIA-1A5 of *L. plantarum* IIA-1A5 against *S. aureus* ATCC 25923.

Medium components	Inhibition zone (mm)*
(K) MRS broth (control)	9.59 ± 0.32[b]
(A) Corn Steep Liquor (CSL)	9.63 ± 0.00[b]
(B) Molasses (10% v/v)	9.62 ± 0.11[b]
(C) A+B	7.92 ± 0.02[c]
(D) A + nutrients (peptone, D(+) glucose, minerals)	11.36 ± 0.40[a]
(E) B + nutrients (peptone, meat extract, minerals)	10.46 ± 0.17[a]
(F) A + B + nutrients (peptone, minerals)	9.13 ± 0.17[b]

*Mean inhibition zone diameters, including the diameter of the paper disc (5 mm). Different superscript following value on the same line indicated statistically significant differences (p<0.05).

Table 3: Antibacterial activity of plantaricin IIA-1A5 crude extract produced in selected CSL medium.

Medium	Inhibition zone (mm)*			
	S. aureus ATCC 25923	*L. monocytogenes* ATCC 7644	B. cereus	*E. coli* ATCC 25922
MRSB	9.72 ± 0.55[a]	9.59 ± 0.17[a]	8.78 ± 0.31[a]	9.31 ± 0.20[a]
CSL	9.81 ± 0.53[a]	9.61± 0.16[a]	8.97 ± 0.29[a]	9.23 ± 0.82[a]

*Mean inhibition zone diameters, including the diameter of the paper disc (5 mm). Different superscript following value on the same line indicated statistically significant differences (p<0.05).

Stability of plantaricin IIA-1A5 activity to pH and heat

The stability of plantaricin IIA-1A5 to pH and heat is particularly important for application in food product. In this study, plantaricin IIA-1A5 crude extract produced by *L. plantarum* IIA-1A5 show remained stable after incubation at room temperature for 1 h, at pH 2, 4 and 6 (Table 4). According to Todorov *et al.* (2011), that the bacteriocin activity was stable after being treated with an inhibition zone diameter of more than 2 mm after deducting the well inhibition zone. In this study, the diameter of the inhibition zone produced due to pH ranged from 5 mm to 8 mm after deducting the diameter of the paper discs.

Similar stabilities had been demonstrated by bacteriocin of *L. plantarum* F1 and *L. plantarum* K25 which were stable at pH 2-6 (Ogunbanwo *et al*., 2003; Wen *et al.*, 2016). The stability to low pH of plantaricin IIA-1A5 was promising for application in acidified food product. Plantaricin IIA-1A5 was also heat stable at

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Table 4: Stability activity of plantaricin IIA-1A5 by *L. plantarum* IIA-1A5 to heat and pH.

Treatment	Inhibition zone (mm)*							
	S. aureus ATCC 25923		*L. monocytogenes* ATCC 7644		*B. cereus*		*E. coli* ATCC 25922	
	Control	CSL	Control	CSL	Control	CSL	Control	CSL
pH 2.0	9.14 ± 0.33^{b}	8.19 ± 0.15^{b}	7.97 ± 0.27^{b}	8.04 ± 0.18^{b}	7.92 ± 0.18^{a}	8.18 ± 0.25^{a}	8.07 ± 0.32^{a}	8.21 ± 0.28^{a}
pH 4.0	9.55 ± 0.15^{b}	8.54 ± 0.03^{b}	8.01 ± 0.07^{b}	8.71 ± 0.13^{b}	7.98 ± 0.18^{a}	8.22 ± 0.29^{a}	8.25 ± 0.07^{a}	8.36 ± 0.08^{a}
pH 6.0	9.69 ± 0.15^{b}	9.14 ± 0.06^{b}	8.20 ± 0.11^{b}	8.63 ± 0.15^{b}	8.20 ± 0.37^{a}	8.39 ± 0.09^{a}	8.20 ± 0.07^{a}	8.52 ± 0.08^{a}
80 °C, 30 min	10.27 ± 0.48^{a}	10.06 ± 0.38^{a}	9.29 ± 0.04^{b}	9.20 ± 0.39^{b}	9.21 ± 0.22^{b}	9.10 ± 0.05^{b}	9.28 ± 0.38^{b}	9.38 ± 0.34^{b}
100 °C, 15 min	10.17 ± 0.50^{a}	10.22 ± 0.17^{a}	9.14 ± 0.25^{b}	9.31 ± 0.33^{b}	9.25 ± 0.34^{b}	9.00 ± 0.27^{b}	9.30 ± 0.25^{b}	9.50 ± 0.25^{b}
121 °C, 15 min	10.39 ± 0.48^{a}	10.41 ± 0.77^{a}	9.06 ± 0.10^{b}	9.22 ± 0.77^{b}	9.16 ± 0.15^{b}	9.06 ± 0.23^{b}	9.61 ± 0.27^{b}	9.11 ± 0.18^{b}

*Mean inhibition zone diameters, including the diameter of the paper disc (5 mm). Different superscript following value on the same line indicated statistically significant differences (p<0.05).

80 °C for 30 min, 100 °C for 15 min and 121 °C for 15 min. These findings were consistent with a number of bacteriocins produced by *L. plantarum* AA135, *L. paracaseii*, *L. lactis*, *L. plantarum*, *L. pentosus* and *L. plantarum* K25 (Abo-Amer, 2007; Sankar *et al*., 2012; Arief *et al*., 2013; Wen *et al*., 2016). The characterization of plantaricin IIA-1A5 purified with cation exchange chromatography was also heat stable at 80 °C and 121 °C for 30 and 15 min and digestible by trypsin (Arief *et al*., 2013). These results suggested that the antibacterial activities were attributed to the heat and acid stable of proteinaceous compound (Wen *et al*., 2016).

Determination of the molecular weight of the plantaricin IIA-1A5 crude extract

The molecular weight of the plantaricin IIA-1A5 crude extract was found approximately 8.0 kDa and 9.9 kDa for plantaricin IIA-1A5 produced in control (MRS broth) and CSL medium, respectively, as determined by SDS-PAGE (Figure 1). Based on the molecular weight, plantaricin IIA-1A5 was classified as typical for class II bacteriocins (<10 kDa) (Arief *et al*., 2013). Arief *et al*. (2015) found smaller value for molecular weight of plantaricin IIA-1A5 purified by cation exchange chromatography (6.4 kDa). Besides, bacteriocin ST16Pa crude extract by *L. plantarum* ST16Pa was determined to be near 6.5 kDa (Todorov *et al*., 2011). The bacteriocin Lp6SH produced by *L. plantarum* Lp6SH isolated from “sha’a”, a maize-based traditionally fermented beverage from Cameroon was partially purified using ammonium sulfate precipitation, gel filtration and cation exchange chromatography had small molecular weight of 2.3 kDa (Marie *et al*., 2012). These differences may be due to different strain and or purification method.

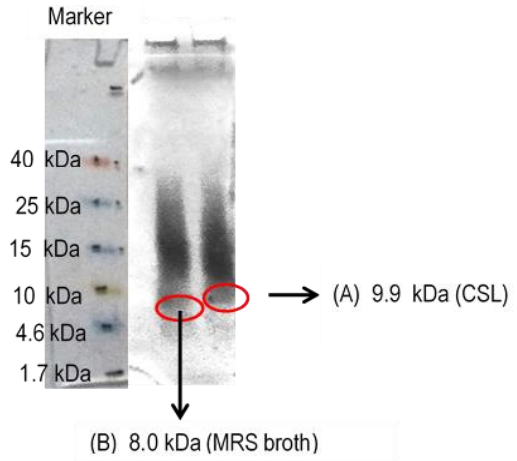

Figure 1: Molecular weight of plantaricin IIA-1A5 crude extract produced using medium (A) CSL (B) MRS broth (control).

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Mode of action of plantaricin IIA-1A5 crude extract

Mode of action of plantaricin IIA-1A5 crude extract was observed by measuring the growth of *S. aureus* ATCC 25923 and *L. monocytogenes* ATCC 7644 over 10 h period. The changes of the OD_{600nm} of both indicator bacteria were shown in Figure 2. The growth of both pathogen without the presence of plantaricin IIA-1A5 increased from OD_{600nm} 0.1 to 1.9. After addition of plantaricin IIA-1A5 crude extract from CSL medium, *L. monocytogenes* ATCC 7644 and *S. aureus* ATCC 25923 did not grow as indicated by relatively constant OD_{600nm} (0.7 to 0.9 for *S. aureus* ATCC 25923 and from 0.8 to 1.1 for *L. monocytogenes* ATCC 7644) which signify plantaricin IIA-1A5 can reduce both patogens. Lower OD_{600nm} was also observed for bacteria treated with plantaricin IIA-1A5 crude extract produced in control medium (MRS broth).

The addition of plantaricin IIA-1A5 crude extract results in reduction of *S. aureus* ATCC 25923 and *L. monocytogenes* ATCC 7644 was expressed in CFU/mL. It shows plantaricin can repressed the growth of both pathogens up to 2 log CFU/mL after 10 h. Cell viability of *S. aureus* ATCC 25923 decreased significantly from 8.05 log CFU/mL to 6.13 log CFU/mL and of *L. monocytogenes* ATCC 7644 also decreased from 8.75 log CFU/mL to 6.42 log CFU/mL. These results suggested that plantaricin IIA-1A5 crude extract has bacteriostatic mode of action. Todorov *et al*. (2011) reported that addition of bacteriocin ST16Pa by *L. plantarum* ST16Pa to 3 h old culture of *E. faecalis* ATCC 19443, *L. innocua* 2030C or *L. sakei* 15521 repressed cell growth of the indicator strain over 10 h. After treatment with bacteriocin ST16Pa, no viable cells of indicator bacteria were detected after 10 h. Based on this result, bacteriocin ST16Pa was considered exhibiting a bactericidal mode of action (Todorov *et al*., 2011). While Marie *et al.* (2012) considered the mode of action of bacteriocin Lp6SH produced by *L. plantarum* Lp6SH as bactericidal based on decrease 2 log CFU/mL of the cell viability of *S. tyhpimurium* 6539 within 5 h.

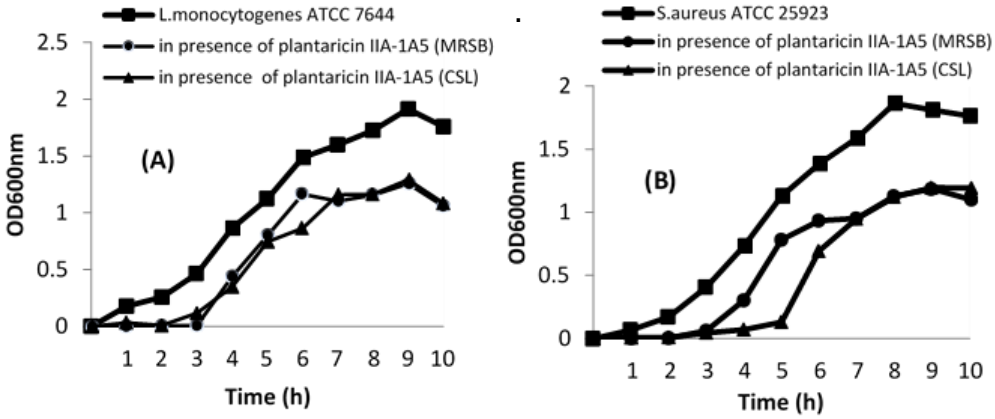

Figure 2: Effect of plantaricin IIA-1A5 crude extract against the growth of (A) *L. monocytogenes* ATCC 7644 and (B) *S. aureus* ATCC 25923.

Comparison of antibacterial activity of plantaricin IIA-1A5, sodium benzoate and sodium nitrite

The antibacterial activity of plantaricin IIA-1A5 was compared with 1000 ppm sodium benzoate and 300 ppm sodium nitrite. Sodium benzoate and sodium nitrite are synthetic preservatives that widely use in food. The results showed that antibacterial activity of plantaricin IIA-1A5 crude extract against *S. aureus* ATCC 25923 (10.09 mm) was not significantly different with 1000 ppm sodium benzoate (9.70 mm) and 300 ppm sodium nitrite (9.82 mm) (Table 5). Based on Table 5, it shows plantaricin IIA-1A5 crude extract is more effective in inhibiting *S. aureus* ATCC 25923 compared to *L. monocytogenes* ATCC 7644. Application of purified plantaricin IIA-1A5 at the concentration of 300 ppm in meatballs tested with *S. aureus* demonstrated a comparable inhibition activity with 300 ppm nitrite (Kia *et al*., 2016). This result indicated that plantaricin has the potency to replace the use of synthetic preservatives in food.

Table 5: Comparison of the antibacterial activity plantaricin IIA-1A5 with synthetic preservatives sodium benzoate and sodium nitrite.

Preservative	Inhibition zone (mm)*	
	S. aureus ATCC 25923	*L. monocytogenes* ATCC 7644
Sodium nitrite (300 ppm)	9.82 ± 0.09[a]	8.21 ± 0.63[b]
Sodium benzoate (1000 ppm)	9.70 ± 0.48[a]	8.44 ± 0.43[b]
Plantaricin IIA-1A5 (Control)	10.07 ± 0.48[a]	9.50 ± 0.13[c]
Plantaricin IIA-1A5 (CSL)	10.09 ± 0.50[a]	9.51 ± 0.14[c]

*Mean inhibition zone diameters, including the diameter of the paper disc (5 mm). Different superscript following value on the same line indicated statistically significant differences (p<0.05).

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CONCLUSION

CSL could be utilized as main component in production medium which contributed as much as 80% of nitrogen source consisted in MRS broth, supplemented with 3% yeast extract and Tween 80 to produce plantaricin IIA-1A5 crude extract by *L. plantarum* IIA-1A5. The plantaricin crude extract obtained in this CSL medium showed similar antimicrobial activity against *S. aureus* and *L. monocytogenes* to control medium (MRS broth). Other characteristics of plantaricin IIA-1A5 crude extract produced in selected CSL medium such as molecular weight, stability to different temperatures (80 °C, 100 °C and 120 °C) and pH (2-6) were also not different significantly compared to control. Mode of action of plantaricin IIA-1A5 crude extract had been shown as bacteriostatic. Comparison of the inhibition activity of plantaricin IIA-1A5 crude extract against *S. aureus* and *L. monocytogenes* with synthetic preservatives indicated that plantaricin IIA-1A5 crude extract have the potency to replace synthetic preservatives including sodium benzoate and sodium nitrite.

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