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OPTIMIZATION OF BACTERIOCIN PRODUCTION BY *Lactococcus lactis* ssp. *lactis* CN1.10a ORIGIN FROM RUSIPS

Optimasi Produksi Bakteriosin yang dihasilkan oleh *Lactococcus lactis* ssp. *lactis* CN1.10a Asal Rusip

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ABSTRACT

Previous study of bacteriocin production on laboratory scale (100 ml) that used MRS broth medium produced unstable activity of bacteriocin. This study aims to determine the optimum growth conditions and media for production of bacteriocin. Bacteria used in this research was a lactic acid bacteria (LAB) *Lactococcus lactis* ssp. *lactis* CN1.10a isolated from rusip, a traditional Bangkanese fermented fish product. The bacteria was first cultivated for subsequent use of bacteriocins production on intermediate scale (2 L), followed by the optimization of temperature, pH and medium for the bacteriocin production, determination of cell growth curve, bacteriocin production curve, bacteriocin activity and stability of bacteriocin during storage. Results showed that the optimum temperature and pH for the growth of producer cell were 28 °C and pH 6. The greatest activity of bacteriocin was produced on CM medium (1% sucrose, 0.45% peptone, 1% yeast extract, 2.84% KH_2PO_4, 0.2% NaCl and 0.02% $MgSO_4.7H_20$) in addition of sucrose as carbohydrate source. Based on the growth curve performed on CM medium with KH_2PO_4, the *L. lactis* ssp. *lactis* CN1.10a was relatively stable up to 48 hours. Bacteriocin produced by the cell was 8000 AU/ml at 24th hour. Bacteriocin was relatively stable when stored at -20 °C for 1 month with a relative activity of 69.4%.

Keywords: optimization, cell growth curve, production of bacteriocins, *Lactococcus lactis*, rusip

ABSTRAK

Studi terdahulu mengenai produksi bakteriosin skala laboratorium (100 ml) yang menggunakan media MRS cair menghasilkan aktivitas bakteriosin yang tidak stabil. Oleh karena itu penelitian ini bertujuan untuk mengetahui kondisi dan media pertumbuhan optimum untuk produksi bakteriosin. Bakteri yang digunakan dalam penelitian ini adalah bakteri asam laktat (BAL) *Lactococcus lactis* ssp. *lactis* CN1.10a yang diisolasi dari rusip, produk fermentasi ikan tradisional Bangka. Terlebih dahulu bakteri dikultivasi untuk selanjutnya digunakan untuk produksi bakteriosin skala intermediet (2 L). Kemudian dilanjutkan dengan optimasi suhu, pH dan media untuk produksi bakteriosin, penentuan kurva pertumbuhan sel, kurva produksi bakteriosin, aktivitas bakteriosin serta stabilitas bakteriosin selama penyimpanan. Hasil penelitian menunjukkan bahwa suhu dan pH optimum untuk pertumbuhan sel produser adalah 28 °C and pH 6. Aktivitas bakteriosin terbaik diproduksi pada media CM (1% sucrose, 0,45% peptone, 1% yeast extract, 2,84% KH_2PO_4, 0,2% NaCl and 0,02% $MgSO_4.7H_20$) dengan sukrosa sebagai sumber karbohidrat. Berdasarkan kurva pertumbuhan pada CM medium dengan KH_2PO_4, maka *L. lactis* ssp. *lactis* CN1.10a relatif stabil hingga 48 jam. Bakteriosin yang diproduksi oleh sel tersebut adalah sebesar 8000 AU/ml pada jam ke-24. Bakteriosin relatif stabil ketika disimpan pada suhu -20 °C selama 1 bulan dengan aktivitas relatif 69,4%.

Kata Kunci: optimasi, kurva pertumbuhan sel, produksi bakteriosin, *Lactococcus lactis*, rusip

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1. Introduction

Bacteriocins are ribosomally synthesized antimicrobial compounds that are produced by many different bacterial species including many members of the lactic acid bacteria (LAB). Bacteriocins are proteinaceous compounds produced by bacteria that exhibit a bactericidal or bacteriostatic mode of action against sensitive bacterial species. These proteins are often active against species closely related to the

producing microorganism (Klaenhammer, 1988; Kaiser et al., 1993). The primary target of bacteriocins produced by lactic acid bacteria is most probably the cytoplasmic membrane, since they initiate reactions which alter the membrane permeability disturbing membrane transport or dissipating the proton motive force and thus inhibiting energy production and biosynthesis of proteins or nucleic acids. Therefore, the bacteriocin often displays a transmembrane helix or an amphiphilic a-helix (Nissen-Meyer et al.,1992 *in* De Vyust & Vandamme, 1994).

Naturally traditional fermented products contain bacteriocins have been consumed for centuries (Smith, 1992 *in* Bhugaloo-vial et al., 1997). Indonesia has many kinds of fermented fish products, one of them is rusip. Rusip is composed of fish, salt, brown sugar and dried rice but sometimes without rice. It is produced by spontaneous fermentation during 7-14 days anaerobically (Yuliana, 2007). Rusip is mainly produced in Bangka Belitung Provinces and widely used as a condiment or mixed with chilli sauce and consumed with boiled rice and vegetables. Rusip contains mainly lactic acid bacteria (LAB) such as *Lactobacillus, Streptococcus, Leuconostoc, Pediococcus, and Enterococcus* (Dessi, 1999 *in* Yuliana, 2007; Wijaya, 2007 *in* Sakti, 2009). Our previous study showed that the lactic acid bacteria which was isolated from rusip was identified as *Lactococcus lactis* ssp. *lactis* CN1.10a. This bacteria could produced bacteriocin. Characteristics of this bacteriocin were sensitive to proteolytic enzymes, i.e. proteinase K and papain but not to RNAse. The bacteriocin has wide target activity of inhibition against Gram-positive and negative bacteria such as *Esherichia coli, Listeria monocytogenes* and *Lactobacillus plantarum.*The activity of bacteriocin could be stimulated by sodium dodecyl sulphate (SDS), lauryl sarcosine and EDTA but strongly inhibited by Tween 20, Tween 80, Triton X-100 and urea. It was stable at pH 2.0 to 6.0, and also was heat stable (121 ºC) (Kusmarwati et al., 2013).

Recently, nisin, produced by strains of *Lactococcus lactis***,** was the only bacteriocin with commercial applications in the food industry. Nisin is a natural antimicrobial peptide produced by *Lactococcus lactis* subsp. *lactis* that effectively inhibits Gram-positive and Gram-negative bacteria and also the out growth of spores of *Bacilli* and *Clostridia*. Additionally it has been used as a biopreservative and a potential agent in pharmaceutical,veterinary and health care products (de Arauz et al., 2009).

Our previous study on lab scale production (100 ml) of bacteriocin from *L. lactis* ssp. *lactis* CN1.10a resulted bacteriocin with activity of 3.453 AU/ml and was stable at high temperature (121 ºC) (Kusmarwati et al., 2013). In that production, we used MRS broth medium. However, bacteriocin activity produced was unstable. Therefore we need to find a good medium that always produce stable bacteriocin by optimation.

Bacteriocin production is usually proportional to growth and shows primary metabolite, however the correlation is weak (Delgado et al., 2005a *in* Delgado, A., 2007). Therefore for producing of bacteriocin, it requires a better understanding of the relationship between growth and bacteriocin production, as well as relationship between bacteriocin production and medium.

Studies on optimation and production of bacteriocin produced by Indonesian fermented food are still limited. Some of the results was reported that production of bacteriocin from meat and milk associated lactic acid bacteria (Usmiati & Marwati, 2007; Syahniar, 2009). Optimal bacteriocin production in batch fermentation usually using complex media and well-controlled physical conditions, such as temperature and pH. Most currently complex media are the concentration of the carbon source, nitrogen source and Tween 80 (Biswas et al., 1991 *in* Callewaert & De Vyust, 2000; Keren et al., 2004 *in* Delgado et al., 2007; Mandal et al., 2008; Anthony et al., 2009; Biscola et al., 2013). Maximum production of bacteriocin ST664BZ was 12,80AU/ml after 23 h and production remainedstable to the end of growth (Todorov & Dicks, 2006).

The objectives of this work were to evaluate the effects of the medium components on cell growth, optimum condition and optimum medium composition for bacteriocin production.

2. Material and Methods

2.1. Bacterial Strains and Media

The bacteriocin producing strain *Lactococcus lactis* subsp. *lactis* CN1.10a was isolated from rusip sampel, fermented fish products of Bangkanese origin. *Staphylococcus aureus*, *Listeria monocytogenes* and *Lactobacillus plantarum* was used as indicator organism in the bioactivity of bacteriocin. Bacterial stocks were kept chilled (-4 °C) in MRS medium. This bacteria were inoculated in 10 ml CM lactose/ CM medium(1% lactose, 0.45% soybean peptone, 1% yeast extract, 2.84% KH_2PO_4, 0.2% NaCl and 0.02% $MgSO_4.7H_2O$) medium (Li et al., 2002) and incubated during 48 hours at 35 °C, propagated three times before experiment.

MRS broth was used as a medium for optimization of pH and temperature for bacteriocin production. CM medium contained different carbohydrate source

(glucose, sucrose and lactose) was optimized to obtain optimum medium for bacteriocin production. CM medium was also used as a culture medium for *Lactococcus lactis* ssp. *lactis CN1.10a*, a bacteriocin-producing strain.There was two initial pH media. The one was adjusted to pH 6 by adding 1 N NaOH and the other was not. *Listeria monocytogenes* was used as the indicator organism in bacteriocin assay and it was grown on medium TSB (Tryptic soy broth), CM medium and TSB medium were autoclaved at 121 °C for 15 menit, respectively.

2.2. Cultivation

The cells of *L. lactis* ssp. *lactis* CN1.10a were pregrown in 50 ml CM medium overnight at 35 °C, and then 10 ml of the culture was added aseptically to 500 ml CM medium for the optimization in 1 L bottle with a rubber plug. The culture was shaken at 170 rpm and (28±2) °C aerobically. Samples were taken from the bottle with sterile pipet every three hours during incubation time (48 hour).

2.3. Bacteriocin Activity

The agar well diffusion method (Tagg & Mcgiven, 1971; Ogunbanwo et al., 2003) was used to detect the antibacterial spectrum of crude bacteriocin from *L. lactis* ssp. *lactis* CN1.10a. Each of 100 µl indicator strain (*Staphylococcus aureus, Listeria monocytogenes* and *Lactobacillus plantarum*) was inoculated into 15 ml Mueller Hinton Agar (0,5 McFrland), and wells (6 mm diameter) were punched in the plate. Each wells was filled with 30 µl of crude bacteriocin *L. lactis* CN1.10a, *L. lactis* UGM and *Pediococcus acidilactici* F-11.The plates were incubated at 35 °C for 24 h. The diameter of inhibition zones (mm) around the wells was measured. This procedure was repeated three times. The antimicrobial activity recorded as positive if a transparent halo zone was observed around the well. The indicator strains were grown in MRS broth for *L. plantarum* and BHI broth for *L. monocytogenes* and *S. aureus* and incubated at 37 ºC for 24 h. Antimicrobial activity was expressed as arbitrary unit (AU) per ml. Using this method, one AU was defined as the area of inhibition zone per volume of bacteriocin sample (mm^2/ml) (Usmiati & Marwati, 2007).

Bacteriocin activity (mm^2/ml) = AU/ml

$$= \frac{Lz - Ls}{V}$$

Note:

Lz = The area of transparent zone (mm^2)

Ls = The area of well (mm^2)

V = Volume of sample (ml)

2.4. Optimum Temperature for Bacteriocin Production

Ten (10) ml of active culture of the strain *L. lactis* ssp. *lactis* CN1.10a was incubated at 30, 35, 37 °C for 24 hours on MRS medium. The culture was sentrifuged at 4 °C, 9.632 xg for 20 minutes; and the supernatant obtained was heated at 80 °C for 15 minutes. Bacteriocin activities of the supernatants of the cultures were calculated as described above.

2.5. Optimum pH for Bacteriocin Production

Samples of MRS broth were prepared by adjusting pH to 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 with 0.1 N or 1 N HCl and 0.1 N or 1 N NaOH before sterilization. The tubes containing 10 ml of pH adjusted MRS broth were inoculated with 100 µl of active culture of the strain *L. lactis* ssp. *lactis* CN1.10a and incubated at 37 °C for 24 h. The culture was sentrifuged at 4 °C, 9.632 xg for 20 minutes, and the supernatant which was obtained was heated at 80 °C for 15 minutes. Bacteriocin activities of the supernatants of the cultures were calculated as described above.

2.6. Optimization of Media for Bacteriocin Production

Each of 50 ml of active culture of the strain *L. lactis* ssp. *lactis* CN1.10a in CM lactose, CM glucose and CM sucrose was incubated at 35 °C for 24 hours. After that, the culture was sentrifuged at 4°C, 9.632 xg for 20 minutes. The supernatant which was obtained was heated at 80 °C for 15 minutes. Antibacterial activity of bacteriocin was assayed to some tested bacteria (*L. plantarum, L. monocytogenes,* and *Staphylococcus aureus)* using agar well diffusion method (Ogunbanwo et al., 2003; Udhayashree et al., 2012).This procedure was repeated three times.

2.7. The Growth Curve and Bacteriocin Production During Growth

To evaluate the growth of the bacteria in CM broth pH 5 and temperature 35 °C, a total plate count method was used (BSN, 2006). Total bacterial analysis of culture was determined at 0, 3rd, 6th, 9th, 12th, 15th, 18th, 21th, 24th, 27th, 30th, 33th, 36th, 39th, 42th, 45th, and 48th hour after incubation. For determining bacteriocin production curve, the strain was inoculated into CM broth (with and without KH_2PO_4) and incubated at 35 °C for 48 h. The samples were aseptically withdrawn, in duplicates, from the culture vessel at 3-h intervals throughout the incubation period. The samples was centrifuged at 4 °C, 9.632 xg for 20 minutes. The free cell supernatant which was obtained

after heated at 80 °C for 15 minutes was crude bacteriocin.The bacteriocin activities of each sample were calculated and the results were presented along with the growth curve (Van Reenen et al., 1998 *in* Altuntas et al., 2010).

2.8. Stability of Bacteriocin During Storage

Crude bacteriocin was stored at –20, 4, and 37 ºC. The reason of bacteriocin storage at 37ºC was to determine whether the activity of bacteriocin remain stable at high temperature. After 30 days, samples were taken from the stored material to determine bacteriocin activity (Ogunbanwo et al., 2003). *L. monocytogenes* was used as the indicator organism in bacteriocin activity assays.

3. Results and Discussions

3.1. Optimum Temperature of Bacteriocin Production

Using the MRS medium, based on Figure 1, the optimal growth conditions which supported maximum bacteriocin production was when it was grown at 35 ºC for 24 hours. Bacteriocin at this condition had high activity of 8,500 AU/ml.

3.2. Optimum pH for Bacteriocin Production

Temperature and pH as well as nutrient availability play a crucial role in bacteriocin production (Lejeune et al. *in* De Vyust & Leroy, 2007). Optimal bacteriocin production usually requires complex media and well-controlled physical conditions, such as temperature and pH (Anthony et al., 2009; Biscola et al., 2013).

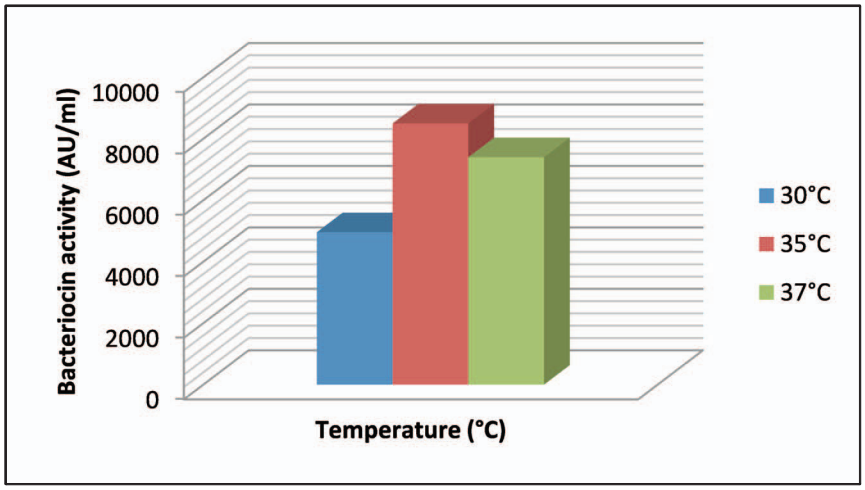


Figure 1. Optimum temperature of bacteriocin productionin MRS medium.

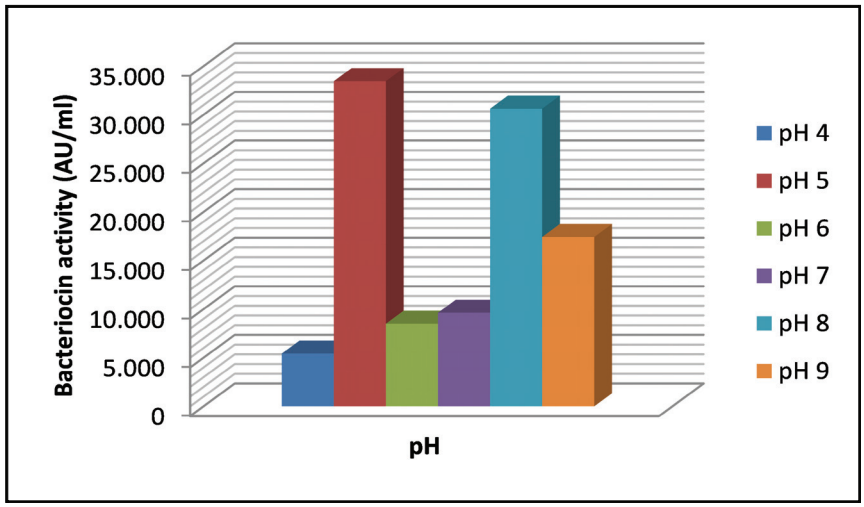


Figure 2. Optimum pH for bacteriocin production in MRS medium.

Using MRS medium and optimum temperature for bacteriocin as above result, bacteriocin production of *L. lactis* ssp. *lactis* CN1.10a was influenced by pH (Figure 2).

The pH suitability for better production of bacteriocin at different pH condition, using MRS medium was in the range of pH 4-9, with optimum pH at 5 (Figure 2). Bacteriocin at this condition had an activity of 33,456 AU/ml. Meanwhile, the activity of bacteriocin on the other pH was lower than others. Mandal et al. (2008) reported pH condition for better production of bacteriocin by *Pediococcus acidilactici* LAB 5 was in the range of pH 5-7, with optimum pH at 6.6. In the contrary, Anthony et al. (2009) reported that alkaline pH (8.0) and high temperature (43 ºC) favoured the production of antibacterial peptide by *B. licheniformis* AnBa9.

Based on optimization using MRS medium, it was showed that optimum bacteriocin production was at temperature 35 °C and pH 5. Therefore for further study, we used temperature 35 °C and pH 5 to optimize of production medium of bacteriocin.

3.3. Optimization of Media for Bacteriocin Production

Based on this research, MRS broth is one of good medium for improving cell growth of *L. lactis* ssp*. lactis* CN1.10a, but the bacteria grown at this medium did not always produce bacteriocin or stable bacteriocin. *Pediococcus acidilactici* LAB 5 failed to produce bacteriocin significantly in MRS medium (Mandal et al., 2008). Therefore, on this work, we used CM medium; and the effects of carbon source (lactose, glucose, and sucrose) in CM medium were evaluated to obtain optimal medium composition for attaining a higher bacteriocin activity (Table 1, 2, 3). CM medium was optimum medium for bacteriocin production by *L. lactis* ATCC 11454 (Li et al., 2002).

Lactococcus lactis ssp. *lactis* CN1.10a could produce bacteriocin on CM medium with lactose and sucrose as carbohydrate source, but the one that grown on CM medium with glucose could not produce bacteriocin activity. Meanwhile, the activity of bacteriocin on CM sucrose at pH 6 was higher than CM lactose (Table 2, 3).The reason for *L. lactis* ssp. *lactis* CN1.10a could produce the highest bacteriocin activity on sucrose-CM medium was most probably corresponded with sugar consumption. Sucrose was consumed faster than lactose or glucose when present as the sole carbohydrate source (Neysens et al., (2003).

3.4. Cell Growth of Bacteriocin Producer and Bacteriocin Production

Bacteriocin production on intermediate scale 2 L was conducted by using shaker at room temperature (28±2) °C for 24 hour. Figure 1 showed cell growth curve of *L. lactis* ssp*.* CN1.10*a lactis* on CM medium, medium added with KH_2PO_4 and medium without KH_2PO_4. The cell growth of bacteriocin producer on CM medium resulted a higher total bacteria.The concentration of KH_2PO_4 strongly affected the cell growth. The function of KH_2PO_4 maybe as buffer to stabilize pH in medium. De Vuyst and Vandamme (1993) *in* Li et al., (2002) reported that potassium dihydrogen phosphate was able to improve cell growth

Table 1. Activity of bacteriocin (inhibition zone) produced by *L. lactis* ssp. *lactis* CN1.10a cultivated on glucose CM medium

Isolate	pH condition	Inhibition zone (mm)		
		SA	LM	LP
L. lactis ssp. *lactis* CN1.10a	Adjusted pH 6	0	0	0
L. lactis ssp. *lactis* CN1.10a	Not adjusted	0	0	0
L. lactis (UGM)	Adjusted pH 6	7.83	8.67	7.50
L. lactis (UGM)	Not adjusted	15.67	14.33	11.00
P. acidilactici	Adjusted pH 6	0	0	0
P. acidilactici	Not adjusted	0	0	0

Note : SA = *Staphylococcus aureus*
LM = *Listeria monocytogenes*
LP = *Lactobacillus plantarum*

Table 2. Activity of bacteriocin (inhibition zone) produced by *L. lactis* ssp. *lactis* CN1.10a cultivated on lactose-CM medium

Isolate	pH condition	Inhibition zone (mm)		
		SA	LM	LP
L. lactis ssp. *lactis* CN1.10a	Adjusted pH 6	8.17	7.33	7.50
L. lactis ssp. *lactis* CN1.10a	Not adjusted	6.83	6.83	6.83
L. lactis (UGM)	Adjusted pH 6	8.33	6.83	8.17
L. lactis (UGM)	Not adjusted	7.00	7.17	6.83
P. acidilactici	Adjusted pH 6	0	0	0
P. acidilactici	Not adjusted	0	0	0

Note : SA = *Staphylococcus aureus*
LM = *Listeria monocytogenes*
LP = *Lactobacillus plantarum*

Table 3. Activity of bacteriocin (inhibition zone) produced by *L. lactis* ssp. *lactis* CN1.10a cultivated on sucrose-CM medium

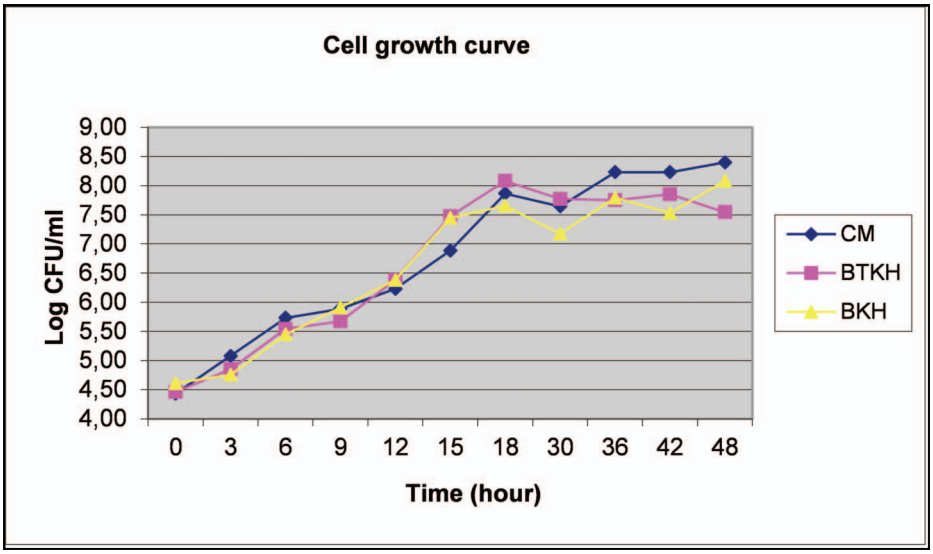
Isolate	pH condition	Inhibition zone (mm)		
		SA	LM	LP
L. lactis ssp. *lactis* CN1.10a	Adjusted pH 6	21.00	19.50	12.33
L. lactis ssp. *lactis* CN1.10a	Not adjusted	19.67	19.50	12.33
L. lactis (UGM)	Adjusted pH 6	13.50	12.50	9.00
L. lactis (UGM)	Not adjusted	12.50	12.33	9.00
P. acidilactici	Adjusted pH 6	0	0	0
P. acidilactici	Not adjusted	0	0	0

Note : SA = *Staphylococcus aureus*
LM = *Listeria monocytogenes*
LP = *Lactobacillus plantarum*

and nisin synthesis. Li et al. (2002) reported the concentration of KH_2PO_4 strongly affected nisin production. High level of KH_2PO_4 concentration (15 g/L) allowed the strain to produce a greater nisin concentration than low level of KH_2PO_4 concentration (5 g/L). Potassium dihydrogen phosphate, yeast extract, sucrose, and soybean peptone were found to be significant at the probability level of 99 or 95%.

For cell growth of *L. lactis* ssp. *lactis* CN1.10a, sucrose, soybean peptone, yeast extract, and potassium dihydrogen phosphate were positively significant factors, but NaCl and $MgSO_4.7H_2O$ were not found to be significant (Li et al., 2002). Therefore, the different components in medium had different effects on cell growth. According to the research, the highest bacteriocin production at intermediate scale was attained at 24th hour at room temperature (Figure 1).

Generally, bacteriocin activity was detected early in the exponential-growth phase and was produced continuously during this phase. The highest activity was reached at the end of the exponential phase, and corresponded with the maximal total cell. The activity of bacteriocin decreased rapidly when cells entered the stationary phase. Therefore, bacteriocin production follows primary metabolite. Similar metabolite kinetics were previously reported for the bacteriocin amylovorin I471, a bacteriocin produced by *Lactobacilli* and *L.*



Note: CM (culture medium); BKTH (Mix medium without KH_2PO_4); BKH (Mix medium with KH_2PO_4).

Figure 3. Cell growth curve of *L. lactis* ssp. *lactis* on different media.

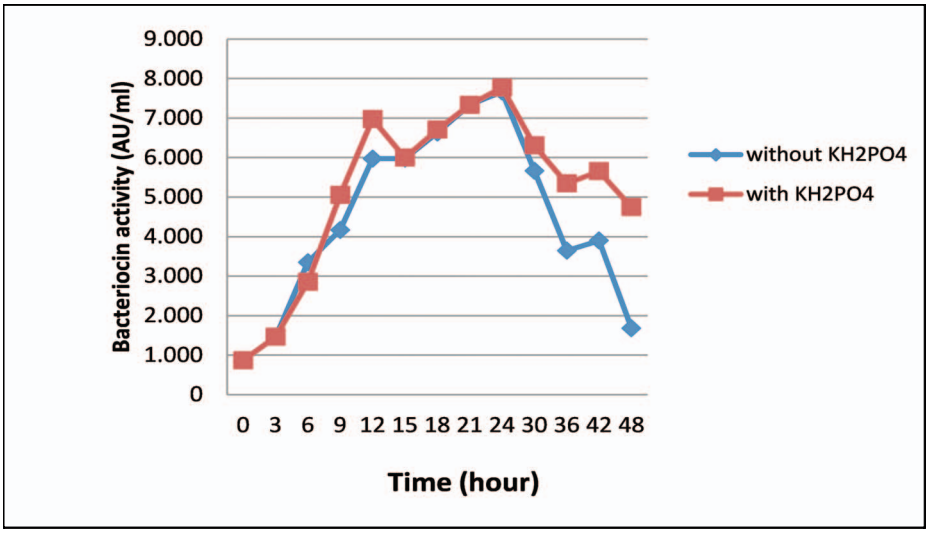


Figure 4. Bacteriocin production curve on different media.

pentosus 31-1 (Neysens et al., 2004; Avonts et al., 2004; Liu et al., 2008). De Vuyst et al. In De Vyust and Leroy (2007) also reported that bacteriocin production is a growth–dependent physiology trait and hence follows primary metabolite. Generally bacteriocin production occurs only in the active growth phase (Parente et al., 1994 *in* Callewaert & Vuyst, 2000). Decreasing in activity levels after logarithmic growth has been observed for lactacin B, and helveticin J (Barefoot et al., 1984; Joeger et al., 1986). In many of these cases, loss of activity has been ascribed to proteolytic degradation, protein aggregation, adsorption to cell surfaces and feedback regulation (Parente et al., 1994 *in* Todorov & Dicks, 2006).

Based on the result obtained, sucrose-CM medium with KH_2PO_4, has been choosen and used it as bacteriocin production medium. Decreasing of total cell slowly on sucrose-CM medium with a present of KH_2PO_4 showed that the function of KH_2PO_4 was a buffer to mantain stability of pH in medium.Todorov and Dicks (2004) claimed bacteriocin production was dependent on pH, nutrients source and temperature. Nisin production is affected by several cultural factors such as producer strain, nutrient composition of media,

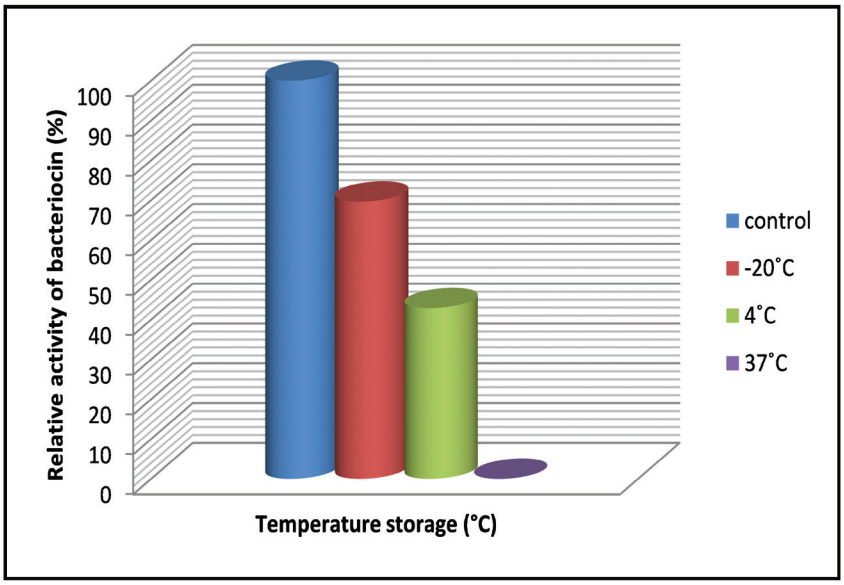


Figure 5. Effect of temperature storage on relative activity of bacteriocin.

pH, temperature, agitation and aeration (Chandrapati & O'Sullivan, 1998 *in* Jozala et al., 2011). In addition, the concentration of KH_2PO_4 strongly affected bacteriocin production.The same with this research, Li et al.(2002) reported that the concentration of KH_2PO_4 strongly affected nisin production. The high level of KH_2PO_4 concentration (15 g/L) allowed the strain to produce a greater nisin concentration than the low level of KH_2PO_4 concentration (5 g/L). Also, nisin production was affected by the level of soybean peptone, decreasing with the elevated concentration of soybean peptone. The other components in the media did not significantly influence nisin production. For bacteriocin production, carbohydrate source from sucrose was able to result in high biomassa and bacteriocin production. Whereas it was different with De Vuyst and Vandamme (1992) in that a high concentration of sucrose was able to result in high biomass, but did not increase the bacteriocin production.Therefore, it was possible to obtain an optimal medium by optimizing the components of medium.

Both CM medium with KH_2PO_4or without KH_2PO_4 produced the highest bacteriocin activity of *L. Lactis* ssp. *lactis* CN1.10a at 24th hour. However, the activity of bacteriocin on CM medium without KH_2PO_4 decreased faster than with KH_2PO_4.

3.5. Stability of Bacteriocin During Storage

The stability test of bacteriocin was conducted by storing the bacteriocin at -20 °C, 4 °C, and 37 °C for 30 days (Figure 5).

Bacteriocin produced by *L. lactis* ssp. *lactis* CN1.10a remained stable (69.4% activity) after storage for 30 days at -20 °C,but not detectable after storage for 30 days at 37 °C, indicating the cold temperature may be the most appropriate preservation technique.In addition to the high anti-Listeria activity showed by this bacteriocin and its stability on low temperature, it showed that it was potential use as a food preservative, especially for lightly preserved seafood where *Listeria* could be a serious problem for its capacity to tolerate refrigerated conditions. Similar activity displayed by pentocin 31-1 produced by *L. pentosus* 31-1 isolated from the traditional China fermented Xuan-Wei Ham were previously reported (Liu et al., 2008).Therefore, more research are needed to study application of this bacteriocin in seafood stored at refrigerated conditions.

4. Conclusion

Bacteriocin was produced optimally at temperature 35 °C and pH 5. Production of bacteriocin on CM medium was better than those produced in MRS, with lactose and sucrose as carbohydrate source. The optimum condition for bacteriocin production using sucrose-CM medium was (28 ±2)°C, pH 6 with added KH_2PO_4. The best storage temperature for bacteriocin was at -20 °C with a relative activity of 69.4%.

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