PRODUCTION AND CHARACTERIZATION OF BACTERIOCIN PRODUCED BY LACTIC ACID BACTERIA ISOLATED FROM RUSIP

Produksi dan Karakterisasi Bakteriosin yang Dihasilkan oleh Bakteri Asam Laktat yang Diisolasi dari Rusip

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ABSTRACT

Research was conducted to produce and characterize bacteriocin produced by lactic acid bacteria (LAB) isolated from rusip, a traditional Bangkanese fermented fish product. Experiment was started by isolation of lactic acid bacteria from rusip, followed by screening to obtain the best isolate which has the highest bacterial inhibition activity. The selected isolate was then identified and used to produce crude bacteriocin. The crude bacteriocin was characterized through its stability in high temperature and proteolytic enzymes, inhibitory spectrum, pH sensitivity and effect of surfactants. The result showed that CN1.10a isolate which was identified as *Lactococcus lactis* subsp *lactis* has the highest bacterial inhibition activities; therefore it was selected to produce crude bacteriocin. The bacteriocin produced was heat stable, sensitive to proteolytic enzymes *i.e.* proteinase-K and papain but not to RNase. It inhibited *Escherichia coli, Listeria monocytogenes* and *Lactobacillus plantarum.* It stable at pH 2.0 to 6.0. Among surfactans used sodium dodecyl sulphate (SDS), lauryl sarcosine and EDTA were able to stimulate bacteriocin production, while the production were strongly inhibited by Tween 20, Tween 80, Triton X-100 and urea. Based on the above characteristic, the bacteriocin was suitable to be used as a preservative of food which has to be processed at high temperature.

Keywords: heat stable, bacteriocin, *Lactococcus lactis*, rusip

ABSTRAK

Penelitian ini dilakukan untuk memproduksi dan mengkarakterisasi bakteriosin yang dihasilkan oleh bakteri asam laktat hasil isolasi dari rusip, produk ikan fermentasi tradisional Bangka. Penelitian dimulai dari isolasi bakteri asam laktat dari rusip, dilanjutkan dengan skrining untuk memperoleh isolat terbaik yang menghasilkan aktivitas penghambatan bakteri tertinggi. Isolat terpilih selanjutnya diidentifikasi dan digunakan untuk memproduksi bakteriosin kasar. Bakteriosin kasar dikarakterisasi melalui pengujian stabilitasnya terhadap suhu tinggi dan enzim proteolitik, spektrum penghambatan, sensitivitas pH dan pengaruh surfaktan. Hasil penelitian menunjukkan bahwa isolat CN1.10a yang kemudian diidentifikasi sebagai *Lactococcus lactis* subsp *lactis* merupakan satu-satunya isolat yang menghasilkan bakteriosin dengan aktivitas penghambatan bakteri tertinggi, sensitif terhadap enzim proteolitik seperti proteinase-K dan papain namun tidak terhadap RNase. Bakteriosin *L. lactis* subsp *lactis* tersebut dapat menghambat *Esherichia coli, Listeria monocytogenes* dan *Lactobacillus plantarum,* dan stabil pada pH 2-6. Di antara surfaktan yang digunakan, sodium dodecyl sulphate (SDS), lauryl sarcosine dan EDTA mampu menstimulasi produksi bakteriosin sedangkan Tween 20, Tween 80, Triton X-100 dan urea sangat kuat menghambat produksi bakteriosin. Berdasarkan karakteristik tersebut maka bakteriosin sesuai untuk digunakan sebagai pengawet pangan yang harus diproses pada suhu tinggi.

Kata Kunci: tahan panas, bakteriosin, *Lactococcus lactis,* rusip

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INTRODUCTION

Bacteriocins are defined as proteins or protein complexes antagonistic to bacteria genetically closely related to the producer organisms (De Vuyst & Vandamme, 1994; Klaenhammer, 1988). They are ribosomally-synthesized peptides or proteins with antimicrobial activity, produced by different groups of bacteria (Galvez et al., 2007).

Many lactic acid bacteria produce bacteriocins with rather broad spectra of inhibition (Galvez et al., 2007). Bacteriocins from lactic acid bacteria (LAB) are more interested than other resources, because

most of LAB are related to fermented foods. Many novel bacteriocin-producing LAB from fermented food have been isolated, produced and characterized. For example, bacteriocin produced by LAB isolated from Sucuk, Turkish fermented meat, vacuum-packed meat product, Pentocin 31.1, bacteriocin produced by *Lactobacillus pentosus* 31-1 isolated from Xuan-Wei Ham, a traditional China fermented meat product, China fermented cabbage, artisanal dry sausages, and bacteriocin produced by *Lactococcus lactis* strain isolated from charqui, a Brazilian fermented, salted and dried meat product (Hilmi CE on *& GoE kalp*, 2000; Budde et al., 2003; Gao et al., 2010; Abrams et al., 2011; Castro et al., 2011; Biscola et al., 2013). The other bacteriocins were also produced by strains *Lactobacillus plantarum*, *L. brevis*, *Pediococcus acidilactici*, *L. pentosus* and *L. lactis* (Osmanagaoglu et al., 1997; Ogunbanwo et al., 2003; Todorov & Dicks, 2005; Omar et al., 2006; Liu et al., 2008; Alegria et al., 2010; Rajaram et al., 2010). Although many bacteriocins are produced by those species, only nisin has GRAS (generally recognized as safe) status and remains the only commercially important bacteriocin being approved as a food preservative (Cleveland et al., 2001). The bacteriocin is commonly used as natural preservatives in meat products, processed cheese and canned vegetables (Abrams et al., 2011; Albano et al., 2008 *in* Karthikeyan *& Santhosh*, 2009; De Arauza et al., 2009). The FAO/WHO Codex Committee on milk and milk products has accepted nisin as a food additive for processed cheese at a concentration of 12.5 mg pure nisin per kilogram product (Ross et al., 2002 *in* De Arauz et al., 2009).

Indonesia has many kinds of fermented fish products, one of them is rusip. Rusip composed of fish, salt, brown sugar and soaked water from dried rice but sometimes without rice. It produced by spontaneous fermentation during 7-14 days anaerobically (Yuliana, 2007). Rusip mainly produced in Bangka Belitung Provinces and widely used as a condiment or mixed with chili 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). *Staphylococcus aureus* sometimes has also been found in rusip (Kusmarwati et al., 2001).

Studies on the exploration of bacteriocin produced by Indonesian fermented food are still limited. Some of the results that have been reported are bacteriocin from meat product and milk-associated lactic acid bacteria (Rahayu et al., 1999; Kusmiati & Malik, 2002; Nugroho & Rahayu, 2003; Usmiati & Marwati, 2007; Syahniar, 2009). This present work was aimed to screen lactic acid bacteria from rusip, produce and characterize the bacteriocin produced by the selected bacteria isolate which has potential to produce a high stable activity of bacteriocin.

MATERIALS AND METHODS

Sample Collection

Rusip samples were collected between the year of 2011 and 2012 from Bangka, Belitung and Palembang regions, Indonesia. They were obtained from different producers which applied different methods of production. The samples were stored at 4ºC in Microbiology Laboratory at the Research and Development Center for Marine and Fisheries Product Processing and Biotechnology (RDCMFPPB), Jakarta, where the experiments were conducted.

Isolation and Screening of Lactic Acid Bacteria

Lactic acid bacteria were isolated from rusip, using dilution method followed by pour plate method on MRS (deMan Rogosa and Sharpe) agar contained 1 % (w/v) $CaCO_3$ (Rahayu & Margino, 1997). Twenty-five grams of sample were added to 225 ml of sterile butterfield phosphate buffer (BPF) and shaken for 1 min. An appropriate decimal dilutions were prepared in 9 ml of BPF, 1 ml of each dilution was poured into a sterile petri dish followed by MRS agar media. Cultures were then incubated at 37ºC for 48 h. Bacterial colonies that exhibited clear zone on MRS agar plate were individually picked and streaked on MRS agar containing 1% (w/v) $CaCO_3$. This procedure was repeated to obtain pure isolates. Each of the isolate was first Gram stained and examined microscopically. Gram positive isolates were then tested for catalase, and isolates which showed catalase negative were identified as lactic acid bacteria. Catalase test was conducted by placing a drop of 3% hydrogen peroxide solution on the isolate. Immediate formation of bubbles indicated the presence of catalase in the cells (catalase positive). Those isolates which have catalase negative were then maintained in MRS broth containing 20% glycerol and 10% skim milk at –20ºC for 3 months. The isolates were subcultured onto MRS agar slant, incubated at 37ºC for 48 hours before used for the next steps of the experiment.

Screening of Lactic Acid Bacteria for Antimicrobial Activity

Escherichia coli ATCC 25922 and *Listeria monocytogenes* ATCC 19115 which obtained from Oxoid, America; *Lactobacillus plantarum* and *Lactobacillus fermentum* were obtained from culture

collection of the RDCMFPPB, Jakarta. Those microorganisms were used as tested bacteria in antimicrobial activity assay. Antimicrobial activity against tested bacteria was determined by agar spotted on the lawn method (Abrams et al., 2011) under aerobic condition. Muller Hinton Agar (MHA) plate were inoculated with 100µl of each tested bacteria after growing them in nutrient broth for *E. coli,* brain hearth infusion (BHI) for *Listeria monocytogenes* and MRS broth for *L. plantarum*. MHA plate contained each of the tested bacteria were then spotted on lawns of 10 µl LAB culture in MRS broth and incubated overnight at 37ºC. The isolate recorded as positive to have antimicrobial activity if a transparent halo zone is observed around the spot.

Production of Crude Bacteriocin

Production of crude bacteriocin followed the method of Ogunbanwo et al. (2003).The isolates were grown in MRS broth at 37ºC for 48 h. After incubation, the broth was centrifuged at 10.000 x g for 15 min at 4ºC. The cell free supernatant was adjusted to pH 6.0 by adding 1M NaOH to exclude the antimicrobial effect of organic acid, followed by filtration of the supernatant through 0.2 µm pore-size cellulose acetate filter and heated at 100ºC for 15 min. The cell free supernatant was regarded as crude bacteriocin.

Bacteriocin Assay

Crude bacteriocin produced were tested against the same tested bacteria using the same method as antimicrobial test for LAB. Bacteriocin assay was determined by agar spotted on the lawn method under aerobic condition (Abrams et al., 2011). The inhibitory activity was tested on MHA. MHA plate contained each of tested bacteria were then spotted on lawns of 10 µl LAB culture in MRS broth and incubated overnight at 37ºC. The crude bacteriocin is recorded to have antimicrobial activity if a transparent halo zone is observed around the spot. The bacteriocin which showed the highest antimicrobial activity was selected for sensitivity test againts proteolytic enzyme.

Sensitivity of Bacteriocin to Proteolytic Enzymes

Crude bacteriocin was assayed to various proteolytic enzymes *i.e*. proteinase-K in 0.05 M Tris hydrochloride (pH 8.0); papain in 1 N NaOH (pH 6.5) and ribonuclease-A in 0.05 M Tris hydrochloride (pH 8.0). About 200 µl aliquot of crude bacteriocin was added with 2 mg/ml of each enzyme and incubated for 60 min at 37ºC. Bacteriocin without the addition of enzyme was used as a control. All tubes were assayed for antimicrobial activity using agar well diffusion method (Ogunbanwo et al., 2003; Udhayashree et al., 2012). MHA plate were inoculated with 100µl of *L. plantarum* which was previously grown in MRS broth at 37ºC for 24 h. Wells (6 mm) were prepared in MHA plate and 10 µl of crude bacteriocin was added into each well. Plates were incubated at 37ºC for 24 h. Only those cell free supernatants which are sensitive to proteinase-K and papain were considered as real bacteriocin and chosen for following steps. The cell free supernatant that was considered sensitive to proteolytic enzyme, *i.e* no transparent halo zone surrounded the wells, were recorded as negative result.

CHARACTERIZATION OF BACTERIOCIN

Effect of pH

About 5 ml aliquot of crude bacteriocin was put in test tube and the pH was adjusted to 2, 4, 6, 8, 10, and 12 individually using either diluted NaOH or HCl. After the samples incubated for 4 h at room temperature, the antimicrobial activity was assayed by using agar well diffusion method. 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) = 1 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)

Heat Stability

About 2 ml aliquot of crude bacteriocin in tube was heated at 80ºC and 115ºC for 20 min and 121ºC for 15 min. The heat treated bacteriocins were then assayed for antimicrobial activity using agar well diffusion method as was described on point sensitivity of bacteriocin to proteolytic enzymes. Crude bacteriocin is considered as heat stable when there is transparent halo zone surrounded the wells (recorded as positive). Quantification of bacteriocin activity (AU/ml) was described above.

Effect of Surfactans

The surfactants tested were Triton X-100, Tween 20, Tween 80 (non ionic), sodium dodecyl sulphate/ SDS (anionic), EDTA, Urea and N-Laurylsarcosine (dipolar ionic). The surfactants were added to each aliquot of crude bacteriocin at the concentration of 0,1 ml or 0,01 g of surfactant per ml crude bacteriocin. Aliquot of the crude bacteriocin without surfactant was

used as a control. After all samples and control were incubated at 37ºC for 60 min, the bacteriocin activity was assayed as described above.

Antimicrobial Activity of Bacteriocin

Antibacterial activity of bacteriocin was assayed to some tested bacteria (*L. plantarum, L. monocytogenes, S. typhimurium* and *E. coli*) using agar well diffusion method as described on point sensitivity of bacteriocin to proteolytic enzymes. MHA plate were inoculated with each 100µl of the tested bacteria after growing them in MRS broth for *L. plantarum* and nutrient broth for *L. monocytogenes, S. thypimurium* and *E. coli* at 37ºC for 24 h. Wells (6 mm) were prepared in MHA plate and 10 µl of crude bacteriocin was added into each well. Plates were incubated at 37ºC for 24 h. The antimicrobial activity recorded as positive if a transparent halo zone was observed around the well. Quantification of bacteriocin activity (AU/ml) was described above.

Identification of Selected Isolate

The selected isolate was identified using API 50 CH strips and API 50 CHL medium (API systems, Biomerieux Sa, French).

RESULTS AND DISCUSSIONS

Isolation of Lactic Acid Bacteria from Rusip

Twenty seven isolates of the lactic acid bacteria were isolated from rusip originated from Palembang,

Table 1. The morphology and Gram stain of lactic acid bacteria isolated from rusip

No	Origin of Rusip	Isolate	Cell Morphology	Gram Stain
1	Palembang	SW1.12	rod	+
2	Palembang	SW1.1b	rod	+
3	Palembang	SW2.1bI	rod	+
4	Palembang	SW2.1c	rod	+
5	Palembang	SW2.12d	rod	+
6	Palembang	SW2.11	rod	+
7	Palembang	SW2.1b II	rod	+
8	Palembang	SW2.12b	rod	+
9	Palembang	SW2.1dI	rod	+
10	Bangka	CN1.10a	rod	+
11	Bangka	CN11a	rod	+
12	Bangka	CN1.8	rod	+
13	Bangka	CN1.7b	rod	+
14	Belitung	BL2.12	coccus	+
15	Belitung	BL1.2	coccus	+
16	Belitung	BL1.9b	coccus	+
17	Belitung	BL2.4	coccus	+
18	Belitung	BL2.13	coccus	+
19	Belitung	BL1.4	coccus	+
20	Belitung	BL1.11	coccus	+
21	Belitung	BL2.10a	coccus	+
22	Belitung	BL2.16a	coccus	+
23	Belitung	BL1.6	coccus	+
24	Belitung	BL2.16b	coccus	+
25	Belitung	BL1.10	Coccus	+
26	Belitung	BL2.18	Coccus	+
27	Belitung	BL2.6	Coccus	+

Bangka and Belitung (Table 1). Gram stained analysis showed 13 strains as Gram-positive rod and 14 strains as Gram-positive coccus.

Screening of Lactic Acid Bacteria for Anti-microbial Activity

Antimicrobial activity against Gram negative bacteria is an unusual phenomenon and has rarely been reported (Abrams et al., 2011). However 17 isolates in this experiment exhibited activity against *E. coli* during the first screening by the development of clear zones (inhibition zone) around the spot. Roth & Keenan (1971) *in* Hwanhlem et al., 2011 reported that lactic acid was able to cause sub-lethal injury to *E. coli*, and similar properties have also been assigned to acetic acid (Przybylski & Witter, 1979 *in* Hwanhlem et al., 2011). Indirect evidence suggests that such injury involves disruption of the lipopolysaccharide (LPSP) layer. Out of 17 isolates, 7 strains were also active against *L. plantarum*, 10 isolates were active against *L. fermentum* and *5* isolates were only active against *L. plantarum* and *L. fermentum* (Table 2). Based on this first screening result, some isolates showed large spectrum of inhibition to Gram positive (*L. plantarum* and *L. fermentum)* and negative bacteria (*E. coli)*. The inhibition zone may be due to the competition for nutrients on the production of bacteriocin, lactic acid, diacetyl, bacteriocin or hydrogen peroxide during culture (Klaenhammer, 1988 *in* Liu et al., 2008; Abrams et al., 2011).

Production and Characterization of Bacteriocin

Crude bacteriocins produced by 19 isolates were obtained from the supernatant of all strains after grown in MRS media at 37ºC for 48 hours.

Among 19 supernatant from rusip, CN1.10a and SW2.11 supernatant showed a larger inhibition zone than others (Table 3). Hence, both CN1.10a and SW2.11 supernatants were tested to the sensitivity againts the proteolytic enzymes to confirm those supernatants as bacteriocin.

Bacteriocin is defined as proteins or protein complexes antagonistic to bacteria (De Vuyst & Vandamme, 1994). As shown in Table 4, the activity of CN1.10a bacteriocin was lost after being treated

Table 2. Result of antimicrobial activity of the lactic acid bacteria

No	Isolates	Inhibition zone (mm)		
		Escherichia coli ATCC 25922	*L. plantarum*	*L. fermentum*
1	SW1.12	5	0	6
2	SW1.1b	6	0	0
3	SW2.1bI	6	0	7
4	SW2.1c	6	0	0
5	SW2.12d	6	7	0
6	SW2.11	7	6	6
7	CN1.10a	7	0	9.5
8	SW2.1b II	6	0	0
9	SW2.12b	9	0	0
10	SW2.1dI	6	0	0
11	BL2.12	7	16	4
12	BL1.2	7	0	4
13	BL1.9b	6	0	0
14	BL2.4	6	0	0
15	BL2.13	0	26.5	7
16	BL1.4	6	7	6
17	BL1.11	6	0	8.5
18	BL2.10a	0	15	0
19	CN11a	5	7.5	2

Table 3. Results of antimicrobial activity of crude bacteriocin

Isolate	Inhibition Zone (mm)		Bacteriocin Activity (AU/ml)	
	E. coli	*L. plantarum*	*E. coli*	*L. plantarum*
SW1.12	7.83 ± 1.04	12.67 ± 0.58	1.265	6.226
SW1.1b	7.67 ± 0.76	10.33 ± 1.15	1141	3535
SW2.1bI	7.67 ± 0.29	9.67 ± 0.29	1141	2875
SW2.1c	8.50 ± 0.50	11.00 ± 1.00	1813	4250
SW2.12d	6.33 ± 2.89	11.33 ± 0.58	203	4618
SW2.11	10.00 ± 0.87	10.33 ± 1.53	3200	3535
CN1.10a	10.25 ± 2.47	11.67 ± 2.89	3453	5009
SW2.1b II	9.67 ± 0.29	9.67 ± 1.61	2875	2875
SW2.12b	9.00 ± 0.87	10.17 ± 1.04	2250	3371
SW2.1dI	8.00 ± 0.00	10.17 ± 1.89	1400	3371
BL2.12	6.83 ± 1.04	8.67 ± 0.58	532	1958
BL1.2	7.00 ± 0.50	8.67 ± 1.15	650	1958
BL1.9b	7.33 ± 0.58	10.50 ± 0.87	886	3713
BL2.4	7.67 ± 0.29	9.00 ± 1.00	1141	2250
BL2.13	8.50 ± 0.50	9.83 ± 2.02	1813	3031
BL1.4	6.83 ± 1.61	10.00 ± 0.00	532	3200
BL1.11	7.33 ± 1.15	10.17 ± 0.76	886	3371
BL2.10a	7.50 ± 0.50	10.67 ± 0.58	1013	3892
CN11a	8.50 ± 1.50	9.00 ± 0.00	1813	2250

with proteolytic enzymes proteinase-K and papain. This condition indicated that the antimicrobial compound of CN1.10a has similar characteristic as bacteriocin. The inhibitory mechanism as shown by CN1.10a is namely bacteriocinogenic (Osmanagaoglu *et al*., 1997). Todorov & Dicks (2005) reported complete inactivation or significant reduction in bacteriocin activity of cell free supernatant after treated with proteinase K, pronase and trypsin. Proteinase-K and pepsin were strongly inhibited the activity of bacteriocin, while α-amilase, DNase, RNase and lipase were unable to inhibit the activity (Rajaram et al., 2010). On the other hand, SW2.11 isolate showed high antimicrobial activity, its activity however was not lose after being treated with proteolytic enzymes. Therefore SW2.11 isolate was not considered as bacteriocin. The inhibitory mechanism of this strain may be be due to the substrate competition or acidification of the media (Abrams et al., 2011). Based on this result, only bacteriocin produced by CN1.10a was further characterized.

Several studies reported similar results. The activity of bacteriocin was completely inhibited by proteolytic enzyme (*i.e* papain, proteinase-K, trypsin, chymotrypsin) indicating that the bacteriocin is proteinaceous in nature, such as Pediocin FA (Osmanagaoglu et al., 1997), bacteriocin from *L. plantarum* F1 and *L. brevis* OG1 (Ogunbanwo *et al*., 2003), bacteriocin from *L. plantarum* ST194BZ (Todorov and Dicks., 2005), bacteriocin from *Leuconostoc mesenteroides* E131 (Xiraphi et al., 2008), bacteriocin bacPPK34 (Abrams et al., 2011), bacteriocin UN01 and bacteriocin from *L. fermentum* (Udhayashree et al., 2012).

Bacteriocin produced by CN1.10a isolate was active at pH 2-6 after incubation for 4 h (Table 5). The activity might be lost by proteolytic degradation, protein aggregation or instability of proteins (Aasen et al., 2000 *in* Todorov et al., 2011). Different observations have been previously reported where bacteriocin R1333 and ST16Pa remain stable after incubation (30ºC) at pH 2, 4, 6, 8, 10 and 12, while

Table 4. Effect of proteolytic enzyme on bacteriocin activity

Treatments	Concentration (mg/ml)	Bacteriocin activity*)	
		CN1.10a	SW2.11
Control (without enzyme)	-	+	+
Proteinase-K	0.1	-	+
Ribonuklease-A	0.1	+	+
Papain	0.1	-	+

*) *L. plantarum* was used as tested bacteria

Table 5. Effect of pH on bacteriocin activity

pH	Bacteriocin activity (AU/ml) *)
2	2.056
4	2.023
6	2.307
8	0
10	0

*) *L. plantarum* was used as tested bacteria

bacteriocin from *L. brevis* OG1 is stable at pH 2 to 8 (Ogunbanwo et al., 2003; Todorov et al., 2011).

As shown in Table 6, bacteriocin produced by CN1.10a had strong heat stability even after being autoclaved at 121ºC for 15 min. Based on this characteristic, bacteriocin from CN1.10a has a potential to be used as a preservative for high temperature processed food. It might offer another application in the processing and preservation of various foods (Gao et al., 2010) such as fermented fish sausage, salami, etc. Similar results were reported, *L. brevis* OG1 bacteriocin was very heat stable, the activity was not reduced after heating at 121ºC for 60 min, while bacteriocin from *L. plantarum* F1 exhibit full activity after heating at 121ºC for 10 min. Other research reported that bacteriocin ST16Pa remains stable after 2 h heating at 25, 30, 37, 45, 60, 80 or 100°C and no decrease in activity after heat treatment at 121°C for 20 min at pH 6.0 (Ogunbanwo et al., 2003; Todorov & Dicks, 2006; Todorov et al., 2011).

Figure 1 showed the effect of surfactant on bacteriocin activity. Among the surfactants, EDTA, SDS and laurylsarcosine stimulated CN1.10a bacteriocin activity, but not Triton X-100, Tween 20, Tween 80 and Urea. In the contrary, Todorov & Dicks (2006); Todorov et al. (2011) & Castro et al., (2011) reported that treatment with Tween 20, Tween 80, urea, Triton X-100 and Triton X-114 have no effect on the activity of bacteriocin R1333, *L. sakei* ACU and plantaricin C19. However, Tween 20 enhances bacteriocin activity.

In the present study, EDTA had effect on CN1.10a bacteriocin activity against *S. aureus*. EDTA is used in food product to prevent oxidation, it also has antimicrobial activity especially against Gram negative bacteria. EDTA has ability to disturb the highly structure of the outer membrane and allow access of hydrophobic molecules to the cytoplasmic membrane (Vaara, 1992 *in* Castro et al., 2011). Therefore, it can be assumed in this case, it might sensitizes *S. aureus* membrane. Because bacteriocin do not act equally against target species, many researchers have examined the affinity of bacteriocin to specific species and strains (Castro et al., 2011).

Meanwhile non-ionic surfactant Triton X-100, Tween 80 and Tween 20 had no effect on bacteriocin activity, although other research was reported that addition of non-ionic surfactants to various food constituents could induce production of bacteriocins. Tween-treated cultures also increased the supernatant activity relative to total activity, probably by deadsorption and disaggregation. However the effects were strain dependent (Reese & Maguire,1969 *in* Castro et al., 2011). In addition, the exposure of CN10a bacteriocin to surfactants resulted an increasing of

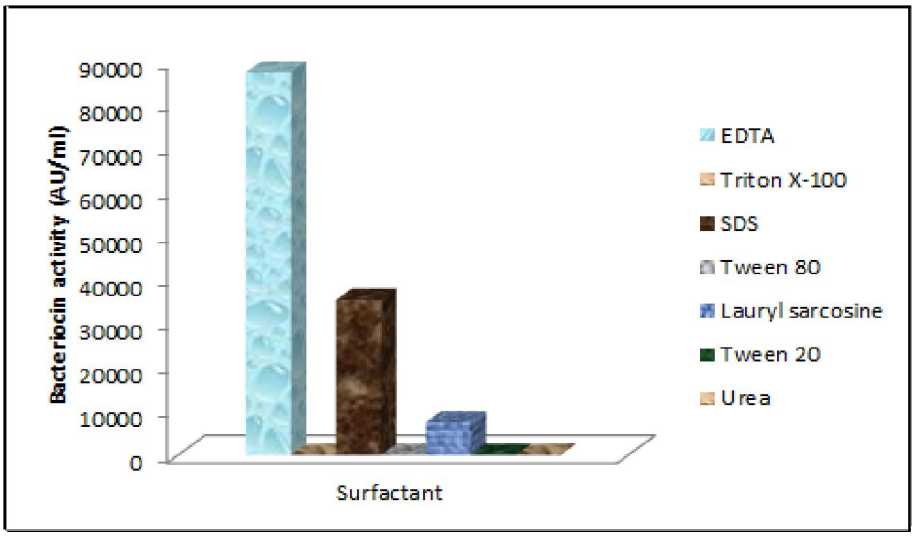

Figure 1. Effect of surfactant on the activity of CN1.10a bacteriocin to *S. aureus.*

Table 6. Effect of temperature on bacteriocin activity

Treatment	Bacteriocin activity (AU/ml)
80°C for 20 min	10.244
115°C for 20 min	8.605
121°C for 15 min	3.197

*) *L. plantarum* was used as tested bacteria

Table 7. Spectrum of antibacterial activity

Indicator strain	Inhibition zone (mm)	Bacteriocin activity (AU/ml)
L. plantarum	11.7	5.045
L. monocytogenes	9.0	2.25
S. thypimurium	0	0
E. coli	10.3	3.505

bacteriocin titre. This increase might be due to the effect of surfactant on the permeability of the cell membrane (Ogunbanwo et al., 2003).

Antibacterial activity of CN1.10a bacteriocin exhibited a small target activity (Table 7). It was able to inhibit *L. plantarum*, *L. monocytogenes, E. coli* and but not *S. thypimurium.* LAB bacteriocin tend to be active against a wide range of mostly Gram positive bacteria, while Gram negative bacteria are generally insensitive to bacteriocin from LAB because they have a thinner layer (10% of cell wall), and an outer membrane (OM) which providing a permeability barrier. Outer membrane on contains lipids and is separated from the cell wall by the periplasmic space. The permeability barrier property of the OM is largely due to the presence of a specific lipopolysaccharide (LPS) layer on the membrane surface. LPS molecules consist of a lipid part, termed lipid A, and a hydrophilic heteropolysaccharide chain protruding outward and providing the cell with a hydrophilic surface (Hwanhlem et al., 2011). Meanwhile Gram-positive bacteria only have a thick mesh-like cell wall, made of peptidoglycan which constructs about 90% of the cell wall (Helander et al., 1996 *in* Omar et al., 2006).

Table 8. Physiological and biochemical characteristics of bacteriocin producing strain

Physiological and biochemical characteristic	Result
Colony morphology	Creamy, smooth round colonies
Gram staining	Gram positive, coccus
Growth in MRS broth	turbid
Fermentation reaction	ribose, galactose, D-glucose, D-fructose, D-mannose amygdalin, arbutin, esculin ferric citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-saccharose, D-trehalose, D-gentobiose,
Catalase	Negative
Oxidase test	Negative
Identification	*Lactococcus lactis* subsp. lactis

Identification of Isolate CN1.10a

Based on the physiological and biochemical characteristic tested using API 50 CHL, CN1.10a isolate was identified as *Lactococcus lactis* ssp. *lactis*. The CN1.10a isolate was Gram positive, coccus, catalase negative and oxidase negative. The strain was capable in fermenting sugar such as ribose, galactose, glucose, fructose, mannose, cellobiose, maltose, lactose, saccharose, trehalose and gentobiose (Table 8).

CONCLUSION

CN1.10a which was identified as *Lactococcus lactis* sp. *lactis* which was isolated from rusip, a fermented fish from Bangka, produced bacteriocin that sensitive to proteolytic enzymes, *i.e.* proteinase K and papain but not to RNAse. The bacteriocin has wide inhibition activity against Gram positive and negative bacteria such as *Esherichia coli, Listeria monocytogenes* and *Lactobacillus plantarum.* The bacteriocin was stable at pH 2.0 to 6.0 and its activity could be stimulated by sodium dodecyl sulphate (SDS), lauryl sarcosine and EDTA surfactans but strongly inhibited by Tween 20, Tween 80, Triton X-100 and urea. This bacteriocin was heat stable (121ºC), therefore it has potential to be used as a preservative for high temperature processed foods.

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