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Original Article

Detection and preliminary characterization of a narrow spectrum bacteriocin produced by *Lactobacillus pentosus* K2N7 from Thai traditional fermented shrimp (*Kung-Som*)

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Abstract

A total of 48 lactic acid bacteria (LAB) exhibited antagonistic activity against *Lactobacillus sakei* subsp. *sakei* JCM 1157 or *Staphylococcus aureus* DMST 8840. Only strain K2N7 was selected for characterization of bacteriocin activity. It was identified as *Lactobacillus pentosus* based on 16S rDNA analysis. The maximum bacteriocin production was detected in early stationary phase of growth. It was found to be sensitive to proteolytic enzymes (trypsin, proteinase K, pronase E and α-chymotrypsin). The bacteriocin K2N7 was heat stable (2 h at 100ºC) and retained activity over a wide pH range (2.0-12.0). Bacteriocin K2N7 has a narrow inhibitory spectrum restricted to genus *Lactobacillus* including *Lactobacillus plantarum* D6SM3, a bacterial strain known to cause overfermentation in *Kung-Som*. The peptide was purified by 60% ammonium sulphate precipitation followed by sequential cation exchange chromatography and hydrophobic interaction characteristic. The molecular mass of bacteriocin K2N7 (2.017 kDa) was determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis (MALDI-TOF MS).

Keywords: bacteriocin, *Kung-Som*, fermented shrimp, narrow spectrum, *Lactobacillus pentosus*

1. Introduction

In spontaneously fermented foods, lactic acid bacteria (LAB) derived from the raw materials or the environment are responsible for fermentation of carbohydrates leading to lactic acid production, and also a low pH value (König and Fröhlich, 2009). The use of LAB in foods has a long history, and they have a generally recognized as safe (GRAS) status (Saito, 2004). In addition, some of them produce an array of antimicrobial agents including bacteriocins substances to inhibit some pathogenic and spoilage bacteria (Ammor *et al*., 2006).

Bacteriocins are antimicrobial compounds of a peptidic nature, extracellularly released low molecular mass peptide or protein (usually 30-60 amino acids) (Savadogo *et al*., 2006) active against different indicator bacteria, including food-poisoning microorganisms and LAB (Ghrairi *et al*., 2008). For the application of bacteriocin-producing isolates, their suitability as starter organisms has to be demonstrated with respect to their performance in the respective process and the product quality (Zacharof and Lovitt, 2012).

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Kung-Som is a traditional fermented shrimp widely distributed in the south of Thailand. It is made from banana shrimp (*Penaeus merguiensis*), sugar and table salt and is spontaneously fermented with the natural micro flora at ambient temperature (25-30°C) for 7-14 days. The principal microorganisms found in *Kung-Som* are various LAB such as *Lactobacillus reuteri*, *L. plantarum* and *L. pentosus* (Sanchart *et al*., 2015).

Overfermentation in *Kung-Som* can occur during storage, while *L. plantarum* was the dominant strain in the finished product (Hwanlem *et al*., 2011), which causes the overfermentation and unacceptable flavor. A narrow spectrum bacteriocin-producing LAB isolated from *Kung-Som*, which could affect *L. plantarum* was of interest to stop the overfermentation.

In this study, we report the screening, production, purification and characterization of a narrow spectrum bacteriocin against *Lactobacillus* strain produced by *L. pentosus* K2N7 isolated from *Kung-Som*.

2. Materials and Methods

2.1 Screening for antagonistic activity of LAB

Samples of *Kung-Som* were spread-plated onto MRS agar (Hi-Media, India) and incubated for 24-48 h at room temperature (30±2°C). Screening for antagonistic activity of LAB was performed by the agar overlay method. Colonies were overlaid with soft MRS or BHI (Hi-Media, India) (0.7% agar) containing 10^6 CFU/ml of an actively growing culture of *Lactobacillus sakei* subsp. *sakei* JCM 1157 or *Staphylococcus aureus* DMST 8840 respectively. After 24 h incubation at 37°C, the colonies with clear zone were picked, re-streaked, checked for catalase activity, Gram stained and examined microscopically.

Production of an antimicrobial substance was tested by the drop plate method (Noonpakdee *et al*., 2003). Briefly, overnight cultures in MRS broth were spotted (5 µl) on MRS agar. After 24 h of incubation at 37°C, the plates were overlaid with soft agar inoculated with the indicator strains. After overnight incubation at 37°C, the strains that exhibited clear zone of radius greater than 5 mm were selected for characterization of antimicrobial compounds.

2.2 Characterization of the antimicrobial compounds

Selected LAB were grown individually for 24 h in MRS broth and centrifuged (Hettich ZENTRIFUGEN, Germany) at 8,500g for 15 min at 4°C. The supernatants obtained were used in the experimental trials as follows (Aslim *et al*., 2005): (1) the cell free supernatants (CFS) were used directly; (2) pH of CFS was neutralized to pH 6.5 with 1 M NaOH to eliminate the antimicrobial effect of organic acid (NCFS); (3) Catalase at a concentration of 300 unit/ml was added to the NCFS to exclude the inhibitory effect of hydrogen peroxide (Ammor *et al*., 2006); (4) Trypsin or α-chymotrypsin or pronase E or proteinase K (1 mg/ml) was added to NCFS and incubated at 37°C for 3 h. All samples were filter-sterilized before use. Inhibition activity was tested by broth microdilution assay (BMDA) (Shelburne *et al*., 2007). The supernatants were added to 96-well microtiter plates in the first column. Then, two-fold serially dilutions with BHI broth were made by drawing up the supernatants into lower column and moving on until the final concentrations. The indicator strains were inoculated and measured by microplate reader at 660 nm (Biotek, Wenooski, VT, USA) after 24 h cultivation. One bacteriocin unit was defined as the reciprocal of the highest dilution showing inhibition higher than 50% (50% of the turbidity of the control culture without bacteriocin). The arbitary unit (AU) of antibacterial activity per milliliter of the supernatant was calculated as

$$\frac{1{,}000 \times 2^n}{\text{supernatant volume (microliter)}}$$ (Perez *et al*., 1990).

Variable n is a reciprocal of the highest dilution that resulted in inhibition to inhibitor strain.

2.3 Identification of bacteriocin-producing LAB

The selected LAB isolates were sent to the Institute of Plant Science and Resources, Okayama University, Japan, for identification along the full length of the 16S rDNA sequence. The nucleotide sequences were identified using NCBI compared to database of GenBank via BLASTn analysis (http://www.ncbi.nlm.nih.gov/).

2.4 Growth and bacteriocin production

The *L. pentosus* K2N7 overnight culture 1% (v/v) was inoculated into 1,000 ml of MRS broth. The culture was incubated at 37°C for 36 h with stirring (100 rpm) under uncontrolled pH condition. At 3 h intervals, 10 ml samples were collected to determine the viable count on MRS plate and bacteriocin activity by BMDA against *L. sakei* subsp. *sakei* JCM 1157.

2.5 Purification of the bacteriocin

L. pentosus K2N7 was cultivated for 12 h at 37°C and centrifuged (6,000g, 15 min, 4°C). The supernatant was then heated at 80°C for 15 min. The ammonium sulphate precipitation (60% saturated) was performed, and the pellets were collected and re-suspended in 50 mM acetate buffer (pH 3.0). The protein was desalted using a 1 kDa cutoff dialysis membrane (Spectra/Por®, USA). After dialysis, the dialysate was lyophilized and kept at -20°C (partially purified), and protein measurement was performed by Lowry's method (Lowry *et al*., 1951). The antagonistic activity was determined by agar well diffusion assay (AWDA) (Perez *et al*., 1990); plates were prepared from *L. sakei* subsp. *sakei* JCM 1157 at the final concentration of 10^6 CFU/ml, then 5 millimeters diameter wells were punched using a cork borer. Fifty micro-

litters of bacteriocin was dispensed into wells and incubated at 37°C for 24 h. The inhibition zone was measured using vernier caliper. The titer was defined as 2^n, where n is the reciprocal of the highest dilution that resulted in inhibition of inhibitor strain. Thus, the arbitary unit (AU) of antibacterial activity per milliliter of the supernatant was calculated as

$$\frac{1{,}000 \times 2^n}{\text{supernatant volume (microliter)}}$$ (Apolônio *et al*., 2008).

The freeze-dried bacteiocin was re-suspended in 50 mM acetate buffer pH 3.0 (10 mg/ml) and applied onto SP-Sepharose Fast Flow column (GE Healthcare Bio-Sciences AB, Sweden), equilibrated with the same buffer with a flow rate of 1 ml/min. Elution was performed with the gradient 0-1.0 M NaCl in equilibration buffer. The protein fractions were measured at 280 nm and the antagonistic activity was tested using AWDA. The active fractions were pooled and subjected to Sep-Pak C18 cartridge column (Millipore, USA) to desalt. The column was equilibrated with 50 mM acetate buffer containing 1 M NaCl (pH 3.0), the active fractions were loaded manually using a 10 ml of syringe and washed with 20% (v/v) in 50 mM acetate buffer (pH 3.0). The protein was eluted using 60% (v/v) in 50 mM acetate buffer (pH 3.0) and the antimicrobial activity tested using the AWDA after evaporation by rotary evaporator and storing at -20°C for further use.

2.6 Spectrum of antimicrobial activity

The partially purified bacteriocin (2.5 mg/ml) in distilled water was tested against reference strains by AWDA. Reference strains were activated and transferred to MRS or BHI soft agar (1% agar) for LAB and the other bacteria, respectively. Wells were cut and filled with 50 µl of bacteriocin. The plates were checked for presence of 1 mm inhibition zone or larger.

2.7 Effect of pH, heat treatment and proteolytic enzymes on bacteriocin activity

The 2.5 mg/ml of freeze-dried bacteriocin in distilled water pH 2.0-12.0 was incubated at 4°C for 1 and 24 h and the activity determined by AWDA. The pH showing the highest stability of bacteriocin activity during 24 h was chosen for the following experiment.

The effect of temperature was tested by incubating the 2.5 mg/ml of partially purified bacteriocin in distilled water pH 3.0 at 4°C, 25°C, 30°C, 37°C, 60°C, 80°C, 100°C for 60 and 120 min and autoclaving at 110°C for 20 min, 121°C for 15 min and then the activity tested by AWDA.

To test the effect of enzymes, bacteriocin K2N7 (2.5 mg/ml) was treated with the following enzymes at a final concentration of 1 mg/ml: trypsin (Fluka, Switzerland); α-chymotrypsin (Fluka, USA); pronase E (Fluka, Japan); proteinase K (Fluka, Switzerland); lipase (Amano, Japan) or 300 unit/ml of catalase (Fluka, USA). All samples were adjusted to pH 7.0 and then incubated at 37°C for 3 h. Residual enzyme activity was finally stopped by boiling for 5 min and then the antibacterial activity screened using AWDA.

2.8 Molecular weight determination

The proteins from each purification step were separated by Tricine-SDS-PAGE as described by Schägger and Von Jagow (1987). Protein solutions were mixed at 2:1 (v/v) ratio with the Tricine-SDS-PAGE sample buffer and boiled for 5 min. The samples (30 µl) were loaded onto the gel (16% separating gels) and subjected to electrophoresis at a constant 100 volts using Mini-PROTEIN Tetra Electrophoresis System (Bio-rad). The 2.0 to 212.0 kDa protein marker (Biolabs, England) was used. After electrophoresis, SDS gel was fixed for 30 min in 12.5% (w/v) trichloroacetic acid and rinsed with water. The gel containing the molecular marker was cut and stained with Coomassie Brilliant Blue G-250; the other half of the gel was overlaid with *L. sakei* subsp. *sakei* JCM 1157 and embedded in MRS agar. The position of the active bacteriocin was visualized by an inhibition zone around the active protein band, as described by van Reenen *et al*. (1998).

The active purified bacteriocin on the Tricine-SDS-PAGE as a single band was cut and sent for MALDI-TOF MS determination (Ultraflex, Bruker Daltonics).

3. Results

3.1 Screening of potential bacteriocin producing lactic acid bacteria

A total of 48 isolates producing inhibition zones against the indicator strains by agar overlay method were selected for further study (data not shown). However, after secondary screening by drop plate method only the 17 isolates exhibiting inhibition zones larger than 5.0 mm radius were selected for further characterization.

After excluding inhibition due to organic acids and hydrogen peroxide, only strain K1N1 and K2N7 exhibited antimicrobial activity. No change in activity was recorded when treated with catalase. The strain K2N7 was selected for further study as to strain K1N1 showed an unstable antibacterial activity in some experiments; this problem was reported on Lactocin S, which had neutral pI at 7.1 and exhibited the activity at pH lower than 6.0 (Mortvedt-Abildgaa *et al*., 1995).

3.2 Identification of bacteriocin producing LAB

Bacteriocin-producing LAB strain K2N7 was identified by comparing the full length of 16S rDNA sequences. Strain K2N7 was identified as *Lactobacillus pentosus* (99%). The sequence was deposited in DDBJ/EMBL/GenBank as accession number AB533229.

3.3 Growth and bacteriocin production

Bacteriocin production (AU/ml) during growth of *L. pentosus* K2N7 in MRS broth at 37ºC is shown in Figure 1. Optical density of supernatant increased from the initial value at 0.012 (10^4 CFU/ml) during 12 h of incubation. The OD 660 nm then stabilized at around 0.5 (10^9 CFU/ml). Bacteriocin production was recorded after 9 h of cultivation, and the maximum levels of antimicrobial activity were found at 15 h of cultivation. This was at the beginning of the stationary phase of growth, the level of inhibition thereafter remaining constant (Figure 1).

3.4 Purification of bacteriocin

Purification of bacteriocin from *L. pentosus* K2N7 is summarized in Table 1. The first step of purification by ammonium sulphate precipitation (partially purified bacteriocin) resulted in an approximately 58-fold concentration and 99.2% recovery. Only a single active peak was observed from SP-Sepharose Fast Flow eluted fraction (Figure 2). After SP-Sepharose Fast Flow and Sep-Pak C18 cartridge step, a total activity of approximately 74.6% remained, while 90% of protein was removed, and a purity of 440 fold was obtained. This led to obtaining high purity bacteriocin substance after the hydrophobic interaction chromatography step was performed.

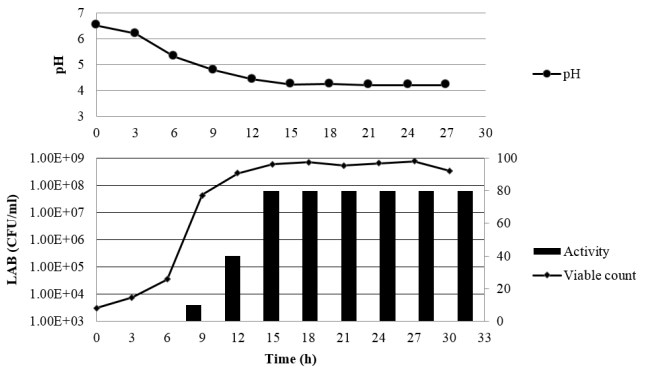

Figure 1. Production of bacteriocin K2N7 in MRS broth (at 37ºC) is indicated by the histrogram. Antimicrobial activity is presented as AU/ml against *Lactobacillus sakei* subsp. *sakei* JCM1157. Growth of *Lactobacillus pentosus* K2N7 on MRS agar plates recorded as log CFU/ml (◆). Changes in pH is shown by (●)

3.5 Effect of enzymes, pH and heat treatment on activity of bacteriocin K2N7

Treatment of the partially purified bacteriocin K2N7 with trypsin, proteinase K, pronase E or α-chymotrypsin resulted in complete inactivation of antimicrobial activity (Table 2). Treatment with catalase did not affect to bacteriocin activity (Table 2), discarding clearly the involvement of H_2O_2 in the antagonism process. In addition, treatment with lipase did not affect the antimicrobial activity, suggesting that bacteriocin K2N7 does not belong to the complex bacteriocins, which contain lipids attached with amino peptide (Chen and Hoover, 2003).

The activity of bacteriocin K2N7 was stable throughout the pH ranging from 2.0 to 12.0 for 1 h incubation (Table 2). However, when the experiment was conducted at pH 2.0 with the bacteriocin 2.5 mg/ml, a substantially higher activity was recorded. Activity was still present after exposure for 24 h to pH 2.0-12.0.

Bacteriocin K2N7 remained stable after 2 h at 25, 30, 37, 45, 60, 80, 100°C (Table 2). Decrease in activity was observed upon heat treatment at 110°C for 20 min and 121°C for 15 min at pH 2.0.

3.6 Spectrum of activity determination of partially purified bacteriocin K2N7

The bacteriocin K2N7 exhibited inhibitory activity against only closely related bacteria including *L. pentosus* K1N1, *L. sakei* subsp. *sakei* JCM 1157 and *L. plantarum* D6SM3. Bacteriocin K2N7 displayed a relatively narrow spectrum of antimicrobial activity as it inhibited only 3 of the 17 reference strains (Table 3).

3.7 Electrophoresis and molecular weight determination

The molecular weight of bacteriocin was estimated to be lower than 6.5 kDa (Figure 3), that is, within the range of most bacteriocins reported from the genus *Lactobacillus* (De Vuyst and Vandamme, 1994). For silver staining, purified bacteriocin appeared as a single band (Figure 4), indicating the homogeneity of bacteriocin. The silver staining is used to detect proteins after electrophoresis on polyacrylamide gels. It has excellent sensitivity (in the very low nanogram range). Electrophoresis results were indicative of a large amount of contaminating proteins removed during purification by SP-

Table 1. Purification of bacteriocin K2N7

Purification step	Total volume (ml)	Total protein (mg)	Total activity (AU)	specific activity (AU/mg)	Yield (%)	Purification (fold)
Supernatant	1,000.00	7,417.45	10,000.00	1.35	100.00	1.00
60% ammonium sulphate	31.00	126.68	9,920.00	78.31	99.20	58.08
SP Sepharose FF and C18	23.33	12.56	7,456.60	594.30	74.65	440.82

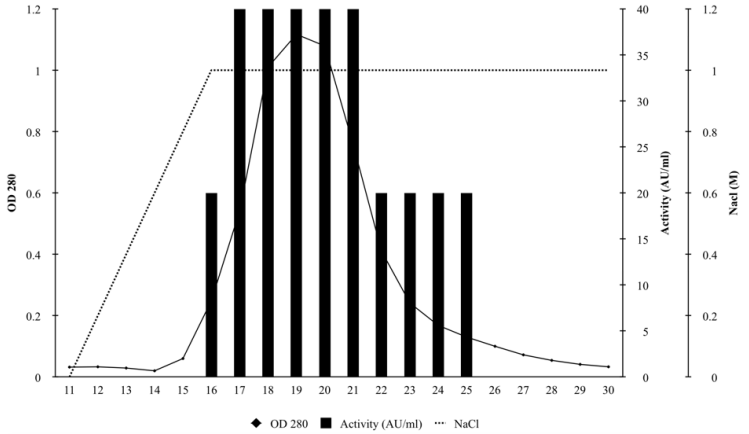

Figure 2. Elution profile of bacteriocin K2N7 on SP-Sepharose Fast Flow column. The 30 mg/ml protein of eluent from 60% ammonium sulphate precipitation was applied onto 5 ml SP-Sepharose Fast Flow column equilibrated with 20 mM acetate buffer pH 3.0. Protein was eluted with a gradient 0-1.0 M NaCl at a flow rate 1 ml/min. Elution profile at 280 nm (—) and a gradient of NaCl (-----) were detected

Table 2. Stability of bacteriocin K2N7 after action of enzymes, and pH and heat treatments against *L. sakei* subsp. *sakei* JCM 1157 using agar well diffusion assay.

	Concentration	Bacteriocin activity (AU/ml)
Enzymes		
Lipase	1 mg/ml	160
Proteinase K, Pronase E		0
α-Chymotrypsin, Trypsin		0
Catalase	300 Units/ml	160
Temperatures		
0, 4, 25, 30, 37, 45, 60, 80,100ºC for 2 h		160
110ºC for 20 min		80
121ºC for 15 min		0
pH		
pH 2.0-12.0		160
Control		160

Sepharose Fast Flow and Sep-Pak C18 cartridge column. After tryptic digestion, MALDI-TOF MS of the purified bacteriocin in the positive mode gave a mass of 2073 m/z (Figure 5).

4. Discussion

Bacteriocin production from *L. pentosus* has been reported on *L. pentosus* ST712BZ from Boza (Brazillian fermented food). It produced bacteriocin ST712BZ which inhibited growth of *Lactobacillus casei, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Klebsiella pneumoniae* and *Lactobacillus curvatus* (Todorov and Dicks, 2007). In addition, pentocin 31-1 from *L. pentosus* 31-1 isolated from the traditional China fermented Xuan-Wei Ham with the size 14 kDa was also reported (Liu *et al*., 2008).

The bacteriocin production from LAB usually occurs throughout the growth phase and ceases at the end of the exponential phase or sometimes before the end of growth. This trend was reported on bacteriocin bacST8KF (Powell *et al*., 2007), ST28MS, ST26MS (Todorov and Dicks, 2005) and the bacteriocin K2N7 in the present study. However, the activity of some bacteriocins decreased during the stationary phase due to the proteolytic enzymes (Bogovic *et al*., 2001) or re-adsorption of bacteriocins to the cell surfaces at low pH (Franz *et al*., 1996). From these descriptions, it can be concluded that bacteriocin K2N7 was stable during fermentation.

Bacteriocin K2N7 in this study was thermo stable, which is the dominant characteristic of bacteriocin. The complex pattern of monosulfide and disulfide intramolecular bonds of bacteriocin helps in the stabilization of secondary

Table 3. Antimicrobial spectrum of bactreriocin produced by *Lactobacillus pentosus* K2N7

Indicator strains	Culture medium	Activity
Lactococcus lactis	MRS	-
Enterococcus gallinarum VanC	MRS	-
Enterococcus faecalis Van B	MRS	-
Enterococcus faecium 174	MRS	-
Enterococcus faecium 139	MRS	-
Streptococcus salivarius LD219	MRS	-
Enterococcus faecium 348	MRS	-
Lactobacillus pentosus K1N1	MRS	+
Lactobacillus plantarum D6SM3	MRS	+
Lactobacillus sakei subsp. *sakei* JCM 1157	MRS	+
Bacillus cereus DMST5040	BHI	-
Salmonella Typhimuirum DMST16809	BHI	-
Vibrio parahaemolyticus DMST5665	BHI	-
Escherichia coli DMST4212	BHI	-
Staphyloccus aureus DMST8840	BHI	-
Salmonella Stanley 42	BHI	-
Listeria monocytogenes DMST17303	BHI	-

+ : Clear inhibition zone
- : Non clear inhibition zone

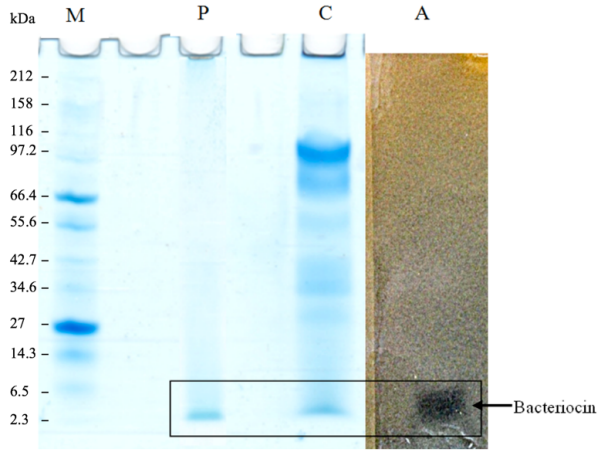

Figure 3. Tricine-SDS-PAGE analysis of protein profile from each purification step of bacteriocin K2N7 purification. Lane C : crude ammonium sulphate precipitation peptide band stained with Coomassie brilliant blue R250; Lane P : purified peptide band of bacteriocin after ammonium sulphate precipitation SP-Sepharose Fast Flow and Sep-Pak C18 cartridge, Lane A : antimicrobial zone of bacteriocin, corresponding to the position of the peptide band (30µl loaded); Lane M : molecular weight marker

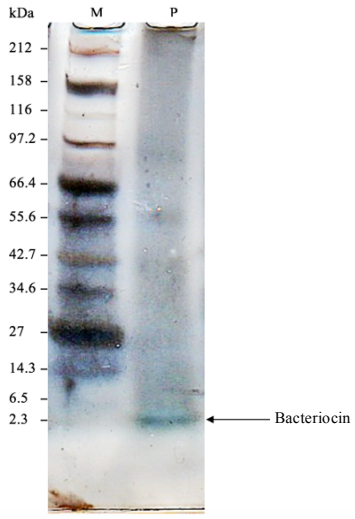

Figure 4. Separation of purified bacteriocin K2N7 using Tricine-SDS-PAGE stained with silver staining. Lane P : Peptide band of bacteriocin after purification using ammonium sulphate precipitation, SP-Sepharose and Sep-Pak C18 cartridge

structures by reducing number of possible unfolded structures (entropic effect) (Oscariz and Pisabarro, 2001). Meanwhile, the pH tests showed that bacteriocin K2N7 was active over a wide range of pH (2.0-12.0). Naturally occurring bacteriocins have either a strong positive net charge, with a high isoelectric point, or at least a strong localized positive charge, making them stable and working over wide pH range. Their high isoelectric point allows them to interact at physiological pH values with the anionic surface of Gram positive bacterial membrane (Oscariz and Pisabarro, 2001). Besides, bacteriocin K2N7 showed a higher activity at low pH because at a pH below their pI, bacteriocins carry a net

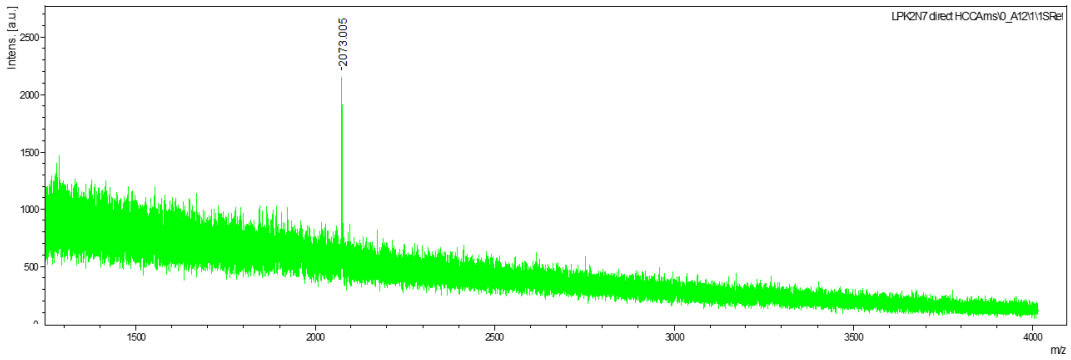

Figure 5. MALDI-TOF chromatogram obtained from tryptic digestion of purified bacteriocin isolated from *Lactobacillus pentosus* K2N7

positive charge and easily attach to the target cell membrane, resulting in higher activity or more stable bacteriocin. The thermotolerant and pH stable characteristics of bacteriocin show the probability for application of bacteriocins as part of a hurdle technology in the food industry (Chen and Hoover, 2003).

Ion exchange chromatography is able to separate the peptides by their electric charge at definite pH. Since most bacteriocins have positive charges at pH lower than iso-electric point, the use of cation exchange resins is appropriate for their purification (Pingitore *et al*., 2007). Bacteriocin K2N7 elution profile showed a single peak of eluted protein using 0-1.0 M NaCl, while contaminated proteins were washed out (Figure 2). The separation of contaminated proteins using SP-Sepharose Fast Flow resin is not only due to the electric charge of the sulfopropyl (SP) group, but also to size exclusion occurring during purification. The agarose gel (size 45-165 µm) has a size exclusion characteristic of 10-4,000 kDa. Hence the high molecular weight contaminant proteins were eliminated, owing to Sepharose Fast Flow resin being agarose with the size of 90 µm (Figure 3).

Tricine-SDS-PAGE was used to determine the mass of the low molecular weight (smaller than 30 kDa) and hydrophobic peptide of bacteriocin. The lower concentrations of acrylamide facilitate electroblotting, which is particularly crucial for hydrophobic proteins (Schagger, 2006). Bacteriocins from *L. pentosus* have been reported to have molecular weights in the range of 3.0 to 14.3 kDa. Todorov and Dicks (2004) found that *L. pentosus* ST151BR produced a small molecular mass bacteriocin at 3.0 kDa. Todorov and Dicks (2007) reported that bacteriocin from *L. pentosus* of Boza had a molecular weight of 14.3 kDa. While, the bacteriocin K2N7 was detected at 2.017 kDa. on MALDI-TOF MS detection; there are no reports about this size of bacteriocin from *L. pentosus*. The novelty of this bacteriocin will be confirmed using amino acid sequencing for further study.

The use of LAB for the production of fermented food is becoming necessary for quality and standardized properties. LAB are GRAS micro-organisms that have been used in the processing of fermented food for centuries (Saito, 2004). During fermentation of *Kung-Som*, *Pediococcus* spp. strain was always found in the early stage of fermentation, during which there is rapid acid production, but the strain not acid tolerant (Roadcharern, 1999), whereas *Lactobacillus* spp. such as *L. plantarum*, *L. delbrueckii* and *L. casei* subsp. *casei* were dominant in middle and final stages of fermentation (Nuphet, 2003).

The application of a narrow spectrum bacteriocin was reported on *L. acidophilus* ATCC 4366, which inhibited *L. sakei* NCDO 2714, an organism known to cause anaerobic spoilage of vacuum-packed meat (Deraz *et al*., 2005). Meanwhile, *L. pentosus* K2N7 exhibited activity against *Lactobacillus* sp. especially *L. plantarum* D6SM3, the dominant LAB in *Kung-Som* finished product and during storage (Hwanhlem *et al*., 2011). Using bacteriocin K2N7 to stop the fermentation process and prevent overfermentation, which causes sour taste, is an available choice. Bacteriocin K2N7 might be applied to the finished product of *Kung-Som* to inhibit *L. plantarum,* resulting in prolonged shelf life and preventing unacceptable flavor by reducing acid production during storage.

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