CHARACTERIZATION OF THE BACTERIOCIN-LIKE SUBSTANCE FROM *LACTOCOCCUS LACTIS* SUBSP. *LACTIS* WX153 AGAINST SWINE PATHOGEN *STREPTOCOCCUS SUIS*

Nopparat Srimark, Nongpanga Khunajakr*

Department of Microbiology, Faculty of Science, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand

ABSTRACT:

Streptococcus suis is one of the most important pathogens in swine, which can infect many organs and has been associated with septicemia, meningitis, arthritis and pneumonia. Moreover, *S. suis* is also an emerging zoonotic pathogen that can infect humans and cause severe disorders such as meningitis and Streptococcal toxic shock-like syndrome (TSLS). Nowadays, the most successful way to treat and prevent *S. suis* infection is to use antibiotics. However, this involves increased risk of antibiotic resistance, and raises issues regarding food safety. There are several potential alternative methods to replace the use of antibiotics. One is to promote pig health by probiotic bacteria and the other is to use bacteriocins, which are antimicrobial proteinaceous compounds, to inhibit the growth of pathogens. *Lactococcus lactis* subsp. *lactis* WX153 is a probiotic lactic acid bacterium isolated from swine feces. The strain was found to produce a bacteriocin-likesubstance (designated as Bacteriocin WX153) that can inhibit the growth of *S. suis*. Bacteriocin WX153 was produced at the stationary phase of *Lc. lactis* WX153 growth and was sensitive to the proteolytic enzyme pronase E, indicating its proteinaceous nature. The Bacteriocin WX153 remained active over a wide pH range from 1 to 12 and still active at high temperature of 121°C for 15 min. The mode of action of Bacteriocin WX153 is bacteriostatic and it has broad antimicrobial spectrum against both Gram-positive and Gram-negative bacteria. Partial purification was performed by ammonium sulfate precipitation, followed by ion-exchange chromatography. The active fraction of Bacteriocin WX153 showed a single protein band with a molecular weight of about 6.5 kDa on SDS-PAGE. Therefore, Bacteriocin WX153 and probiotic bacterium *Lc. lactis* WX153 may have the potential to be used as anti-*S. suis* agents to substitute antibiotics for treatment of, and protection against, *S. suis* infection in swine and become more safety for human.

Keywords: *Streptococcus suis*, *Lactococcus lactis*, Probiotic, Bacteriocin

DOI: 10.14456/jhr.2015.16 *Received September 2014; Accepted December 2014*

INTRODUCTION

Streptococcus suis is considered an important swine pathogen worldwide. *S. suis* infection is often associated with several serious diseases such as meningitis, arthritis, septicemia, pneumonia, and endocarditis [1]. Moreover, *S. suis* is an emerging zoonotic agent that can cause pandemic human infections [2]. *S. suis* is a Gram-positive facultative bacteria, which is an opportunistic pathogens. Normally, it has the natural habitat in the upper respiratory tract, genital tract and alimentary tract of swine [3]. Therefore, infection is easy to spread within the sties because of secretions [3, 4]. Among 35 serotypes of *S. suis* based on capsular antigens, serotype 2 is the most frequently isolated from both infected pigs and humans worldwide. In western countries, infected people with *S. suis* were those who get close contact with swine. In the East Asia and the Southeast Asia, most patients derived this bacterium by eating raw or uncooked pork [3, 5]. The symptoms of *S. suis* infected patients were

* *Correspondence to: Nongpanga Khunajakr*
E-mail: naongpanga.khu@kmutt.ac.th

Cite this article as: Srimark N, Khunajakr N. Characterization of the bacteriocin-like substance from *Lactococcus lactis* subsp. *lactis* WX153 against swine pathogen *Streptococcus suis*. J Health Res. 2015; 29(4): 259-67. DOI: 10.14456/jhr.2015.16

similar to those of infected pigs. In 2005, there was an outbreak of *S. suis* in Sichuan, where 28% of 215 patients, who infected with *S. suis* serotype 2 sequence type (ST) 7, showed an unusual streptococcal toxic shock-like syndrome (STSLS) with 62% mortality rate [6].

Nowadays, *S. suis* infections in pigs and humans are treated with several antibiotics [1, 3]. However, some strains of *S. suis* that recently isolated are found to be resistant to antibiotics e.g. penicillin, tetracycline, etc. [7, 8]. Moreover, Hu et al. [9] found that *S. suis* N7 has SSUR61 gene, which encodes β-lactamase causing the resistant to β-lactam antibiotic. This gene is on transposon of *Enterococcus* sp., so there is evidently a risk of horizontal antibiotic resistant gene being transferred between pathogenic streptococcal and related species. For food safety and for prevention of increasing antibiotic resistance of pathogenic bacteria reasons, the finding for alternatives to antibiotic is necessary.

Bacteriocin is a ribosomally-synthesized anti-microbial peptide, which produced from bacteria that are active against other bacteria using a specific immune mechanism [10]. Most bacteriocin producing strains belong to lactic acid bacteria including *Pediococcus*, *Lactobacillus* and *Lactococcus* [11, 12], and some of them are classified as probiotic bacteria. *Lactococcus lactis* subsp. *lacis* is one of lactic acid bacteria, which can produce many types of bacteriocin include a well-known bacteriocin called nisin. Nisin is commercially used as food preservative [13, 14]. Not only applied in food, but nisin also shows its efficiency in bovine mastitis treatment caused by *Staphylococcus aureus* [15]. Moreover, Label et al. [16] found that nisin can counter *S. suis* and beat the cells more effectively when synergistically used with antibiotic.

The aim of this study is to characterize and purify an antimicrobial compound, produced from *Lc. lactis* subsp *lactis* WX153, which is a potential probiotic bacterium isolated from swine intestine. Knowledge on anti-*S. suis* compound may increase the potential usage of antibacterial compound for treatment and prevention of *S. suis* infection in the swine industry.

MATERIALS AND METHODS

Bacterial strains and growth conditions

Lactococcus lactis subsp. *lactis* WX153, isolated from swine feces [17], was grown in De Man, Rogosa and Sharpe (MRS) broth at 37°C. *Streptococcus suis* DMST18783, isolated from infected pig was derived from Department of Medical Science, Ministry of Public Health, Thailand (DMSC). *S. suis* was routinely grown in Brain Heart Infusion (BHI) medium (Conda, spain) at 37°C.

Determination of antimicrobial activity

The antimicrobial activity was evaluated by agar well diffusion assay as described by Schiliger and Lücke [18]. *S. suis* DMST 18783 was used as the indicator microorganism for antimicrobial assays. *S. suis* was grown for overnight and diluted to an OD 600 of 0.5, then 1% of diluted culture was inoculated into fresh BHI broth (~ 1 x 10^6 cfu/mL) and incubated for 6 – 8 h. A sterile cork borer was used to make a 7 mm diameter wells. The bacteriocin was prepared by growing *Lc. lactis* WX153 in MRS broth for 18 h at 37°C and centrifuged at 10,000 rpm for 5 min at 4°C to remove the cells. The supernatants then were filtered through a syringe filter with a pore size of 0.2 μm. To rule out the possibility of inhibition due to organic acids and hydrogen peroxide produced by *Lc. lactis* WX153, the pH of supernatant was adjusted to 6.5 with 1 N NaOH and treated with catalase (1 mg/mL) (Sigma) at room temperature for 30 min, respectively. Bacteriocin activity was determined by loading 50 μL of 2-fold serially diluted supernatant fluid into a 7 mm agar wells prepared as described above. The zone of inhibition was examined after incubation at 37°C for 24 h. The bacteriocin activity was expressed in Arbitrary Unti per milliter (AU/mL) which calculated as (the highest dilution giving a zone of growth inhibition) x (1000 μL) x (50^{-1} μL).

Growth and bacteriocin production by *Lc. lactis* WX 153

Lc. lactis WX 153 was grown in 100 mL MRS broth and incubated at 37°C. Samples were taken every 4 h for 48 h to evaluate for growth by using a standard plate count method. Antimicrobial activity in culture supernatants was also determined as described above. The experiment was done in triplicate.

Sensitivity of bacteriocin to proteolytic enzyme

The proteinaceous nature of bacteriocin was investigated by treatment the cell-free supernatants with proteolytic enzymes as described by Kojić et al. [19]. *Lc. lactis* WX153 was grown in MRS broth at 37°C for 18 h. Cells were removed by centrifugation, and the pH was adjusted to 6.5. The cell-free supernatants then were incubated for 1 h at 37°C in the present of protease enzymes, Pronase E (Type XIV- Calbiochem), proteinase K (TypeII - Sigma),

Table 1 Antimicrobial spectrum of Bacteriocin WX153 against the indicator strains

Indicator strains	Strains	Source	Activity
Aeromonas hydrophilia	TISTR 1321	TISTR	-
Bacillus cereus	TISTR 121	TISTR	+
Bacillus megaterium	TISTR 003	TISTR	-
Bacillus subtilis	TISTR 010	TISTR	-
Campylobacter jejuni	DMST 15190	DMSC	+
Enterococcus faecalis	TISTR 579	TISTR	-
Enterococcus hirae	TISTR 928	TISTR	-
Escherichia coli O157:H7	DMST 12743	DMSC	+
Listeria monocytogenes	DMST 4553	DMSC	+
Pseudomonas aeruginosa	DMST 15501	DMSC	+
Salmonella enterica	DMST 8014	DMSC	-
Salmonella Typhimurium	TISTR 292	TISTR	-
Staphylococcus aureus	TISTR 118	TISTR	+

TISTR: Thailand Institute of Science and Technology Research Culture Collection Center
DMSC: Department of Medical Sciences, Ministry of Public Health, Thailand

trypsin (Type XIII - Sigma), α-chymotrypsin (Type II - Sigma) and pepsin (Sigma) at a final concentration of 1 mg/mL. Antimicrobial activity was tested using agar well diffusion assay. The same buffers used for each proteolytic enzyme preparation were used as negative control and the bacteriocin containing supernatant was used as positive control.

Effect of pH and temperature on bacteriocin activity

The effect of temperature and pH on the bacteriocin activity was examined according to Tolinački et al. [20]. One mL. of *Lc. lactis* WX153 cell- free supernatants were incubated at 40, 50, 60, 70, 80, -90, 100 and 121°C for 15 min and tested for the remaining activity of bacteriocin. To evaluate the effect of pH, the supernatants were adjusted to pH 1 – 12 using NaOH and HCl. After incubation for 1 h at room temperature, the supernatants were neutralized to pH 7. The bacteriocin activity using agar well diffusion assay was also tested as described above.

Mode of action of bacteriocin

S. suis was grown in 30 mL BHI broth until it reached early exponential phase (3 h of cultivation). A 6 mL aliquot of cells–free supernatant containing bacteriocin from *Lc. lactis* WX153 was added to *S. suis* culture at a final concentration of 32 Units/mL. Growth of *S. suis* before and after addition of bacteriocin was monitored every hour for 12 h using viable plat count method.

Anti-microbial spectrum of bacteriocin

Culture supernatant obtained from *Lc. lactis* WX153 grown in MRS broth for 18 h at 37°C was tested for antimicrobial activity against Gram-positive and Gram-negative bacteria listed in Table 1 using agar well diffusion assay as described above.

Bacteriocin purification

The bacteriocin purification was performed by growing *Lc. lactis* WX153 in MRS broth for 16 h and cells were removed by centrifugation at 10,000 rpm for 10 min. Partial purification was performed by precipitation of bacteriocin from culture supernatant at 60% ammonium sulfate saturation overnight at 4°C. The floating pellicles were collected and re-suspended in 10 mL of 20 mM sodium phosphate buffer, pH 7.2, and dialyzed against the same buffer at 4°C for 48 h using dialysis tubing with a molecular weight cut-off of 3.5 kDa. The dialysate was filtered through 0.45 μM filter membrane and was further purified using the fast protein liquid chromatography system (FPLC) (ÄKTAprime plus, GE Healthcare) equipped with anion exchange Hitrap Q FF Sepharose column (GE Healthcare). Gradient elution with a NaCl concentration at 0 – 1 mM in 20 mM sodium phosphate buffer was performed at a flow rate of 1 mL/min. Fractions were collected and tested for bacteriocin activity using agar well diffusion method. Protein concentrations were examined by Lowry-Folin method [21].

RESULTS

***Lc. lactis* WX 153 growth and antimicrobial activity**

The relationship between cell growth and antimicrobial activity was evaluated in MRS medium. As shown in Figure 1, growth of *Lc. lactis* WX153 reached stationary phase after 8 h then started to decline after 12 h of incubation. Antimicrobial activity was detected in the exponential growth phase and reached the highest

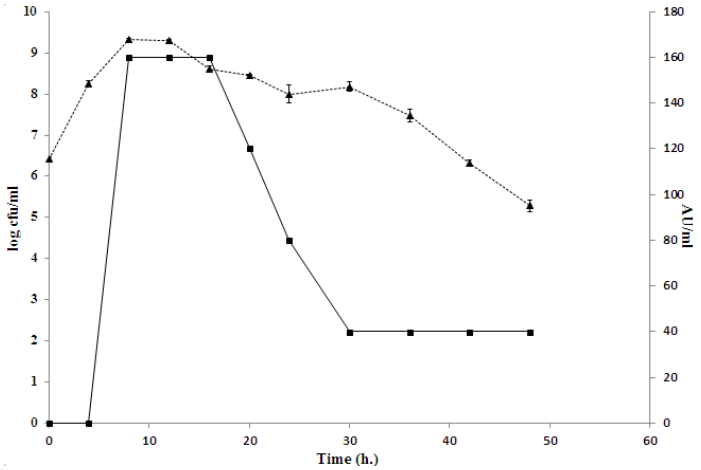

Figure 1 The growth of *Lc. lactis* subsp. *lactis* WX153 and bacteriocin production. Dash line with triangle (▲) represent bacterial growth using plate count agar method, solid line with square (■) represent bacteriocin activity.

activity of 160 AU/mL at 8 h of incubation and produced continuously until early decline phase. The activity decreased rapidly when cells enter death phase after 16 h.

Proteolytic sensitivity of bactriocin

To determine if the antimicrobial compound has a proteinaceous nature, the cell-free supernatant was tested for its sensitivity toward 5 proteolytic enzymes. Complete inactivation of antimicrobial activity was observed after treatment of the cell-free supernatant with PronaseE (Table 2). Inhibition due to the effect of organic acids and hydrogen peroxide was excluded by treatment the cell-free supernatant with catalase and neutralized to pH 6.5 with NaOH. It was found that, the cell-free supernatant retained its activity after treatment with either catalase or hydrogen peroxide. Inactivation of antimicrobial compound with proteolytic enzyme indicated proteinaceous nature of the compound. As the results mentioned above, the inhibitory compound produced by *Lc. lactis* WX15 was tentatively identified as bacteriocin-like compound and was designated as Bacterocin WX153.

Effect of pH and temperature on bacteriocin activity

Effect of pH and temperature on antimicrobial activity of Bacteriocin WX153 was carried out using *S. suis* as indicator strain. Bacteriocin WX153 retains its antimicrobial activity after exposure to temperature of 40 to 80 °C for 15 min. Heat treatment at 90 to 121°C for 15 min reduced its antimicrobial activity to 50% (Table 2).

Bacteriocin WX153 was stable at pH 2 to 5, while 40 to 60 % residual antimicrobial activity was observed at pH range from 6 to 11. The residual activity of 20% was detected at pH 1 and 12 (Table 2).

Table 2 Effect of temperature, pH and proteolytic enzymes on Bacteriocin WX153 activity

Treatment	Bacteriocin Activity (AU/mL)
Temperature (°C)	
Control	80
40	80
50	80
60	80
70	80
80	80
90	40
100	40
121	40
pH	
1	15
2	75
3	75
4	75
5	75
6	45
7	30
8	45
9	45
10	30
11	30
12	15
Proteolytic enzymes	**Diameter of inhibition zone (mm)**
Non (control)	11
Pronase E	7*
Proteinase K	11
α-chymotrypsin	9
Pepsin	9
Trypsin	10

* Agar well diameter = 7 mm.

Mode of action of bacteriocin

Addition of Bacteriocin WX153 to the exponential

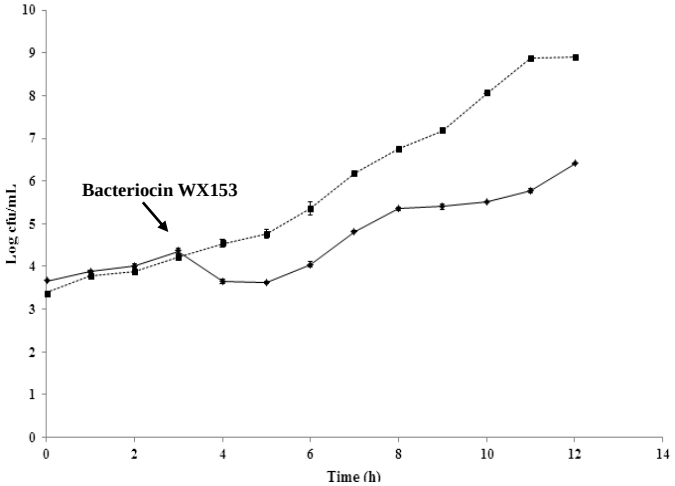

Figure 2 Growth of *Streptococcus suis* in brain heart infusion medium at 37 °C in the presence of 32 AU/mL Bacteriocin WX153 at early log phase (♦) in the absence of Bacteriocin WX153(■). Arrow indicated the time of addition of cells–free supernatant containing bacteriocin from *Lc. lactis* WX153

Table 3 Purification of Bacteriocin WX153

Fraction	**Vol (mL.)**	**Protein (mg/mL)**	**Bacteriocin activity (AU/mL)**	**Total bacteriocin activity** (AU)	**Recovery (%)**	**Specific bacteriocin activity (AU/mg)**	**Increase in specific activity (fold)**
Cell-free supernatant	1000	24.26	160	160,000	100	6.59	1.0
Ammonium sulfate precipitation	12	9.64	2,560	30,720	19.2	265.56	40.30
Filtrate after filtration	11	9.08	1,280	14,080	8.8	140.97	21.39
Ion-exchange chromatography	45	0.42	111.11	5,000	3.1	261.50	39.68

phase cells culture of *S. suis* (3-h old) resulted in growth inhibition of *S. suis*. A reduction in viable cell count of 0.7 log unit was observed after 1h of addition of bacteriocin and remained constant for 2 h compared with the control without bacteriocin (Figure 2). However, upon further incubation, growth resumed after 5 h and continued to grow at the same rate of the control. These observations indicated that Bacteriocin WX153 has bacteriostatic effects on *S. suis*.

Antimicrobial spectrum of bacteriocin

The results of antimicrobial spectrum of Bacteriocin WX153 against Gram-positive and Gram- negative bacteria are shown in Table 1. Bacteriocin WX153 had a broad spectrum of activity as it can inhibit the growth of both Gram-positive bacteria and Gram-negative bacteria. Interestingly, among the 3 Bacillus strains tested, only *Bacillus cereus* is sensitive to Bacteriocin WX153. Some Gram-negative food- borne pathogens such as *Campylobacter jejuni*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 were also susceptible to this antimicrobial compound.

Purification of bacteriocin

Ammonium sulfate precipitation and anion exchange chromatography using a FPLC system were applied for the purification of bacteriocin from *Lc. lactis* WX153. The percentage of recover and degree of purification at each step of purification are summarized in Table 3. *Lc. lactis* WX153 was grown in 1 L MRS broth for 16 h. The cell–free supernatants were collected for purification using ammonium sulfate at 60% saturation. A total bacteriocin activity of 160,000 AU was obtained in culture broth and the activity of 30,720 AU, which account for only 19.2%, was recovery after ammonium sulfate precipitation.

The fraction then was filtered through 0.45 μm membrane filter for further purification by anion-exchange column chromatography. Loss of bacteriocin activity was observed in this filtering step and 45% of the activity retained after filtration. After anion-exchange chromatography step, only 3.1% recovery of bacteriocin was recorded. However, as shown in Table 3, considerable loss of protein concentration in each purification step

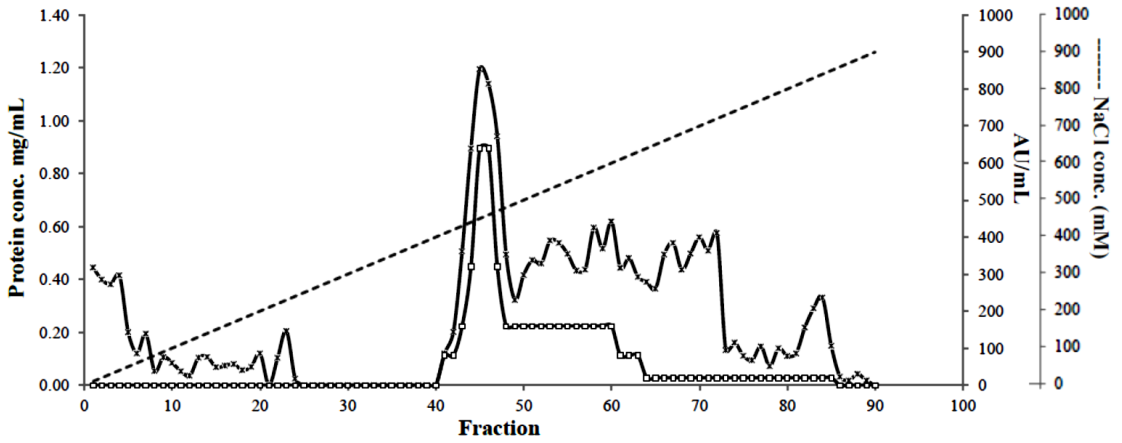

Figure 3 Gradient elution profile of Bacteriocin WX153 using FPLC system equipped with anion-exchange Q-FF column: solid line with asterisk (*) represent protein concentration (mg/mL) by Lowry-Folin method, solid line with square (□) represent bacteriocin activity by agar well diffusion assay, and dash line represent gradient elution of NaCl concentration (mM)

resulted in an increase in specific activity. Figure 3 shows the elution profile of crude antimicrobial compound using a FPLC system equipped with anion-exchange column. One major protein peak was detected with several minor peaks. All fractions in the major protein peak were found to exhibited antimicrobial activity against *S. suis* with the highest antimicrobial activity of 640 AU/mL detected in fractions 45 and 46.

DISCUSSION

Streptococcus suis is an important epidemic swine pathogen and also emerging zoonotic pathogen [1-3]. There are evidences of increasing resistance to antibiotics of pathogenic bacteria due to overuse and inappropriate use of antibiotic for treatment, prevention of disease and also for growth promotion in livestock [10, 22]. This inappropriate use of antibiotics leads to the spread of antibiotic resistant bacteria and antibiotic residual in meat [23]. Therefor the use of antibiotics in livestock was banned in many countries [7-10]. Antimicrobial compounds produced from microorganism now received more attention as alternatives to antibiotics [10, 11]. Bacteriocins are natural proteinaceous antimicrobial compounds produced from bacteria especially some lactic acid bacteria which were classified as GRAS (Generally Recognized As Safe) and usually used for food production [24, 25]. *Lactococcus lactis* subsp. *lactis* is one of GRAS lactic acid bacteria which has been used in food industry and as feed additive for livestock to promote animal health [25, 26]. *Lactococcus lactis* subsp. *lactis* WX153 isolated from swine feces was found to be a potential probiotic bacterium [17, 23]. This strain can be efficiently used to produce bacteriocin named Bacteriocin WX153 which inhibited the growth of *S. suis*. The pattern of growth and bacteriocin production by *Lc. lactis* WX153 indicated that Bacteriocin WX153 is a secondary metabolite bacteriocin as it was produced during exponential phase and had maximum level at stationary phase then decline at early dead phase Whereas the bacteriocin which display primary metabolite kinetic will be produced during the exponential growth phase and reach maximum level during the middle of the exponential phase to the beginning of stationary phase [27]. *Lactococcus lactis* subsp. *lactis* reported to produce either primary or secondary metabolite bacteriocin such as *Lc. lactis* subsp. *lactis* ST1 [28] and *Lc. lactis* subsp. *lactis* PD 6.9 [29] was produced the primary and secondary metabolite bacteriocins respectively. The antimicrobial activity of Bacteriocin WX153 was detected during the exponential phase and decreased after the cell entered late stationary phase to death phase. Reduction of bacteriocin production might due to the effect of extracellular protease secreted into the culture medium [29]. Loss of activity due to adsorption of bacteriocin to cell surface of producer strains has been reported in *Lc. lactis* subsp. *lactis* ST1 [28], *Lactobacillus plantarum* AMA-K [30] and *Lactococcus* sp. GM005 [31]. Further investigation is required to elucidate the causes of reduction in bacteriocin activity during the late stationary phase and death phase. Inactivation of Bacteriocin WX153 by Pronase E indicated proteinaceous nature of bacteriocin from *Lc. lactis* WX 153. Even though bacteriocins are protein, however, they are susceptible to a certain group of

proteolytic enzymes [29]. Nisin, a class I bacteriocin, is a food grade bacteriocin produced by *Lc. lactis* subsp. *lactis* and is generally known for its sensitivity to trypsin [29, 32, 33]. Since Bacteriocin WX 153 was inactivated by Pronase E but not trypsin, the bacteriocin from *Lc. lactis* WX153 may be differ from Nisin. Bacteriocin WX 153 may be classified as class II bacteriocin, The characteristics of bacteriocin in this group are heat stable, remained active in wide pH range and it has low molecular weight protein (< 10 kDa) [34].

Not only bacteriocin, lactic acid bacteria also produced other antimicrobial compounds including organic acids and hydrogen peroxide [22, 35]. In this experiment, the effect of organic acids and hydrogen peroxide was excluded by treatment of the cell-free supernatant with catalase and neutralized with NaOH, respectively. This is to confirm that the antimicrobial activity detected in the cell-free supernatant is not due to the effect of either hydrogen peroxide or organic acids but bacteriocin.

In mode of action experiment, *S. suis* exhibited a rapid decrease in viable cell counts after 1 h of Bacteriocin WX153 addition, but the reduction of *S. suis* less than 99.9% indicating it has bacteriostatic mode of action [36]. There are some bacteriocins which have bacteriostatic mode of action such as bacteriocin Bac UB9 [20] and Bozacin 14 [32] but, most of bacteriocin produced from lactic acid bacteria especially class-II bacteriocins has bactericidal mode of action [23, 32, 35, 37-39] because its hydrophobic residues in their structures interacts and inserts into the membrane of target bacteria, leading to pore formation and membrane leakage. [16, 39-41]. Moreover, the mode of action of bacteriocin is depended on the bacteriocin concentration. Zanfir et al. [35] showed that bacteriocin Acidophilin 801 had bacteriostatic effects when the concentration lower than 250 AU/mL but when it was over 250 AU/mL, Acidophilin 801 was exhibited the bactericidal mode of action. So, the mode of action of Bacteriocin WX153 needs to be elucidated at higher concentration to confirm its mode of action. Bacterocin WX 153 had broad antimicrobial spectrum against both Gram-positive and Gram-negative bacteria, whereas most of bacteriocin produced by lactic acid bacteria has narrow antimicrobial spectrum [12, 37, 38]. Additionally, there are some bacteriocins produced by lactic acid bacteria such as Bac UB9 [20], Bozacin 14 [32] and Enterocin E760 [42] can inhibit both Gram-positive and Gram-negative bacteria.

Purification of Bacteriocin WX153 was achieved by combining ammonium sulfate precipitation and FPLC system equipped with anion-exchange column. Precipitation with 60% ammonium sulfate saturation resulted in floating pellicle and become incompletely soluble in phosphate buffer. Similar observations of floating pellicle have been reported for bacteriocin Lactacin F produced by *Lactobacillus acidophilu*s 11088 [43] and lactobin A produced by *Lactobacilus amylovorus* LMG-P 13139 [44]. This might be due to the highly hydrophobic nature of bacteriocin, which is usually found in class II bacteriocin [11, 39, 45]. Because of the low solubility of the floating pellicle, the recovery percentage of the bacteriocin in aqueous fraction is quite low (Table 3). However, the purification using ammonium sulfate precipitation followed by anion exchange chromatography successfully purified Bacteriocin WX153. SDS-PAGE of active fractions eluted from anion exchange column was also carried out. A single protein band of around 6.5 KDa was observed. However, the band was not clearly visible and the data is not shown here. Bacteriocins with similar molecular weight as Bacteriocin WX 153 such as acidophilin 801 [35] and lactacin B [46] were also reported.

To avoid the use of antibiotics in animal husbandry, searching for new alternative antimicrobial compounds have been extensively studied. Cao et al. [15] reported the use of nisin for treatment of mastitis disease caused by *Straphylococcus aureus* in cattle. Lebel et al. studied the use of commercial nisin and a novel bacteriocin suicin to treat *S. suis* [16, 41]. Line et al. [42] found the significantly reduction of *Campylobacter jejuni* colonization in broiler chickens fed with bacteriocin. Cotter et al. [13] reported the inhibition of *Listeria monocytogenes* infection in mice which fed with a bacteriocin producing *Lactobacillus salivarus* UCC118. Therefore, further studies to evaluate the effectiveness of *Lc. lactis* subsp. *lactis* WX153 and Bacteriocin WX153 in prevention of *S. suis* infection are also essential.

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