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Isolation and optimization to enhance anti-*Streptococcus suis* bacteriocin production by *Lactobacillus plantarum* RB01-SO

Showpanish, K.[1], Sonhom, N.[1], Janyaphisan, T.[2], Woraprayote, W.[2] and Rumjuankiat, K. [1*]

[1]College of Agricultural Innovation and Food Technology, Rangsit University, Lak Hok, Mueang Pathum Thani District, Pathum Thani 12000, Thailand; [2]Functional Ingredients and Food Innovation Research Group, National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Thailand Science Park, Phahonyothin Road, Pathum Thani 12120, Thailand.

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Abstract One hundred and twenty lactic acid bacteria (LAB) isolated from traditional Thai fermented vegetable products were tested against *Streptococcus suis* an important food borne pathogen causing severe disease in pig farming and consumers. Only one isolate designated as "RB01-SO" inhibited *S. suis*, and also *Bacillus subtilis*, *Enterococcus faecalis*, *Lactobacillus sakei*, *Lactococcus lactis*, *Listeria innocua*, *Listeria monocytogenes*, *Micrococcus luteus*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Aeromonas veronii*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* Typhimurium and *Vibrio harveyi*. Inhibitory activities of RB01-SO cell free supernatant (CFS) were completely destroyed by various proteolytic enzymes including trypsin, α-chymotrypsin and pepsin, indicative of the proteinaceous or bacteriocin nature of the antimicrobial substance of RB01-SO. Bacteriocin production was highest when strain RB01-SO was cultured in MRS broth supplemented with 1% NaCl and initial pH of 7.0. Highest anti-*S. suis* activity of 400 AU/mL was obtained from the CFS after the bacterium was incubated at 30℃ for 12 h at the above mentioned condition. Anti-*S. suis* activity of the CFS still remained after freeze-drying, suggesting its stability under the drying process. LAB that produced anti-*S. suis* agent with promising characteristics were successfully screened and isolated and showed potential for use in the food and feed industries.

Keywords: Bacteriocin, *Sterptococcus suis*, Fermented vegetable products, Bacteriocin production

Introduction

Streptococcus suis is an important zoonotic pathogen, commonly found in the upper respiratory tract of pigs, especially the tonsils and nasal cavities (Yongkiettrakul *et al.*, 2019). Infection by *S. suis* is a major global problem in

***Corresponding Author:** Rumjuankiat K.; **Email:** kittaporn.r@rsu.ac.th

the swine industry (Zhang *et al.,* 2012b). *S. suis* infection is also of significant public health concern because it can be transmitted to humans by direct contact with swine or consuming pork by-products (Goyette-Desjardins *et al.,* 2014; Takeuchi *et al.,* 2017; Thu *et al.,* 2021). This disease can lead to bacteremia, sepsis, septic arthritis and meningitis with severe neurological sequelae, including permanent hearing loss (Gottschalk *et al.,* 2010; Kerdsin *et al.,* 2011; Takeuchi *et al.,* 2017). Human infections caused by *S. suis* have been reported in both Western and Asian countries including China, Thailand, Vietnam, Hong Kong and Japan (Arai *et al.,* 2015; Cheung *et al.,* 2008; Ho *et al.,* 2011; Ip *et al.,* 2007; Jiang *et al.,* 2020; Pachirat *et al.,* 2012; Takeuchi *et al.,* 2017; Wang *et al.,* 2005). In Thailand, the Bureau of Epidemiology, Ministry of Public Health, reported an annual incidence rate of 0.51/100,000 people in 2020 (accessed on 15 July 2021). Provinces with the highest recorded cases of infection were Lampang, Phayao, Uttaradit, Nakhon Ratchasima and Sukhothai

Antibiotics are the preferred treatment of choice to prevent *S. suis* infection in humans and swine (Seitz *et al.,* 2016). However, *S. suis* has shown antibiotic resistance (Yongkiettrakul *et al.,* 2019; Zhang *et al.,* 2012b). To prevent increased antibiotic resistance of this pathogenic bacterium, finding alternative therapies to replace antibiotics is necessary. Bacteriocins are a class of ribosomally synthesized antimicrobial peptides produced from lactic acid bacteria (LAB). They are usually antagonistic to genetically closely related and/or broad range organisms (Woraprayote *et al.,* 2016). LAB are a heterogeneous group of Gram-positive bacteria characterized by their capacity to convert sugars into lactic acid (Ullah *et al.,* 2017). LAB can be found and isolated from any environment that is rich in carbohydrates, including plants, fermented food and the mucous membranes of humans and animals (Florou-Paneri *et al.,* 2013; Liu *et al.,* 2014). LAB are an important group of bacteria that have multiple applications in the food and feed industries by providing health benefits for humans and animals (Hernández-González *et al.,* 2021). Bacteriocins produced from LAB are usually classified as generally recognized as safe (GRAS) since they can be easily degraded by proteolytic enzymes without regard to the occurrence of antibiotic-resistant bacteria (Todorov *et al.,* 2012). Therefore, bacteriocins are rapidly gaining attention as an alternative to prevent *S. suis* infection (Nandakumar and Talapatra, 2014; Zhang *et al.,* 2012b).

The objectives of this study were to isolate and identify LAB from Thai fermented vegetable products, and optimize the production of bacteriocins against *S. suis* for use as potential candidates for food and feed applications.

Materials and methods

Isolation of LAB from fermented vegetable products

LAB were isolated from six Thai fermented vegetable products including fermented spring onion (Ton-Hom-Dong), pickled lettuce (Phak-Kad-Dong), fermented spider weed (Phak-Sen-Dong), fermented garlic pear (Phak-Kum-Dong), pickled bamboo shoots (Hnomi-Dong) and fermented phak hnam (Phak-Hnam-Dong). Twenty-five gram samples were homogenized in 225 mL of sterile 0.85% normal saline buffer using a stomacher (400C stomacher circulator, Seward, England). All samples were 10-fold serially diluted and 100 µL of each dilution was uniformly spread directly onto de Man, Rogosa and Sharpe (MRS, Difco, USA) agar containing 0.5% $CaCO_3$ (Sigma-Aldrich, St. Louis, MO, USA), and incubated at 30 ℃ for 24 h (Bautista-Gallego *et al.*, 2013; Hwanhlem *et al.*, 2014). Single bacterial colonies producing a clear zone were selected and stored in 20% glycerol at -80 ℃.

Screening and determination of LAB producing antimicrobial substance

Screening of 120 LAB isolates producing antimicrobial substance (AMS) was carried out using the slightly modified spot-on-lawn method as described by Ennahar *et al.* (2001). Single colonies of LAB were cultured in MRS broth and incubated at 30 ℃ for 16 h without agitation. The cultures were centrifuged at 10,000 rpm at 4 ℃ for 10 min. Cell free supernatant (CFS) was collected and filtrated through a membrane filter (0.2 µm; Advantec, Japan). The CFS of LAB was two-fold serially diluted in sterilized distilled water. Ten microliters of each diluted sample were spotted onto the surface of a medium agar plate previously uniformly swabbed with an overnight culture of indicator strains, and adjusted to 0.5 McFarlane (Lalitha, 2004). The various indicator strains were propagated according to the growth conditions shown in Table 1. The *S. suis* bacteria were obtained from the Functional Ingredients and Food Innovation Research Group, National Center for Genetic Engineering and Biotechnology, Thailand. After overnight incubation, the titer of the inhibition zone was expressed as arbitrary units (AU/mL) (Afrin *et al.*, 2021).

Bacterial identification by MALDI-TOF MS analysis

LAB isolates were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Single colonies of LAB isolates were sequenced by Betagro Science Center Co., Ltd., Pathum Thani,

Thailand. In brief, a single colony was smeared onto a steel MALDI-TOF target plate and covered with 1 µL of ready to use matrix solution. The plate was dried at room temperature and automatically analyzed using a MALDI-TOF mass spectrometer (Microflex, Bruker, Germany). MALDI-TOF MS data were analyzed by Biotyper software to compare each sample mass spectrum to the reference mass spectra in the database. The identification scores were interpreted as described by Somsri *et al.* (2017).

Table 1. List of indicator strains and their growth conditions

Indicator strain[1/]	Medium[2/]	Growth condition
Gram-positive bacteria		
Bacillus subtilis ATCC 6633	TSB	30 ℃
Bacillus subtilis JCM 1465^T	TSB	30 ℃
Enterococcus faecalis JCM 5803^T	MRS	37 ℃
Lactobacillus sakei JCM 1157^T	MRS	30 ℃
Lactococcus lactis ATCC 19435	MRS	30 ℃
Listeria innocua ATCC 33090	TSB	37 ℃
Listeria monocytogenes ATCC 19115	TSB	37 ℃
Micrococcus luteus NBRC 12708	TSB	30 ℃
Staphylococcus aureus DMST 20635	TSB	37 ℃
Streptococcus suis NaH	THY	37 ℃
Streptococcus suis P1/7	THY	37 ℃
Streptococcus agalactiae 1611	TSB	30 ℃
Gram-negative bacteria		
Aeromonas veronii 1755	TSB	30 ℃
Escherichia coli ATCC 25922	TSB	37 ℃
Escherichia coli ATCC 35401	TSB	37 ℃
Escherichia coli F18	TSB	37 ℃
Escherichia coli O157:H7	TSB	37 ℃
Pseudomonas aeruginosa ATCC 27853	TSB	37 ℃
Salmonella Typhimurium ATCC 13311	TSB	37 ℃
Vibrio harveyi AQVH 01	TSB+1.5%NaCl	30 ℃

[1]/ATCC = American type culture collection, Rockville, Md, USA; JCM = Japanese collection of microorganisms, Wako, Japan; DMST = Department of medical sciences, Thailand; NBRC = NITE biological resource center, Chiba, Japan

[2]/MRS = De man, Rogosa and Sharpe (Difco, USA), TSB = Tryptic soy broth (Difco, France), THY = Todd-Hewitt broth (Oxoid, England) supplemented with 2% yeast extract (Difco, USA)

Effect of proteolytic enzymes on antimicrobial activity

The AMS was treated with three proteolytic enzymes, consisting of trypsin (pH 7.5), α-chymotrypsin (pH7.5) (Sigma-Aldrich, St. Louis, MO, USA) and pepsin (pH 3.0) (Fisher Scientific Inc., Pittsburgh, PA, USA) at a final concentration of 1 mg/mL. The enzymatic reaction was incubated at 37 ℃ for 3 h and consequently stopped by heating at 100 ℃ for 5 min (Gao *et al.*,

2010). The residual antimicrobial activity of CFS was evaluated by the spot-on-lawn method using *S. suis* P1/7 as an indicator strain. The antimicrobial activity of untreated CFS was defined as 100% residual activity.

Bacteriocin production in different growth media, pH and NaCl

To compare the efficiency of various culture media on bacteriocin production, an overnight culture (16 h) of LAB at approximately 9 log CFU/mL was inoculated (1%) into five different culture media including M17 (Oxoid Ltd., England), Brain Heart Infusion (BHI, Merck, Darmstadt, Germany), LAPT (10 g/L yeast extract, 15 g/L peptone, 10 g/L tryptone, 1 mL/L Tween 80 and 10 g/L glucose) (Marianelli *et al.,* 2014), de Man, Rogosa, and Sharpe (commercial MRS, Difco, USA) and modified MRS broth (mMRS) without Tween 80. All samples were incubated at 30℃ for 16 h without agitation. Finally, an aliquot of each sample was removed to test the AMS against *S. suis* P1/7 using the spot-on-lawn method as previously described.

The effect of pH and NaCl concentration on bacteriocin production was investigated according to the method of Mahrous *et al.* (2013) and Sarika *et al.* (2010), respectively. For pH assay, bacteriocin production was assessed in 10 mL of MRS broth, with pH adjusted to 2-10 by 6 M HCl or 6 M NaOH solution, whereas 1-12% NaCl concentration was added to MRS broth for NaCl assay. All treatments were then inoculated with 1% *Lb. plantarum* RB01-SO (9 log CFU/mL) and incubated at 30℃ for 16 h, without agitation. The RB01-SO strain was also cultured in MRS medium (pH 6.72) without any pH and NaCl adjustment and used as the control. Bacteriocin production was examined by the spot-on-lawn method as previously described.

Growth temperature profiling of Lb. plantarum RB01-SO

Production of anti-*S. suis* bacteriocins was evaluated according to the modified method of Huang *et al.* (2009) with slight modifications. In brief, 1% (v/v) of an overnight culture of *Lb. plantarum* RB01-SO was inoculated into MRS broth containing 1% NaCl (pH 7) and incubated at 30, 37 and 40℃ for 24 h. Throughout the incubation period an aliquot of culture broth was removed every 3 h to determine cell density by absorbance at 600 nm using a microplate reader (SpectraMax Plus384, Molecular Devices, USA). The drop plate method was performed in triplicate on MRS plates for bacterial viability enumeration (Herigstad *et al.,* 2001) and anti-*S. suis* activity was measured by the spot-on-lawn method as previously described.

Preparation of the anti-S. suis bacteriocin powder

The anti-*S. suis* substance was prepared by culturing RB01-SO in MRS at 30 ℃ for 12 h. The CFS was collected by centrifuging at 10,000 rpm at 4 ℃ for 10 min, and sterilized by heating at 100 ℃ for 10 min. The sample was then frozen in a 95% ethanol bath and freeze-dried under vacuum at -80 ℃, 0.1 mBar using a Labconco Corporation Cascade Console Freeze-Drying System (Labconco Corporation, Kansas, MO, USA) and kept at 4 ℃ before use (Woraprayote *et al.*, 2013; Zendo *et al.*, 2003). Anti-*S. suis* activity of freeze-dried bacteriocin was measured by the spot-on-lawn method, as described above. Protein content of powdered bacteriocin was determined according to Lowry *et al.* (1951).

Statistical analysis

Data were presented as mean ± standard deviation (SD) of duplicate determination from two independent experiments.

Results

Isolation, screening and evaluation of antimicrobial activity of AMS producing LAB

A total of 120 LAB colonies were collected from six Thai fermented vegetable products as shown in Table 2.

Table 2. LAB bacteriocins isolated from Thai fermented vegetables

Fermented product	Number of LAB isolates	Number of bacteriocin producing LAB	Name
Fermented spring onion	57	3	RB01-SO, RB08-SO, RSU-SO2
Pickled lettuce	0	0	-
Fermented spider weed	32	1	RB01-SW
Fermented garlic pear	19	0	-
Pickled bamboo shoots	0	0	-
Fermented phak hnam	12	0	-

These isolates were determined for antimicrobial substance (AMS) using neutralized cell free supernatant (CFS) against various indicator strains. Among these, four LAB isolates, namely RB01-SO, RB08-SO, RSU-SO2 and RB01-SW exhibited broad antimicrobial spectra against 12 Gram-positive bacteria

(*Bacillus subtilis* ATCC 6633, *B. subtilis* JCM 1465^T, *Enterococcus faecalis* JCM 5803^T, *Lactobacillus sakei* JCM 1157^T, *Lactococcus lactis* ATCC 19435, *Listeria innocua* ATCC 33090, *Listeria monocytogenes* ATCC 19115, *Micrococcus luteus* NBRC 12708, *Staphylococcus aureus* DMST 20635, *S. suis* NaH, *S. suis* P1/7 and *Steptococcus agalactiae* 1611) and eight Gram-negative bacteria (*Aeromonas veronii* 1755, *Escherichia coli* ATCC 25922, *E. coli* ATCC 35401, *E. coli* F18, *E. coli* O157:H7, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella* Typhimurium ATCC 13311 and *Vibrio harveyi* AQVH 01). However, the RB01-SO strain displayed anti-*S. suis* activity, as shown in Table 3.

Table 3. Antimicrobial spectra of isolated bacteriocins from fermented prodcuts

Indicator strain	Bacteriocin activity (AU/mL)			
	RB01-SO	**RB08-SO**	**RSU-SO2**	**RB01-SW**
Gram-positive bacteria				
Bacillus subtilis ATCC 6633	400	100	100	200
Bacillus subtilis JCM 1465^T	1,600	100	100	200
Enterococcus faecalis JCM 5803^T	1,600	800	4,800	400
Lactobacillus sakei JCM 1157^T	3,200	3,200	1,600	3,200
Lactococcus lactis ATCC 19435	6,400	1,600	3,200	3,200
Listeria innocua ATCC 33090	400	200	200	200
Listeria monocytogenes ATCC 19115	800	100	100	0
Micrococcus luteus NBRC 12708	800	100	100	100
Staphylococcus aureus DMST 20635	400	200	200	200
Streptococcus suis NaH	400	0	0	0
Streptococcus suis P1/7	200	0	0	0
Streptococcus agalactiae 1611	800	100	200	100
Gram-negative bacteria				
Aeromonas veronii 1755	200	100	100	100
Escherichia coli ATCC 25922	200	400	200	400
Escherichia coli ATCC 35401	200	100	100	0
Escherichia coli F18	100	0	0	0
Escherichia coli O157:H7	200	0	0	0
Pseudomonas aeruginosa ATCC 27853	100	100	100	100
Salmonella Typhimurium ATCC 13311	200	100	100	200
Vibrio harveyi AQVH 01	100	0	0	0

Bacterial identification by MALDI-TOF MS analysis

LAB species of four isolates including RB01-SO, RB08-SO, RSU-SO2 and RB01-SW were identified using MALDI-TOF MS. MALDI-TOF analysis for these isolates exhibited an intermediate score of ≥2.00-2.29 as a precise

identification between the genus and the species level. Therefore, the four isolates were identified as *Lactobacillus plantarum* (data not shown).

Sensitivity of bacteriocin to proteolytic enzymes

The effect of proteolytic enzymes on bacteriocin activity was investigated using *S. suis* P1/7 as an indicator strain. Sensitivities of AMS produced by *Lb. plantarum* RB01-SO to trypsin, α-chymotrypsin and pepsin are shown in Table 4. Inhibitory activity of *Lb. plantarum* RB01-SO was completely inactivated by these proteolytic enzymes, while the control treatment gave 200 AU/mL. Inactive antimicrobial activity after treatment with proteolytic enzymes indicated the proteinaceous nature of the bacteriocins.

Table 4. Antimicrobial activity of bacteriocins produced by *Lb. plantarum* RB01-SO after treatment with proteolytic enzymes

Proteolytic enzymes	**Residual activity[1/] (AU/mL)**
Untreated (control)	200
Trypsin	0
α-chymotrypsin	0
Pepsin	0

[1]/Residual activity was determined using *S. suis* P1/7 as an indicator strain

Effect of different growth media, pH and NaCl on bacteriocin production

Five growth media (M17, BHI, LAPT, MRS and mMRS) were determined for bacteriocin productivity using *S. suis* P1/7 as an indicator strain. Highest bacteriocin activity of *Lb. plantarum* RB01-SO was obtained at 200 AU/mL, cultured in commercial MRS and mMRS media at 30 ℃. However, *Lb. plantarum* RB01-SO did not produce bacteriocins in the other three media including M17, BHI and LAPT although viable cells of this strain were detected at approximately 9 log CFU/mL, as shown in Table 5.

Table 5. Bacteriocin production of *Lb. plantarum* RB01-SO in five culture media consisting of M17, BHI, LAPT, commercial MRS and modified MRS

Culture medium	**pH of CFS**	**Viable cell (log CFU/mL)**	**Anti-microbial activity (AU/mL)**
M17	6.22 ± 0.02[1/]	9.16 ± 0.10	0
BHI	4.99 ± 0.05	9.28 ± 0.09	0
LAPT	3.98 ± 0.01	9.26 ± 0.04	0
Commercial MRS	3.75 ± 0.01	9.64 ± 0.07	200
Modified MRS (mMRS)	3.86 ± 0.02	9.65 ± 0.12	200

[1]/Values are reported as the mean ± standard deviation

To investigate the effect of pH and NaCl concentration on bacteriocin production, *Lb. plantarum* RB01-SO was cultivated in MRS broth at various pH levels and 30 ℃ for 16 h (Table 6). Production of anti-*S. suis* activity was highest at 400 AU/mL, when RB01-SO was cultivated in MRS broth with initial pH of 7 (Table 6). Anti-*S. suis* bacteriocins were not produced in MRS broth at pH 2 since RB01-SO was not detected.

Table 6. Effect of pH and NaCl concentration of culture media on bacteriocin production produced *Lb. plantarum* RB01-SO

Sample	Viable cell (log CFU/mL)	Activity (AU/mL)
control	9.75±0.13[1/]	200
pH		
2	N.D.	0
3	6.19±0.04	100
4	9.00±0.14	100
5	9.78±0.07	200
6	10.05±0.02	200
7	9.70±0.22	400
8	9.67±0.19	200
9	9.85±0.29	200
10	9.44±0.07	100
NaCl concentration (%)		
1	10.09±0.15	400
2	10.21±0.08	200
3	10.11±0.06	200
4	10.46±0.02	200
5	9.78±0.07	100
6	9.56±0.07	100
7	9.64±0.15	100
8	6.97±0.10	100
9	7.36±0.08	100
10	7.15±0.03	100
11	6.95±0.05	100
12	7.11±0.10	100

[1]/Values are reported as the mean ± standard deviation; N.D. means not detected at the detection limit of 100 CFU/mL

The effect of NaCl concentration (1%-12%) in growth medium on AMS production was also studied. *Lb. plantarum* RB01-SO showed an increase in anti-*S. suis* activity (400 AU/mL) when cultivated in 1% NaCl concentration MRS broth, whereas the control (without NaCl addition) and 2%-12% NaCl added caused a decrease in anti-*S. suis* activity, as shown in Table 6.

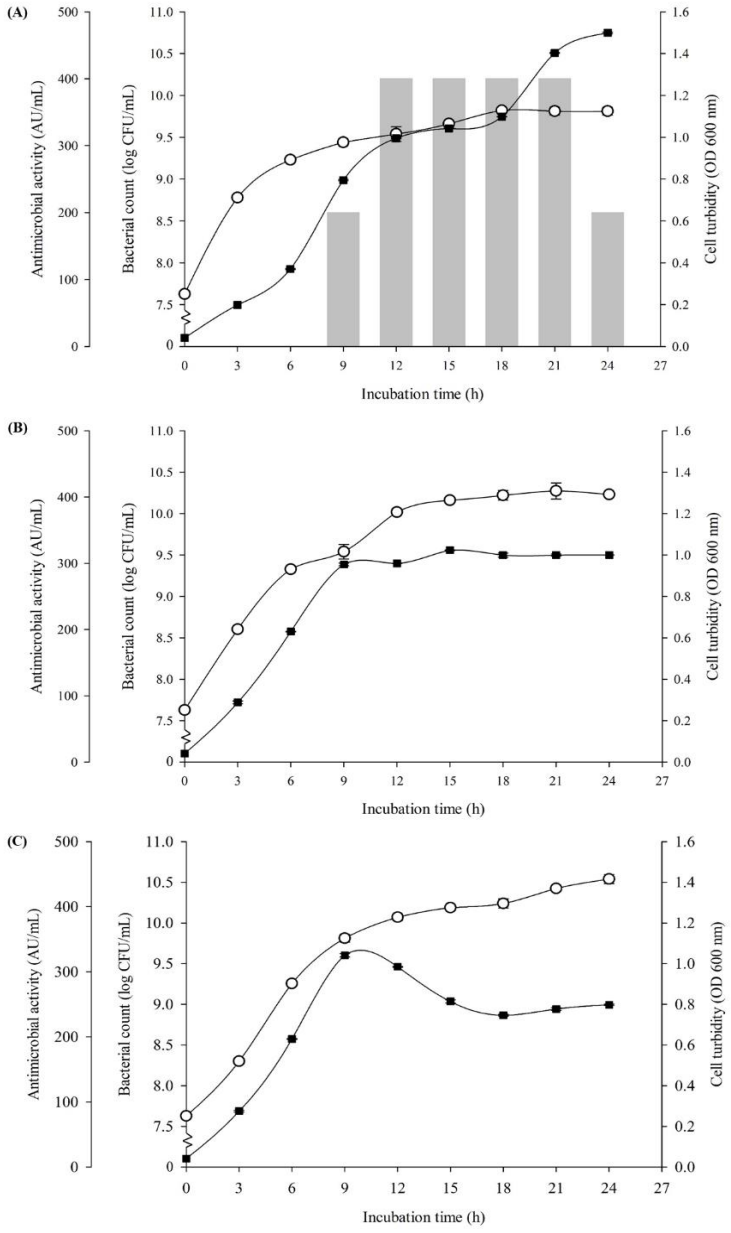

Figure 1. Growth profile of *Lb. plantarum* RB01-SO and anti-*S. suis* bacteriocin production at 30 ℃ (A), 37 ℃ (B) and 40 ℃ (C). Cellular growth (-o-), OD 600 nm (-■-) and bacteriocin production (bar). Data points are means of duplicate determinations from two independent experiments. Data are presented without standard deviation bars as there was very little variation (<5%)

Production of anti-S. suis bacteriocins at different temperatures

Lb. plantarum RB01-SO was tested in MRS broth containing 1% NaCl, pH 7 at various temperatures including 30, 37 and 40 ℃. Its antimicrobial activity against *S. suis* P1/7 was examined using the spot-on-lawn assay. As shown in Figure 1, viable cell counts of *Lb. plantarum* RB01-SO increased from 7.62 log CFU/mL to 9.81 log CFU/mL at 30 ℃ for 24 h after incubation, while bacteriocin activity was produced during late-exponential-growth phase to stationary growth phase (9-24 h). Highest antimicrobial activity (400 AU/mL) was recorded at 12 h and remained constant during 12-21 h after incubation. However, no bacteriocin activity was observed in the CFS, although *Lb. plantarum* RB01-SO was able to grow at 37 and 40 ℃.

Determination of antibacterial activity of freeze-dried anti-S. suis bacteriocins

Antimicrobial efficiency of anti-*S. suis* bacteriocins produced by *Lb. plantarum* RB01-SO was also determined after freeze-drying. Results showed that AMS produced by RB01-SO still exhibited antimicrobial activity against *S. suis* after passing through the freeze-drying process. Specific anti-*S. suis* activity of freeze-dried AMS was 78.74 ± 0.12 AU/mg.

Discussion

LAB have been used in food and feed for centuries, and play an important role in food fermentation and preservation (Chen *et al.,* 2010). Fermentation is one of the oldest methods for food preservation, through the process of digested grains and vegetables by Lactobacilli (Lal *et al.,* 2010). LAB enhance the preservation of fermented products and also promote nutrition by providing vitamins, minerals and bacteriocins (Choi *et al.,* 2013). Bacteriocins produced by LAB have attracted special interest because they are generally recognized as safe (GRAS) (Kumar *et al.,* 2017; Namasivayam *et al.,* 2014). Bacteriocin-producing bacteria can be isolated from various fermented vegetable products as *Lactobacillus brevis* (Kang and Kim, 2010), *Lactobacillus casei* (Ullah *et al.,* 2017), *Lb. plantarum* (Hu *et al.,* 2013; Zhao *et al.,* 2016), *Lb. sakei* (Gao *et al.,* 2010; Jiang *et al.,* 2012), *Lactobacillus spicheri* (Gautam and Sharma, 2015), *Lactococcus garvieae* (Gao *et al.,* 2015) and *Pediococcus pentosaceus* (Huang *et al.,* 2009; Shin *et al.,* 2008; Suganthi and Mohanasrinivasan, 2015). In this study, three LAB strains were isolated from fermented spring onion and identified as *Lb. plantarum* RB01-SO, RB08-SO and RSU-SO2, while other

LAB strains were isolated from fermented spider weed and identified as *Lb. plantarum* RB01-SW. The CFS of all isolates showed broad antimicrobial spectra against both Gram-positive and Gram-negative pathogens. However, only CFS of *Lb. plantarum* RB01-SO exhibited antimicrobial activity against *S. suis*. A few anti-*S. suis* producing strains have previously been reported including *Lactobacillus pentosus* (Zhang *et al.*, 2012b), *Lc. lactis* (LeBel *et al.*, 2013; Srimark and Khunajakr, 2015), *S. aureus* (Crupper *et al.*, 1997) and *S. suis* (Melancon and Grenier, 2003). To the best of our knowledge, this paper documents a newly reported anti-*S. suis* bacteriocin-producing *Lb. plantarum*. The antimicrobial substance (AMS) of *Lb. plantarum* RB01-SO was destroyed by three proteolytic enzymes including trypsin, α-chymotrypsin and pepsin, suggesting that the AMS produced was a proteinaceous antimicrobial substance, defined as a bacteriocin (Shin *et al.*, 2008; Srimark and Khunajakr, 2015).

Growth conditions of Lactobacilli including pH and temperature directly influenced bacteriocin production (Sabo *et al.*, 2014). Culture medium composition was also reported to significantly affect bacteriocin production (Dyaee and Luti, 2019). Here, the production of bacteriocins by *Lb. plantarum* RB01-SO was highest in MRS and mMRS media. Several studies reported MRS broth as an optimal culture medium for enhancing growth and bacteriocin biosynthesis by LAB since it was rich in organic nitrogen sources, with high sugar content as a carbon source and also contained vitamins and minerals (Cheigh *et al.*, 2002; Han *et al.*, 2011; Li *et al.*, 2002; Woraprayote *et al.*, 2015). Production of bacteriocins by other strains of *Lb. plantarum* was also performed in MRS broth (Todorov and Dicks, 2005; Mollendorff *et al.*, 2009). Bacteriocin production by *Lb. plantarum* ST13BR improved when grown in MRS broth compared with M17 broth and BHI broth (Todorov *et al.*, 2004), while removal of Tween 80 from the growth medium of *Lb. plantarum* YJG and *Lb. plantarum* ATM11 did not remarkedly affect their bacteriocin production (Han *et al.*, 2011; Thirumurugan *et al.*, 2015). This result concurred with our observation in *Lb. plantarum* RB01-SO.

pH, NaCl concentration, growth temperature and microbial growth phase all play an important role in bacteriocin production (Todorov *et al.*, 2012; Turgis *et al.*, 2016; Zhang *et al.*, 2012a). Several studies found that production of bacteriocins was pH-dependent (Todorov *et al.*, 2000; Jiménez-Díaz *et al.*, 1993; Kelly *et al.*, 1996). While pH of the growth medium also impacted the expression of bacteriocin biosynthetic genes and the associated absorption of bacteriocins on bacterial cell membranes of bacteriocin producers (Yang *et al.*, 2018). Generally, optimal pH of the culture medium for bacteriocin production by LAB ranged from pH 5.5 to 5.6 (Abbasiliasi *et al.*, 2017). The NaCl

concentration on bacteriocin production was reported to depend on the strain and isolation source of the bacteriocin producer (Delgado *et al.,* 2005a; Delgado *et al.,* 2007; Leal-Sánchez *et al.,* 2002). High concentration of NaCl reduced the productivity of bacteriocins by some LAB (Herranz *et al.,* 2001; Iyapparaj *et al.,* 2013). Optimal concentration of NaCl produced a stressful environment that enhanced bacteriocin production (Abbasiliasi *et al.,* 2017). Changes in environmental factors, especially temperature, significantly affected bacteriocin production by LAB (Delgado *et al.,* 2005b; Leal-Sánchez *et al.,* 2002; Mataragas *et al.,* 2004). Several studies observed that the optimal temperature for bacteriocin production was similar to the optimal temperature for bacterial growth (Abo-Amer, 2011; Mataragas *et al.,* 2003; Rajaram *et al.,* 2010). In our study, the strain RB01-SO grew at various temperatures ranging from 30 ℃ to 40 ℃. However, this strain only produced bacteriocins at 30 ℃. This result concurred with Iyapparaj *et al.* (2013) and Todorov *et al.* (2011). They reported that bacteriocin production was highest at 30 ℃ and decreased when growth temperature increased. At low temperature, bacterial growth rate decreased, facilitated by the accumulation and availability of important metabolites for bacteriocin biosynthesis (Abbasiliasi *et al.,* 2017).

Bacteriocin production was reported to be triggered by a quorum sensing mechanism which was highest at late log phase or early stationary growth phase (Sabo *et al.,* 2014; Martinez *et al.,* 2013). Here, production of anti-*S. suis* bacteriocins by *Lb. plantarum* RB01-SO was highest at the stationary growth phase and then decreased at 24 h of cultivation. Abanoz and Kunduhoglu (2018) and Todorov and Dicks (2006) reported that decrease in antimicrobial activity after 24 h of cultivation or at the extended stationary phase may be caused by the action of proteolytic enzymes secreted by the producer.

Stability of the bacteriocin RB01-SO during freeze-drying suggested the feasibility of using this downstream drying process to produce bacteriocin powder for use as a natural food and feed preservative.

In conclusion, a bacteriocinogenic *Lb. plantarum* RB01-SO isolated from traditional Thai fermented vegetable produced bacteriocins with antimicrobial activity against *S. suis* and other Gram-positive and Gram-negative pathogens, and showed economic potential for food and animal farming. The optimal condition for production of anti-*S. suis* bacteriocin was culture in MRS broth supplemented with 1% NaCl and initial pH of 7.0. Stability of the bacteriocin after the freeze-drying process suggested feasibility for use as a bacteriocin powder, with natural preservative application in the food and feed industries.

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