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Original Article

Characterization of an antimicrobial substance produced by *Lactobacillus plantarum* NTU 102

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KEYWORDS
Lactobacillus plantarum NTU 102;
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Abstract *Background/Purpose:* Lactic acid bacteria (LAB) are used in a variety of bio-industrial processes, including milk fermentation, and have been reported to have bactericidal activities. We previously isolated *Lactobacillus plantarum* NTU 102 from homemade Korean-style cabbage pickles. The aims of this work were to perform a screen of the antimicrobial substances produced by *L. plantarum* NTU 102 and to characterize it.
Methods: In this study, we investigated the bactericidal activity of this LAB strain and demonstrated that the cell-free supernatant of *L. plantarum* NTU 102 had antimicrobial activity.
Results: The antibacterial activity was significantly decreased by proteolytic enzymes, including pepsin, proteinase K, and trypsin, suggesting that the antimicrobial substance had proteinaceous properties. Additionally, this activity was heat stable and not affected by alterations in the pH from 1.0 to 4.0. The antibacterial substance produced by *L. plantarum* NTU 102, which we named LBP102, exhibited a broad inhibitory spectrum. The active compound was identified by nuclear magnetic resonance (NMR) techniques (^{1}H NMR and ^{13}C NMR). The IUPAC name was 2-(2-1 mino-1-hydroxyethoxy) ethyl 2-methylpropanoate. The substance showed antibacterial activity against *Vibrio parahaemolyticus*, and completely inhibited the growth of *V. parahaemolyticus* on agar plates at a concentration of 75 μg/mL.
Conclusion: The proposed antimicrobial substance, LBP102 was found to be effective against *V. parahaemolyticus* BCRC 12864 and *Cronobacter sakazakii* BCRC 13988. The remarkable effects of LBP102 against this and other pathogens indicated its potential as a natural preservative/food additive.

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Introduction

Lactic acid bacteria (LABs) are widely employed in fermented milk, in industrial fermentation, and as starter cultures in the dairy industry.[1] LAB-mediated fermentation is believed to be the oldest means of food preservation and has been shown to suppress the microbe-dependent spoilage of food and prolong the shelf life of foods.[2] LABs produce various compounds, such as organic acids (acetic, propionic, butyric, and formic acid), hydrogen peroxide, diacetyl, antifungal compounds (fatty acids and phenyllactic acid), and different bacteriocins to inhibit pathogenic bacteria and bacteria responsible for food spoilage.[3] These bactericidal substances exert their activities through a range of different mechanisms. However, many of these compounds have not yet been identified or characterized.[4]

Since LABs and its by-products were classified as Generally Regarded as Safe (GRAS) as a food ingredient, the bacteriocins produced by LABs are of particular interest to the food industry.[5] For food application, bio-preservation refers to the use of antagonistic microorganisms or their metabolic products to inhibit (or destroy) undesired microorganisms in food products, thereby enhancing food safety and extending the shelf life of foods. There are three approaches for potential application of LABs and bacteriocins for biopreservation of foods in the food industry: inoculation of LABs that produce the bacteriocin into foods during processing; application of the purified or crude bacteriocin directly onto the food product; and applications of a previously fermented product from a bacteriocin-producing strain.[4,6]

The previous studies have indicated that bio-protective LABs isolated from meat and meat products can contribute to the safety of fermented meat products through bacteriocin production.[7] The antimicrobial activities of bacteriocins are affected by the number of microorganisms in food, conditions of the application, interaction with/inactivation by food components, and pH and temperature of the product.[8] Previous studies have helped clarify the effects of specific conditions from food environments on the production of bacteriocins. Moreover, bacteriocin titers can be dramatically changed by altering environmental conditions, and optimum production may require a certain combination of influencing factors.[9]

In a previous study, we isolated *Lactobacillus plantarum* NTU 102 from homemade Korean-style cabbage pickles. In our previous studies, this strain showed good survival at low pH, tolerance to high bile concentrations, pathogen inhibition activity, and good ability to decrease low-density lipoprotein cholesterol (LDL-C) to high-density lipoprotein cholesterol (HDL-C) ratios.[10,11] *L. plantarum* NTU 102 also showed the ability to induce immune modulation and enhance the immune ability of *Litopenaeus vannamei*. This strain also increased its resistance to *Vibrio alginolyticus* infection.[12] However, the precise factors responsible for these effects are unknown.

The aims of this work were to perform a screen of the bacteriocins produced by *L. plantarum* NTU 102 and to characterize this antibacterial substance and its potential applications in the food industry.

Materials and methods

Microorganisms

L. plantarum NTU 102 was grown in MRS broth (Difco, USA) at 37 °C for 24 h. The source and growth media of indicator strains used in this study are shown in Table 1. Before testing, cultures from frozen stocks (−80 °C) were grown for 24 h in suitable media.

Preparation of the cell-free supernatant (CFS)

L. plantarum NTU 102 was incubated in MRS (de Man, Rogosa and Sharpe) broth at 37 °C for 24 h. Bacterial cells were removed by centrifugation at 8000*g* at 4 °C for 5 min. The CFS was filtered through a sterilized 0.22-μm Millex-GV filter (Millipore, Billerica, MA, USA) and stored at 4 °C.

Extraction of the antimicrobial substance from *L. plantarum* NTU 102

In this study, the solvent extraction method was modified to concentrate the antimicrobial substance from the CFS.[13] The CFS (1000 mL) was concentrated by lyophilization and rehydrated with 100 mL phosphate buffer (0.06 M Na_2HPO_4, 0.03 M KH_2PO_4, pH 4.0). After ethyl acetate extraction, the water layer was discarded, and the ethyl acetate in the organic layer was removed by a rotary vacuum evaporator (Buchi, Flawil, Switzerland). Fractions with antimicrobial activity were fractionated by silica gel G60 column chromatography (30 mm ID × 400 mm L) using the mobile phase isoamyl alcohol: EtOH (6:1) and hexane: ethyl acetate (1:5). Antimicrobial activities of the fractions were preliminarily screened by well diffusion method. We then carried out isolation and characterization of LBP102, the antimicrobial substance from this LAB strain.

Antimicrobial assay

The antimicrobial activities of the *L. plantarum* NTU 102 CFS was assessed against gram-negative bacteria, gram-

Table 1 The indicator strains used in this study.

Strains	Source[a]	Media[b]
Staphylococcus aureus BCRC 14958	BCRC	TSB
Streptococcus mutans BCRC 15255	BCRC	BHI
Klebsiella pneumoniae subsp. *pneumoniae* BCRC 10692	BCRC	TSB
Salmonella enterica subsp. *enterica* BCRC 10747	BCRC	TSB
Vibrio parahaemolyticus BCRC 12864	BCRC	TSB
Pseudomonas aeruginosa BCRC 11864	BCRC	TSB
Cronobacter sakazakii BCRC 13988	BCRC	TSB
Candida albicans BCRC 20511	BCRC	YM

[a] BCRC, Bioresource Collection and Research Center, Hsinchu, Taiwan.
[b] TSB, Tryptic Soy Broth; BHI, Brain Heart Infusion; YM, Yeast Malt Broth.

positive bacteria, and yeast (Fig. 1). A modified agar well diffusion method was used to evaluate antimicrobial activities.[14] The cultures, containing approximately 10^9 colony-forming units (CFU)/mL of indicator bacteria, were inoculated on Mueller-Hinton agar plates (Difco). Wells (7 mm in diameter) were punched in the plates using a sterile 1-mL micropipette tip. The wells, which were bored into the agar plate, were filled with 60 μL of CFS extract and then allowed to diffuse into the agar during a 4-h pre-incubation period at room temperature and incubated aerobically overnight at 37 °C for 24 h. The antimicrobial activities were determined by the inhibition zone around the wells. The percentage of growth inhibition was calculated using the formula: I (%) = Hinton (Inhibition zone of treated CFS/Inhibition zone of untreated CFS) × 100.

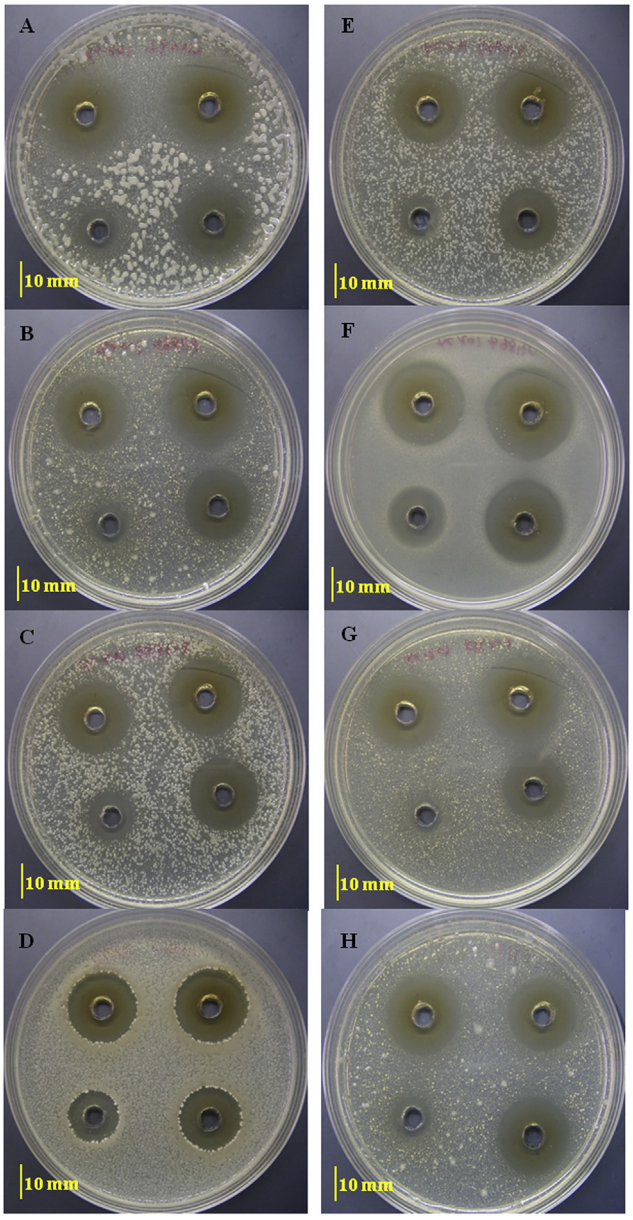

Figure 1. Antibacterial activity of the *Lactobacillus plantarum* NTU 102 cell-free supernatant (CFS) against pathogenic strains: (A) *Klebsiella pneumoniae* subsp. *pneumoniae* BCRC 10692, (B) *Pseudomonas aeruginosa* BCRC 11864, (C) *Cronobacter sakazakii* BCRC 13988, (D) *Candida albicans* BCRC 20511, (E) *Salmonella enterica* subsp. *enterica* BCRC 10747, (F) *Vibrio parahaemolyticus* BCRC 12864, (G) *Staphylococcus aureus* BCRC 14958, and (H) *Streptococcus mutans* BCRC 15255. The wells contained 60 μL CFS, and the concentrations of the CFS were 200 mg/mL (upper right well), 150 mg/mL (upper left well), 100 mg/mL (bottom right well), and 75 mg/mL (bottom left well).

Sensitivity of antimicrobial substance to protease, pH values, and heat treatment

The heat sensitivity of the *L. plantarum* NTU 102 CFS and LBP102 were investigated with heat treatment at 30, 50, 70, 100 and 121 °C (autoclave) for 15 min. The effects of protease on antimicrobial activity were determined by incubation of the CFS with 1 mg/mL proteinase K, trypsin, and pepsin (Sigma–Aldrich, St. Louis, MO, USA) at 37 °C for 2 h. For the pH stability assay, the CFS was adjusted to pH values ranging from 1.0 to 11.0 by 1 M HCl or 1 M NaOH. After pH adjustments, samples were filtered through a sterilized 0.22-μm Millex-GV filter (Millipore), and antimicrobial activity was determined by the agar well diffusion method.

Minimum inhibitory concentration (MIC) assay

Seventy-five microliters of diluted indicator microbial culture (10^6 CFU/mL) was deposited into each well of a 96-well plate for MIC assays.[15] The CFS extract was serially diluted, and 75 μL of each dilution was added per well. The 96-well plates were incubated aerobically at 37 °C overnight, and optical densities were measured at 600 nm with an enzyme-linked immunosorbent assay (ELISA) reader (Thermo Lab system, Waltham, MA, USA).

Identification of the antimicrobial substance LBP102

For chromatographic separation, a Luna C18 column (25 cm × 4.6 mm i.d., particle size 5 μm, Phenomenex, Torrance, CA, USA) was used at 35 °C. When the analytical HPLC column was used, it sufficed to collect enough material for NMR confirmation of the structure expected. The mobile phases were ethyl acetate with 0.1% formic acid (A) and hexane with 0.1% formic acid (B). The active fraction was eluted with a linear gradient of solvent A decreasing from 5% to 0% at a flow rate of 1 mL/min for 20 min. The detectors for high-performance liquid chromatography (HPLC) were PDA (220–450 nm) and fluorescence (excitation wavelength, 340 nm; emission wavelength, 450 nm). The structure of the active fraction was determined using a Bruker A VIII 500 MHz Fourier transform-nuclear magnetic resonance (FT-NMR) instrument (Bruker, Billerica, MA, USA) in the Instrumentation Center of National Taiwan University. NMR spectra were recorded on a spectrometer equipped with a 2.5-mm microprobe using methanol-d4 (CD_3OD) as a solvent. Chemical shifts are reported relative to the solvent peaks (methanol-d4 ^1H δ3.3 and ^{13}C δ49.2).

RT-PCR analysis

Total RNA was extracted from the cells of *Vibrio parahaemolyticus* BCRC 12864.[16] Reverse transcription polymerase chain reaction (RT-PCR) was performed with SuperScript™ III First-strand Synthesis SuperMix (Invitrogen, Carlsbad, CA, USA). The Quantitative PCR was performed with Platinum® SYBR® Green qPCR SuperMix-UDG w/ROX (Invitrogen) in an ABI StepOne® (Life Technologies, Grand Island, NY, USA). The primers were vmeB RT-F, vmeB RT-R; vmeD RT-F, vmeD RT-R; 16S rRNA RT-F, 16S rRNA RT-R.[17] The reaction mix included template cDNA (100 ng), 200 nM of each primer, and SYBR-Green mix.[18] The expression levels of the genes tested were normalized relative to the expression of the 16S rRNA gene of *V. parahaemolyticus*.

Statistical analysis

Statistical comparisons were performed using one-way analysis of variance (ANOVA) with Duncan's test. Differences with p values of less than 0.05 were considered statistically significant.

Results

Antimicrobial activity spectrum of the CFS

The CFS of *L. plantarum* NTU 102 displayed potential antimicrobial activity against *Staphylococcus aureus* BCRC 14958, *Streptococcus mutans* BCRC 15255, *Klebsiella pneumoniae* subsp. *pneumoniae* BCRC 10692, *Salmonella enterica* subsp. *enterica* BCRC 10747, *V. parahaemolyticus* BCRC 12864, *Pseudomonas aeruginosa* BCRC 11864, *Cronobacter sakazakii* BCRC 13988, and *Candida albicans* BCRC 20511. Among these pathogens, the CFS of *L. plantarum* NTU 102 (75 mg/mL) had strong antimicrobial activity against *K. pneumoniae* subsp. *pneumoniae* and *V. parahaemolyticus* (Fig. 1). Analysis of the antimicrobial activities of this CFS against gram-positive bacteria, gram-negative bacteria, and yeast revealed that this preparation had a broad inhibitory spectrum.

Effects of proteases, pH, and heat treatments on the antimicrobial activity of the antimicrobial substance

Next, we analyzed the effects of heat, pH, and enzymes on the bacteriocin activity of *L. plantarum* NTU 102 CFS against *V. parahaemolyticus* BCRC 12864 (Table 2). The antimicrobial activity of this sample was significantly decreased by all the proteolytic enzymes tested here, including pepsin, proteinase K, and trypsin. These results suggested that the antibacterial substance in the *L. plantarum* NTU 102 CFS had proteinaceous characteristics. Interestingly, when we tested the effects of pH on the antibacterial activities of this sample, we found that the antimicrobial activity remained constant within the pH range from 1.0 to 4.0. Complete loss of activity was observed at pH values ranging from 5.0 to 11.0. Although the antimicrobial activity was completely lost after 15 min of treatment at 121 °C, it was relatively stable at 30, 50, 70, and 100 °C for 15 min.

MIC assays

Table 3 shows the MIC values of LBP102 against *S. aureus*, *S. mutans*, *K. pneumoniae*, *S. enterica*, *V. parahaemolyticus*, *P. aeruginosa*, *C. sakazakii*, and *C. albicans*. The MIC values varied depending on the strain of bacteria, and methods for the determination. In this study, we used a modified assay[15] to evaluate MIC values. Among the test strains, *V. parahaemolyticus* and *C. sakazakii* were more sensitive to LBP102, whereas *S. mutans*, *K. pneumoniae* subsp. *pneumoniae*, and *S. enterica* subsp. *enterica* exhibited moderate sensitivity.

To investigate mode of action of the antibacterial substance produced by *L. plantarum* NTU 102, LBP102 (5, 25, 50, 75, 100, 125, and 150 μg/mL) was added to culture of *V. parahaemolyticus* suspended in MH broth. The number of viable cells was determined by standard plate counting after 24 h of incubation (Fig. 2). Increasing concentrations of the LBP102 led to a marked decrease in the number of viable *V. parahaemolyticus* cells. For the control culture, viable cell counts increased from 5 log units when the absorbance was 3.92 at 600 nm. The viable cell counts of *V. parahaemolyticus* decreased to 3 log units of after exposure to 50 μg/mL LBP102 when the absorbance at 600 nm was 2.02. However, a reduction in the viable cell count was observed in the presence of 75 μg/mL when the absorbance at 600 nm was 1.05. Thus, this result indicated that LBP102 had bactericidal activity.

Table 2 The effects of enzymes, pH, and heat treatment on antimicrobial activity of the *Lactobacillus plantarum* NTU 102 CFS.

Treatment		Inhibition zone (diameter in mm)[a,b]	The percentage of growth inhibition, I (%)[c]
Control		9.0	100
Enzymes	Pepsin	1.2	13*
	Trypsin	3.2	36*
	Proteinase K	3.0	33*
Heat	30	9.0	100
(°C, 15 min)	50	8.5	94
	70	8.0	89
	100	8.0	89
	121	—	—
pH	1.0	9.0	94
	2.0	8.5	94
	3.0	8.0	88
	4.0	8.0	82
	5.0	—	—
	6.0	—	—

[a] *Vibrio parahaemolyticus* BCRC 12864 was used as the indicator strain.
[b] The inhibition zone (diameter in mm) does not include the disc diameter (7 mm). —, no inhibition zone.
[c] I (%) = (Inhibition zone of treated CFS/Inhibition zone of untreated CFS) × 100.
Significance: *$p < 0.05$ vs. control.

Table 3 Minimum inhibitory concentrations (MICs) of the *Lactobacillus plantarum* NTU 102 CFS and antimicrobial substance LBP102.

	MIC (μg/mL)	
	LBP102	NTU 102 CFS
Staphylococcus aureus BCRC 14958	70.0	3350.0
Streptococcus mutans BCRC 15255	35.0	877.5
Klebsiella pneumoniae subsp. *pneumoniae* BCRC 10692	35.0	3350.0
Salmonella enterica subsp. *enterica* BCRC 10747	35.0	1775.0
Vibrio parahaemolyticus BCRC 12864	17.5	1775.0
Pseudomonas aeruginosa BCRC 11864	70.0	>3350.0
Cronobacter sakazakii BCRC 13988	17.5	1775.0
Candida albicans BCRC 20511	70.0	3350.0

CFS: the cell-free supernatant.
LBP102: the antimicrobial substance purified from the CFS.

Identification of the antimicrobial substance LBP102

From a total of 1 L of culture, approximately 12 mg of the antibacterial substance was obtained. This substance was identified by HPLC. The HPLC retention time was 10.24 min (Fig. 3). The active fraction was subjected to NMR analysis. The chemical shifts of ^{1}H NMR (500 MHz, methanol-d4) were δ1.22 (H-a), 3.61 (H-b), 4.08 (H-c), 2.00 (H-d), 4.22 (H-e), and 1.36 (H-f), while that of ^{13}C NMR (500 MHz, methanol-d4) were δ20.48 (C-1), 30.32 (C-2), 178.42 (C-3), 68.89 (C-4), 71.22 (C-5), 62.02 (C-6), 49.17 (C-7), and 17.20 (C-8) (Fig. 4). These data were analyzed using the NMRShiftDB web site (http://nmrshiftdb.nmr.uni-koeln.de/), including the spectrum of ^{13}C, ^{1}H, ^{15}N, ^{31}P, and other nuclei, spectral patterns, structures, and other properties. The similarities of ^{13}C and ^{1}H were about 81.07% and 63.65% respectively. The prediction of LBP102 was identified as shown in Fig. 4C. The chemical structure was drawn by using MarvinSketch (http://www.chemaxon.com/products/marvin/) and the IUPAC name was 2-(2-1 mino-1-hydroxyethoxy) ethyl 2-methylpropanoate.

RT-PCR analysis of the RND-type efflux transporter genes

We investigated the expression of Resistance nodulation cell division (RND)-type efflux transporter genes in *V. parahaemolyticus* BCRC 12864 using the RT-PCR method. RND-type efflux transporters play the main role in intrinsic resistance to various antimicrobial agents in many gram-negative bacteria. In this RND family, VmeCD and VmeAB are central players of *V. parahaemolyticus*. The transporters gene encoded by *vmeB*, and *vmeD*. The LBP102 at 17.5 μg/mL significantly suppressed expression of *vmeB*, and *vmeD* compared with expression in the untreated control ($p < 0.05$) (Fig. 5).

Discussion

We previously isolated *L. plantarum* NTU 102 from homemade Korean-style cabbage pickles. In this study, we investigated the bactericidal activity of this LAB strain.

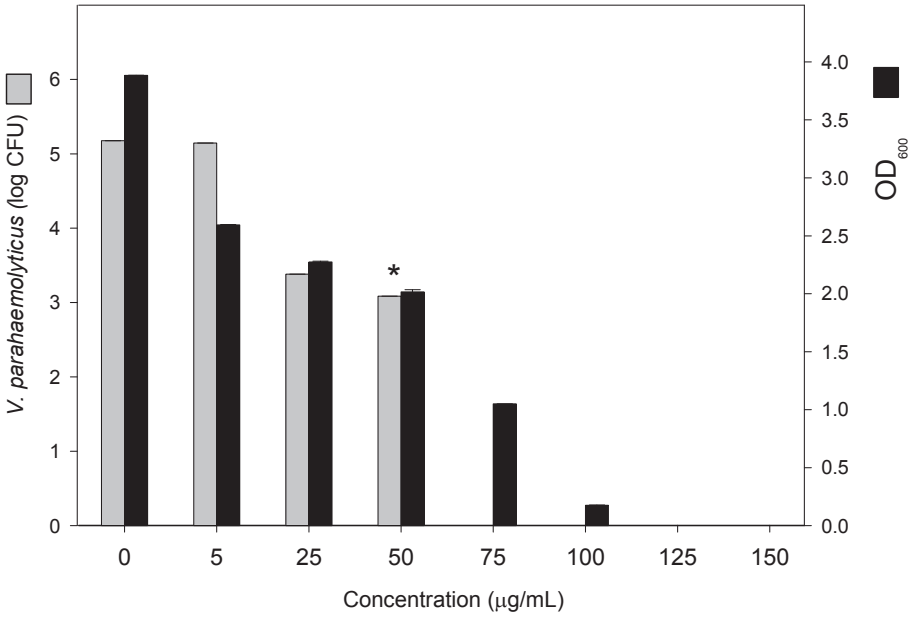

Figure 2. Viable cell counts of *V. parahaemolyticus* BCRC 12864 during growth in MH broth in the presence of the antibacterial substance produced by *Lactobacillus plantarum* NTU 102. ■: viable counts; ■: absorbance at 600 nm. Significance: *$p < 0.05$ vs. control.

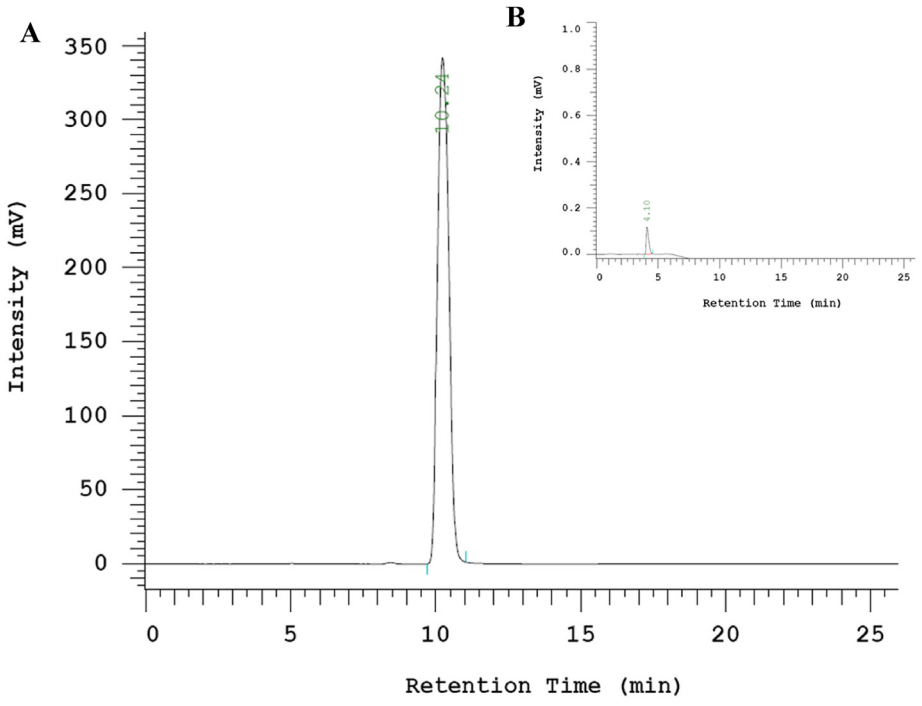

Figure 3. HPLC profile of the antimicrobial substance LBP102 produced by *Lactobacillus plantarum* NTU 102. (A) The detectors for HPLC were fluorescence (excitation wavelength: 340 nm, emission wavelength: 450 nm) and (B) PDA (220—450 nm).

Interestingly, we demonstrated that the cell-free supernatant of *L. plantarum* NTU 102 had antimicrobial activity.

Many *L. plantarum* strains have been reported to produce antibacterial substances (bacteriocins) called plantaricins.[19] These compounds are highly diverse in their activities and structures and have been shown to be particularly active against food-borne pathogens and gastrointestinal pathogens.[20] A previous study reported the characterization of plantaricin D, produced by *L. plantarum* BFE 905, from ready-to-eat salad.[21] Additionally, a number of bacteriocins, such as plantaricin A, B, and C, have been shown to be produced by *L. plantarum*,[22] and the bacteriocin produced by *L. plantarum* BS has activity against *S. aureus* and *Escherichia coli* O157:H7. Many bacteriocins isolated from LABs are active against gram-positive bacteria, while gram-negative bacteria generally exhibit little sensitivity to bacteriocins. The difference in resistance between gram-positive and gram-negative may be due to differences in the cell envelopes.[23]

In our previous studies, we successfully isolated *L. plantarum* NTU 102 from homemade Korean-style cabbage pickles. This strain showed tolerance to acid and bile, exhibited potent antimicrobial activities, and had decreased LDL-C/HDL-C ratios.[10,11] Moreover, this strain also induced immune modulation, enhanced the immune ability of *L. vannamei* (whiteleg shrimp, also known as Pacific white shrimp), and increased the resistance of *L. vannamei* to *V. alginolyticus* infection.[12] Importantly, the *L. plantarum* NTU 102 CFS had broad-spectrum activity against gram-positive bacteria, gram-negative bacteria, and even yeast (Fig. 1). Therefore, the antibacterial substance produced by *L. plantarum* NTU 102 may have potential applications as a bio-preservative and food additives.

Some LAB bacteriocins exhibit strong heat stability. For example, plantaricin LP84 remains stable, with no loss of activity, after 20 min of treatment at 121 °C and brevicin SD-22 maintains bacteriocin activity after autoclaving at 121 °C for 15 min. The heat stability of bacteriocin is thought to be due to the formation of small globular structures, strongly hydrophobic regions, and stable cross-linkages.[24] Because the antibacterial substance isolated from *L. plantarum* NTU 102 exhibited heat stability, it may be useful as a food preservative during the manufacturing of processed foods subjected to high heat treatment.

Enzyme sensitivity varies depending on the type of bacteriocin. Enterocins produced by *Enterococcus* spp. and plantaricin produced by *L. plantarum* ST28MS are sensitive to proteolytic enzymes, such as proteinase K, trypsin, papain, and pepsin, whereas bacteriocin activity is not affected by lipase, lysozyme, and catalase.[25] Thus, bacteriocins are probably degraded by digestive enzymes in the gastrointestinal tract and should therefore be nontoxic. Furthermore, a number of bacteriocins produced by LAB are relatively stable under treatment with organic solvents (chloroform, acetonitrile, methanol, ethanol, and isopropanol) and conditions such as storage at −20 °C, 4 °C, or room temperature. There are bacteriocins that exhibit stable activity at acidic, neutral, and even alkaline pH values. The antibacterial activity of bacteriocin produced by *Lactobacillus curvatus* SE1 is stable at pH 2.0—11.0.[26] The antibacterial substance LBP102 which was identified as shown in Fig. 4C, and the structure revealed partial

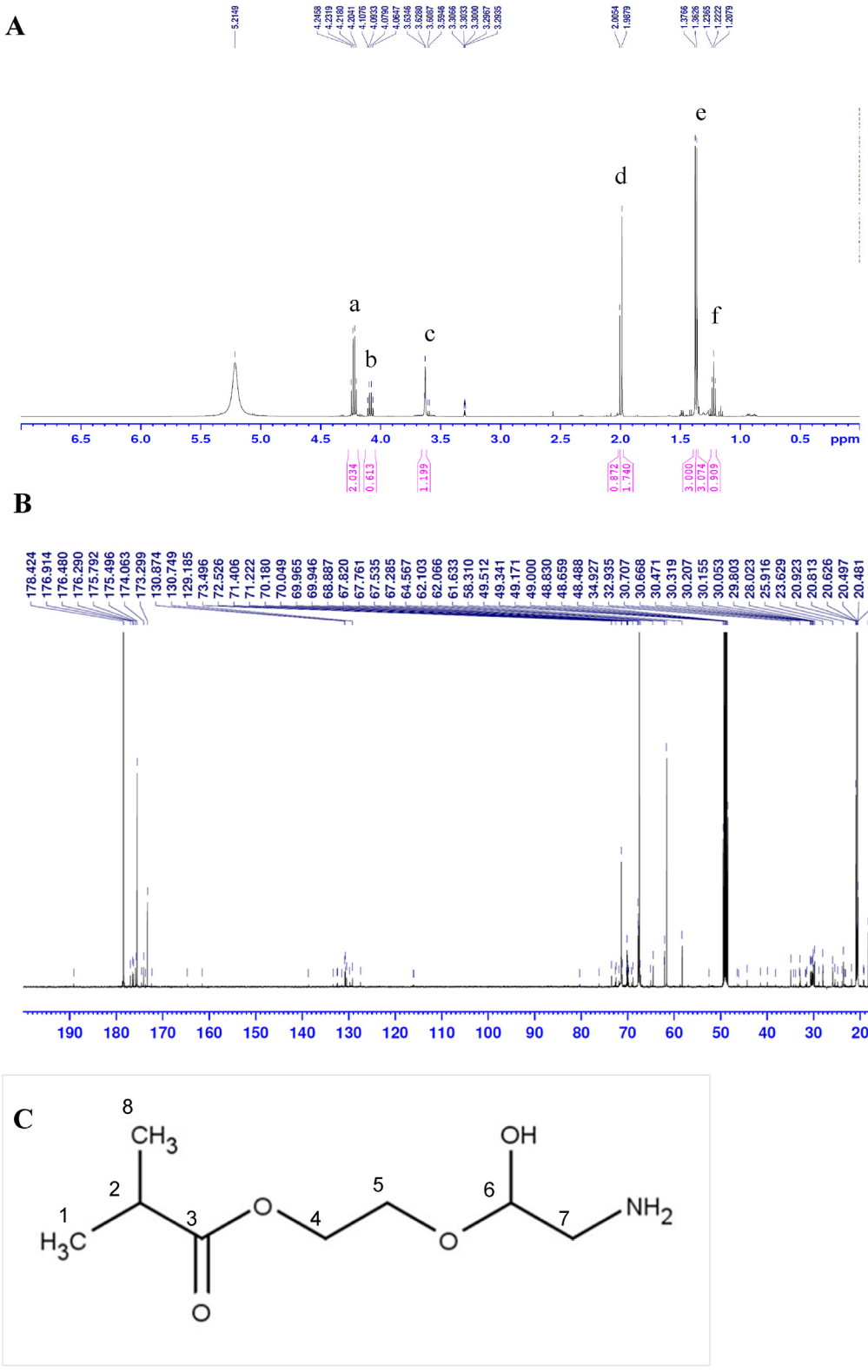

Figure 4. NMR profile of the antimicrobial substance LBP102 produced by *Lactobacillus plantarum* NTU 102. The NMR spectra were recorded on a spectrometer equipped with a 2.5-mm microprobe. The active fraction was dissolved in d solvent. The chemical shifts are reported relative to the solvent peaks (methanol-d4 ^{1}H δ3.3 and ^{13}C δ49.2). The chemical shifts of ^{1}H NMR (500 MHz, methanol-d4) (A) were δ1.22 (H-a), 3.61 (H-b), 4.08 (H-c), 2.00 (H-d), 4.22 (H-e), and 1.36 (H-f), while that of ^{13}C NMR (500 MHz, methanol-d4) (B) were δ20.48 (C-1), 30.32 (C-2), 178.42 (C-3), 68.89 (C-4), 71.22 (C-5), 62.02 (C-6), 49.17 (C-7), and 17.20 (C-8). (C) The prediction of LBP102 was identified and the chemical structure was drawn.

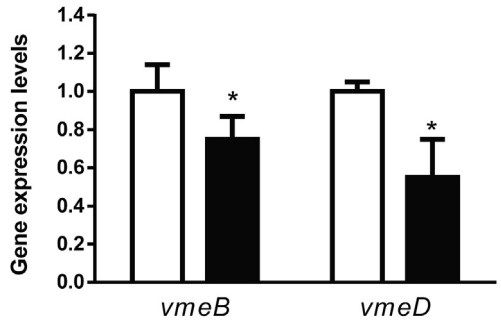

Figure 5. Effects of MIC level of LBP102 on the gene expression of (RND)-type efflux transporter in *V. parahaemolyticus* BCRC 12864 using the RT-PCR method. Relative gene expression compared with untreated control in MH broth supplemented with 17.5 μg/mL LBP102. □: untreated control; ■: LBP102 (17.5 μg/mL). Significance: $*p < 0.05$ vs. untreated control.

structural similarity with leucine and lysine. The result implicated that LBP102 has enzyme sensitivity and is unstable with pH value changes. The LBP102 exhibited a broad inhibitory spectrum, and inhibition of bacterial growth (especially for *V. parahaemolyticus* and *C. sakazakii*). Although the antibacterial activity of *L. plantarum* NTU 102 was stable in the narrow pH range 1.0—4.0, the antibacterial activity of *L. plantarum* NTU 102 may have applications in food preservation. In previous study, the bacteriocins from *L. plantarum* have been shown to induce cell death by means of inhibition of cell-wall synthesis, nucleic acid synthesis, and protein synthesis or enzymatic activity.[27] In this study, the relationship between LBP102 structure and antibacterial activity was not clear and need for further study.

In previous studies, Matsuo et al. (2013)[17] have reported on the cloning and properties of two transporters in the RND family, VmeB and VmeD from *V. parahaemolyticus*. The results showed that there were about 50 multidrug efflux transporters, as shown by the primary structure similarities of the transporters obtained from the databases of genome sequences in *V. parahaemolyticus*.[28] The *E. coli* KAM33, which harbors pRHR229 (carrying *vmeAB*) or pSVP201 (carrying *vmeCD*), encodes the multidrug efflux transporter VmeAB or VmeCD. Cells possessing VmeAB or VmeCD confer much higher MICs than control cells for a variety of antimicrobial agents. Moreover, gene-deficient *V. parahaemolyticus* shows a reduced survival rate in the presence of deoxycholate.[17,29] The *vme* suppression of *V. parahaemolyticus* BCRC 12864 observed in our study, suggests that the inhibitory effect of LBP102 on RND-type efflux transporter at the transcriptional levels. The expression of RND-type efflux transporters was shown to be controlled with several transcriptional regulators; therefore, the expression of other genes may be induced when cells are in the intestine or are exposed to antimicrobial agents.

In conclusion, the antibacterial substance produced by *L. plantarum* NTU 102 had antibacterial activity against *V. parahaemolyticus*. We suggest that this substance may have potential applications as a starter of fermented foods or as a food preservative for controlling food-borne pathogens. Additional studies are required to determine the effects of complex nutrients on the synthesis of the antibacterial substance and to elucidate the mechanisms and genetics of the bactericidal inhibition through this substance.

Conflict of interest

No conflict of interest declared.

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