Characterization of an Amylase-sensitive Bacteriocin DF01 Produced by *Lactobacillus brevis* DF01 Isolated from *Dongchimi*, Korean Fermented Vegetable

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Abstract

A DF01 strain that inhibits tyramine-producing *Lactobacillus curvatus* KFRI 166 was isolated from Dongchimi, a traditional Korean fermented vegetable, and identified as *Lactobacillus brevis* by biochemical analysis and reverse transcriptase sequencing of 16S rRNA. The antimicrobial compound produced by *L. brevis* DF01 was secreted at a maximum level of 640 AU/mL in late exponential phase in MRS broth, and its activity remained constant during stationary phase. The activity of bacteriocin DF01 was totally inactivated by α -chymotrypsin, pronase E, proteinase K, trypsin, and α -amylase, but not by catalase, which indicates the compound was glycoprotein in nature. The activity was not affected by pH changes ranging from 2 to 12 or heat treatment (60, 80, and 100°C for 30 min), but was reduced after autoclaving. Bacteriocin DF01 had bacteriolytic activity and a molecular weight of approximately 8.2 kDa, as shown by tricine-SDS-PAGE analysis. Therefore, bacteriocin DF01 can be used in the manufacture of fermented meat products due to its inhibition of tyramine-producing *L. curvatus* and non-inhibition of *L. sake*, which is used as a starter culture for meat fermentation.

Key words: amylase-sensitive bacteriocin, *Lactobacillus brevis*, dongchimi

Introduction

Lactic acid bacteria (LAB) are widely used as starters in manufacture of fermented foods, such as yogurt, fermented sausages and vegetable, and provide beneficial effects for the sensory and hygienic quality of the final products. Most of them related to fermented foods have acquired the GRAS (Generally Regarded As Safe) status.

Wild bacterial strains need to create a competitive niche to survive in their hostile natural environment, so they often produce antimicrobial compounds. With regard this fact, LAB are focused on their antagonistic effects on food-borne pathogens and spoilage bacteria by organic acids, hydrogen peroxide, carbon dioxide, diacetyl, and bacteriocins (Schillinger and Lücke, 1989; Salminen *et al.*, 2004).

Bacteriocins are a heterogeneous group of ribosomally synthesized antimicrobial peptides that usually inhibit the growth of microorganisms genetically closely related to

producer strains by bactericidal or bacteriostatic effects (Klaenhammer, 1993). Some studies suggested that some bacteriocin-producing LAB or purified bacteriocins may be used as natural food preservatives since bacteriocins, which have proteinaceous nature, would be degraded in the gastrointestinal tract of man and animals (Eckner, 1992; Cleveland *et al.*, 2001). The potential of using bacteriocins as preservatives could be achieved by using bacteriocin-producing bacteria as starters or by adding bacteriocins as food additives. The latter will necessarily need optimizations of their production because it is usually dependent on various strain-specific factors (Leal-Sánchez *et al.*, 2002). It was documented that these compounds are produced and secreted by almost all the bacterial species examined to date (Riley and Wertz, 2002).

Dongchimi is one of the water-based kimchi, Korean traditional fermented vegetable, fermented in brine by LAB. Recently, there are some interests to make Dongchimi juice as soft drinks to promote human health due to its particular taste and anti-adipogenic activity (Ko *et al.*, 1994; Ann, 2001; Kong *et al.*, 2006).

Biogenic amines in food are synthesized by microbial decarboxylation of precursor amino acids (Ten Brink *et*

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al., 1990). Christensen *et al.* (1999) described that fermentative bacteria, like *Lactobacillus* spp., may catabolize amino acids to obtain energy in nutrient-limited environments. Biogenic amines, such as histamine, putrescine, tyramine, tryptamine, b-phenylethylamine and cadaverine, are considered as undesirable compounds due to their potential toxic effects attributed to vasoactive and psychoactive properties (Ten Brink *et al.*, 1990) and due to an index of quality deteriorations in meat products because the longer meat products stored, the more contents of biogenic amines are formed during storage (Rokka *et al.*, 2004). Cadaverine and putrescine, called as secondary amines, are much less toxic than histamine and tyramine, but they could be converted into carcinogenic compounds such as nitrosamines by nitrosation reaction during curing process (Saccani *et al.*, 2005).

Many fermented foods such as salt-fermented fish products, fermented soybean products, and fermented vegetable products have been traditionally developed in Korea. Recently, it was reported that many fermented foods such as, cheeses (Gianotti *et al.*, 2008), fermented sausage (Latorre-Moratalla *et al.*, 2008), and sauerkraut (Kalaè *et al.*, 2000) contained comparable contents of biogenic amines. For that reason, Korean fermented foods are likely to contain biogenic amines. Therefore it is necessary to examine the contents of biogenic amines in those foods and to investigate the relationship between those foods and biogenic amine-producing bacteria.

The initial bacteriological composition and population in food are critical factors influencing formation of biogenic amines (Bover-Cid *et al.*, 2000; Kalaè *et al.*, 2000). Therefore, the aim of this study was to isolate bacteriocin-producing LAB from *dongchimi* and its characteristics. We expect the bacteriocin which inhibits biogenic amine-producing bacteria could contribute to decrease contents of biogenic amines in fermented foods as an agent used to control the bacteriological composition and population.

Materials and Methods

Bacterial strains used in this study

All microorganisms were stored in 15% glycerol stock at -60 until required, and subcultured twice in appropriate broth before use. The media used in this study were as follow; Lactobacilli MRS Broth (Difco, Sparks, MD, USA), Nutrient Broth (Acumedia Manufacturers, Inc., Lansing, MI, USA), Brain Heart Infusion Broth (Acumedia Manufacturers, Inc., Baltimore, MD, USA), Tryptic

Soy Broth (Difco), and Reinforced Clostridial Medium (Difco). LB (Luria Bertani) broth, YM broth, and LBS (*Lactobacillus* selective medium) broth were made in our laboratory according to the DifcoTM and BBLTM Manual.

L. brevis DF01 was isolated from *dongchimi* and the other microorganisms were purchased from ATCC (American Type Culture Collection, Rockville, MD, USA), IFO (Institute for Fermentation of Osaka, Osaka, Japan), KCCM (Korean Culture Center of Microorganisms, Seoul, Korea), or KCTC (Korea Collection for Type Cultures, Daejeon, Korea), or obtained from KFRI (Korea Food Research Institute, Songnam, Korea), and they are listed in Table 1.

Isolation and identification of bacteriocin-producing LAB

Dongchimi was purchased from a local market and putative bacteriocin producers were isolated using a modified sandwich method, also called triple agar layer method, developed by Kim *et al.* (1993). The 0.1 mL of sample serially diluted was pour-plated with 5 mL MRS bottom agar (1.5%), left to solidify, overlaid with MRS soft middle agar (0.75%), and incubated at 37°C for 24 h. The plates were overlaid with 5 mL of MRS agar seeded with *L. curvatus* KFRI 166 as an indicator bacterium ($\leq 10^7$ CFU/ml) and incubated at the same condition above. The colonies which showed inhibition zone against indicator bacteria were transferred on three MRS plates, one of them was for master plate and the others are for deferred and simultaneous assays. The number of indicator *L. curvatus* KFRI 166 in 5 mL of MRS top agar was ca. $\leq 10^7$ CFU/g and incubated at the same condition above. The colonies which showed inhibition zone against indicator bacteria were purified by streaking on MRS plate and each colony was confirmed using agar-well diffusion assay. For agar-well diffusion assay, *L. brevis* DF01 was cultivated in MRS broth at the same condition above, and the culture was centrifuged at 10,000 g for 15 min. The supernatant was neutralized to pH 6.2 with 3N NaOH and filtered by membrane filter (pore size of 0.20 μ m, ADVANTEC DISMIC-13CP, Toyo-Rochi, Tokyo, Japan). Each aliquot of 100 μ L of cell-free supernatant was placed into wells (diameter of 0.8 mm) of the agar plate pre-inoculated with indicator strains ($\leq 10^7$ CFU/g), and the plates were kept at 4°C for 4 h to allow for diffusion before incubation.

Isolated strains were identified by Gram staining, a catalase test, morphology, and carbohydrate fermentation pattern using the API 50 CHL kit (BioMrieux, Marcy

Table 1. Antimicrobial spectrum of *L. brevis* DF01.

Microorganism	Media	Temp(°C)	Inhibition ¹⁾		
			SA	DA	ADT
<i>Aeromonas hydrophila</i> ATCC 7966	NA	30	-	-	-
<i>Bacillus cereus</i> KCTC 3624	NA	30	-	+	-
<i>Bacillus mycoides</i> KCTC 3453	NA	30	-	-	-
<i>Bacillus natto</i> KFRI 170	NA	30	-	+	-
<i>Bacillus subtilis</i> KCTC 2023	NA	35	-	+	-
<i>Bacillus thuringiensis</i> KCTC 3452	NA	30	-	+	-
<i>Escherichia coli</i> ATCC 25922	LB	37	-	.	-
<i>Escherichia coli</i> O157:H7 ATCC 23514	LB	37	-	-	-
<i>Lactobacillus acidophilus</i> KCTC 3179	MRS	37	-	-	-
<i>Lactobacillus bulgaricus</i> IFO 3533	MRS	37	-	-	-
<i>Lactobacillus casei</i> KFRI 704	MRS	37	-	-	-
<i>Lactobacillus curvatus</i> KFRI 166	MRS	37	+	+++	+++
<i>Lactobacillus delbrueckii</i> KFRI 154	MRS	37	-	-	-
<i>Lactobacillus sake</i> ATCC 11506	MRS	37	-	-	-
<i>Leuconostoc mesenteroides</i> ATCC 10830	MRS	37	-	-	-
<i>Listeria innocua</i> ATCC 33090	BHI	37	-	-	-
<i>Listeria monocytogenes</i> KCTC 3569	BHI	37	-	.	-
<i>Pediococcus acidilactici</i> IFO 3884	MRS	37	-	++	-
<i>Pediococcus pentosaceus</i> KFRI 834	MRS	37	-	-	-
<i>Propionibacterium acnes</i> KCCM 41747	RCM	37	-	-	-
<i>Pseudomonas aeruginosa</i> ATCC 27853	NA	37	-	+	-
<i>Salmonella enteritidis</i> KCCM 12021	NA	37	+	+++	-
<i>Salmonella typhimurium</i> KCTC 1925	NA	37	+	++	-
<i>Shigella flexneri</i> ATCC 12022	NA	37	++	+++	-
<i>Staphylococcus aureus</i> ATCC 13565	NA	37	-	-	-
<i>Staphylococcus aureus</i> KCTC 3881	NA	37	-	-	-
<i>Staphylococcus epidermidis</i> KCTC 3958	NA	37	-	-	-
<i>Streptococcus mutans</i> ATCC 25175	BHI	37	-	++	-
<i>Yersinia enterocolitica</i> ATCC 23715	TSA	37	-	-	-
<i>Candida albicans</i> ATCC 13264	YM	25	-	-	-
<i>Saccharomyces cerevisiae</i> ATCC 13264	YM	25	-	-	-

Abbreviations: BHI, Brain Heart Infusion agar; LB, Luria Bertani agar; MRS, Lactobacilli MRS agar; NA, Nutrient agar; RCM, Reinforced Clostridial Medium agar; TSA, Tryptic Soy Agar; YM, YM agar.

¹⁾ -, absence of inhibition zone; +, 1 to 10 mm; ++, 11 to 20 mm; +++, above 21 mm (diameter of inhibition zone); SA, Simultaneous Assay; DA, Deferred Assay; ADT, agar-well-diffusion test.

I'Etoile, France). And the identity of the isolate was confirmed by reverse transcriptase sequencing of 16S rRNA (Macrogen, Inc., Seoul, Korea)

Growth kinetics and bacteriocin production of *L. brevis* DF01

MRS broth was inoculated with *L. brevis* DF01 (1% of inoculum) of an overnight culture and incubated at 37°C. The viable cell count (CFU/g), changes in pH, and bacteriocin activity (AU/mL) against *L. curvatus* KFRI 166 using agar-well diffusion assay were recorded every two hours for 36 h. Arbitrary units (AU/mL) were defined as the reciprocal of the highest dilution at which the growth inhibition was detectable.

Antimicrobial spectrum of *L. brevis* DF01

Three methods (simultaneous, deferred, and agar well diffusion assays) were used to determine antimicrobial activity of *L. brevis* DF01. The antimicrobial activity against indicator strains was demonstrated by measuring diameter of clear zone around the well.

Induction of bacteriocin activity

According to the method of previous study (Rojo-Bezares *et al.*, 2007), *L. brevis* DF01 was co-cultivated with heat-treated (55°C for 60 min or 100°C for 15 min) or autoclaved (121°C for 20 min) indicator bacteria, or cell-free supernatant of indicator bacteria. The initial bacterial concentrations of the *L. brevis* DF01 and heat-killed indi-

cator bacteria were 10^8 CFU/g and 10^7 CFU/g, respectively. Cell-free supernatants were obtained and submitted to agar-well diffusion assay to evaluate induction of antimicrobial activity against indicator bacteria.

Effect of enzymes, pH, heat and detergents treatments

Each aliquot of cell-free supernatant was treated with 0.1 mg/mL of α -amylase, α -chymotrypsin, catalase, pronase E, proteinase K, and trypsin according to the suppliers' instructions. All enzymes were purchased from Sigma-Aldrich (St. Louis, MO, USA). To test thermal stability, cell-free supernatants were adjusted to pH 6.2 and each aliquot was heated 60, 80, or 100°C for 10 min or 30 min, or autoclaved (121°C for 15 min). Sensitivity to different pH values was examined by adjusting cell-free supernatants to different pH values ranging from pH 2 to pH 12 with 3 N HCl or 3 N NaOH, and then the samples were incubated at 37°C for 2 hr. These samples were readjusted to pH 6.2 before antimicrobial activity test. The effect of detergents on the bacteriocin was tested by adding Tween 20 (1%, w/v, final concentration), Tween 80 (1%, w/v) and sodium dodecyl sulphate (SDS, 1%, 2%, w/v). After completing each of four treatments, the remaining activity was determined by agar-well diffusion assay.

Mode of action

MRS broth (160 mL) was inoculated with an overnight culture of *L. curvatus* KFRI 166 (1% of inoculum) and incubated at 37°C. Forty mL of bacteriocin-containing cell-free supernatant neutralized to pH 6.2 were added when the growth of *L. curvatus* KFRI 166 reached early exponential phase (6 h old). Each of samples was taken every 1 h to determine optical density value under 600 nm wavelength using spectrophotometer (Smart Plus SP-1900PC, Woongki Science, South Korea).

Molecular weight determination of bacteriocin DF01

L. brevis DF01 was inoculated in 200 mL of LBS (*Lactobacillus* selective medium) broth (2% of inoculum) and incubated at 37°C for 24 h. Cells were harvested by centrifugation (Sorvall RC-5C Plus, Dupont Co., USA) at 6,000 g for 10 min at 4°C. The bacteriocin in the supernatant was precipitated by adding ammonium sulfate to 70% saturation and gathered by centrifugation under the same condition as above. The precipitate was resuspended in 10 mL of 25 mM ammonium acetate buffer

(pH 6.5), desalted by using a 1200 molecular weight cut-off dialysis sack (Sigma-Aldrich, St. Louis, MO, USA), and submitted to filtration through a 0.20 μ m pore size membrane filter (ADVANTEC DISMIC-13CP, Toyoco, Tokyo, Japan).

The proteins in the concentrated supernatant were visualized using Tricine-SDS-PAGE, described by Schägger (2006), because this method was effective for the resolution of proteins smaller than 30 kDa. The sample was reduced by mercaptoethanol, incubated at 37°C for 15 min, and subjected to SDS-polyacrylamide gel electrophoresis (Ezcell electrophoresis system, Komabiotech Inc., Seoul, Korea). The 10 μ L of sample was loaded on wells of 13% T and 3% C acrylamide gels (1.0 mm thick, without SDS for silver staining) overlaid with 4% T and 3% C of stacking gel; T is the total acrylamide concentration and C is the cross-linker concentration. The gels were run at 30 V until the dye of sample buffer reached the bottom of stacking gel, after that, volt was raised to 90 V and carried out until the dye moved out of the gel.

After completion of electrophoresis, the gels were cut into two pieces; one is for silver staining described by Schägger (2006), and the other is for gel overlay assays as described previously (Bhunia *et al.*, 1987).

For gel overlay assays, the gels were fixed for 2 h in fixing solution (10% acetic acid, 50% methanol, 100 mM ammonium acetate), rinsed three times over 2 h, stored overnight in ddH₂O at 4°C, overlaid with 10 mL of MRS soft agar (0.75% agarose) seeded with *L. curvatus* KFRI 166 ($\leq 10^7$ CFU/g) and incubated at 37°C for 36 h.

Results

Identification of bacteriocin-producing LAB

Strain DF01 was isolated from *dongchimi* and exhibited an antimicrobial activity against *L. curvatus* KFRI 166. Isolate DF01 showed Gram positive, rod shape, catalase negative, and lowered to pH 4.30 at their stationary phase, and it was tentatively identified as *L. brevis* based on biochemical test by API 50 CHL kit (99.4% identity). This isolate was further analyzed by sequencing of 16S rRNA and was determined by *L. brevis* DF01 (Fig. 1).

Growth and antimicrobial activity of *L. brevis* DF01

Fig. 2 shows the growth profile of *L. brevis* DF01 grown in MRS broth at 37°C and antimicrobial activity against *L. curvatus* KFRI 166 by agar-well diffusion assay. The viable cell counts were increased from 8×10^6

Table 2. Physico-chemical stability of the bacteriocin produced by *L. brevis* DF01.

Treatment	Bacteriocin activity
Enzymes	
ProteinaseK	-
PronaseE	-
Trypsin	-
α -Chymotrypsin	-
α -amylase	+
Catalase	
PH	
2~12	+
Heat	
60°C, 80°C, 100°C for 10 min	+
60°C, 80°C, 100°C for 30 min	+
121°C for 15 min	+
Detergent (m/v)	
SDS (1%)	+
SDS (2%)	+
Tween 20 (1%)	+
Tween 80 (1%)	+

ity of crude bacteriocin DF01 are summarized in Table 2. In short, the activity of bacteriocin DF01-containing supernatant was totally inactivated after treatments with proteolytic enzymes such as α -chymotrypsin, pronase E, proteinase K and trypsin, and also with α -amylase, which gave the evidence of its glycoprotein nature. In contrast, no change in activity was observed after treatment of catalase, indicating the antimicrobial effect was not derived from hydrogen peroxide. In different pH, heat, and detergent treatment experiments, the antimicrobial activity was not affected after incubation of supernatants containing bacteriocin adjusted to pH 2, 3, 5, 7, 9, 11, and 12 for 2 h, incubation of supernatants heated at 60, 80 and 100°C for 10 or 30 min, and adding Tween 20 (1%, w/v), Tween 80 (1%, w/v), and SDS (1%, 2%, w/v). The activity was partly decreased after autoclaving (121°C for 15 min) of supernatant.

Mode of action

Addition of bacteriocin DF01-containing supernatant to the 6-h old culture of *L. curvatus* resulted in growth inhibition. The turbidity of indicator bacteria culture was decreased immediately after adding cell-free supernatant and the turbidity was determined by optical density value under 600 nm wavelength using spectrophotometer. The optical density (OD_{600nm}) was decreased from 0.269 to 0.101 for 5.5 h (Fig. 3). The decreased turbidity means that bacteriocin DF01 has a bacteriolytic characteristic which induces killing by cell lysis.

Fig. 3. Effect of bacteriocin DF01 on the growth of *L. curvatus* KFRI 166; Control (empty triangles) and treated cells (black squares) with crude bacteriocin DF01.

Molecular weight of bacteriocin DF01

Concentrated cell-free supernatant of *L. brevis* DF01 culture was separated by Tricine-SDS-PAGE and the gels were subjected to gel overlay assay. A band showed inhibition zone against *L. curvatus* KFRI 166 in overlaid plate, corresponding to molecular weight of about 8.2 kDa (Fig. 4).

Fig. 4. Direct detection of bacteriocin DF01 from the concentrated supernatant by Tricine-SDS-PAGE; Lane M, molecular mass marker (Mark 12™ Unstained Standard, Invitrogen) stained by silver nitrate; 1, Concentrated bacteriocin stained by silver nitrate; 2 and 3, overlaid onto plate seeded with *L. curvatus* KFRI 166.

Discussion

The bacteriocin-producing LAB isolated from Dongchimi was identified as *L. brevis*. This bacteria was reported a strain of microbial composition in *dongchimi* in previous study (Kim and Kim, 1999).

The antimicrobial activity of *L. brevis* DF01 may be due to the production of organic acids which cause a consequent reduction in pH or due to the production of hydrogen peroxide, diacetyl, bacteriocins or bacteriocin-like substances (Schillinger and Lcke, 1989; Salminen *et al.*, 2004). Hence antimicrobial activity of bacteriocin was confirmed by agar-well diffusion assay using neutralized supernatant of the *L. brevis* DF01 culture, and its proteinaceous nature was examined by treatments of enzymes. Bacteriocin DF01 was totally inactivated by proteolytic enzymes and α -amylase. The sensitivity to α -amylase implies the presence of an essential carbohydrate moiety with protein portion for its antimicrobial activity, therefore bacteriocin DF01 could be considered as a compound belonging to the fourth class of bacteriocins, as suggested by Klaenhammer (1993). However Nes *et al.* (1996) affirmed that the fourth class of bacteriocins has not yet been characterized adequately at the biochemical level, therefore they suggested recognition of this class seems premature. The following was the reason that plantaricin S produced by *L. plantarum* LPCO10 was first examined as a glycoprotein which was totally inactivated after α -amylase treatment (Jiménez-Díaz 1993), but plantaricin S was confirmed that its bacteriocin activity was associated with two distinct peptides without carbohydrate moiety after purification by reverse-phase chromatography (Jiménez-Díaz, 1995), which means that cell-free supernatant containing glycoprotein bacteriocin should be further investigated after purification for its exact composition. Like the case of plantaricin S, bacteriocin DF01 should be also examined its characteristic after purification.

Bacteriocins which have glycoprotein nature were also reported by Lewus *et al.* (1992), Mathot *et al.* (2003), and Hernández *et al.* (2005); leuconocin S from *Leuconostoc paramesenteroides*, unnamed bacteriocin from *Streptococcus thermophilus* 580, and plantaricin TF711 from *Lactobacillus plantarum*, respectively. But there is no common characteristic in their antimicrobial spectrum, heat stability, and pH stability. Bacteriocin DF01 showed very narrow antimicrobial spectrum, while plantaricin TF711 inhibited gram-positive bacteria such as, *Lactobacillus plantarum*, *Lactobacillus curvatus*, *Lactobacillus*

lactis, *Clostridium sporogenes*, and even gram-negative bacteria such as *Shigella sonnei* and *Klebsiella pneumoniae*. With regards to heat-stability and pH-stability, bacteriocin DF01 was the most stable among these glycoprotein bacteriocins; the activity of leuconocin S was reduced by 75% after heat treatment (60°C for 60 min), the bacteriocin produced by *Streptococcus thermophilus* 580 maintained its activity only up to 10 min at 60°C, and plantaricin TF711 was led to complete inactivation by autoclaving (121°C for 20 min) and incubation at above pH 11.

Rojo-Bezares *et al.* (2007) described that the production of bacteriocin secreted by *L. plantarum* strain J23 was only detected when *L. plantarum* strain J23 was co-cultured with intact cells or heated cells (55°C for 60 min) of inducer strains (*L. lactis* MG1363, *L. hilgardii* J81, and *P. pentosaceus* FBB63), but not detected with pure-culturing of *L. plantarum* strain J23 and co-culturing with heated (100°C for 15 min) or autoclaved (121°C for 20 min) inducer strains. On the other hand, Mathot *et al.* (2003) found that the production of bacteriocin produced by *S. thermophilus* 580 was doubled by co-culturing with *L. delbrueckii* subsp. *lactis*. In our study, we designed the induction tests of bacteriocin activity, but there were not any positive results in this experiment. Therefore, we could reach a conclusion that *L. brevis* DF01 produce bacteriocin in constitutive way.

The optical density (OD_{600nm}) of indicator bacteria culture was decreased after adding cell-free supernatant of *L. brevis* DF01, revealing that bacteriocin DF01 has a bacteriolytic characteristic which induces killing by cell lysis (Fig. 3). Some authors proposed that release of autolytic enzymes could be one of the reasons of bacteriocin-induced cell lysis. In their studies, they subjected most bacteriocins are cationic, so they can displace the binding site between anionic polymers (teichoic and lipoteichoic acids) and autolytic enzymes (Bierbaum and Sahl, 1987; Jack *et al.*, 1995). But bacteriocin DF01 showed a very narrow antimicrobial spectrum so its activity may specifically interact with receptors on sensitive bacteria.

The molecular weight of bacteriocin DF01 determined by Tricine-SDS-PAGE was approximately 8.2 kDa. Before this experiment, *L. brevis* DF01 was previously cultured in MRS, modified MRS without beef extract which was replaced by peptone, or LBS (*Lactobacillus* selective medium) broth. We compared resolutions of each of the three concentrated cell-free supernatants through Tricine-SDS-PAGE and the best resolution was obtained using the supernatant from LBS broth (data not

shown). This should be the reason that LBS medium has less protein contents, which ease to purify the peptide.

In fermented meat products, *L. curvatus* and *L. sake* are found as predominant microorganisms. But some *L. curvatus* strains produce up to five different biogenic amines such as, tyramine, putrescine, phenylethylamine, tryptamine, and cadaverine (Straub *et al.*, 1995; Bover-Cid *et al.*, 2001). *L. curvatus*, thus, causes accumulation of biogenic amines within products. Hence Roig-Sagués and Eerola (1997) suggested *L. sake* strains which do not have any activities of amino acid decarboxylation could be more suitable than *L. curvatus* when it comes to using a starter in meat manufacture.

We can conclude that bacteriocin DF01 produced by *L. brevis* DF01 is a novel bacteriocin that have a good potential for an agent which can inhibit biogenic amine-producing bacteria in meat products, and we expect that it can lead to decrease of the contents of biogenic amines especially tyramine in manufacture of fermented meat product.

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