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Bactericidal Effect of Bacteriocin of *Lactobacillus plantarum* K11 Isolated from Dongchimi on Escherichia coli O157

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ABSTRACT – Among 68 strains of lactic acid bacteria (LAB) isolated from *Dongchimi*, a strain K11 was selected due to its bactericidal activity against *Escherichia coli* O157. The strain K11 was identified as *Lactobacillus plantarum*, based on physiological and biochemical characteristics. In the late exponential phase, *La. plantarum* K11 showed maximum bacteriocin activity (12,800 BU/mL) and maintained until the early stationary phase. The bacteriocin activity was completely inactivated by all the proteolytic enzymes such as pepsin, protease, proteinase K, papain, chymotrypsin, and trypsin, but the activity was not affected by catalase, a-amylase, lysozyme, and lipase, suggesting proteinaceous nature of the bacteriocin. Additionally, this activity was not affected in the pH range from 3.0 to 9.0 and under storage conditions like 30 days at -20, 4, or 25°C. Although the bacteriocin activity was absolutely lost after 15 min treatment at 121, it was relatively stable at 70°C for 60 min or 100°C for 30 min. The activity was disappeared by treatment with acetone, benzene, ethanol, or methanol, but it was not affected by treatment with chloroform or hexane. The antibacterial activity of the bacteriocin was good against some LAB including *Lactobacillus* spp., *Enterococcus* spp., and *Streptococcus* spp., but not against food-borne pathogens such as *Bacillus* spp., *Listeria* spp., and *Staphylococcus* spp. as well as yeasts and molds. Especially, some intestinal bacteria such as *Enterobacter aerogenes* and *E. coli* were significantly affected by the bacteriocin of *La. plantarum* K11. Furthermore, the addition of 640 BU/mL resulted in the complete clearance of *E. coli* O157 after 10 hr.

Key words: *Escherichia coli* O157, *Lactobacillus plantarum*, bacteriocin

Introduction

Escherichia coli O157:H7 is recognized as the most important cause of serious human diseases such as hemorrhagic colitis, hemolytic-uremic syndrome, or thrombotic thrombocytopenic purpura[1]. Since first reported as a pathogen in 1982, the Centers for Disease Control and Prevention (CDC) have estimated that 73,000 patients infected with shiga toxin-producing *E. coli* O157:H7 occur annually in the United States, more than 2,000 enter hospital, and about 60 die[2]. The growth of *E. coli* O157:H7 was markedly suppressed by organic acid such as acetic, propionic, and butyric acids and spices such as garlic, ginger, and mustard[3-5]. In addition, some evidence suggests that probiotic bacteria such as *Lactobacillus* spp. and *Propionibacterium* spp., and bacteriocin-producing lactic acid bacteria (LAB) exert growth-inhibitory and bactericidal activities on *E. coli* O157:H7[6-8].

Because bacteriocins have a growth-inhibitory potential on microorganisms, they are using as biopreservatives of meat, fish, dairy, and vegetable products[9]. Bacteriocin application in the food industry could decrease consumer's concern for food-borne pathogens and artificial chemical preservatives, causing cancer, deformity, and mutation[10]. Bacteriocins often have synergistic effect on other treatments such as modification of temperature, gas, and pressure, application of pulsed electric field, or addition of sodium diacetate and sucrose fatty acid ester. Therefore, they can be used as a hurdle technology to improve the safety of food and veterinary medicine[11-13].

In this study, a strain having bactericidal activity against *E. coli* O157 was isolated from *Dongchimi*, a Korean fermented food. And antimicrobial spectrum and physical and chemical characteristics of the bacteriocin produced by the selected strain was investigated to assess its usefulness as a natural food additive and application as a starter culture for fermentation of vegetables and sausage products.

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Materials and Methods

Media and reagents

All media were purchased from Difco Co. (Spaks, MD, USA). The API CH 50 kit was obtained from bioMerieux Co. (March l'Etoile, France) and reagents and enzymes from Sigma-Aldrich (St. Louis, MO, USA).

Incubation condition of indicator microorganisms

We used *E. coli* O157 ATCC 43889 for measurement of bacteriocin activity of isolated LAB from *Dongchimi*. In order to find antimicrobial spectrum, Gram-positive bacteria (*Bacillus* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Enterococcus* spp., *Listeria* spp., *Streptococcus* spp.), Gram-negative bacteria (*Enterobacter* spp., *Escherichia coli*, *Salmonella* spp., *Vibrio* spp.), and some yeasts and molds were used as indicator microorganisms. All strains were obtained from American Type Culture Collection, Korean Collection for Type Cultures, and Korean Culture Center of Microorganisms. Bacteria were propagated in brain heart infusion (BHI) broth at 37°C under aerobic condition. Mold and yeast were cultured in potato dextrose agar (PDA) and YM agar at 25°C, respectively.

Isolation of LAB and preparation of crude bacteriocin

Radishes were purchased from the local oriental grocery store and *Dongchimi* was prepared with radish and Chinese cabbage in our laboratory. All samples were homogenized in sterile phosphate buffer solution (PBS, pH 7.2) for 5 min with a blender. Appropriate dilutions were prepared with PBS and 1 mL of serial 10-fold dilutions was spread onto Lactobacilli MRS agar plates containing 1% $CaCO_3$. Colonies forming clear zones on the MRS agar plate were selected as LAB. Cell-free culture supernatant of LAB grown in MRS broth at 37°C for 12 hr was obtained from a culture of LAB by centrifugation at 7,000 × g for 10 min at 4 and the pH was adjusted to 6.5-7.0 using 1 M NaOH. Ammonium sulfate to 50% (w/v) saturation (at 4) was added to the cell-free culture supernatant, the mixture had been stirred for overnight at 4°C, and the protein precipitate was centrifuged at 10,000 × g, for 20 min at 4°C. And then the pellet was solubilized in 10 mL of sodium phosphate buffer (10 mM; pH 6.5), desalted by cellulose dialysis membrane (Spectrum Labs., LA, USA) and filtered through a 0.22 μm membrane filter (Millipore Corp., Billerica, USA).

Bacteriocin activity assay

Bacteriocin activity was quantified by the microtitre plate assay[14]. Each well of the microtitre plate (BD Falcon, Franklin Lakes, USA) contained 200 μL MRS broth, 50 μL crude bacteriocin solution serially twofold diluted, and 100 μL bacterial suspension of the indicator organisms, *E. coli* O157 ATCC 43889 (10^6 CFU/mL). After incubation at 37°C for 12 hr, the growth-inhibitory effect was determined by recording the bacterial growth by means of absorbance measurements at 660 nm using an enzyme linked immunosorbent assay (ELISA) reader (Spectrocount, Packard Instruments, Meriden, CT, USA). One bacteriocin unit (BU) was arbitrarily defined as the reciprocal of the highest dilution fold that inhibited the growth of the indicator bacteria by 50% of turbidity of the control culture without bacteriocin. The highest dilution was multiplied by 20 (1 mL : 50 μL) to obtain the activity units per mL (BU/mL).

Identification of the bacteriocin-producing LAB

Selected LAB strains were characterized by physiological and biochemical tests according to the criteria of Bergey's Manual of Systematic Bacteriology[15]. Cell morphology was determined by optical microscopy (DW-THN, DONGWON). Carbohydrate fermentation patterns were determined using the API 50 CH system (BioMerieux, France) according to the manufacturer's instructions.

Physical and chemical characterization of bacteriocin

To test whether the bacteriocin is sensitive to heat, bacteriocin solution was exposed to heat treatment under conditions like 60 min at 70°C, 30 min at 100°C, or 15 min at 121. To test the effect of pH on the stability of the bacteriocin, pH of crude bacteriocin solution was adjusted to various values from pH 2 to 12. After incubation at 37°C for 1 hr, the reaction mixture was brought to neutral pH (6.5) and the bacteriocin activity was assessed. Furthermore, it was tested whether the bacteriocin is digested by different types of enzymes. Crude bacteriocin solution was mixed with the following enzyme solution at a final concentration of 1 mg/mL: catalase (10 mL potassium phosphate, pH 7.0), α-amylase (50 mM sodium acetate, pH 6.0), pepsin (10 mM citrate, pH 2.0), protease (50 mM Tris-HCl, pH 7.5), proteinase K (50 mM Tris-HCl, pH 7.5), chymotrypsin (50 mL Tris-HCl, pH 8.0), trypsin (50 mL Tris-HCl, pH 8.0), papain (10 mM potassium phosphate, pH 7.0), lysozyme (50 mL Tris-HCl, pH 7.0), and lipase (50 mL Tris-HCl, pH 7.5). After 1 hr incubation at 37°C, the enzymes were inactivated by heating at 80 for 10 min. The influence of organic solvents was also tested. Crude bacteriocin solution was mixed with 50% of the organic solvents such as acetone, benzene, chloroform, ethanol, hexane, and methanol. After leaving at 4°C for 2 hr, organic solvents were removed from sample by an evaporator (BUCHI, Rotavapor R-205, Flawil Switzerland), and the bacteriocin activity was tested. Finally, to test the stability during storage, crude bacteriocin was stored at -20, 4, or 25°C for 30 days. After each treatment,

the bacteriocin activity was determined.

Antimicrobial spectrum

Antimicrobial activity was tested against a wide spectrum of LAB as well as food-borne pathogens, yeast, and mold by the paper disk diffusion assay[16]. Appropriate agar (1.0%, w/v) media were inoculated (1%, v/v) with indicator microorganism suspension. 50 μL crude bacteriocin was loaded to a paper disk (F 8 mm, Toyo), and the plates were incubated at optimal conditions and the diameter of the inhibition zones was measured.

Concentration dependence of the crude bacteriocin

Concentration-dependent inhibitory effect of the bacteriocin on *E. coli* O157 was studied. Cells of *E. coli* O157 ATCC 43889 grown up to log-phase in BHI broth were harvested by centrifugation (7,000×g, for 10 min), washed twice in PBS, and resuspended in their appropriate broth to yield 1.0×10^6 CFU/mL. Crude bacteriocin was added to give final concentrations of 160, 320, and 640 BU/mL and the bacteriocin-treated cells and cells without the treatment were incubated for 10 hr at 37°C. Then, the number of viable *E. coli* O157 ATCC 43889 cells was determined by serial 10-fold dilution in PBS, and 1 mL aliquots were poured evenly on MacConkey Agar. Plates were incubated aerobically at 37°C for 24 hr and the number of colony forming units was estimated.

Results and Discussion

Selection and identification of a bacteriocin-producing LAB

Sixty-eight LAB isolated from *Dongchimi*, a Korean fermented food, were investigated for screening of bacteriocin-producing strains. Only K11 of these strains was found to produce a compound with an antibacterial activity against *E. coli* O157 ATCC 43889. The major antibacterial mechanisms of LAB on pathogenic bacteria include production of acids, hydrogen peroxide, and bacteriocins[17]. Because neutralization of the crude bacteriocin of K11 and treatment with catalase did not modify the initial antibacterial activity, action of hydrogen peroxide or lactic acid from K11 strain was excluded as an antibacterial mechanism (data not shown). Therefore, antibacterial activity against *E. coli* O157 ATCC 43889 was supposed to be resulted from bacteriocin produced by K11.

The strain K11 was identified as *La. plantarum*, based on physiological and biochemical characteristics and the utiliza-

Table 1. Phenotypic characteristics and the utilization of various sugars by *La. plantarum* K11

Contents	Results	Sugar	Results	Sugar	Results
Cell shape	Rod	Glycerol	-	Salicine	-
Gram staining	+[a]	Erythritol	-	Cellobiose	+
Spores staining	-[b]	d-Arabinose	-	Maltose	+
Acid-fast staining	-	l-Arabinose	+	Lactose	+
Motility	-	Ribose	+	Melibiose	+
Gas from glucose	-	d-Xylose	-	Saccharose	+
H_2S production	-	l-Xylose	-	Trehalose	+
Lactic acid	L[c]	Adonitol	-	Inuline	-
Nitrate reduction	-	β-Methyl-xyloside	-	Melezitose	+
Methyl red	+	Galactose	+	d-Raffinose	-
Voges-Proskauer	-	d-Glucose	+	Amidon	-
Horse blood hemolysis	-	d-Fructose	+	Glycogene	-
Sheep blood hemolysis	-	d-Mannose	+	Xylitol	-
Catalase	-	l-Sorbose	-	β-Gentiobiose	-
Oxidase	-	Rhamnose	-	d-Turanose	+
Urease	-	Dulcitol	-	d-Lyxose	-
Arginine hydrolysis	-	Inositol	-	d-Tagatose	-
Growth in aerobic condition	+	Mannitol	+	d-Fucose	-
anaerobic	+	Sorbitol	+	l-Fucose	-
Growth at 15-40°C	+	α-Methyl-d-Mannoside	+	d-Arabitol	-
Growth at pH 2.0-4.0	-	α-Methyl-d-glucoside	-	l-Arabitol	-
5.0-9.0	+	N-Acetyl glucosamine	-	Gluconate	-
10.0-12.0	-	Amygdaline	-	2-ceto-gluconate	-
Growth in 0-5% NaCl	+	Arbutine	-	5-ceto-gluconate	-
10% NaCl	-	Esculine	-		

[a] positive reaction; [b] negative reaction; [c] configuration of lactic acid produced from glucose.

tion of various sugars determined using the API 50 CHL profiles (Table 1). The strain consisted of Gram-positive, non-spore forming rod with the ability to grow at 15-40°C, pH 5.0-9.0, less than 5% NaCl, and facultatively anaerobic condition. And K11 fermented glucose into L-lactic acid did not hydrolyase arginine and not produce catalase, oxidase, or urease. In addition, the strain fermented maltose, melibiose, trehalose, D-mannose, sorbitol, and L-arabinose, but did not ferment erythritol, inositol, D-xylose, rhamnose, cellobiose, inuline, and D-raffinose.

Gonzalez *et al.*[18] and Franz *et al.*[19] reported characterization of plantaricin C, a bacteriocin produced by a strain of diary origin and plantaricin D produced by *La. planatrum* BFE 905 from ready-to-eat salad. Also, there are a number of bacteriocins such as plantaricin A, B, F, S, and T produced by *La. plantarum*[20-23]. Although *La. plantarum* WHE 92, a producer of pediocin AcH, had anti-*L. monocytogenes* activity[24], a bacteriocin produced by *La. planatrum* K11 inhibited growth of *E. coli* O157.

Generally, many bacteriocins of LAB are active against Gram-positive bacteria, but Gram-negative bacteria are a little insensitive to bacteriocins[25]. The difference in resistance between Gram-positive and Gram-negative may be due to the differences in the cell envelopes of these bacteria[26]. If the outer membrane of Gram-negative cells was injured, the permeation of bacteriocins to the cytoplasmic membrane could be facilitated[27]. However, some papers reported that pediocin-producing *La. lactis* inhibited *E. coli* O157:H7 as well as *L. monocytogenes* and *S. aureus* in cheese[28], and combination of nisin and cinnamon greatly contributed to the inactivation of *E. coli* O157:H7[29].

Effect of incubation time on bacteriocin production

Fig. 1 shows relationship between growth phases and bacteriocin production. Bacteriocin activity was detectable in the culture supernatant after 8 hr when an absorbance of 0.454 at 660 nm and pH 4.87 of the culture broth. In the late exponential phase, *La. plantarum* K11 showed maximum bacteriocin activity (12,800 BU/mL) and maintained until the early stationary phase. But since then, bacteriocin activity declined and completely disappeared after growth for 22 hr. In the stationary phase, extracellular pH was maintained, however, bactericidal activity decreased, excluding a possibility of lactic acid as a bactericidal mechanism.

According to other studies[18,25], the maximum inhibitory activity of plantaricin C and plantaricin ST31 was observed in the stationary phase, and Suma *et al.*[30] founded that the antimicrobial activity observed for *La. plantarum* NCIM 2084 occurred between the late log and early stationary phases, wherein the cell numbers had reached the maximum level like our results. However, unlike the bacteriocin of *La.*

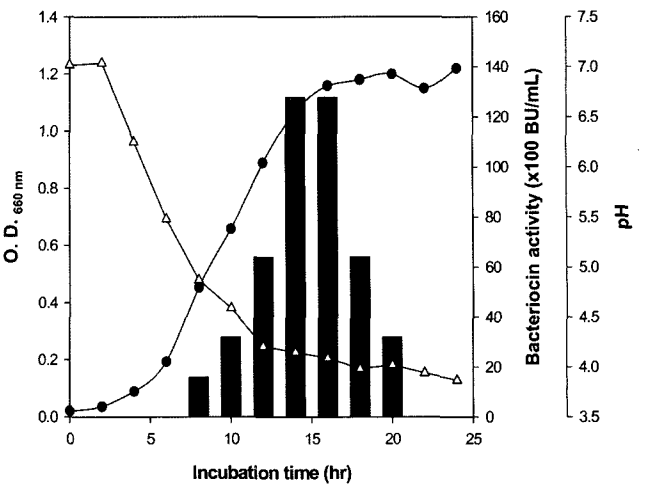

Fig. 1. Relationships among growth phases, lactic acid production, and bacteriocin production by *La. plantarum* K11 cultured at 37°C in MRS broth. -●-, Bacterial growth; -△-, pH; ■, bacteriocin activity.

*plantarum*K11, *E. casseliflavus* IM 416K1 isolated from Italian sausages started to produce antilisterial bacteriocin (enterocin 416K1) at 4 hr and the bacteriocin activity remained constant after 60 hr of incubation[31].

As shown in other bacteriocins, the bacteriocin is supposed to be produced as a secondary metabolite during the bacterial growth phase. Tagg and Wannamaker[32] reported that creation of an acidic pH in the course of carbohydrate fermentation was essential for the recovery of detectable streptococcin A-FF22, nisin-like antibiotic substance. The cause of that bacteriocin activity declined at the stationary phase could be due to proteolytic degradation by the enzymes released from cells[33].

Physical and chemical characterization of bacteriocin

Influences of heat, pH, storage temperature, organic solvents, and enzymes on the bacteriocin activity of *La. plantarum* K11 against *E. coli* O157 ATCC 43889 were investigated (Table 2). The bacteriocin activity was completely inactivated by all the proteolytic enzymes such as pepsin, protease, proteinase K, papain, chymotrypsin, and trypsin, but not affected by catalase, α-amylase, lysozyme, and lipase. Therefore, it could be suggested that the bacteriocin has proteinaceous nature but does not require a carbohydrate or a lipid moiety for the activity. And also the activity was found not to be affected by the pH range from 3.0 to 9.0 and under storage conditions like for 30 days at -20, 4, or 25°C. Although the bacteriocin activity was absolutely lost after 15 min treatment at 121, it was relatively stable at 70°C for 60 min or 100°C for 30 min. The activity was disappeared by treatment with acetone, benzene, ethanol, or methanol, but it was not affected by treatment with chloro-

Table 2. Physico-chemical stability of the bacteriocin produced by *La. plantarum* K11

	Treatment	Bacteriocin activity
Enzymes	Catalase	+
	α-amylase	+
	Pepsin	-
	Protease	-
	Proteinase K	-
	Chymotrypsin	-
	Trypsin	-
	Papain	-
	Lysozyme	+
	Lipase	+
Heating	70°C, 60 min	+
	100°C, 30 min	+
	121°C, 15 min	-
pH	2.0	-
	3.0	+
	4.0	+
	5.0	+
	6.0	+
	7.0	+
	8.0	+
	9.0	+
	10.0	-
	11.0	-
	12.0	-
Organic solvents	Acetone	-
	Benzene	-
	Chloroform	+
	Ethanol	-
	Hexane	+
	Methanol	-
Storage for 30 days	-20°C	+
	4°C	+
	25°C	+

form or hexane.

Some LAB bacteriocins are very heat-stable. For example, plantaricin LP84 has stability with no loss even after 20 min treatment at 121[30]. Heat stability of bacteriocin could be due to the formation of small globular structures and the occurrence of strongly hydrophobic regions, and stable cross-linkage[34]. Because the bacteriocin of *La. plantarum* K11 has heat stability, it would be favored to use as a food preservative during manufacturing of processed foods with a heating procedure.

Enzyme sensitivity varies depending on kinds of bacteriocin. Enterocins produced by *Enterococcus*spp. and ST28MS and ST26MS produced by *La. plantarum* were sensitive to proteolytic enzymes such as proteinase K, trypsin, papain, and pepsin, whereas the bacteriocin activity was not affected by lipase, lysozyme, and catalase[35,36]. However, the bacteriocin activity produced by *La. plantarum* LPCO10 was sensitive to various glycolytic and lipolytic enzymes as well as proteolytic enzymes, suggesting that it was a glycolipoprotein[23]. Thus, bacteriocins would be degraded by digestive enzymes of the gastrointestinal tract and seem to be nontoxic. Furthermore, a number of bacteriocins produced by LAB are relatively stable under treatment with organic solvents (methanol, chloroform, acetonitrile, ethanol, iso-propanol, and cyclohexane) and conditions such as storage at -20, 4°C, or room temperature[18,25,37]. Although plantaricin UG1 was stable in the narrow pH range 4.5-7.0, some bacteriocin activity was stable at acidic and neutral pHs (2.0-8.0), in addition, the antibacterial activity of bacteriocin produced by *La. curvatus* SE1 was stable even at alkaline pH[37-39]. The bacteriocin of *La. plantarum* K11 like other bacteriocins has considerable stability to long-term storage at low temperature or at a wide pH range, therefore, it can be used to prevent the growth of pathogenic bacteria such as *E. coli* O157 in refrigerated, frozen, and acidic foods.

Antimicrobial spectrum

The antimicrobial spectrum exhibited by the bacteriocin of *La. plantarum* K11 is shown in Table 3. The bacteriocin showed an antibacterial activity that was effective against some LAB including *Lactobacillus* spp., *Enterococcus* spp., and *Streptococcus*spp., but ineffective against food-borne pathogens such as *Bacillus* spp., *Listeria* spp., and *Staphylococcus* spp. among tested Gram-positive bacteria as well as yeasts and molds. Especially, by the bacteriocin of *La. plantarum* K11 showed strong bactericidal effects against intestinal bacteria as like *Enterobacter aerogenes* and *E. coli.*

Plantaricin 423, plantaricin UG1, and antibacterial substance produced by *La. plantarum* ST31 are shown to be effective against Gram-positive bacteria including *Lactobacillus* spp., *Lactococcus* spp., *Listeria* spp., *Bacillus* spp., and *Clostridium* spp, contrasting to present results[25,38,40]. Furthermore, some bacteriocins of *La. plantarum* have a wide antibacterial spectrum against Gram-negative microbes such as *E. coli, Pseudomonas aeruginosa, Acinetobacter baumanii, Shigella flexneri, Salmonella typhimurium, Helicobacter pylori* as well as Gram-positive microbes[36,39,41]. Whereas, plantaricin D has a narrow antibacterial spectrum; it showed antibacterial effects only against *Lactobacillus* spp. and *Listeria* spp., but not against *Bacillus* spp. and *Staphylococcus* spp.[19].

Bactericidal action of the bacteriocin

To investigate mode of action of the bacteriocin produced by *La. plantarum* K11, the bacteriocin (160, 320, or 640 BU/mL) was added to the *E. coli* O157 ATCC 43889 which was

Table 3. Antimicrobial spectrum of the bacteriocin produced by *La. plantarum* K11

Micro-organisms	Indicator species	Inhibition zone[a]
Gram-positive bacteria	*Bacillus cereus* ATCC 11778	-
	B. licheniformis KCTC 1918	-
	B. stearothermophilus ATCC 10149	-
	B. subtilis ATCC 35421	-
	Lactobacillus acidophilus KCTC 3168	+++
	La. brevis KCTC 3102	+
	La. casei ATCC 25302	-
	La. paracaei ATCC 25302	-
	La. plantarum KCTC 1048	-
	Leuconostoc mesenteroides KCTC 3719	++
	Enterococcus faecalis KCTC 3206	-
	E. faecium KCTC 2022	++
	E. faecium KCCM 11028	++
	Listeria monocytogenes KCTC 3569	-
	L. innocua ATCC 33090	-
	L. ivanovii ATCC 19119	-
	Streptococcus lactis ATCC 1913	+
	Stapylococcus aureus ATCC 6538	-
Gram-negative bacteria	*Enterobacter aerogenes* ATCC 13480	+
	Escherichia coli ATCC 11229	++
	Salmonella enteritidis ATCC 13076	-
	Sal. typhimurium KCTC 2514	-
	Vibrio parahaemolyticus KCTC 2471	-
	V. vulnificus KCTC 2982	-
Molds	*Aspergillus oryzae* KCTC 6983	-
	Penicillium roqueforti KCCM 11269	-
Yeasts	*Candida albicans* KCTC 7965	-
	Saccharomyces cerevisiae KCTC 7246	-
	Kluyveromyces marxianus KCCM 35458	-

[a] +, 8.5-10.0 mm; ++, 10.1-12.0 mm; +++, more than 12.1 mm including paper disk diameter.

suspended in BHI broth. The number of viable cell counts determined by standard plate counting at indicated incubation times was shown in Fig. 2. The addition of increasing concentrations of the bacteriocin led to a marked decrease in the number of viable cells of *E. coli* O157. For the control culture, viable cell counts increased from 6 log units to about 9 log units within 10 hr at 37°C. However, viable cell counts of *E. coli* O157 decreased about 2 log units from an initial cell counts within 8 hr after expose to 160 BU/mL of the bacteriocin, and the addition of 640 BU/mL resulted in the complete clearance of cells of *E. coli* O157 after 10 hr. Thus, this result indicates that the bacteriocin produced by *La. plantarum* K11 has strong bactericidal activity. The bacteriocin of *La. plantarum* K11 has higher activity than colicin Hu194 produced by *E. coli* Hu 194. *E. coli* O157:H7 43890 was successfully eliminated (5 log CFU/g) from

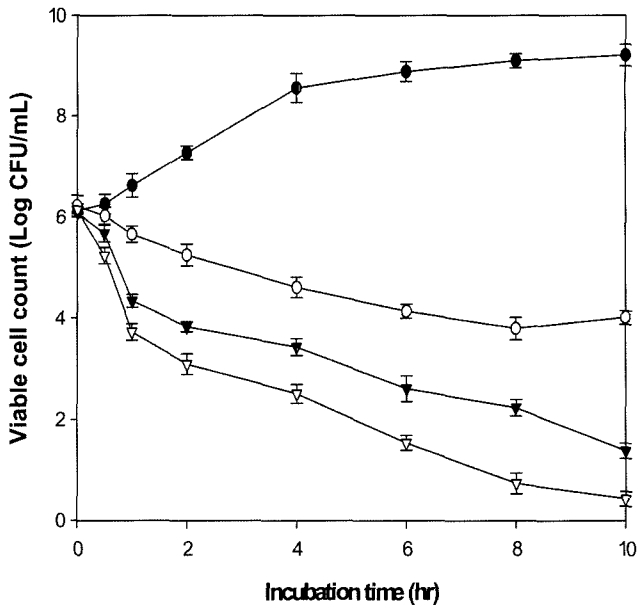

Fig. 2. Viable counts of *E. coli* O157 during the growth in BHI broth in the presence of the bacteriocin produced by *La. plantarum* K11. -●-, control; -○-, bacteriocin 160 BU/mL; -▼-, bacteriocin 320 BU/mL; -▽-, bacteriocin 640 BU/mL.

inoculated alfalfa seeds after soaking in a colicin Hu194 suspension at a concentration of 10,000 AU/g[42].

A bacteriocidal mode of action has been described for plantaricin S and T produced by *La. plantarum* LPCO10[23] and plantaricin UG1[38], and also for bacteriocin of *La. plantarum* inhibiting the growth of *L. monocytogenes*[37]. A number of bacteriocins induce cell lysis by means of binding to the cytoplasmic membrane, insertion of bacteriocin molecules into the membrane, and formation of a complex which leads to dissipation of the proton motive force and efflux of intracellular components such as various enzymes and ions[43,44].

In conclusion, because the bacteriocin produced by *La. plantarum* K11 isolated from *Donchimi* has antibacterial activity against *E. coli* O157, we suggest that it has potential applications as a starter of fermented foods or food preservative for controlling food-borne pathogens. In the future, influence of complex nutrients and different incubation conditions on synthesis of the bacteriocin has to be investigated along with elucidation of bactericidal action mechanism and genetic characterization of bacteriocin.

요 약

동치미로부터 분리한 유산균 (68 균주) 중 *Escherichia coli* O157에 대한 항균 효과를 나타내는 균주는 *Lactobacillus plantarum* K11로 동정되었다. 분리균주 *La. plantarum* K11이 생산한 박테리오신의 항균 활성은 대수증식기 후반부

에 12,800 BU/mL로 최대 활성에 이르렀다. 항균 활성은 pepsin, protease, proteinase K, papain, chymotrypsin 및 trypsin 처리에 의해 완전히 소실되었으나, catalase, α-amylase, lysozyme 및 lipase에 의해서는 영향을 받지 않았으므로 단백질성 물질임을 확인하였다. 게다가, 이 활성은 pH 3.0-9.0의 조건하에서나 -20, 4 및 25°C에서 30일간의 저장 동안에도 안정하였다. 또한 100°C에서 30분간 가열처리에도 비교적 안정한 편이었고, chloroform이나 hexane 처리에도 활성에 변함이 없었다. 분리 균주의 박테리오신은 *Bacillus* spp., *Listeria* spp. 및 *Staphylococcus* spp. 등의 일부 식중독균의 억제효과는 나타나지 않았으나, *Enterobacter aerogenes*와 *E. coli* 등의 장내세균의 억제에는 효과적이었으며, 특히 640 BU/mL의 박테리오신 처리에 의해서 10시간 배양 만에 *E. coli* O157이 완전하게 사멸되었다.

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