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Characterization of Antimicrobial Substance Produced by *Lactobacillus paraplantarum* KNUC25 Isolated from Kimchi

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The KNUC25 strain isolated from over-fermented whole Chinese cabbage kimchi was examined for its physiological characteristics using API 50 CHL system assay and identified as *Lactobacillus paraplantarum* by analysis of whole-cell protein SDS-PAGE pattern assay and similarity of 16S rDNA sequence. *L. paraplantarum* KNUC25 had a broad antimicrobial activity spectrum from Gram positive to Gram negative bacteria. Scanning electron micrograph analysis showed that KNUC25 might attack to cell surface of indicator cells and destruction can lead to inhibition of the cell growth. The antimicrobial substance of the KNUC25 strain was stable to various degrading enzymes and at high temperature and not a plasmid-born matter. Resistance to proteolytic enzymes showed that an antimicrobial activity of KNUC25 might not be caused by proteinous substance. Maximum production of antimicrobial substance was the exponential growth phase at 30°C.

Key words: Lactic acid bacteria, *Lactobacillus paraplantarum*, antibacterial activity, Kimchi

Introduction

Kimchi is a Korea's most representative traditional food with its rich flavor, nutritional value, and preservable property and produced by fermentation carried out by lactic acid bacteria (LAB).

LAB are a heterogeneous family of microorganisms that can ferment a variety of nutrients [28] primarily into lactic acid. They are mainly Gram-positive, anaerobic, non-sporulating, and acid tolerant bacteria. LAB are used as 'natural' or 'selected' starters in food fermentations in which they perform both acidification, flavour-compound production [16, 18], as well as protection of the food form spoilage and pathogenic microorganisms by producing organic acids, lactic acid, hydrogen peroxide, diacetyl [2, 25], antifungal compounds such as fatty acids [8] or phenyllactic acid [19], and bacteriocin [30]. Historically, the traditional roles for many LAB have been as starter cultures to drive food and dairy fermentations, leading to their widespread human consumption and generally recognized as safe (GRAS) status.

Various microorganisms originally present in the raw materials initiate their growth during Kimchi fermentation process. But gradually lactic acid bacteria become dominant species and involved at different stages of the ripening process and producing antimicrobial substances and other metabolites [6]. *Leuconostoc mesenteroides* has been known to be the most important organism and dominant during the early and middle stages. *Lc. mesenteroides* endows Kimchi its desirable and characteristic refreshing flavor by producing organic acids and carbon dioxide [24]. *Lc. mesenteroides* cells, however, quickly disappear in the late stage because of accumulation of acids. More acid resistant lactobacilli such as *Lactobacillus plantarum* and *Lactobacillus brevis* become the dominant flora and they accelerate the deterioration of Kimchi by producing more acids. And *Lactobacillus paraplantarum* is also one of the deteriorating lactobacilli in late Kimchi fermentation process.

L. paraplantarum was first isolated from beer and human feces by physiological characteristics similar to those of *Lactobacillus plantarum* [10]. *L. paraplantarum* do not catabolize alpha-methyl-d-mannoside. However, because they exhibit little DNA relatedness to *L. plantarum* and *Lactobacillus pentosus*, the strains were classified as members of a new species, *Lactobacillus paraplantarum*.

A *Lactobacillus paraplantarum* KNUC25 was isolated from over-fermented whole Chinese cabbage Kimchi [1].

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The isolate was identified as *Lactobacillus paraplantarum* on the basis of morphology with Scanning electron microscopy (SEM), methods of Bergey's manual of systematic bacteriology and 16S rDNA analysis [14]. Previously, KNUC25 was misidentified as *Leuconostoc carnosum* by 16S rDNA sequencing in 2003 [1]. After an accurate reappraisal, it was determined that the 16S rDNA of the KNUC25 strain had 99.2% identity with those from *Lactobacillus paraplantarum* TKR17B (accession number: AJ878739) in 2005 [14]. And the KNUC25 strain was registered to GenBank in NCBI (accession number: EF20067). The isolated *L. paraplantarum* KNUC25 showed significant antibacterial activities to pathogenic bacteria, for example, *Bacillus subtilis*, *Escherichia coli*, *Salmonella enteritidis*, *S. typhi*, *Staphylococcus aureus*, *Shigella boydii*, and *S. sonnei*.

L. paraplantarum C7, which produces a novel bacteriocin that strongly inhibits *L. plantarum*, was previously isolated from Kimchi [20] and specific characteristics of paraplantaricin C7, a bacteriocin produced by *L. paraplantarum* C7, were reported [22]. *L. paraplantarum* KNUC25 has wider inhibition spectrum and there could be another antimicrobial substance or inhibitive mechanism which has not been identified yet. Therefore, characterization of antimicrobial substance produced by *Lactobacillus paraplantarum* KNUC25 isolated from Kimchi was tried.

Materials and Methods

Bacterial strains and culture conditions

For cultivation of lactic acid bacteria strains, MRS [11] broth and agar plate were used. *L. paraplantarum* was cultured for 24 hrs at 30°C either in MRS broth (Merck, Germany) or on the equivalent solid media prepared by adding 1.5% agar and 0.5% $CaCO_3$. The strain was stored at -70°C in MRS broth containing 15% glycerol. The composition of MRS medium was as follows: peptone from casein 1%; meat extract 0.8%; yeast extract 0.4%; d(+)-glucose 2%; di-potassium hydrogen phosphate 0.2%; tween 80 0.1%; di-ammonium hydrogen citrate 0.2%; sodium acetate 0.5%; magnesium sulfate 0.02%; and manganese sulfate 0.004%.

Streptococcus mutans KCTC3065 was incubated in BHI (BD, France) broth. Other strains were cultured in LB medium (1% bactotryptone, 0.5% yeast extract, 0.5% NaCl and 0.1% glucose).

Analysis of the isolate based on whole-cell protein SDS-PAGE pattern

A microbial cell expresses many different proteins that form a rich source of information of characterization, classification, and identification. SDS-PAGE fingerprinting of whole-cell proteins method was reported that it can be used for the identification of LAB in Kimchi [5]. Therefore, for further confirmation of identification of *L. paraplantarum* KNUC25, SDS-PAGE of the whole-cell protein of the isolate was performed by the modified method [5]. The reference strains and the isolate for SDS-PAGE of whole-cell proteins were cultured at 30°C in MRS broth for overnight. The pellet of the sample was collected by centrifugation at 12,000 × g for 3 min at 4°C and washed twice with deionized water and suspended in 200 μL of 50 mM Tris-HCl buffer (pH 7.6). And the sample was frozen by liquid nitrogen and thawed at 40°Cwater bath several times and then sonicated. After centrifugation at 10,000 × g for 10 min, the supernatant was removed and resuspended by adding SDS sample buffer. For protein denaturation, samples were heated for 5 min at 100°C. The cell debris was removed by centrifugation, and the supernatants were collected for analysis by SDS-PAGE. The concentration of protein was measured by the Bradford assay [17]. After electrophoresis, the gel was stained for 1 h with 0.1% Coomassie brilliant blue R-250 (Bio Basic Inc., Canada), and destained with 10% acetic acid and 30% methanol solution for overnight.

Strain typing by API 50 CHL system

The carbohydrates fermentation patterns and biochemical characteristics were identified through API 50 CHL system (BioMerieux, France) [4]. Before testing, the strain was subcultured twice overnight in MRS broth at 30°C. The test was carried out according to the manufacturer's instructions and the results were read after the strain was incubated at 30°C for 2 days. The API LAB program was used to analyze the data.

Plasmid isolation

The plasmid mini-prep procedure used was devised by modifying steps from *E. coli* mini-prep procedures to meet the specified needs of lactobacillus plasmid isolation [27]. First of all, overnight cultured cells were collected by centrifugation (8,000 × g, 5 min) and supernatant was removed. The pellet was resuspended in 25% sucrose

containing 30 mg/mL lysozyme to final volume of 200 μL and incubated 37°C for 15 min. After that, 400 μL of alkaline SDS solution (3% SDS in 0.2 N NaOH) was added and mixed immediately. After incubation at room temperature in 7 minutes, 300 μL of ice-cold 3 M sodium acetate (pH 4.8) was added and immediately mixed and centrifuged at max speed for 15 minutes at 4°C. Supernatant was transferred and added 650 μL of isopropanol and centrifuged. And all liquid and resuspended pellet was removed in 320 μL H_2O. Two hundreds μL of 7.5 M ammonium acetate containing 0.5 mg/mL ethidium bromide and 350 μL phenol/chloroform solution were added. After centrifugation, upper phase was transferred and added 1 mL absolute ethanol. After that, sample was centrifuged again and pellet was washed in 70% ethanol. All liquid was removed and pellet was resuspended in 40 μL TER (TE + 0.1 mg/mL RNase).

Preparation of cell-free supernatant of *L. paraplantarum* KNUC25

The cell-free supernatant was obtained from a 24 hr culture by centrifugation for 30 min at 8,000 × g at 4°C. After filter sterilization (0.22 μm; Millipore, USA), a pH 4.5 was observed for cell-free supernatant over time. Proper volume of cell-free supernatant was divided into a tube (Corning, Mexico), frozen at -70°C and lyophilized. Samples were kept in -70°C deep freezer before use.

Antimicrobial activity

The antimicrobial activity of *L. paraplantarum* KNUC25 was detected by two methods, the plate diffusion assay and a microtiter plate assay [15]. In the first case, the indicator strain was inoculated into 5 mL LB broth (1%, v/v) and incubated at 37°C. When optical density at 600 nm of indicator cells was reached at 0.2~0.3, 100 μL of cells were spreading onto LB agar plate and 10 μL of 30-fold concentrated cell-free supernatant was spotted onto an overlaid sterilized filter paper disc (diameter 6 mm, Toyo Roshi Kaisha, Ltd., Japan). One mg Nisin/250 μL of 0.02 M HCl was used as a positive control. After incubation at 37°C, the presence of an inhibition zone was examined. And the second case, the 100 μL indicator cells which were prepared as the same method mentioned above were mixed with concentrated cell-free supernatant in 96 well culture plate (Falcon, USA) and incubated at 37°C. Change of absorbance value (OD_{580}) was measured by ELISA (Merck, Germany) at appropriate intervals.

Preparation of bacterial specimen for SEM observation

The isolates were cultured in 5 mL of LB broth until the OD_{600} reached to 0.6~0.7 and treated with 30-fold concentrated cell-free supernatant. After incubation at 37°C, indicator cells were harvested by centrifugation at 4°C for 5 min at 8,000 rpm. Cell pellet was washed 2~3 times with 0.1 M potassium phosphate buffer (pH 7.2), treated with 2.5% glutaraldehyde and kept on ice for 90 min. Then, it was centrifuged at 4°C for 5 min at 8,000 rpm and the supernatant was removed gently by pipetting. The cell pellet was washed 2~3 times with 0.1 M potassium phosphate buffer (pH 7.2), treated with 1% OsO_4solution and kept at room temperature for 1 hr. Centrifugation and washing step were then repeated once more. Next, dehydration of the bacterial specimen was carried out sequentially using 50%, 70%, 80%, 90%, 95% and 100% ethanol. The last step, bacterial specimen was treated with isoamyl acetate and kept at room temperature for 1 hr. The removal of supernatant was performed by centrifuging. The bacterial specimen was frozen at -70°C and then lyophilized. After plantinum coating (20 mA, 90 sec), morphology of the bacterial specimen was observed finally using FE-SEM S-4300 (Hitachi, Japan).

Effect of different treatments on the antimicrobial activity produced by *L. paraplantarum* KNUC25

The stability of the active substance to enzymes, pH and heat treatment was tested on cell-free supernatant of 24 h culture of *Lactobacillus paraplantarum* KNUC25, incubated at 37°C against *Bacillus subtilis* 168. After each treatment, the residual antimicrobial activity was determined by the plate diffusion test [32]. All determinations were carried out in triplicate.

For test of stability at different temperature, the cell-free supernatant of *L. paraplantarum* KNUC25 was exposed to various heat treatments: 60°C, 100°C, and 121°C for 10, 20, and 30 min. In order to determine the sensitivity of the substance to pH, the supernatant was adjusted to pH levels ranging from 2, 4, 5, 6, 7, 8, 9, 10, or 12 with 1 N HCl and 1 N NaOH. After treatment, the supernatant was frozen at -70°C and lyophilized. The residual antimicrobial activity was determined by the supernatant concentrated to 30 times of its original volume.

The sensitivity of the cell-free supernatant of *L. paraplantarum* KNUC25 to various enzymes was assayed by incubating the supernatant with: proteinase K in 10 mM Tris-HCl-50 mM NaCl-5 mM EDTA (pH 7), pepsin in 50 mM citrate (pH 2), catalase in 10 mM potassium phosphate (pH 7), trypsine in 50 mM Tris-HCl (pH 7), or α-amylase in 0.1 M sodium phosphate (pH 7). The enzymes (all from Sigma, USA) were used at a final concentration of 1 mg/mL and incubated at 37°C, except the catalase at 25°C, for 2 hours. The supernatant in buffer without enzymes as well as the enzyme solutions were exposed to the same conditions as control.

Kinetics of antimicrobial substance production

The optimal temperature of antimicrobial substance production in MRS broth was determined by inoculating *L. paraplantarum* KNUC25 at 5% (v/v) and incubating at 4°C, 18°C, 25°C, 30°C and 37°C for 24 hours. The growth was followed by measuring the optical density at 600 nm.

The kinetics of antimicrobial substance production was also studied. The MRS broth was inoculated with 5% (v/v) precultured *L. paraplantarum* KNUC25 and incubated at 30°C for 48 hours. Culture samples were removed at 6 hourly intervals and filtrated (0.22 μm; Millipore, USA). After lyophilization, dried supernatant was resolved by 1×PBS buffer and used for antimicrobial activity test.

Results and Discussion

Identification of *Lactobacillus paraplantarum* KNUC25

Previously, the isolates, KNUC25, was misidentified as *Leuconostoc carnosum* [1]. After an accurate reapproaisal, KNUC25 was identified as *L. paraplantarum* by 16S rDNA sequencing [14]. The protein patterns of SDS-PAGE of whole cell protein and API 50 CHL system offered a complementary result of identification.

The identification of strain by SDS-PAGE technique was investigated by electrophoretic runs of duplicate protein extracts of strains and the whole cell protein patterns are displayed in Fig. 1. SDS-PAGE profiles of whole cell proteins were highly reproducible and showed similar patterns among the strains of the same species. Identification of the isolated strain, KNUC25, by SDS-PAGE was performed with the comparison of the protein fingerprints that were derived from reference strains with almost known species of lactic acid bacteria in Kimchi. SDS-PAGE of whole cell proteins of the reference strains and the strain, KNUC25, showed their different band patterns, which were able to discriminate between *Lactobacillus* strains and other lactic acid bacteria, *Leuconostoc* and *Weissellastrains*. The SDS-PAGE patterns of whole cell proteins of the KNUC25 had significant similarity to those of *Lactobacillus paraplantarum*.

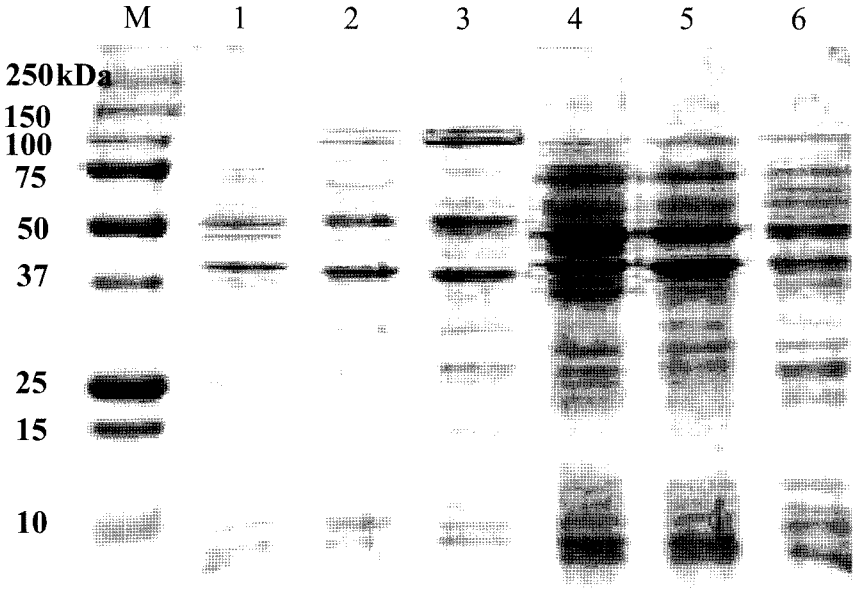

Fig. 1. SDS-PAGE profiles of whole cell proteins of reference strains and the KNUC25 strain tested. Lanes: M, Protein molecular weight markers (kDa); 1, *Lactobacillus sakei* subsp. *sakei* KCTC3603; 2, *Leuconostoc mesenteroides* subsp. *mesenteroides* KCTC3505; 3, *Weissella Kimchii* CHJ3 KCTC3746; 4, *Lactobacillus plantarum* KCTC3104; 5, *Lactobacillus paraplantarum* KCTC5045; 6, *Lactobacillus paraplantarum* KNUC25.

The API 50 CHL system is a one of the methods for identification of lactic acid bacteria isolated from Kimchi [13, 23, 26] by physiological reactions. The identification result of KNUC25 was *Lactobacillus plantarum* (Table 1). The test result was inconsistent with the identification by SDS-PAGE of whole cell protein. Misidentifications like this result also were reported [12, 31]. Although the result of API 50 CHL was not an accurate identification method, it gave information about physiological characteristics of KNUC25.

No plasmids were detected from *L. paraplantarum* KNUC25 (data not shown).

Moreover, an analysis of % similarity of *L. paraplantarum* KNUC25 with other *Lactobacillus* species (Table 2) offered a definite basis for the identification of KNUC25 as *Lactobacillus paraplantarum*. An analysis of % similarity was supported by the Biological Resource Center in Korea Research Institute of Bioscience and Biotechnology in Daejeon. Another report about an isolation of *Lactobacillus paraplantarum* strain from Kimchi [20] suggests that *L. paraplantarum* is one of the lactic acid bacteria involved in Kimchi fermentation.

Antimicrobial activity

The inhibition spectrum of the cell-free supernatant of *L. paraplantarum* KNUC25 against other microorganisms is presented in Table 3. The test microorganisms were various Gram-positive, or Gram-negative bacteria including other LAB and fungi. The KNUC25 inhibited most of test strains. Especially, KNUC25 could inhibit anaerobic bacteria such as *Streptococcus mutans* causing dental caries. Moreover, there were also antibacterial activities against lactic acid bacteria. But *Salmonella paratyphica* ATCC11511 was insensitive. The results indicate that the cell-free supernatant had a broad inhibition spectrum.

Scanning electron micrograph of the indicator bacteria treated by supernatant of KNUC25 showed (Fig. 2) that KNUC25 attacks to cell surface of indicator cells and

Table 1. Physiological characteristics of *L. paraplantarum* KNUC25 isolated from Kimchi.

Characteristics	*L. plantarum* KCTC5045	*L. paraplantarum* KCTC3104	KNUC25
N-acetyl-glucosamine, Amygdalin, Arbutin, Cellobiose, Esculin, Fructose, Gentiobiose, Glucose, Maltose, Mannose, Salicin, Sorbitol, Sorbose	+[1]	+	+
Adonitol, D-arabinose, L-arabinose, D-arabitol, L-arabitol, Dulcitol, Erythriol, D-fucose, L-fucose, Galactose, Glycerol, Glycogen, 2-keto-gluconate, 5-keto-gluconate, Inositol, D-Lyxose, Mannitol, Rhamnose, Starch, D-Tagatose, D-Turanose, Xylitol, D-xylose, β-methyl-D-xyloside	-	-	-
Lactose	+	+	-
Melibiose	+	+	-
Trehalose	+	+	-
Gluconate	+	+	-
Melezitose	+	+	-
Ribose	+	-	-
L-xylose	+	-	-
α-methyl-D-mannoside	+	-	-
α-methyl-D-glucoside	+	-	-
Raffinose	+	-	-
Inulin	-	-	-

[1] Symbols: +, positive; -, negative.

Table 2. Analysis of % 16S rDNA sequences similarity of *L. paraplantarum* KNUC25 with other *Lactobacillus* species.

Strain	Accession No.	% Similarity	nt differences/compared
Lactobacillus paraplantarum DSM 10667T	AJ306297	99.40	8/1323
Lactobacillus plantarum JCM 1588	D79211	98.64	18/1321
Lactobacillus plantarum JCM 1149	D79210	98.56	19/1321
Lactobacillus plantarum str. Lp 39 NCDO 1752 (T)	X52653	97.85	28/1300

Table 3. Antimicrobial activity spectrum of the cell-free supernatant of *L. paraplantarum* KNUC25.

Indicator strain	Antimicrobial activity[1]
Bacillus sutbilits 168	+[2]
Leuconostoc mesenteroides subsp. mesenteroides KCTC3505	+
Lactobacillus plantarum KCTC3104	+
Weissella Kimchii CHJ3 KCTC3746	+
Lactobacillus paraplantarum KCTC5045	+
Streptococcus mutans KCTC3065	+
Listeria monocytogenesis KCTC3710	+
Salmonella enteritidis ATCC13076	+
Escherichia coli sØ990	+
Salmonella typhi ATCC19430	+
Salmonella paratyphica ATCC11511	-
Staphylococcus aureus ATCC25923	+
Candida albicans TIMM 1768	+
Trichophyton rubrum KCTC6345	+

[1] Antimicrobial activity was determined by the spot-on-the-lawn assay as described in materials and methods.
[2] Antimicrobial activity; +, positive; - negative.

destruction can lead to inhibition of the cell growth. However, a more specific study about antimicrobial mechanism of KNUC25 is needed.

Stability of the KNUC25 culture supernatant

Inhibitory activity of the KNUC25 culture supernatant was not affected by heat treatment at 60, 100, and 121°Cfor 30 min (Table 4). There was no decrease of inhibition by increasing temperature and treatment time.

Variation of pH between 2 and 6 did not affect the activity, but the activity was rapidly reduced at pH 7, 8, 9, 10, and 12. Though a clear inhibition zone was detected by cell-free supernatant of KNUC25, rather small inhibition zone was appeared by pH 7 to 12 adjusted KNUC25. This result indicated that a part the antimicrobial activity could be caused by accumulation of acids which produced during fermentation process.

And the activity of the antibacterial compound produced by *L. paraplantarum* KNUC25 was resistant to protease treatment (pepsin, trypsin, and proteinase K). These results demonstrate that the antibacterial compound is not proteinous nature and a stable agent against proteolytic enzymes. Moreover, stable antibacterial activity by catalase-treated supernatant to the indicator bacteria means hydrogen peroxide is not a major substance of inhibition [21]. Amylase did not cause any antibacterial inactivation may imply that carbohydrate is not an essential moiety for

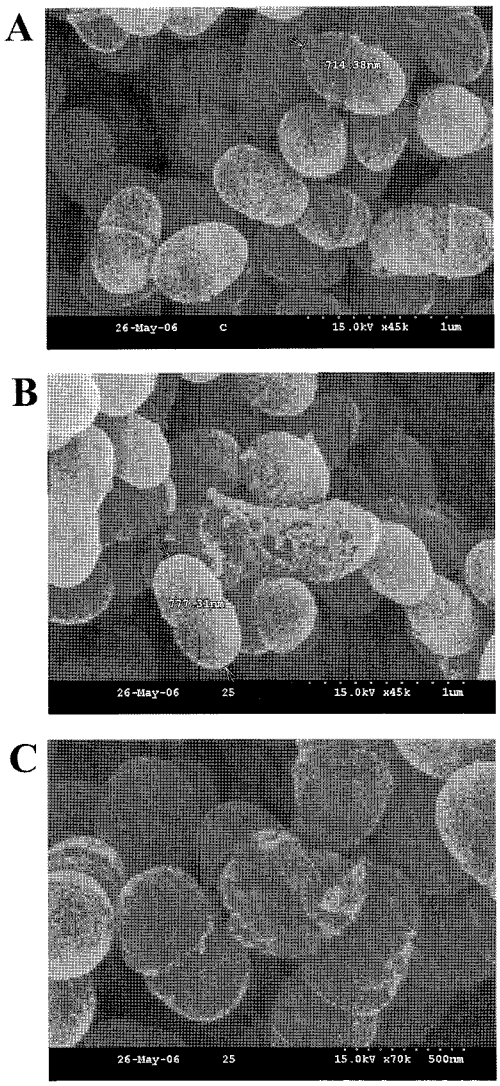

Fig. 2. Scanning electron micrograph of the indicator bacteria treated by supernatant of KNUC25. The morphology of bacteria was observed by SEM (Scanning electron microscope). Panel (A), *Streptococcus mutans* KCTC3065; panels (B) and (C); *S. mutans* KCTC3065 treated by 30-fold concentrated supernatant of KNUC25.

KNUC25. Since α- amylase is an extracellular enzyme, which catalyzes the hydrolysis of α-D-(1, 4) glycosidic bonds in oligosaccharides and polysaccharides.

Bacteriocins are bacterially produced antimicrobial peptides and many bacteriocins are produced by food-grade lactic acid bacteria [9]. Most of the bacteriocin operons are located on plasmid [3, 7, 29]. However, no plasmids were detected from *L. paraplantarum* KNUC25. Therefore, the antimicrobial activity of *L. paraplantarum* KNUC25 might be caused by not a plasmid-born antimicrobial substance, but acids or other metabolites which were made during fermentation.

Table 4. Effect of various treatments on the antibacterial activity of KNUC25.

Treatments		Activity[1]
Control		+[2]
Temperature		
60°C	10 min	+
	20 min	+
	30 min	+
100°C	10 min	+
	20 min	+
	30 min	+
121°C	10 min	+
	20 min	+
	30 min	+
pH		
2		+
4		+
5		+
6		+
7		±
8		-
9		-
10		-
12		-
Enzyme		
Pepsin		+
ProteinaseK		+
Trypsin		+
Catalase		+
α-amylase		+
Nisin[3]		+

[1] *B. subtilis* 168 was used as an indicator.
[2] Antibacterial activity: +, high positive; ±, moderate positive; -, negative.
[3] Positive control.

These results indicate that the antimicrobial compound of KNUC25 is significantly stable, not protein nature material, and not a plasmid-born which could be a desirable property when incorporated to foods.

Effect of conditions on the production of antimicrobial substance of KNUC25

The optimal temperatures for cell growth and antimicrobial substance production were examined. The maximum inhibition activity was obtained at 30°C while the cell growth was alike at 25°C and 30°C (Fig. 3). Fig. 4 shows that production of antimicrobial substance was started during the exponential growth phase at 30°C. Six hour was the maximum time to produce the most active KNUC25 at 30°C. After 6 h of incubation, the activity of KNUC25 was

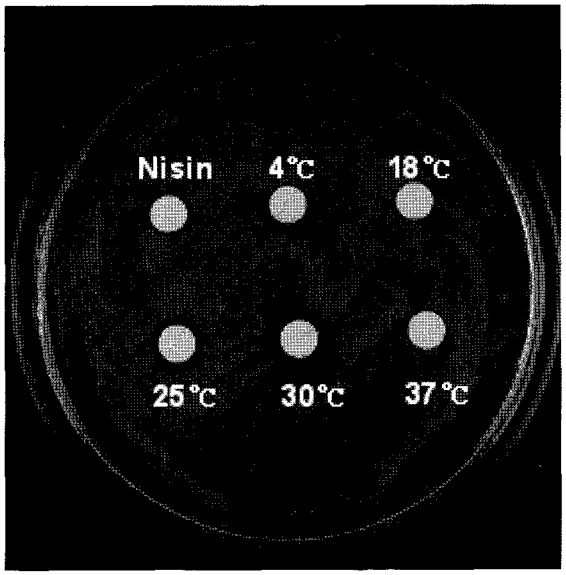

Fig. 3. Optimal temperature for antimicrobial substance production: *L. paraplantarum* KNUC25 was inoculated at 5% (v/v) into MRS broth and incubated at 4°C, 18°C, 25°C, 30°C and 37°Cfor 24 hours. The optimal temperature of antimicrobial substance production in MRS broth was determined by a presence and size of an inhibition zone.

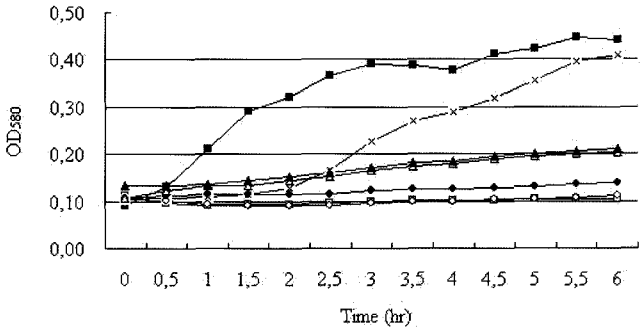

Fig. 4. Detection of antimicrobial activity of supernatant of *L. paraplantarum* KNUC25 after different incubation time (0, 6, 12, 24, and 48 h) at 30°C: *L. paraplantarum* KNUC25 was inoculated with 5% (v/v) precultured KNUC25 cells and incubated at 30°C for 48 hours. Culture samples were removed at 6 hourly intervals. Indicator cells was treated by concentrated cell-free supernatant and incubated at 37°C. Change of absorbance value (OD580) was measured at appropriate intervals. One mg Nisin/250 μL of 0.02 M HCl was used as a positive control: ■, None; □, Nisin; ×, 0 h; ○, 6 h; ●, 12 h; △, 24 h; ▲, 48 h.

almost maintained during the fermentation process. Initial pH of incubation was pH 6 and after 12 h the pH declined to 5 to 4 (data not shown).

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국문초록

김치로부터 분리된 *Lactobacillus paraplantarum* KNUC25가 만드는 항균 물질의 특성

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숙성 정도가 오래된 김장 배추 김치에서 분리되어 16S rDNA 염기 서열 분석을 통해 부분 동정된 KNUC25 분리 균주를 단백질 전기 영동 패턴과 생리적 특징 그리고 염기 서열의 유사성을 비교하여 *Lactobacillus paraplantarum* 로 동정하였다. *L. paraplantarum* KNUC25의 농축 상등액은 그람 양성균과 음성균에 넓은 범위의 항균 활성을 나타냈다. 전자 현미경을 통해 KNUC25가 만들어내는 항균 물질은 세균의 표면에 작용하여 생육을 억제하는 것으로 확인되었다. 항균 활성 물질을 생산하는 최적 온도는 섭씨 30도이고, 높은 온도에서도 항균 활성을 보였으며 단백질 분해 효소에 안정하여 비단백질성 항균 물질일 가능성을 보여주었다.