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Inhibitory Effect of *Lactobacillus plantarum* K11 on the Adhesion of *Escherichia coli* O157 to Caco-2 Cells

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Abstract Inhibitory effect of *Escherichia coli* O157 adhered to Caco-2 cells by the cells of *Lactobacillus plantarum* K11 and the cell-free culture supernatant (CFCS) and bacteriocin prepared from this strain was investigated. As the cell counts of viable *L. plantarum* K11 previously adhered to Caco-2 were increased, the rate of adhesion and adherent cell counts of *E. coli* O157 was lower. However, because the heated *L. plantarum* K11 rarely have the adhesion ability to Caco-2, the adhesion rate and adherent cell counts of *E. coli* O157 were high. In addition, the inhibitory effects of *E. coli* O157 adhesion by the CFCS and bacteriocin of *L. plantarum* K11 were dose-dependent manner. Therefore, the inhibition of adhesion of *E. coli* O157 to Caco-2 may result from the antimicrobial substances such as lactic acid and bacteriocin. Moreover the inhibitory activity of adhesion by the heated bacteriocin for 30 min at 100°C was similar to activity of non-treated bacteriocin, but the activity was disappeared by treatment with protease.

Keywords: *Lactobacillus plantarum*, *Escherichia coli* O157, adhesion, bacteriocin

Introduction

Probiotics are defined as the live microbial food supplements, which beneficially affect the host by improving its intestinal microbial balance (1). The representative probiotic strains include *Lactobacillus acidophilus, Lactobacillus johnsonii, Lactobacillus casei, Lactobacillus gasseri, Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium bifidum, Bifidobacterium infantis, Enterococcus faecalis,* and *Enterococcus faecium* (2,3).

Probiotic bacteria may promote endogenous barrier mechanisms in patients with atopic dermatitis, and by alleviating intestinal inflammation, may act as a useful tool in the treatment of food allergy (4). Probiotics did not cause a statistically significant change in interferon (IFN)-γ, interleukin (IL)-4, tumor necrosis factor (TNF)-α, eosinophil cationic protein or transforming growth factor-β (5). Oral administration of *L. casei* strain Shirota has been found to enhance innate immunity by stimulating the activity of splenic NK cells and it stimulated the production of Th1 cytokines and repressed incidence of tumors and production of IgE antibodies against ovalbumin in experimental mice (6,7). In addition, *Lactobacillus reuteri* CRL 1098 is an effective hypocholsterolemic adjuvant at a low cell concentration for mice and *L. acidophilus* ATCC 43121 is more likely to affect deconjugation and dehydroxylation during cholesterol metabolism than the assimilation of cholesterol into cell membranes (8,9).

Many studies has been reported that probiotics might suppress the growth of the intestinal microflora that convert procarcinogens into carcinogens and produce antitumourigenic or antimutagenic compounds, although the precise mechanism of action is not yet known (10-12). *L. johnsonii* La1-acidified milk ingestion induced a decrease in *Helicobacter pylori* density in the antrum, and reduced inflammation and gastritis activity in the antrum and the corpus, and the spent culture supernatant of the *L. acidophilus* strain LB treatment also inhibits the *H. pylori* urease activity *in vitro* (13,14).

Besides, probiotics have been examined for their effectiveness in the prevention and treatment of a diverse spectrum of gastrointestinal disorders such as antibiotics-associated diarrhea, infectious bacterial and viral diarrhea including diarrhea caused by rotavirus, *Shigella* spp., *Salmonella* spp., enterotoxigenic *Escherichia coli*, *Vibrio cholerae*, and human immunodeficiency virus/acquired immunodeficiency disorder (15). *Lactobacillus* species exert inhibition effect against pathogenic microorganisms by different mechanisms such as production of antimicrobial agents, which include organic acids, hydrogen peroxide, and bacteriocins (ribosomal synthesized antimicrobial peptides) (16,17). *B. breve* 4 and *B. infantis* 1 strains inhibited Caco-2 cell invasion by enteropathogenic *E. coli, Yersinia psedotuberculosis*, and *S. typhimurium* strains and adhesion of *S. typhimurium* was significantly inhibited by probiotic *L. johnsonii* LJ1 and *L. casei* Shirota (18,19). Also, the amount of adherent *Staphylococcus aureus* in human intestinal mucus was reduced 39-44% by *L. rhamnosus* GG, *L. lactis* subsp. *lactis*, and *Propionibacterium freudoenreichii* subsp. *shermanii*, which was probably due to the production of antimicrobial substances (20).

It had already reported that the bacteriocin of *L. plantarum* K11 isolated from *dongchimi* exhibited the bactericidal effect against *E. coli* O157 *in vitro* using the microtitre plate assay (21). In this study, we investigated inhibition of *E. coli* O157 adhesion to Caco-2 cells by the

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cell, cell-free culture supernatant (CFCS), and the bacteriocin of the *L. plantarum* K11.

Materials and Methods

Caco-2 cell cultures The adhesion of the LAB strains was examined using Caco-2 human colon adenocarcinoma epithelial cells which were obtained from the Korean Cell Line Bank (KCLB). Caco-2 cells were grown in 75 cm^2 tissue culture flasks in Dulbecco's modified Eagle medium (DMEM, Gibco, Invitrogen Ltd., Paisley, UK) supplemented with 10%(v/v) fetal bovine serum (FBS, Gibco) 2 mM of glutamine, 1 mM of sodium pyruvate, 100 units/mL penicillin, and 50 μg/mL streptomycin at 37°C in a humidified 5% CO_2 incubator until approximately 90% confluent. Monolayers of Caco-2 cells were used at late post-confluence culture after 15 days, with a change of medium every 2 days.

Adhesion of *L. plantarum* K11 on Caco-2 Prior to the adhesion assay, *L. plantarum* K11 cells obtained from *dongchimi* were centrifuged for 10 min at 7,000×g and washed twice with PBS after cultured for 24 hr at 37°C in MRS broth. To obtain heat-killed, PBS suspensions of *L. plantarum* K11 cells were heated at 95°C for 20 min, washed with phosphate buffered saline (PBS) subsequently, and assayed for adhesion activity. The heated or non-heated bacteria (10^5, 10^6, 10^7, 10^8, and 10^9 CFU/mL) resuspened in DMEM was transferred in 24-well with Caco-2 monolayers and incubated for 2 hr at 37°C in 5% CO_2. After incubation, the cells in each well were washed twice with PBS, fixed with 2% formalin for overnight, stained with 2% eosin Y, again washed twice with 1% acetic acid added in 50% ethyl alcohol, and measured at 570 nm using an enzyme-linked immunosorbent assay (ELISA) reader (Spectrocount, Packard Instruments, Meriden, CT, USA). Individual histograms were made based on the percentage of the control level. Adhesion assays were conducted in triplicate.

Inhibition of *E. coli* O157 adhesion to Caco-2 by *L. plantarum* K11 cells *E. coli* O157 ATCC 43889 was obtained from American Type Culture Collection (ATCC). *L. plantarum* K11 and *E. coli* O157 incubated under optimal condition respectively were harvested by centrifugation, washed twice with PBS, and resuspened in DMEM. *L. plantarum* K11 bacteria resusupened in DMEM was transferred in well with Caco-2 cells, incubated for 2 hr at 37°C in 5% CO_2, and washed 3 times with sterile PBS. And then the *E. coli* O157 suspended in DMEM were added to each well and incubated for 2 hr at 37°C in 5% CO_2. The wells were washed with PBS and assayed for bacterial adhesion as described above. Besides, to determine the numbers of adherent *E. coli* O157 after adhesion to Caco-2 cells, a trypsin-ethylenediamine tetraacetic acid (EDTA) solution (100 μL) was then added to each well washed with PBS and the plate incubated at 37°C for 5 min. A 900 μL aliquot of PBS was then pipetted into each well, the adherent bacterial cells to Caco-2 were completely unattached to well and centrifuged for 10 min at 1,000×g, and collected the pellet was suspended with PBS. The numbers of adherent *E. coli* O157 were determined after serial dilution and plating on MacConkey agar. Plates were incubated at 37°C for 18-24 hr and the adherent cells were expressed as colony forming units (log CFU/mL).

Inhibition of *E. coli* O157 adhesion to Caco-2 by the CFCS and bacteriocin of *L. plantarum* K11 CFCS of *L. plantarum* K11 grown in Lactobacilli MRS broth at 37 °C for 12 hr was obtained from a culture by centrifugation at 7,000×g for 10 min at 4°C. To prepare the bacteriocin, the pH of CFCS was adjusted to 7.0 using 1 M NaOH, and ammonium sulfate to 50%(w/v) saturation was added to the CFCS, 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., Laguna Hills, CA, USA) and filtered through a 0.22-μm membrane filter (Millipore Corp., Billerica, MA, USA). Bacteriocin activity was quantified by the microtitre plate assay (22).

To examine the effect of the CFCS and bacteriocin of *L. plantarum* K11 on adherent *E. coli* O157 to Caco-2, *E. coli* O157 bacteria resusupened in DMEM was transferred in well with Caco-2 cells, incubated for 2 hr at 37°C in 5% CO_2. And then the CFCS (25, 50, 100, and 200 μL/mL) and bacteriocin (80, 160, 320, and 640 BU/mL) were added to each well and incubated for 2 hr, and the reduction rates of *E. coli* O157 adhesion and adherent cell counts were estimated as described above. In addition, the bacteriocin was exposed to heat treatment for 30 min at 100°C, and mixed with protease (50 mM Tris-HCl, pH 7.5) solution at a final concentration of 1 mg/mL and the enzymes were inactivated by heating after 10 min at 80°C. *E. coli* O157 bacteria (10^8 CFU/mL) resusupened in DMEM was transferred in well with Caco-2 cells, incubated for 2 hr at 37°C in 5% CO_2. And then enzymes or heating-treated bacteriocin solution (640 BU/mL) were added to each well and incubated for 2 hr, and the reduction rates of *E. coli* O157 adhesion and adherent cell counts were estimated.

Results and Discussion

Adhesion of *L. plantarum* K11 to Caco-2 cells The rates of adhesion of the viable and heated *L. plantarum* K11 to Caco-2 cells were shown in Fig. 1. The level of adhesion at a 10^5 CFU/mL of viable *L. plarntarum* K11 was 15.7, and 53.2% was shown by 10^9 CFU/mL, therefore, the more the cell counts of viable *L. plantarum* K11 were increased, the more the rate of adhesion to Caco-2 were increased. But the level of adhesion of heated *L. plantarum* K11 ranged from 3.2 to 9.3% irrespective of the cell counts.

Gopal *et al.* (23) reported that *L. rhamnosus* DR20, *L. acidophilus* HN017, and *B. lactis* DR10 showed strong adhesion indices (219±36, 172±16, and 194±25, respectively) with the Caco-2 cell lines *in vitro*. Also, Ouwehand *et al.* (24) suggested that the rate of adhesion ranged from 3 (*L. casei* 01) to 43% (*L. rhamnosus* GG), so that there was a significant variation in adhesion between the strains. In addition, Suegara *et al.* (25) reported that heating treatment of *L. fermenti* C26 considerably decreased their ability to adhere to host cells and also their survival rate.

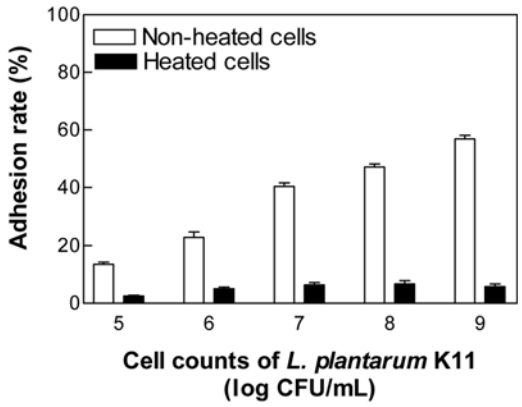

Fig. 1. Adhesion rates of viable and heated *L. plantarum* K11 to Caco-2 cells.

Effects of the viable and heated *L. plantarum* K11 on the adhesion of *E. coli* O157 to Caco-2 cells The effects of the viable and heated *L. plantarum* K11 on the adhesion of *E. coli* O157 to Caco-2 cells were shown in Table 1 and 2. The more the cell counts of viable *L. plantarum* K11 previously adhered to Caco-2 were increased, the more the rate of adhesion and adherent cell counts of *E. coli* O157 was low. The adhesion rate of *E. coli* O157 (10^4 CFU/mL) after adhered with viable *L. plantarum* K11 (10^4 CFU/mL) to Caco-2 was 145±6.7%, and the adherent cell counts was 2.86 log CFU/mL, however the adhesion rate and adherent cell counts of *E. coli* O157 after adhered with 10^9 CFU/mL of viable *L. plantarum* K11 were 105±1.7% and 0.91 log CFU/mL. Meanwhile, the adhesion rate of *E. coli* O157 (10^4 CFU/mL) to Caco-2 ranged from 107±9.2 to 121±12.1% regardless of the cell counts of heated *L. plantarum* K11, and at this time the adherent cell counts of *E. coli* O157 ranged from 1.18 to 1.97 log CFU/mL. Above all, because the heated *L. plantarum* K11 rarely have the adhesion ability to Caco-2, the more the cell counts of *E. coli* O157 which was adhered to Caco-2 were increased, the more the adhesion rate and adherent cell counts were high. The results indicated that because the components (protein) mediating the adhesion of the *Lactobacillus* strain to the host cells are the heat-labile (25), the ability of protein-containing substances connected with the adhesion of *L. plantarum* K11 was lost by heat treatment. Hence, the viable *L. plantarum* K11 previously adhered to Caco-2 have inhibitory effects of the adhesion rate and adherent cell counts of *E. coli* O157, but those effects were not

Table 1. Rates of adherence and adherent cell counts of *E. coli* O157 after adhesion of viable *L. plantarum* K11 to Caco-2

Cell counts of *L. plantarum* K11 (CFU/mL)[1)]	Cell counts *E. coli* O157 (CFU/mL)[2)]							
	10^4		10^5		10^6		10^7	
	Adhesion (%)[3)]	Cell counts (Log CFU/mL)[4)]	Adhesion (%)	Cell counts (Log CFU/mL)	Adhesion (%)	Cell counts (Log CFU/mL)	Adhesion (%)	Cell counts (Log CFU/mL)
10^4	145±6.7	2.86	156±11.2	3.03	159±12.1	3.15	168±14.9	3.58
10^5	127±10.2	2.34	131±9.1	2.57	140±13.1	2.59	133±14.0	3.04
10^6	112±8.1	2.18	124±7.2	2.21	120±8.8	2.27	119±13.8	2.61
10^7	105±2.9	1.83	107±4.3	2.01	111±6.3	1.95	104±3.1	2.25
10^8	107±3.4	1.17	110±2.5	1.27	116±4.1	1.56	111±5.5	1.60
10^9	105±1.7	0.91	104±2.1	1.01	112±3.6	1.24	107±4.0	0.80

[1)]Viable cell counts of viable *L. plantarum* K11 which are adhered to Caco-2.
[2)]Viable cell counts of *E. coli* O157 which are adhered after adhesion of viable *L. plantarum* K11 to Caco-2.
[3)]Adhesion rate of *E. coli* O157 after adhesion of viable *L. plantarum* K11 on Caco-2. Adhesion rate of *L. plantarum* K11 to Caco-2 was considered as 100%. Values are mean±SD of 3 separate experiments.
[4)]Viable cell counts of adhered *E. coli* O157 after adhesion of viable *L. plantarum* K11 to Caco-2.

Table 2. Rates of adherence and adherent cell counts of *E. coli* O157 after adhesion of heated *L. plantarum* K11 to Caco-2

Cell counts of *L. plantarum* K11 (CFU/mL)[1)]	Cell counts of *E. coli* O157 (CFU/mL)[2)]							
	10^4		10^5		10^6		10^7	
	Adhesion (%)[3)]	Cell counts (Log CFU/mL)[4)]	Adhesion (%)	Cell counts (Log CFU/mL)	Adhesion (%)	Cell counts (Log CFU/mL)	Adhesion (%)	Cell counts (Log CFU/mL)
10^4	118±15.0	1.61	120±16.2	1.90	147±5.6	2.52	163±6.2	3.67
10^5	107±9.2	1.97	131±21.5	2.26	141±11.4	3.06	158±5.1	3.34
10^6	116±10.8	1.42	128±12.4	2.66	146±9.5	3.15	170±10.3	3.59
10^7	119±13.2	1.50	130±8.5	1.97	159±10.6	3.29	167±6.7	3.84
10^8	116±7.6	1.18	128±9.3	2.57	149±8.7	2.77	173±8.2	3.49
10^9	121±12.1	1.37	129±10.1	2.46	151±13.5	2.95	174±7.9	3.35

[1)]Viable cell counts of heated *L. plantarum* K11 which are adhered to Caco-2.
[2)]Viable cell counts of *E. coli* O157 which are adhered after adhesion of heated *L. plantarum* K11 to Caco-2.
[3)]Adhesion rate of *E. coli* O157 after adhesion of heated *L. plantarum* K11 to Caco-2. Ahesion ratio of heated *L. plantarum* K11 to Caco-2 was considered as 100%. Values are mean±SD of 3 separate experiments.
[4)]Viable cell counts of adhered *E. coli* O157 after adhesion of heated *L. plantarum* K11 to Caco-2.

shown by the heated *L. plantarum* K11 cannot adhered to Caco-2.

Owing to the inhibitory effects of *L. casei* subsp. *rhamnosus* strain on the adherence of enteropathogenic *E. coli*, enterotoxigenic *E. coli* and *K, pneumoniae*, this probiotic strain could be used to prevent colonization of the gastrointestinal tract by a large variety of pathogens (26). Also, because the pretreatment of intestinal (T84) cells with lactic acid-producing bacteria reduced the pathogen-induced drop in transepithelial electrical resistance, probiotics prevent epithelial injury induced by attaching-effacing bacteria (27).

Hirano *et al.* (28) reported that while the adhesion and colonization of enterohemorrhagic *E. coli* (EHEC) was not affected by any of the *L. gasseri, L. casei*, and *L. plantarum*, the suppressive effect on EHEC internalization was strictly dependent on viable *L. rhamnosus* but could not be observed with the killed *L. rhamnosus*. Besides, Lactobacilli were able to compete with pathogenic gastrointestinal (GI) bacteria when they were incubated together, but the degree of inhibition of adhesion was bacterial strain-dependent (29,30). The differences in adhesion between strains were not related to differences in viability among *Lactobacillus* strains, but that amount of bound bacteria is dependent on the number of bacteria added (31).

Miyoshi *et al.* (32) demonstrated that the adhesion of *L. reuteri* 104R appears due to the binding of adhesion promoting protein to receptor-like molecules on Caco-2 cells. And, the *Lactobacillus* strains that increased extracellular secretion of MUC3 mucin led to reduced adherence of enteropahtogen *E. coli* E2348/69 during co-incubation (33).

Meanwhile, Reid *et al.* (34) suggested that by virtue of a direct correlation between bacterial hydrophilicity and adhesion to uroepithelial cells, *L. rhamnosus* GG was able to compete with *E. coli* and *Salmonella* spp. of low hydrophobicity and high adhesion-receptor interaction to Caco-2 cells. Also, the interference of adhesion of gastrointestinal bacteria by *L. rhamnosus* GG was probably through steric hindrance and the degree of inhibition was related to the distribution of the adhesion receptors and hydrophobins on the Caco-2 surface (30). High cell surface hydrophobicity may favor the colonization of mucosal surface and play a role in the adhesion of bacteria to epithelial cells, thus hydrophobicity may be helpful for adhesion, but it is obviously not a prerequisite for a strong adherence capacity (35,36).

Moreover, the surface topography of *L. crispatus, L. helveticus,* and *L. johnsonii* shows major differences in a crystalline-like protein layer of the cell wall. In force volume images calculated into elasticity and adhesion force maps, *L. crispatus* and *L. helveticus* have a surface with a homogeneous stiffness with no adhesion events, but *L. johnsonii* strains have surface properties which strongly differ from the 2 strains (37).

In addition, a combination of *L. casei* NY1301 and *L. gasseri* NY0509 may have synergistic effects of adhesion to human intestinal mucosa (38), and combinations of probiotic strains are useful and more effective in inhibition of pathogen adhesion than individual strains (39).

Effects of the CFCS and bacteriocin of *L. plantarum* K11 on the adhesion of *E. coli* O157 to Caco-2

Probiotics may reduce the number of pathogens by producing antimicrobial components such as lactic acid, hydrogen peroxide and bacteriocins (40). To examine effects of the lactic acid and bacteriocin of *Lactobacillus* on the adhesion of *E. coli* O157 to Caco-2 cells, we prepared the CFCS and bacteriocin from *L. plantarum* K11. The results of adhesion rate and adherent cell counts of *E. coli* O157 by the antimicrobial substances of *L. plantarum* K11 were shown in Table 3 and 4. When the cell counts of *E. coli* O157 which be adhered to Caco-2 were 10^4 CFU/mL, the reduced adhesion rate and cell counts by 25 μL/mL of CFCS were 9.5±5.2 and 8.9±5.7 %, and the reduction rates by 200 μL/mL were 53.0±15.2 and 35.2±2.1%, respectively. When the cell counts of *E. coli* O157 which be adhered to Caco-2 were 10^8 CFU/mL, the reduced adhesion rate and cell counts by 25 μL/mL of CFCS were 4.5±2.8 and 4.1±1.1%, and the reduction rates by 200 μL/mL were 40.5±8.8 and 28.0±3.6%, respectively. In addition, when the *E. coli* O157 (10^4 CFU/mL) was adhered to Caco-2, the adhesion rate and cell counts were decreased 5.2±2.3 and 4.7±1.4% by 80 BU/mL of bacteriocin, and were declined 35.1±2.6 and 28.7±11.2% by 640 BU/mL. However, the reduction effects of adhesion and cell counts of *E. coli* O157 which was adhered with 10^7 and 10^8 CFU/mL were not appeared by the bacteriocin

Table 3. Effects of the CFCS of *L. plantarum* K11 on the adhesion and adherent cell counts of *E. coli* O157 to Caco-2

Cell counts of *E. coli* O157 (CFU/mL)[1]	CFCS of *L. plantarum* K11 (μL/mL)[2]							
	25		50		100		200	
	Reduction rate (%)							
	Adhesion[3]	Cell Counts[4]	Adhesion	Cell counts	Adhesion	Cell counts	Adhesion	Cell counts
10^4	9.5±5.2	8.9±5.7	16.0±6.7	13.5±3.3	26.0±5.2	22.1±1.8	53.0±15.2	35.2±2.1
10^5	7.1±2.1	7.8±2.3	14.1±8.1	9.8±6.1	25.4±7.6	19.0±2.6	51.7±8.1	28.1±5.2
10^6	8.9±3.6	7.5±3.8	15.8±6.6	11.2±5.0	19.9±10.1	20.1±1.9	49.9±3.6	27.9±4.4
10^7	6.4±1.4	5.7±0.6	11.4±9.1	10.7±2.7	20.5±7.9	21.5±3.3	48.5±9.4	26.1±1.7
10^8	4.5±2.8	4.1±1.1	8.5±3.5	9.6±3.3	17.5±5.3	18.5±1.4	40.5±8.8	28.0±3.6

[1]Viable cell counts of *E. coli* O157 which are adhered to Caco-2.
[2]Added amount of CFCS prepared from *L. plantarum* K11.
[3,4]Results are expressed as reduction % (mean±SD) of adhesion rate and adherent cell count of *E. coli* O157 by the CFCS compared to the control (not added the CFCS).

Table 4. Effects of the bacteriocin prepared from *L. plantarum* K11 on the adhesion and adherent cell counts of *E. coli* O157 to Caco-2

Cell counts of *E. coli* O157 (CFU/mL)[1)]	Bacteriocin of *L. plantarum* K11 (BU/mL)[2)]							
	80		160		320		640	
	Reduction rate (%)							
	Adhesion[3)]	Cell counts[4)]	Adhesion	Cell counts	Adhesion	Cell counts	Adhesion	Cell counts
10^4	5.2±2.3	4.7±1.4	10.0±5.2	11.5±5.6	14.7±5.8	12.8±5.5	35.1±2.6	28.7±11.2
10^5	3.5±1.8	2.8±0.4	7.3±4.7	10.7±1.4	19.7±6.2	13.1±7.6	38.7±3.1	29.1±10.4
10^6	3.2±1.2	1.6±0.7	5.9±3.2	12.1±6.7	16.2±7.2	14.0±2.0	33.5±10.2	32.0±5.8
10^7	-[5)]	-	5.3±1.6	9.9±2.8	13.1±3.6	11.7±3.3	29.7±5.8	25.4±2.3
10^8	-	-	6.5±29.1	7.1±1.5	11.5±5.5	8.9±6.6	37.3±6.7	24.1±4.0

[1)]Viable cell counts of *E. coli* O157 which are inoculated to Caco-2.
[2)]Added amount of CFCS prepared from *L. plantarum* K11.
[3,4)]Results are expressed as reduction percentage (mean±SD) of adhesion rate and adherent cell count of *E. coli* O157 by the bacteriocin compared to the control (not added the bacteriocin).
[5)]Reduction rates of adhesion and adhered cell counts of *E. coli* O157 were not measured.

of 80 BU/mL. In other words, the adhesion inhibitory effects by the CFCS and bacteriocin were dose-dependent manner. Therefore, the inhibition of *E. coli* O157 adhesion to Caco-2 may result from the antimicrobial substances such as lactic acid and bacteriocin.

Meanwhile, when *E. coli* O157 (10^8 CFU/mL) which was adhered to Caco-2 was exposed to 640 BU/mL of bacteriocin, the rate of adhesion and adherent cell counts of *E. coli* O157 were declined 37.3±6.7 and 24.1±4.0%, moreover the inhibitory activity of adhesion by the heated bacteriocin for 30 min at 100°C was similar to activity of non-treated bacteriocin, but the activity was disappeared by treatment with protease (Fig. 2).

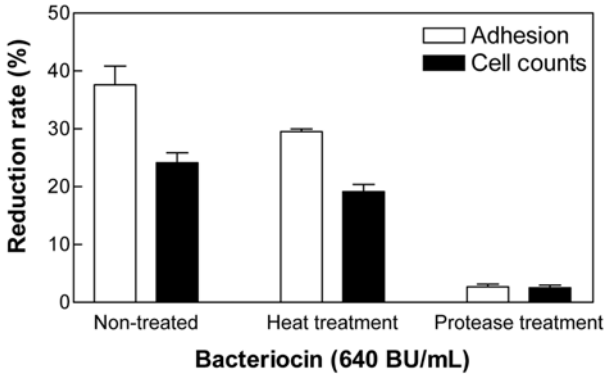

Fig. 2. Effects of the bacteriocin of *L. plantarum* K11 treated with heating or protease on the adhesion and adherent cell counts of *E. coli* O157 to Caco-2.

Adhesion of *S. typhimurium* was reduced in the presence of spent culture supernatant of *Lactobacillus* GG, particularly inhibition effect was observed with acidified fresh MRS broth, but not detected at neutral pH values, therefore the observed inhibition of *S. typhimurium* adhesion to Caco-2 was most likely a pH effect (41). On the other hand, the substances from both the *E. faecium* 18C23 cells and the spent culture supernatant contributed to the inhibition of adhesion of *E. coli* K88 to the small intestine mucus receptors, but the inhibiting effect was not solely a pH effect since considerable inhibitory action was demonstrated after neutralizing the mixture or spent culture supernatant to pH 7.0 (42).

Meanwhile, a surface-binding protein (29 kDa) from *L. fermentum* RC-14 that inhibits adhesion of *E. faecalis* 131 had been purified and characterized (43). Baccigalupi *et al.* (44) reported that at least 2 small (less than 3 kDa) factors were involved in mediating the *in vitro* interaction of *L. fermentum* with Caco-2 cells. Coconnier *et al.* (45) suggested that the adhesion-promoting factor was proteinaceous, since trypsin treatment dramatically decreased the adhesion of the *L. acidophilus* BG2FO4 strain. However, anti-adhesive factors of *Bifidobacterium*, antagonizing the adhesion of *Clostridium difficile* to human enterocytes were thermolabile, active at neutral pH and unaffected by proteolytic cleavage such as proteinase K and chymotrypsin (46). Especially, an adhesion-promoting protein that mediated binding of *L. fermentum* 104R to small intestinal porcine mucus is probably noncovalently bound to the cell wall and is released to the growth medium when the bacterial cells reach stationary phase (47).

Besides, the adhesion of *L. plantarum* Lp6 to rat small intestinal mucus was mainly mediated by the mannose specific adhesins, which might be proteins that reversibly bind to the cell surface components, and cell surface-bound exopolysaccharides were also involved in adhesion (48). The adhesion-promoting factor of *L. acidophilus* to Caco-2 cells was present in the spent culture supernatant, and carbohydrates on the bacterial cell wall appeared to be partly responsible for the interaction between the bacteria and the extracellular adhesion-promoting factor of *Lactobacillus* species (45). Likewise, another result showed that the adhesion of *L. johnsonii* La1 to Caco-2 cells was inhibited in a concentration-dependent way by lipoteichoic acid (LTA) separated and purified from *L. johnsonii* La1 bacteria (49).

In the future, we will study on identification and purification of inhibition substance(s) of *L. plantarum* K11 on adhesion of *E. coli* O157 to human intestinal Caco-2 cells.

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