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Effects of oral intake of *Kimchi*-derived *Lactobacillus plantarum* K8 lysates on skin moisturizing

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Running title: Moisturizing effect of *L. plantarum* K8 lysates

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Abstract

Skin is the soft outer covering of vertebrates that provides protection from pathogenic infection, physical damage, or UV irradiation and controls body temperature and water content. In this study, we examined the effects of oral intake of Kimchi-derived *Lactobacillus plantarum* K8 lysates on skin moisturizing. In an *in vitro* study, we observed that hyaluronic acid content increased in HaCaT cells treated with *L. plantarum* K8 lysates. Oral administration of *L. plantarum* K8 lysates effectively attenuated the horny layer formation and decreased epidermal thickening in DNCB-treated SKH-1 hairless mice skin. The damage to barrier function was reduced after 8 weeks of oral administration of *L. plantarum* K8 lysates as compared to that in the atopic dermatitis mice. For the test with volunteers, we manufactured experimental candy containing 2.1% *L. plantarum* K8 lysates, while control candy did not contain bacterial lysate. A significant increase in hydration in the experimental candy-administered group as compared to control candy-administered group was observed on the face after 4 and 8 weeks, and on the forearm after 4 weeks. Decreases in horny layer thickness and TEWL value were observed on the face and forearm of the experimental group. Together, the *in vitro* cell line and *in vivo* mouse studies revealed that *L. plantarum* K8 lysates have a moisturizing effect. A clinical research study with healthy volunteers also showed an improvement in barrier repair and function when volunteers took *L. plantarum* K8 lysates-containing candy. Thus, our results suggest that *L. plantarum* K8 lysates may help to improve skin barrier function.

Keywords: *Lactobacillus plantarum*, *L. plantarum* K8 lysates, bacterial lysates, atopic dermatitis, aglycone isoflavone, moisturizer.

Introduction

Healthy skin contributes to the prevention of infection, pressure ulcer development, incontinence-associated dermatitis, and skin tears (2, 8). It also prevents skin dryness by inhibiting the evaporation and loss of water. The extracellular lipid matrix is composed of a lipid complex including ceramide (50%), cholesterol (25%), and free fatty acids (10%) (10). Clinically, dry skin is dehydrated and has a low elasticity, which results in a rough and damaged outermost horny layer. It is an uncomfortable condition marked by scaling, itching, and cracking. The water content in skin is affected by environmental factors, skin metabolism, lipid layers, or natural moisturizing factors (5). Psoriasis and atopic dermatitis (AD) are chronic and relapsing inflammatory skin diseases caused by a skin barrier disorder. Although the pathophysiology of AD is not fully known, it involves a complicated interaction of environmental and genetic factors that induce abnormalities in the structure and function of the epidermal barrier and immune system. It is now well established that moisturizers play an important role in preventing skin inflammation in AD, including reducing the amount of topical corticosteroid use (13, 17). The presence of probiotic bacteria in the intestinal microbiota is also known to correlate with protection against atopy. Probiotics, such as lactobacilli, are very promising evidence to recommend the addition of probiotics to foods for the prevention and treatment of allergic diseases (12).

Lactic acid bacteria (LAB) are known for their health-promoting effects such as the non-specific enhancement of the immune system, protection against intestinal infection, reduction of serum cholesterol level, and antioxidant properties (11). Several strains of LAB also have effects on the production of cytokines, such as IL-12, IL-10, TNF- α , TGF- β , IL-8 and RANTES, and on cell proliferation in human intestinal epithelial cells (18). *Lactobacillus*

plantarum is a well-documented probiotic that has been used in clinical trials for the regulation of the immune system and treatment of gastrointestinal diseases with probiotics (6). The beneficial and protective properties of probiotic Gram-positive bacteria might be due to cell wall components including lipoteichoic acid (LTA). The biggest issue in the preparation of single components, however, may be complicated processes, which is time- and cost-consuming works. On the other hand, the preparation of *L. plantarum* K8 lysates are simple and cost-saving. In this study, we tried to identify the role of *L. plantarum* K8 lysates on the regulation of immune activity. For this, we examined the hyaluronic acid production in HaCaT cells, and assessed the attenuation effect of *L. plantarum* K8 lysates on AD mice. The moisturizing effect of *L. plantarum* K8 lysates-containing candy on the human face and forearm was also examined.

Materials and Methods

Preparation of *L. plantarum* K8 lysates

L. plantarum K8 (KCTC 10887BP) was cultured in 10 L MRS broth (Himedia Laboratories, Mumbai, India) containing soy bean peptone at 37°C for 18 h, and then cells were harvested by centrifugation at 8000 rpm for 10 min. After a wash in phosphate-buffered saline (PBS) three times, 50 g cells (wet weight) were disrupted by sonication. To obtain active components from disrupted cells, additional sonication was performed (up to 9 times). Disrupted bacteria (*L. plantarum* K8 lysates) were freeze-dried and packed under clean room conditions. Active components such as LTA and aglycone isoflavone were examined by phosphate assay and LC-MS/MS, respectively, and described in Supplementary Materials.

Manufacturing of *L. plantarum* K8 lysates-contained candies.

We manufactured candies using common ingredients. Experimental candy contained 2.1% *L. plantarum* K8 lysates, while control candy contained no lysates. The information on candy ingredients is described in Table S1.

Mice

SKH-1 hairless female mice (6 weeks old) were purchased from Central Lab, Animal Inc. (Seoul, Korea). They were kept in individual cages at 24±2°C and 50±10% moisture condition, and fed nutritionally balanced rodent food (Central Lab. Animal Inc.) and sterilized water. The mice were cared for and used in accordance with the guidelines of the animal ethics committee of Gyeonggi Bio Center. To develop a mouse model of atopic dermatitis (AD) using skin sensitization, each mouse was exposed to 200 µl immune-disturbing material containing acetone:olive oil (3:1) and 2.5% 2,4-dinitrochloro benzene

(DNCB; Sigma, MO, USA). After 3 days exposure, mice were treated with 150 µl 1.0% DNCB at 3-day intervals. Mice were allocated to three random groups ($n=8/\text{group}$): normal control group, AD group, and atopy-treated group. The treated group was orally administered by gavage with *L. plantarum* K8 lysates (1×10^9 CFU/mouse, day) in 100 µl water.

Histopathological examination

The dorsal skins of the experimental mice were removed on the final day of the schedule and fixed in 10% neutral-buffered formalin and embedded in paraffin. Serial paraffin sections (5 µm) were stained with haematoxylin and eosin (H&E), and the skin layer was examined by NIS-Elements (Nikon, Japan).

Clinical study with healthy volunteers

Forty-one (41) healthy volunteers aged 25 to 60 years with dry and dark skin were recruited from the campus of Kyung Hee University (Yongin, Korea), where the trial was conducted. All volunteers signed an informed consent form before participation. The Medical Ethics Committee of Kyung Hee University approved the trial (KHUSBC 2013-011). Participants were randomly assigned to two groups (control and experimental group), and then randomly assigned to two subgroups groups within each group to examine each side of the face. Participants took four control candies or experimental candies a day (two candies in the morning and two candies in the evening) for 8 weeks. Participants were banned from using certain functional cosmetics, external application, and dietary supplement from two weeks before study to the end of the study.

Skin hydration measurement

Hydration under the dead skin (10-20 μm of the Stratum corneum) was examined using the Corneometer[®] CM825 according to the manufacturer's instructions (Courage+Khazaka Electronic GmbH, Cologne, Germany). To determine whether epidermal permeability barrier function was altered in participants, transepidermal water loss (TEWL) was assessed using a Vapometer[®] (Delfin Technologies Ltd., Kuopio, Finland). Additionally, the interior of the forearm and front cheek were examined with D-squame[®] Black Tape. The image of the collected dead skin samples was magnified 70 times using Charm View (Moritex, Japan) and analyzed with Image-Pro[®] Plus software (Media Cybernetics Inc. MD, USA).

Statistical analysis

The average of the value estimated before and after 4 and 8 weeks of administration of candies was statistically analyzed according to the time and between groups. Data represent the mean $\pm$ SEM and the normality test was confirmed by Shapiro-Wilk test. The statistical significance at different time points was tested by paired *t*-test ($p < 0.05$) when the data fit the normality test (95% confidence) or by Wilcoxon test ($p < 0.05$) when the data did not fit the normality test. Statistical significance between groups was performed by independent *t*-test ($p < 0.05$) when the data fit the normality test or by Mann-Whitney test ($p < 0.05$) when the data did not fit the normality test. SPSS[®] 20.0 software was used for the analysis.

Results

Preparation of *L. plantarum* K8 lysates and candy manufacturing

To obtain *L. plantarum* K8 lysates, a general sonication method was used. About 30~50 g *L. plantarum* K8 (wet weight) were resuspended in distilled water after 3-5 washes, and then subjected to sonication. Cell wall component, e.g., lipoteichoic acid (LTA), was extracted from the disrupted cells. LTA is a major pathogen-associated molecular pattern (PAMP) molecule of Gram-positive bacteria. LTA mediates innate immune and inflammatory responses (14) similar to the recognition of lipopolysaccharide (LPS) in Gram-negative bacterial sepsis (7). Interestingly, the repetition of the sonication step increased the extraction of active components, such as LTA and aglycone isoflavone, within the disrupted bacteria. The amount of LTA (Figure S1A) and aglycone isoflavone such as daidzein and genistein (Figure S1B), for example, increased with number of sonication steps, and peaked after the 8th sonication and decreased with the 9th sonication step. *TNF- α inhibitory effect of the *L. plantarum* K8 lysates against LPS was increased by repeated sonication (7 to 8 times) in THP-1 cells as compared to fewer sonication steps (fewer than 4 times), indicating that more active components might be extracted from the cell wall by increasing the sonication number (data not shown).* *L. plantarum* K8 lysates did not induce cell death (data not shown), while hyaluronic acid contents increased up to 78~85% with cells treated with *L. plantarum* K8 lysates compared to the positive control N-acetyl-d-glucosamine (NAG)-treated cells, indicating that *L. plantarum* K8 lysates may have a moisturizing effect (Fig. 1). The candies used in this study had the same ingredients except that the experimental candy contained 2.1% *L. plantarum* K8 lysates (Table S1). After the manufacture of candies, index components, such as LTA (Figure S2) and aglycone isoflavone (Table S2) of *L. plantarum* K8 lysates, were examined by liquid chromatography-mass spectrometry (LC/MS).

Assessment of clinical and epidermal permeability barrier function in mice

Clinical assessment of AD was performed by measuring the horny layer of the skin. The horny layer increased significantly in the DNCB-treated group compared with the negative control, while oral administration of *L. plantarum* K8 lysates effectively attenuated the horny layer formation (Fig. 2A). Oral administration of *L. plantarum* K8 lysates also decreased epidermal thickening in DNCB-treated skin (Fig. 2A, H&E staining). Epidermal permeability barrier function was assessed by measuring TEWL. TEWL increased significantly in the DNCB-treated group by 600%, 580%, and 440% after oral administration for 4, 6, and 8 weeks, respectively, compared with that in the negative controls. The damage to barrier function after the 8 weeks oral administration was reduced by *L. plantarum* K8 lysates (12.6%, $p=0.0115$) compared with that in the AD mice that received DNCB treatment only (Fig. 2B). The thickness of mouse skin was increased in DNCB-treated group by 450%, 320%, and 250% after oral administration for 4, 6, and 8 weeks, respectively, as compared to control. Oral administration of *L. plantarum* K8 lysates decreased skin thickness by 10%, 35% ($p=0.0365$), and 40% ($p=0.0425$) in comparison to skin thickness in the DNCB-treated group (Fig. 2C). These results suggest that oral administration of *L. plantarum* K8 lysates improves the clinical signs of AD and reduces damage to epidermal barrier function in mice.

L. plantarum K8 lysates-containing candy increased skin hydration

The hydration contents of the front cheek and interior of forearm of the volunteers were examined before initiation of the study, and 4 and 8 weeks after the administration of candy. A statistically significant difference was found using an independent *t*-test and Mann-Whitney test between the control and experimental group. The hydration on the face and forearm increased in the experimental group (*L. plantarum* K8 lysates-containing candy-

administered group) after 4 weeks of administration when compared to hydration prior to testing. Both the control and experimental groups showed that the hydration increased on the face (5.87% in control and 19.01% in experimental) and forearm (5.49% in control and 16.86% in experimental) at 8 weeks after administration of the candy. A significant increase in hydration in the experimental group as compared to control group was shown on the face after 4 and 8 weeks administration, while hydration increased on the forearm after 4 weeks administration (Table 1).

***L. plantarum* K8 lysates-containing candy decreased TEWL values**

A statistically significant difference in TEWL between control and experimental groups was observed at 8 weeks (paired *t*-test, $p < 0.01$; Wilcoxon signed rank test, $p < 0.05$). When compared to the control group TEWL values were significantly decreased in the experimental candy-administered group on both face and forearm (Table 2). The decrease of TEWL in the experimental group was about 8% on the face and 14% on the forearm as compared to the control group. No statistical differences were observed between the TEWL values of the two groups at 4 weeks.

***L. plantarum* K8 lysates-containing candy decreased the thickness of the horny layer**

The horny layer was significantly decreased on the face in the experimental group after 4 and 8 weeks, while both control and experimental groups showed a significant decrease in the horny layer on the forearm. When compared to the control group, the thickness of the horny layer was found to be significantly reduced on the face (18.48%) and forearm (29.61%) of experimental group volunteers after oral administration of *L. plantarum* K8 lysates-containing candy for 4 and 8 weeks as compared to control group (3.53% on face and 14.27%

on forearm) (Table 3). A statistically significant difference was found by using an independent t -test ($p^{**}<0.01$ and $p^{***}<0.0001$) and Mann-Whitney test ($p<0.05$). The image of the horny layer from control and experimental groups was shown in Fig. S3. Skin horny layer was reduced in volunteers who took *L. plantarum* K8 lysates-containing candy on both face (Fig. S3A) and forearm (Fig. S3B).

Discussion

This study was aimed at investigating the effects of oral administration of *L. plantarum* K8 lysates on the AD mouse model and the moisturizing effect of *L. plantarum* K8 lysates-containing candy on the human face and forearm. It is known that AD mouse is a good animal model for the study of miniaturization effects since moisturizing affect AD alleviation (17). AD is a common chronic inflammatory skin disease that is increasing in prevalence, and is frequently found in infants and children (4). Pathogenesis of AD involves a complicated interaction of environmental and genetic factors that induce the abnormalities in the structure and function of the epidermal barrier and immune system (13). Interleukin (IL)-4 switches B cell signals to synthesis IgE, and IgE-mediated mast cell activation leads to the release of various kinds of chemical mediators, which results in infiltration of inflammatory cells into the skin lesion. On the other hand, IL-12 and Interferon (IFN)- γ repress B cell-mediated IgE synthesis (16). The elevated serum IgE levels in patients with AD also suggest that T helper (Th) 2 cytokines may participate in disease pathogenesis (3). In our previous study using *L. plantarum* lysates, we observed decreased IL-4 in peritoneal macrophages and casein-specific IgE level from blood, indicating that *L. plantarum* lysates repress Th2 response. Th1-response cytokines, such as IL-12 and IFN- γ , were increased by *L. plantarum* lysates. The regulation of Th1/Th2 balance by *L. plantarum* lysates may contribute the induction of hyaluronic acid and alleviation of AD lesions.

These day, it is well established that moisturizers play an important role in preventing skin inflammation in AD, including reducing the amount of topical corticosteroid use (11, 13, 15, 17). Thus, the use of moisturizers is considered standard therapy for the treatment of AD. The purpose is to improve dry skin with decreased barrier function and also to prevent recurrence

of inflammation. Moisturizers are externally applied compounds comprising multiple components, aiming to maintain skin integrity and appearance. Previous randomized controlled studies have shown that the use of moisturizers reduces the amount of topical corticosteroid use, and affects the skin barrier function of normal skin by reducing TEWL and susceptibility to irritants (17). In the current study, oral administration of *L. plantarum* K8 lysates cell lysates has been shown to alleviate AD in a mouse model by reducing the TEWL value. The epidermal thickness was also reduced in the *L. plantarum* K8 lysates-administered experimental group. This alleviation may be induced by regulatory dendritic cells and the generation of CD4⁺FoxP3⁺ Treg cells within the mesenteric lymph nodes (9). Treg cells play a critical role in immune-mediated inflammation by preventing T-cell activation. The regulation of Treg cells by probiotic cell lysates, however, has not been studied yet.

A clinical research study with healthy volunteers showed improvement in barrier repair and function when volunteers took *L. plantarum* K8 lysates cell lysate-containing candy. TEWL values were significantly decreased in the experimental group after 8 weeks of administration as compared to the control group. Although the water content on the face and forearm of volunteers increased in both the control and experimental group, a significant increase was shown in the experimental group when it was compared to the control group. Statistically significant differences were found; sample candy containing *L. plantarum* K8 lysates demonstrated significantly higher capacitance readings. In contrast, control candy did not emerge as different when compared to control site values. While the reasons for this difference were not explored in this study, it can be inferred that the active component in *L. plantarum* may contribute to the difference in capacitance readings.

In conclusion, we observed that the hyaluronic acid was significantly increased by *L. plantarum* K8 lysates *in vitro* experiment. Hyaluronic acid is known to restore hydration. The alleviation of AD lesion in mouse model also indicates that *L. plantarum* K8 lysates have a moisturizing effect. The experiments with healthy volunteers who administered *L. plantarum* K8 lysates-containing candies showed increased hydration, less water loss, and decreased horny layers on face and forearm as compared to the control group who administered *L. plantarum* K8 lysates-free candies. Thus, our results suggest that *L. plantarum* K8 lysates may help to improve skin miniaturization.

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274

275 **Conflict of interest**

276 The authors have declared no conflicting interests.

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320

321 Tables

322 Table 1. Comparison of hydration content between groups.

Site	Week	Group	Δ Mean $\pm$ S.D. (A.U)	<i>p-value</i> [†]
Face	4	Control	0.64 $\pm$ 0.48	0.000 ^{***^A}
		Experimental	6.47 $\pm$ 1.14	
	8	Control	2.39 $\pm$ 0.61	0.007 ^{**}
		Experimental	8.10 $\pm$ 1.86	
Forearm	4	Control	0.49 $\pm$ 0.44	0.030 [*]
		Experimental	2.22 $\pm$ 0.62	
	8	Control	1.35 $\pm$ 1.04	0.068 ^A
		Experimental	4.14 $\pm$ 0.84	

323 [†]*p-value* : Independent *t*-test (**p*<0.05, ***p*<0.01, ****p*<0.001)

324 [†]*p-value*^A: Mann-Whitney test

325

326 Table 2. Comparison of epidermal water loss between groups (TEWL value)

Site	Group	Week	Δ Mean $\pm$ S.D. (g/m ² h)	<i>p</i> -value†	IF°(%)
Face	Control	0	13.02 $\pm$ 2.15	-	-
		4	13.21 $\pm$ 2.12	0.392	▲1.46
		8	12.76 $\pm$ 2.08	0.330	▼1.20
	Experimental	0	12.98 $\pm$ 1.83	-	-
		4	12.90 $\pm$ 2.05	0.837	▼0.62
		8	11.93 $\pm$ 1.62	0.008**	▼8.09
Forearm	Control	0	6.89 $\pm$ 1.16	-	-
		4	6.90 $\pm$ 1.16	0.936	▲0.15
		8	6.61 $\pm$ 1.31	0.187	▼4.06
	Experimental	0	7.56 $\pm$ 1.04	-	-
		4	7.35 $\pm$ 1.35	0.303	▼2.78
		8	6.50 $\pm$ 1.33	0.002***^	▼14.02

327 †*p*-value: Paired *t*-test (**p*<0.05, ***p*<0.01, ****p*<0.001)328 †*p*-value ^: Wilcoxon signed rank test.329 ° Intensification factor (IF): (W_x – W₀)/W₀×100, calculated by mean value

330

331 Table 3. Comparison of horny layers between groups

Site	Week	Group	Δ Mean $\pm$ S.D. (pixels)	<i>p-value</i> [†]
Face	4	Control	272.35 $\pm$ 3,795.82	0.002**
		Experimental	-14,696.67 $\pm$ 2,306.89	
	8	Control	-7,373.50 $\pm$ 4,395.46	0.000***
		Experimental	-38,263.71 $\pm$ 3,653.16	
Forearm	4	Control	-29,518.20 $\pm$ 4,504.67	0.007** [^]
		Experimental	-48,557.33 $\pm$ 5,394.31	
	8	Control	-35,369.05 $\pm$ 5,180.61	0.000***
		Experimental	-72,941.62 $\pm$ 5,427.48	

332 [†]*p-value* : Independent *t*-test (**p*<0.05, ***p*<0.01, ****p*<0.001)

333 [^]*p-value*: Mann-Whitney test

334

Figure legend

Figure 1. Hyaluronic acid increased with *L. plantarum* K8 lysates. To examine the level of hyaluronic acid expression, HaCaT cells were treated with *L. plantarum* K8 lysates at the indicated dosages and 20 mM N-acetyl-d-glucosamine (NAG) was used as a positive control. The secretion of hyaluronic acid was examined by ELISA method.

Figure 2. Effect of oral administration of *L. plantarum* K8 lysates on skin histology in the mouse model. (A) AD was induced by DNCB sensitization, and *L. plantarum* K8 lysates were orally administered for 8 weeks. Immunohistochemical staining showed that the skin epidermal layer decreased in the experimental group as compared to the control group. The differences in TEWL value (B) and epidermal thickness (C) between control and experimental groups are shown. AD, atopic dermatitis; NG, non-treated group; CG, DNCB-treated control group; TG, DNCB and *L. plantarum* K8 lysates-treated experimental group.

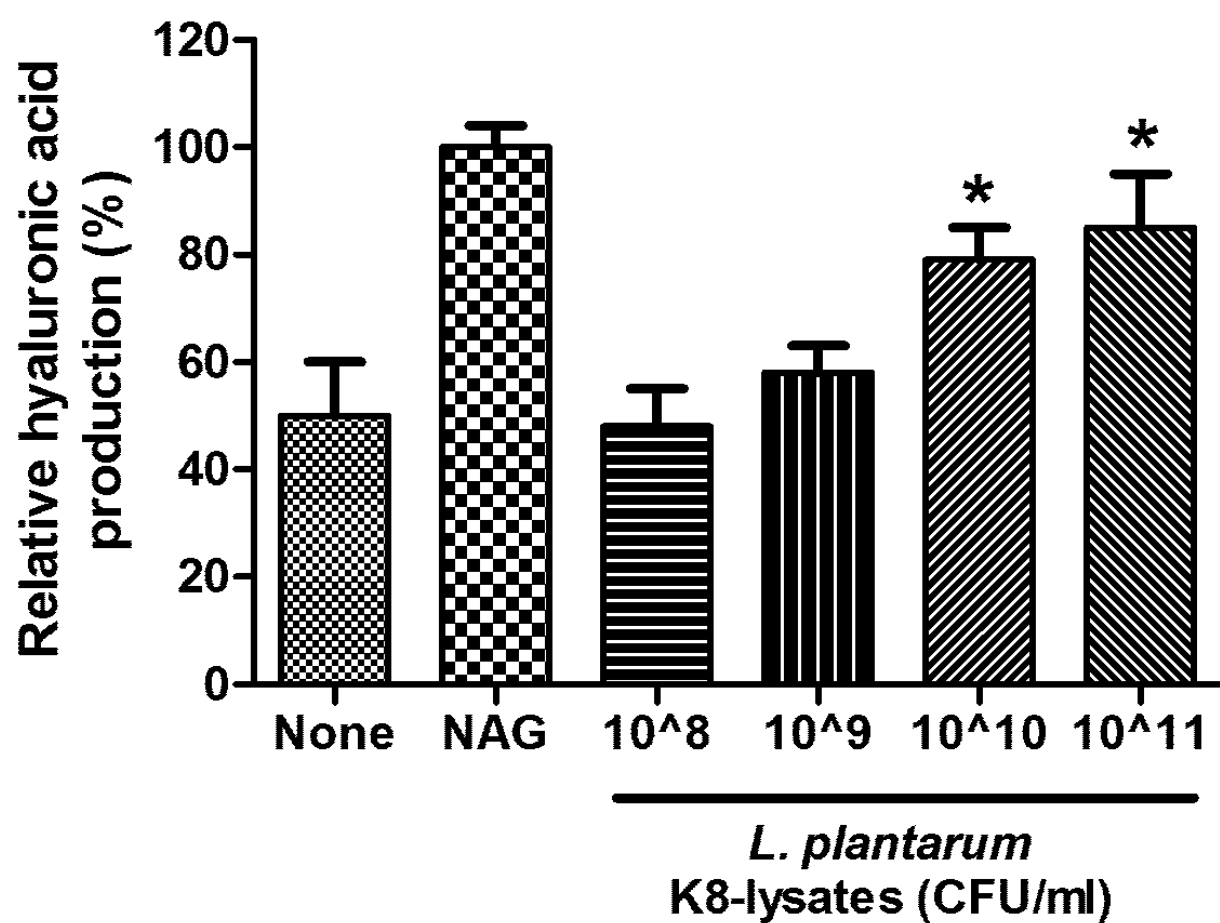


Fig. 1

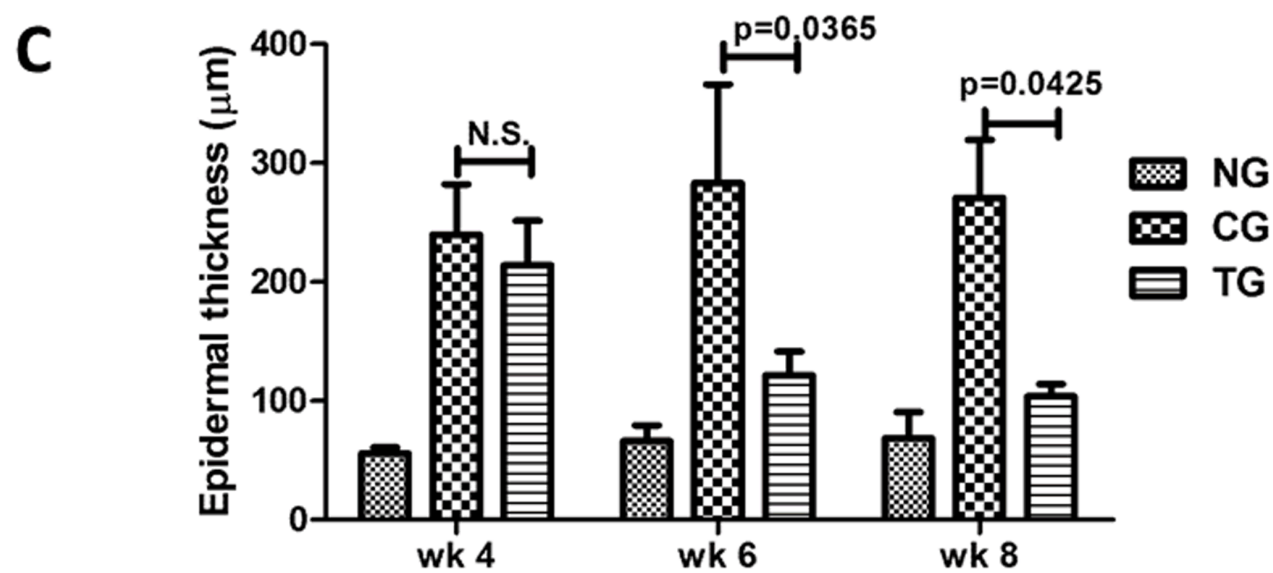
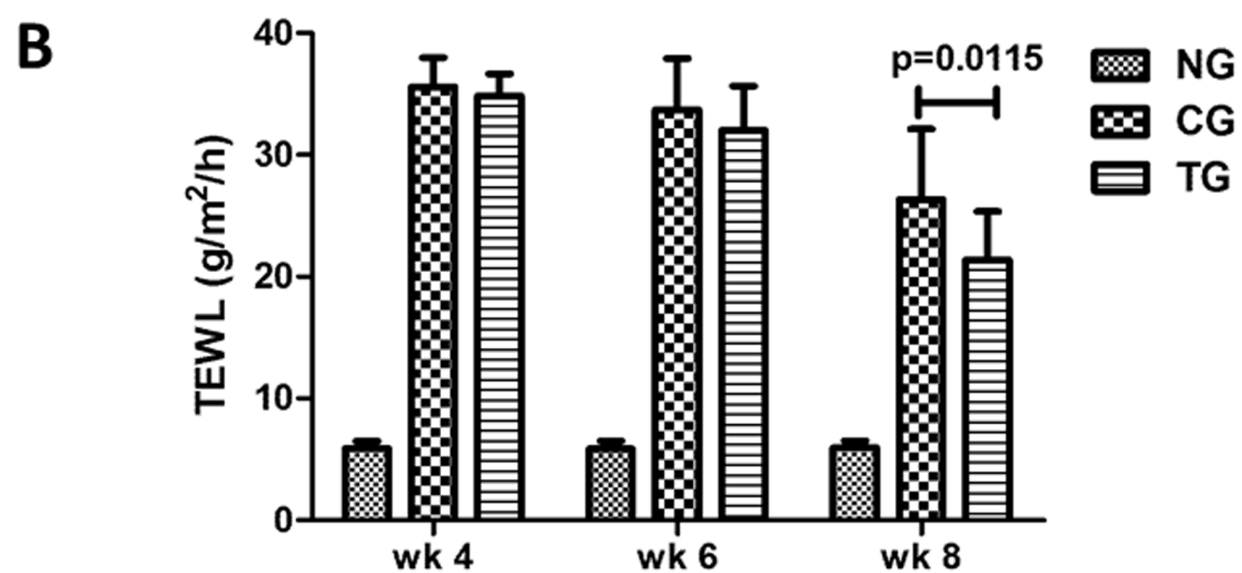
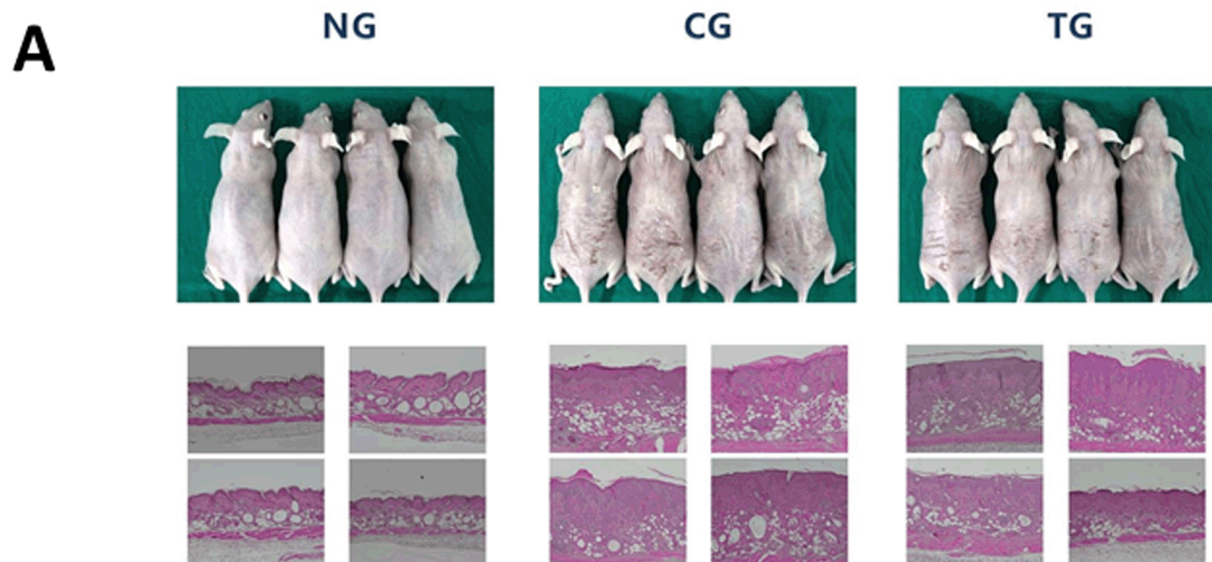
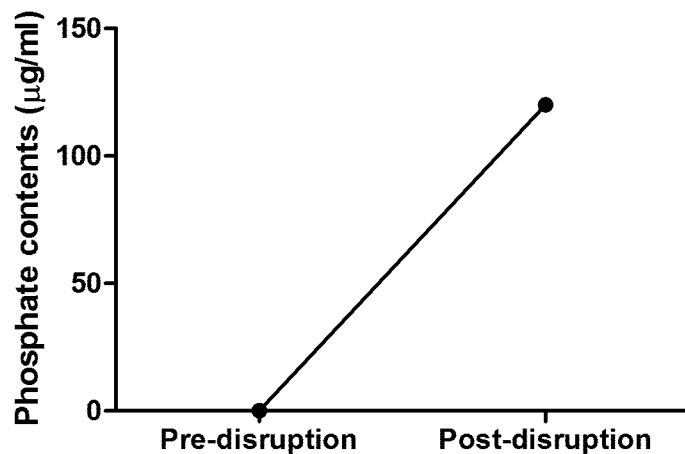


Fig. 2

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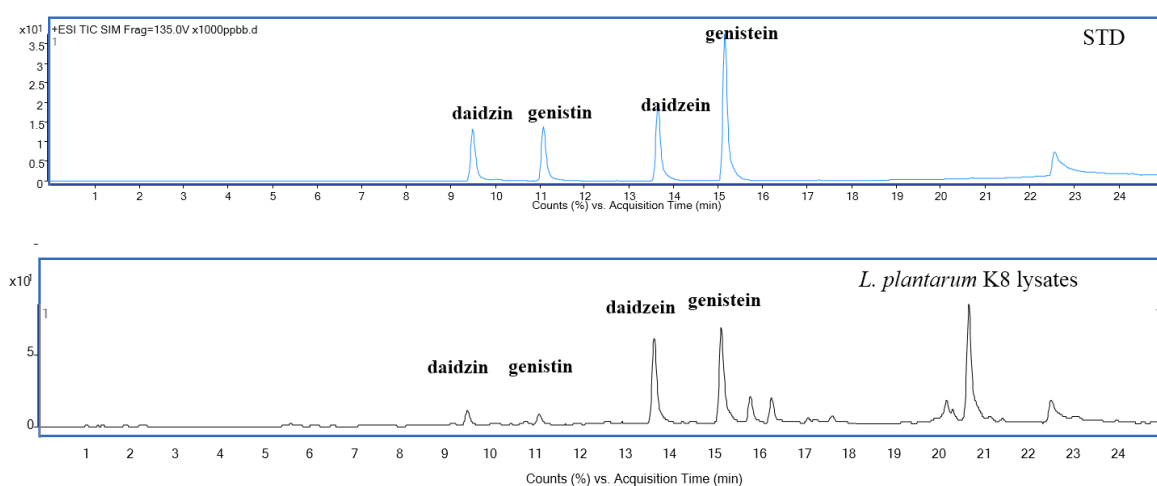
Supplementary Materials

Figure S1. A.



LTA, one of the active ingredients found in *L. plantarum* K8 lysates, increased after **sonication**. To examine the contents of LTA from *L. plantarum* K8, a phosphate assay was performed with intact and disrupted bacteria. The phosphate content increased after bacterial disruption, indicating that soluble LTA was extracted from the cell wall of the bacteria.

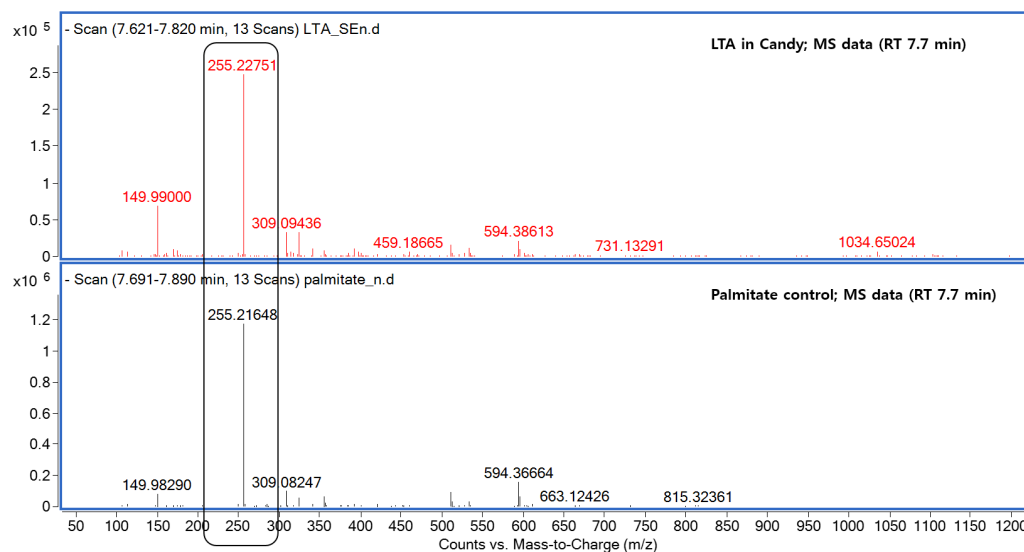
Figure S1. B.



Aglycone, one of the active ingredients found in *L. plantarum* K8 lysates, increased after **sonication**. After disruption of bacteria, isoflavone was detected with LC-MS/MS (Agilent

Technologies 6410 Triple Quad). As shown in Fig. S1B, daidzein and genistein were detected from *L. plantarum* K8 lysates. To exclude undesired detection from cell surface and media, *L. plantarum* K8 was washed with distilled water over three times before disruption, but very low levels of aglycone isoflavone were detected from washed water and soybean-containing media, while media containing a high level of glycoside-conjugated isoflavones decreased after incubation with *L. plantarum* K8 (data not shown). This result suggests that aglycone isoflavone may accumulate within the *L. plantarum* K8 cells and can be extracted from cells by sonication.

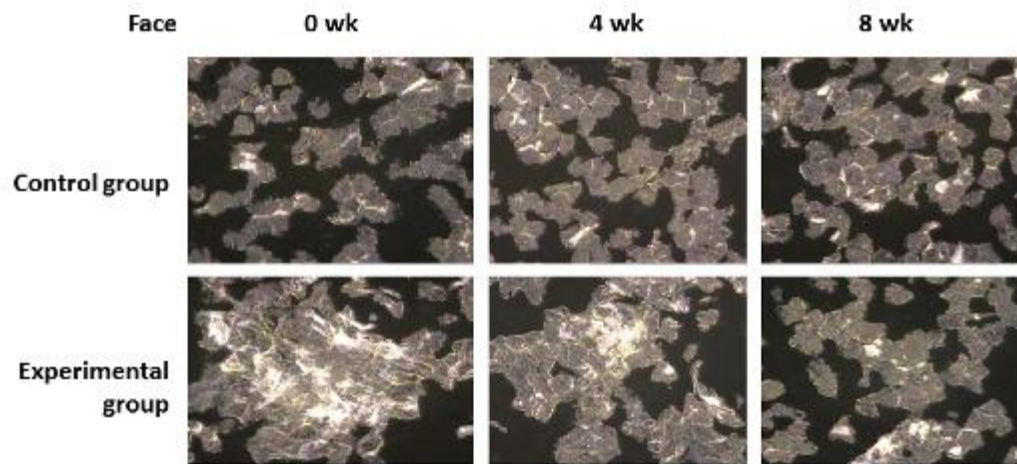
Figure S2.



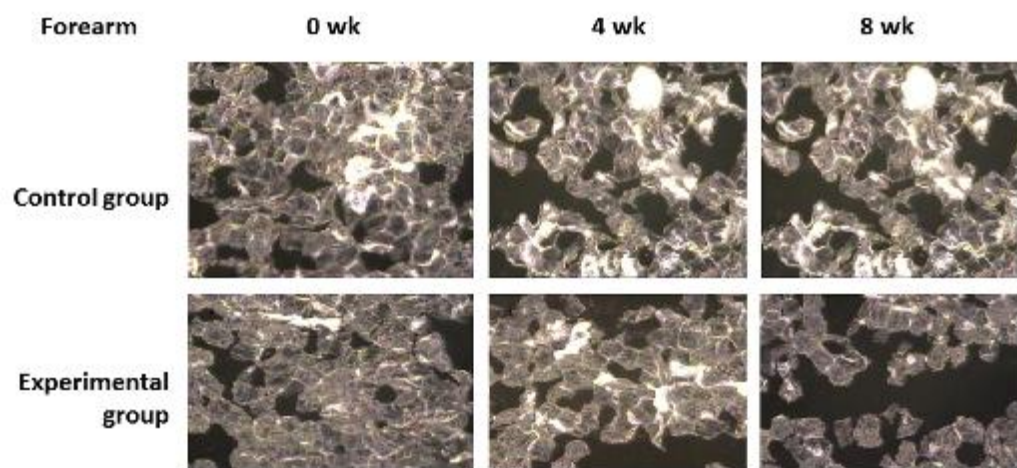
LTA was detected in the manufactured candy sample. Three samples of candies having different lot numbers were grilled and suspended in distilled water. Suspended candy samples were sonicated and then extracted with ethanol. The ethanol fraction was mixed with methanol and subjected to LC/MS-MS analysis. The positive control was palmitate since it is one of the major fatty acids detected from LTA glycolipids. As shown in Fig. S3, palmitate was detected from the experimental candies, indicating that experimental candy contains LTA. A representative result is shown.

Figure S3.

A



B



The image of the horny layer from control and experimental groups. Skin horny layer was reduced in volunteers who took *L. plantarum* K8 lysates-containing candy. The thickness of the horny layer on the face (A) and forearm (B) at different time points is shown.

Table S1. Composition of candy

	Experimental	Control
Title	Probiotic-containing candy	Probiotic-free candy
Label	Experimental	Control
Composition	Glucose, <i>L. plantarum</i> K8 lysates 2.1%, Xylitol 2%, Vitamin C, Blueberry concentrate powder, Citric acid anhydrous, DL-malic acid, combined congener (Blueberry flavor, raspberry flavor), Enzymatically Modified Stevia Glucosyl Stevia, Magnesium Stearate	Glucose, Xylitol 2%, Vitamin C, Blueberry concentrate powder, Citric acid anhydrous, DL-malic acid, combined congener (Blueberry flavor, raspberry flavor), Enzymatically Modified Stevia Glucosyl Stevia, Magnesium Stearate
Property	Mauve candy	Mauve candy
Package	White airtight container	White airtight container
Storage	Room temperature	Room temperature

Table S2. Aglycone isoflavone was detected in the manufactured candy sample.

	Daidzein†	Genistein†
Control Candy	0	0
Experimental Candy	0.103±0.064	0.093±0.021

†Aglycone isoflavone contents in candies: Mean±S.D. (ng/ml)

Three control and sample candies were chosen from different lot numbers. They were grilled and suspended in distilled water. Suspended candy samples were sonicated and then extracted with ethanol. The ethanol fraction was mixed with methanol and subjected to LC/MS-MS analysis. Daidzein and genistein were detected from experimental candies, but not control candies.