

Laminaria japonica Combined with Probiotics Improves Intestinal Microbiota: A Randomized Clinical Trial

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ABSTRACT *Laminaria japonica*—a widely used ingredient in seaweed kimchi—and lactic acid bacteria (LAB)—a main component of traditional fermented Korean food—may alter human intestinal microbiota composition and have a positive effect on various digestive problems. However, few clinical trials have investigated the potential benefits of *L. japonica* when combined with LAB for human intestinal microbiota. Therefore, this study was designed to evaluate the effects of *L. japonica* and representative LAB on the human intestine. Forty participants with no known digestive diseases were randomly assigned to one of the two combination groups: (1) *L. japonica* with LAB and (2) *L. japonica* with placebo LAB. The study agents were administered for 4 weeks with a 2-week follow-up period. The primary outcome measure was the number of each of the seven LAB species in the human intestine, and the secondary outcome measures included the Korean version of the Gastrointestinal Symptom Rating Scale, the World Health Organization Quality of Life, and bowel functions. The primary outcome was evaluated before and after administration of the study agents (0 and 4 weeks), and the secondary outcomes were evaluated at 0, 4, and 6 weeks. Four of the seven LAB species were found to be significantly increased in the *L. japonica* with the LAB group and five species were significantly different from those of the placebo group. The secondary outcome measures did not change significantly. In conclusion, *L. japonica* with LAB facilitated the proliferation of beneficial human intestinal microbiota. (Trial number: ClinicalTrials.gov NCT01651741).

KEY WORDS: • health functional food • intestine • probiotics • seaweeds

INTRODUCTION

KOREAN NATIONAL CUISINE has evolved based on the perspective that “the root of medicine and food is equal,” and is largely founded on fermented food such as kimchi, gochujang (fermented red chili paste), and *doenjang* (fermented bean paste). Korean food is known internationally for its high nutrition, diversity, and taste.¹ Kimchi, the representative traditional Korean fermented food, is made with vegetables such as Chinese cabbage. It is seasoned with a variety of ingredients, including garlic, green onion, and red pepper. The main vegetable ingredients and the degree of fermentation affect the taste of kimchi and determine the different kimchi varieties. *Laminaria japonica*, an edible seaweed, has been used as one of the main ingredients in kimchi for many years. It contains various factors, including alginate and fucoidans, which are known for their dietary benefits and therapeutic effects against constipation² and inflammation.³ Because of the beneficial effect of *L. japonica*,

its consumption has increased annually, reaching a mean 5 kg/year for an ordinary Korean adult.^{4,5}

Lactic acid bacteria (LAB) are primarily responsible for the fermentation of kimchi and more than 10⁸ colony-forming units of LAB are involved.⁶ During the early stages of kimchi fermentation, the LAB profile is comparatively diverse; however, in the later stages, the LAB profile becomes simpler and consists mostly of *Lactobacillus plantarum*.⁷ LAB have been the focus of international research for their potentially beneficial roles in stabilizing the gut microflora.⁸ LAB are also effective for preventing various digestive problems such as colon cancer⁹ and chronic diarrhea.¹⁰

Recent research on *L. japonica* and LAB has shown that *L. japonica* with LAB possesses a high antioxidant activity and protects against hepatic damage, obesity, hypertension, stress, insomnia, and alcoholic liver damage.^{11,12} However, although the beneficial effects of *L. japonica* with LAB are well known, few clinical trials have investigated its effect in human intestine.

This study aimed to evaluate the effect of *L. japonica* with LAB as representative compounds of seaweed kimchi. We examined the microbial communities in the healthy human intestine before and after the administration of *L. japonica*

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with LAB. We also compared the number of intestinal microbiota between the two groups (*L. japonica* with LAB and *L. japonica* with the placebo LAB group). Additionally, questionnaires on gastrointestinal symptoms and quality of life were used to investigate the combined effect of *L. japonica* with LAB.

MATERIALS AND METHODS

Subjects and study design

Forty-three volunteers with no history of disease, aged 18–75 years were recruited from the local community through bulletin board advertising, brochures, and banners. Three subjects failed in the screening test and the remaining 40 participants were enrolled in the study. The current study was reviewed by the Institutional Review Board and Ethics Committee of the Kyung Hee University Hospital at Gangdong (KHNMC-OH-IRB 2012-005). The trial followed the standards of the International Committee on Harmonization of Good Clinical Practice and the revised version of the Declaration of Helsinki. All participants were asked to fill out informed consent forms, and they were allowed adequate time to decide whether they would participate in the trial before signing the consent.

The study was performed as a placebo-controlled and double-blinded trial with 40 participants randomly allocated to two groups: (1) *L. japonica* with Duolac7S (DUO) and (2) *L. japonica* with placebo DUO. Participants were required to complete a 4-week treatment period (weeks 0–4) and a 2-week follow-up period (weeks 4–6). The flowchart of the entire trial is shown in Figure 1.

All eligible participants were free of diseases and clinical symptoms, according to the Korean version of Gastrointestinal Symptom Rating Scale (KGSRS) with scores of

< 3 points and no history of abdominal surgery related to the digestive system. Subjects who took over-the-counter medications that affected gastrointestinal motility or antibiotics, herbal medicine, or probiotics 2 weeks before the start of the trial were excluded. Pregnant women or women who planned to become pregnant during the trial period were also excluded. The study participants were asked not to take any medication that could affect the gastrointestinal tract during the trial, and if medications were taken, the drug name, usage, and duration were recorded on the case report form.

An independent statistician was responsible for the assignment of random numbers that were generated through a creation program. An opaque sealed envelope containing the table of random serial numbers was kept in a locked cabinet. The participants, investigator, and clinical research coordinator (CRC) were blinded to the randomization, and the blinding procedure was monitored by an authorized clinical research associate.

All participants were asked to report any adverse events to the principal investigator or CRC during the entire trial. The values for serum complete blood count, blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase levels, and erythrocyte sedimentation rate were assessed to confirm the safety of the study agents after the treatment period. The safety test analyses were performed at an accredited laboratory, and any adverse events and safety issues were documented on the case report form.

Interventions

The agents used in this trial were water-extracted *L. japonica* (LJE) and DUO. The LJE tablet was an oval-shaped,

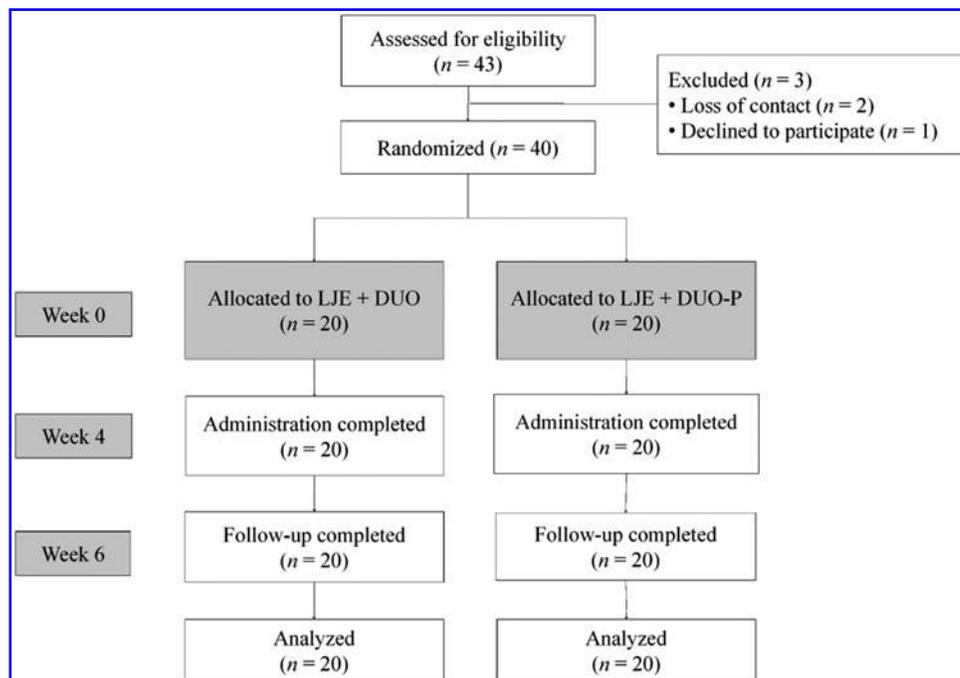


FIG. 1. Flow chart of the trial. LJE, *Laminaria japonica* extract; DUO, Duolac7S; DUO-P, Placebo of Duolac7S.

green tablet comprising 625 mg of LJE and 20 mg of diluting agent. Hanpoong Pharm and Foods Co., Ltd. (Jeonju, Republic of Korea) was in charge of LJE manufacturing according to the Korea Food Manufacturing Practice standards. LJE was packaged in a white plastic bottle, and one bottle of LJE was distributed to each participant.

DUO is a probiotic mixture composed of seven LAB: three strains of *Bifidobacterium* (*B. brevis*, *B. lactis*, and *B. longum*), three strains of *Lactobacillus* (*L. acidophilus*, *L. plantarum*, and *L. rhamnosus*), and one strain of *Streptococcus* (*S. thermophilus*). DUO has been approved by the Korean Food and Drug Administration for improving the balance of the intestinal microbiota. The DUO was packaged in a white ellipse capsule containing 5×10^9 bacteria (7×10^8 viable cells/strain). Placebo DUO was composed of cornstarch and nearly identical to the study agent in color, shape, odor, and taste. DUO and placebo DUO were produced by Cell Biotech Co., Ltd. (Gimpo, Korea). Participants took two LJE tablets and one DUO or placebo capsule twice daily (30 min after the morning and the evening meal) for 28 days. The dosage of LJE was determined based on preliminary experimental research in mice showing that the optimal results were achieved with a dose of 100–300 mg of LJE (data not shown). Considering the human metabolic rate (about 10% of mouse) and mean adult weight (50 kg), we set the dosage of LJE as 1.25 g per day (625 mg/capsule). Compliance was determined by counting the remaining LJE tablets or DUO capsules, and all participants reached the required compliance rate of 80%.

Primary outcome

The primary outcome was the number of each of the above-mentioned seven LAB species in the subject's intestine. Each participant was asked to submit a 5-g fecal sample at baseline (week 0) and at the end of the administration period (week 4). The samples were stored at -70°C until analysis. The qualified laboratory of Cell Biotech Co., Ltd. analyzed the fecal samples for the presence and the number of the seven LAB species.

Secondary outcomes

The severity of gastrointestinal symptoms was assessed by the KGSRS. KGSRS included 15 questions to evaluate various digestive problems, including abdominal pain, heartburn, indigestion, diarrhea, and constipation¹³ and each item was assessed on a five-point Likert scale, with a higher score indicating a severe degree of symptoms. KGSRS has been regarded as reliable and validated by a previous study.¹⁴

The quality of life was evaluated by the World Health Organization Quality of Life Brief Assessment (WHOQOL-BREF), which consists of four categories and 26 questions that yield a multidimensional profile of scores related to the quality of life such as physical function, psychological function, social relationships, and environmental function. The Korean version of the WHOQOL-BREF demonstrated consistency, validity, and reliability in a previous study.¹⁵

Last, stool frequency and consistency by the Bristol stool scale¹⁶ was evaluated. Ease of passage, as defined by the adjectival scale (from manual disimpaction to incontinence) was also assessed. All secondary outcomes were investigated at baseline (week 0), the end of the administration period (week 4), and the end of the follow-up period (week 6).

Statistical analysis

The sample size was calculated considering that the current trial was a pilot study. According to similar previous research,¹⁷ the sample size in this trial was set as 20 in each group. The withdrawal rate was assumed to be 25% and the statistical power was 80% with statistical significance of P value $< .05$.

All data were analyzed by an independent statistician using SPSS version 16.0 (SPSS, Inc., Chicago, IL, USA). A comparison of the baseline characteristics between the two groups was made using an independent t -test for continuous variables and the Fisher's exact test for categorical variables. All continuous variables were presented as mean \pm SEM and categorical variables were presented as percentages. The primary outcome, the comparison of the number of LAB between the two groups at 4 weeks, was analyzed using an independent t -test. A paired t -test was used for comparison of the number of each LAB species before (week 0) and after administration of the study agents (week 4).

KGSRS, WHOQOL-BREF, or bowel function scores were compared between the two groups using an independent t -test. Comparison of subgroup scores on the KGSRS or WHOQOL-BREF was conducted using the Wilcoxon signed-rank test. A P value of $< .05$ was considered statistically significant.

RESULTS

General demographic characteristics

Between May 2012 and November 2012, 43 patients were screened; of these, 3 participants failed to meet the inclusion criteria (Fig. 1). No subject dropped out and all subjects completed the study, possibly because of the short study duration. Baseline demographic characteristics, the KGSRS scores, and bowel function scores were balanced between the two groups at the beginning of the study as shown in Table 1. There were also no significant differences in WHOQOL-BREF scores except for the mind domain ($P = .042$).

Intestinal microbiota

After completing the treatment, there were statistically significant changes in the number of intestinal microbiota in the participants' feces. The numbers of four types of intestinal microbiota (*B. brevis*, *B. lactis*, *L. plantarum*, and *L. rhamnosus*) showed a statistically significant increase after administration of *L. japonica* with DUO, whereas no significant change in the number of any intestinal microbiota was noted in the *L. japonica* with the placebo DUO group

TABLE 1. CHARACTERISTICS OF THE PATIENTS AND THE BASELINE LACTIC ACID BACTERIA, GASTROINTESTINAL SYMPTOM RATING, QUALITY OF LIFE, AND BOWEL FUNCTION SCORES

Variables	LJE+DUO (n=20)	LJE+DUO-P (n=20)	P value
Age (years)	27.05±5.72	29.35±7.90	.298
Body mass index (kg/m ²)	20.56±1.76	21.12±3.65	.540
Male (%)	40.0	40.0	1.000
Smoking (%)	5.0	30.0	.091
Drinking (%)	90.0	90.0	1.000
Coffee (%)	85.0	85.0	1.000
Lactic acid bacteria (log ₁₀ /g)			
<i>Lactobacillus rhamnosus</i>	3.85±0.14	3.95±0.09	.542
<i>Bifidobacterium brevis</i>	3.71±0.15	3.49±0.23	.421
<i>Lactobacillus plantarum</i>	4.27±0.34	4.43±0.33	.740
<i>Bifidobacterium lactis</i>	6.67±0.08	6.29±0.13	.163
<i>Streptococcus thermophilus</i>	4.42±0.16	4.14±0.26	.360
<i>Lactobacillus acidophilus</i>	3.73±0.17	3.74±0.22	.981
<i>Bifidobacterium longum</i>	6.95±0.20	6.78±0.31	.641
KGSRS	7.25±5.41	6.85±5.91	.825
WHOQOL-BREF			
Body	27.80±3.94	26.65±3.03	.308
Mind	22.75±2.69	20.75±3.29	.042
Social relationship	11.25±1.33	10.65±1.73	.226
Environmental function	29.10±2.75	27.05±4.44	.087
Total	9090.90±9.11	8585.10±10.02	.063
Bowel function			
Frequency	1.05±0.46	0.90±0.21	.188
Consistency ^a	3.80±0.83	3.85±0.81	.849
Ease of passage ^b	5.10±1.21	4.70±1.26	.312

Data are presented as mean±SD values or percentages. Baseline values were analyzed by the Fisher's exact test for categorical variables and the independent *t*-test for continuous variables.

^aUsed a scale of 1–7: 1, watery stool; 7, hard, lumpy stool.

^bUsed a scale of 1–7: 1, fecal incontinence; 7, manual disimpaction.

KGSRS, Korean version of gastrointestinal symptom rating scale; LJE, *Laminaria japonica* extract; DUO, Duolac7S; DUO-P, Placebo of Duolac7S; WHOQOL-BREF, The World Health Organization Quality of Life Brief Assessment.

(Fig. 2). There was a significant difference in the numbers of five intestinal microbiota (*B. brevis*, *B. lactis*, *B. longum*, *L. plantarum*, and *L. rhamnosus*) between the *L. japonica* with DUO and *L. japonica* with placebo DUO groups after administration (week 4) as shown in Figure 2.

KGSRS, WHOQOL-BREF, and bowel function scores

There were no statistically significant changes in the KGSRS, WHOQOL-BREF, and bowel function scores (stool frequency, consistency, and ease of passage) after the intake of study agents in either group. Furthermore, no difference in the KGSRS, WHOQOL-BREF, and bowel function scores was noted between the two groups at week 4.

Adverse events and safety

No major adverse events were noted after treatment with the study agents. Liver and kidney functions were evaluated at week 4, and no safety issues were found in either group.

DISCUSSION

In the current study, we examined the effect of *L. japonica*, a main ingredient of kimchi, with DUO, a probiotic

containing kimchi LAB, on human intestinal microbiota. Administration of *L. japonica* with DUO caused an increase in the number of four types of intestinal microbiota (*B. brevis*, *B. lactis*, *L. plantarum*, and *L. rhamnosus*), and that of five microbiota (*B. brevis*, *B. lactis*, *B. longum*, *L. plantarum*, and *L. rhamnosus*) was significantly different compared with the placebo group. In the placebo group, there were no significant changes in the number of intestinal microbiota. Moreover, there were no significant changes in KGSRS, quality of life, or bowel function after the treatment in either group.

Normal intestinal microflora consists of > 1000 bacterial species and their number is the highest in the terminal ileum and colon.¹⁸ In healthy people, there is a good balance between beneficial and pathogenic bacteria in the intestines. Fecal microbial changes associated with aging include reduced number of beneficial bacteria such as *Bifidobacterium*¹⁹ and instability of intestinal microbiota, which may play a role in the pathophysiology of irritable bowel syndrome.²⁰ Other studies have reported that the lack of *Bifidobacteria* and *Lactobacilli* could cause functional abdominal pain and diarrhea.^{21,22} Because the intestinal microbiota plays a significant role in the intestinal metabolism and natural defense of the host, maintaining and

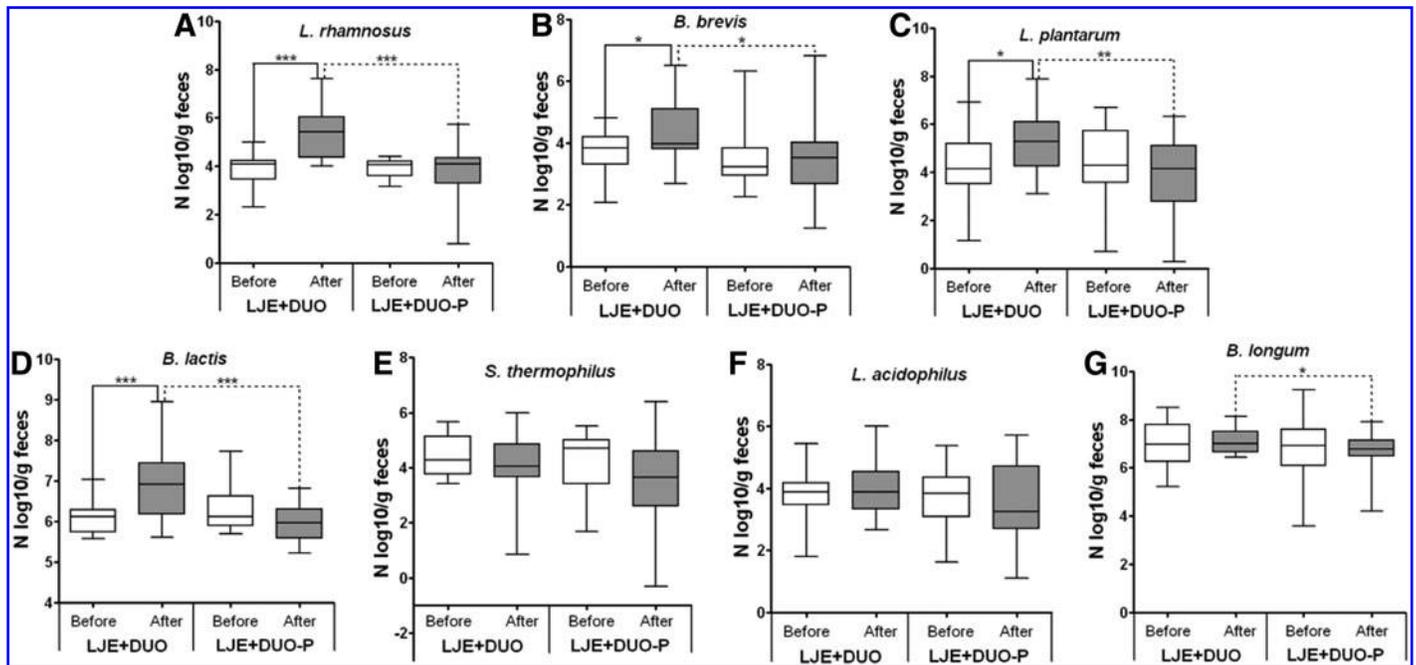


FIG. 2. Effect of 4-week LJE with DUO on intestinal microbiota compared with LJE with DUO-P in subjects who have no organic disease. (A) *Lactobacillus rhamnosus*; (B) *Bifidobacterium brevis*; (C) *Lactobacillus plantarum*; (D) *Bifidobacterium lactis*; (E) *Streptococcus thermophilus*; (F) *Lactobacillus acidophilus*; (G) *Bifidobacterium longum*. Values shown as mean \pm SEM. * $P < .05$, ** $P < .01$, *** $P < .001$: Data were compared before and after 4 weeks by a paired t -test. Data were compared between LJE + DUO and LJE + DUO-P groups after 4 weeks by an independent t -test.

increasing the intestinal microbiota for improved barrier function of the mucosa and resistance to invasion and proliferation of pathogenic agents are vital.²³

Seaweed kimchi is a popular traditional fermented Korean dish made of vegetables and seaweeds such as *L. japonica*, with diverse seasonings.²⁴ Kimchi has been recognized as a healthy functional food with an ability to regulate digestive problems, and has gained popularity after being mentioned in the magazine “Health”.²⁵ East Asian individuals, who consume a greater quantity of seaweed than those in the West, have been reported to experience fewer constipation episodes, and the rates of cancer and obesity are also comparatively lower.⁴ As a fermented food, kimchi contains a number of LAB, in which *L. plantarum* plays a crucial role at the final stage of fermentation.²⁶ LAB in kimchi are important for taste and quality and play a potentially beneficial role as probiotics, which are viable microorganisms that improve the host’s intestinal microbial balance.²⁷ DUO, containing a large number of LAB such as *L. plantarum*, has been a popular dietary supplement in Korea because of its beneficial effects on digestive symptoms and easy accessibility without a prescription.²⁰ Recently, *L. japonica* with LAB has been the focus of clinical research for its beneficial profiles, including gamma-aminobutyric acid and taurine, which prevent metabolic syndrome and mental disorders.^{11,12} This trial is the first study to investigate the change in the number of intestinal microbiota after administration of *L. japonica* with LAB. We tried to standardize and formulate each study agent

(*L. japonica* and LAB), which made it possible to conduct a patient, assessor-blind trial.

Kimchi has a variety of ingredients and contains a great variety of LAB during fermentation. The LAB species in kimchi have not been fully identified and some of them cannot be cultured. Therefore, we designed a study to compare the two main components of kimchi (*L. japonica* and DUO). *L. japonica* has been reported to have anti-inflammatory, anticoagulant, antiangiogenic and antiadhesive effects,³ and it is also the most effective for improving colitis symptoms in mice among eight species of representative seaweeds in our previous experimental research (data not shown). Although DUO does not contain most kinds of kimchi LAB, it includes species of *Lactobacillus* and *Streptococcus* genera that are mainly involved in kimchi fermentation.

In our previous clinical study on the effect of a herbal prescription combined with DUO on intestinal microbiota in subjects without any organic disease, we observed synergistic effects on the numbers of several beneficial LAB (data not shown). In addition, DUO significantly increased the number of several beneficial LAB, and the placebo group showed a slight decrease in the number of beneficial LAB in feces. Considering that temporal instability in the fraction of active microbiota is related to functional disease and fluctuating digestive symptoms,²⁸ and that healthy people tend to show a decrease in the number of intestinal *Bifidobacteria* with advancing age,²⁹ *L. japonica* might potentially be used to prevent the decreases in intestinal bacterial numbers.

Because *L. japonica* with real DUO was associated with a significant increase in the number of intestinal microbiota compared with the placebo group, there might be a synergistic effect of *L. japonica* and DUO. This finding suggests that *L. japonica* might serve as a prebiotic supporting the proliferation of beneficial microbacteria in the human intestine.

There were no significant changes between the two groups in KGSRs, quality of life, and bowel function. Because *L. japonica*, which contains a lot of fiber, has been widely reported to prevent constipation,² it is possible that it might also cause diarrhea or abdominal pain. We therefore investigated secondary variables associated with digestive symptoms and quality of life to confirm the safety profile of *L. japonica*, and no factors that might negatively affect the quality of life or aggravate digestive problems were detected. The statistical difference ($P = .042$) at baseline in the mind domain of WHOQOL-BREF is a negligible level without clinical meaning.

We could not use placebo *L. japonica*, because it is almost impossible to make placebos of a natural product (*L. japonica* in this case) with the same odor, taste, and color. Thus, the most feasible design reflecting real kimchi for the general Korean population was considered to be a two-group (*L. japonica* with LAB and *L. japonica* with placebo LAB) comparison parallel design.

Our study has some limitations. The study agents in this trial did not fully reflect seaweed kimchi consumed in everyday life. Furthermore, more research will be needed to identify new kimchi LAB and their role during fermentation. This study has a small sample size, which might weaken the significance of the beneficial effects of *L. japonica* with LAB. Further studies, including the mechanisms of action and with large sample sizes, are needed.

In conclusion, this study showed that *L. japonica* with LAB might have a beneficial effect on human intestinal microbiota by enhancing the proliferation of beneficial bacteria. DUO as a LAB played a key role in increasing the number of intestinal microbiota and *L. japonica* might also help prevent the reduction in the number of beneficial intestinal microbiota. The synergistic effect of *L. japonica* with LAB might improve the intestinal environment and overall health by alleviating digestive symptoms.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Shin BG: Research articles: the effect of cognition at Korean food on image, attitude, globalization, and purchasing intention. *J Foodserv Manage Soc Korea* 2009;12:129–149.
2. Kuda T, Fujii Saiheki T, Hasegawa A, Okuzumi M: Effects of brown algae on fecal flora of rat. *Nippon Nogeikagaku Kaishi* 1992;58:307–314.
3. Cumashi A, Ushakova NA, Preobrazhenskaya ME, et al.: A comparative study of the anti-inflammatory, anticoagulant, anti-angiogenic, and antiadhesive activities of nine different fucoindans from brown seaweeds. *Glycobiology* 2007;17:541–552.
4. Park TG: Food and Nutrients, Joongang daily news. http://ocatholic.cafe24.com/bbs/zboard.php?id=life_01&no=77 (accessed August 2013)
5. MOMAF: Statistic Database for Fisheries Production. <https://stat.mltn.go.kr/portal/main/portalMain.do> (accessed August 2013)
6. Lee CW, Ko CY, Ha DM: Microfloral changes of the lactic acid bacteria during kimchi fermentation and identification of the isolates. *Korean J Appl Microbiol* 1992;20:102–109.
7. Yeun H, Yang HS, Chang HC, Kim HY: Comparison of bacterial community changes in fermenting kimchi at two different temperatures using a denaturing gradient gel electrophoresis analysis. *J Microbiol Biotechnol* 2013;23:76–84.
8. Gibson SE: Probiotics. In: *Encyclopedia of Food Sciences and Nutrition* (Benjamin C, ed.). Oxford Academic Press, Oxford, 2003, pp. 4792–4798.
9. Al-Rwaily MA, Herzallah SM, Humeid MA, Yamani MI: Effect of dried dates extract on the growth and viability of *Bifidobacteria* in different milk types. *Pak J Nutr* 2005;4:142–147.
10. De Vrese M, Stegman A, Richter B, Fenselau S, Laue C, Scherezenmeir J: Probiotics-compensation for lactase insufficiency. *Am J Clin Nutr* 2001;73:421–429.
11. Lee BJ: Development of functional food using fermented marine organism. *Food Ind Nutr* 2013;18:8–12.
12. Kang YM, Lee BJ, Kim JJ, et al.: Antioxidant effects of fermented sea tangle (*Laminaria japonica*) by *Lactobacillus brevis* BJ20 in individuals with high level of γ -GT: a randomized, double-blind, and placebo-controlled clinical study. *Food Chem Toxicol* 2012;50:1166–1169.
13. Svedlund J, Sjödin I, Dotevall G: GSRs—a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. *Dig Dis Sci* 1988;33:129–134.
14. Kwon SS, Jung HK, Hong JH, Park HS: Diagnostic validity of the Korean Gastrointestinal Symptom Rating Scale (KGSRs) in the assessment of gastro-esophageal reflux disease. *Ewha Med J* 2008;32:73–80.
15. Min SK, Kim KI, Lee CI, Jung YC, Suh SY, Kim DK: Development of the Korean versions of WHO Quality of Life scale and WHOQOL-BREF. *Qual Life Res* 2002;11:593–600.
16. Heaton KW, Radvan J, Cripps H, Mountford RA, Braddon FE, Hughes AO: Defecation frequency and timing, and stool form in the general population: a prospective study. *Gut* 1992;33:818–824.
17. Niedzielin K, Kordecki H, Birkenfeld B: A controlled double-blind, randomized study on the efficacy of *Lactobacillus plantarum* 299V in patients with irritable bowel syndrome. *Eur J Gastroenterol Hepatol* 2001;13:1143–1147.
18. Sears CL: A dynamic partnership: celebrating our gut flora. *Anaerobe* 2005;11:247–251.
19. Ouwehand AC, Tiihonen K, Saarinen M, Putaala H, Rautonen N: Influence of a combination of *Lactobacillus acidophilus* NCFM and lactitol on healthy elderly: intestinal and immune parameters. *Br J Nutr* 2009;101:367–375.
20. Cha BK, Jung SM, Choi CH, et al.: The effect of a multispecies probiotic mixture on the symptoms and fecal microbiota in

- diarrhea-dominant irritable bowel syndrome: a randomized, double-blind, placebo-controlled trial. *J Clin Gastroenterol* 2012;46:220–227.
21. Si JM, Yu YC, Fan YJ, Chen SJ: Intestinal microecology and quality of life in irritable bowel syndrome patients. *World J Gastroenterol* 2004;10:1802–1805.
 22. Kassinen A, Krogius-Kurikka L, Mäkivuokko H, *et al.*: The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. *Gastroenterology* 2007;133:24–33.
 23. Nova E, Viadel B, Wärnberg J, Carreres JE, Marcos A: Beneficial effects of a synbiotic supplement on self-perceived gastrointestinal well-being and immunoinflammatory status of healthy adults. *J Med Food* 2011;14:79–85.
 24. Kim M, Chun J: Bacterial community structure in kimchi, a Korean fermented vegetable food, as revealed by 16S rRNA gene analysis. *Int J Food Microbiol* 2005;103:91–96.
 25. Choi IH, Noh JS, Han JS, Kim HJ, Han ES, Song YO: Kimchi, a fermented vegetable, improves serum lipid profiles in healthy young adults: randomized clinical trial. *J Med Food* 2013;16:223–229.
 26. Park GY, Choi HS: Antimutagenic and anticancer effects of lactic acid bacteria isolated from kimchi. *Bioindustry* 2000;13:11–17.
 27. Lim SM, Im DS: Screening and characterization of probiotic lactic acid bacteria isolated from Korean fermented foods. *J Microbiol Biochemol* 2009;19:178–186.
 28. Durbán A, Abellán JJ, Jiménez-Hernández N, *et al.*: Instability of the faecal microbiota in diarrhoea-predominant irritable bowel syndrome. *FEMS Microbiol Ecol* 2013 Jul 25 [Epub ahead of print]; DOI: 10.1111/1574–6941.12184.
 29. Ouwehand AC, Tiihonen K, Saarinen M, Putaala H, Rautonen N: Influence of a combination of *Lactobacillus acidophilus* NCFM and lactitol on healthy elderly: intestinal and immune parameters. *Br J Nutr* 2009;101:367–375.