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Effect of *Lactobacillus gasseri* BNR17 on irritable bowel syndrome: a randomized, double-blind, placebo-controlled, dose-finding trial

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Abstract *Lactobacillus gasseri* BNR17 is a strain isolated from human breast milk. The objective of this randomized, double-blind, placebo-controlled, and preliminary dose-finding trial was to find the effective dose and evaluate the effect of *Lb. gasseri* BNR17 on irritable bowel syndrome (IBS) symptoms. A total of 55 volunteers aged over 20 years with body mass index over 23 kg/m^2 were randomized to intake a placebo, low-dose (BNR-L, 2×10^8 CFU/day), intermediate-dose (BNR-M, 2×10^9 CFU/day), or high-dose BNR (BNR-H, $2 \times 5 \times 10^9$ CFU/day) for four weeks. Questionnaire for IBS symptoms scores and *Lb. gasseri* BNR17 in feces were assessed at the beginning and end of the trial. Among IBS symptoms scores, abdominal pain score was significantly reduced in BNR-H group. *Lb. gasseri* BNR17 was detected in all intake groups except placebo. In the preliminary study, *Lb. gasseri* BNR17 was confirmed to have probiotic properties.

Keywords *Lactobacillus gasseri* BNR17 · Irritable bowel syndrome · Probiotics

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Introduction

Probiotics are live and nonpathogenic microorganisms with beneficial effects on host's health [1]. Health benefits of probiotics are mostly focused on gut health or treatment efficacy. Among functional gastrointestinal disorders, irritable bowel syndrome (IBS) is a common digestive disorder characterized by abdominal pain and discomfort including diarrhea, constipation, or both without consistently demonstrable structural or biochemical causes [2]. In westernized countries including Korea, IBS is one main reason that lowers quality of life. In a nationwide analysis, 6% of Korean population seeks medication for IBS. Such high prevalence leads to increase medical cost [3]. Due to obscure etiology of IBS, main treatment strategies involve the use of anti-spasmodics or anti-depressants at low dose to relieve abdominal pain [4, 5]. Some probiotics have been investigated to be effective in the management of IBS. However, their benefits are likely to be strain-specific [6]. Moreover, few randomized controlled trials have tested the efficacy of probiotics for IBS with controversial results [6, 7].

Lactobacillus gasseri BNR17 is a strain isolated from human breast milk. Its probiotic properties have been evaluated [8]. Because this strain was isolated in the attempt to find anti-diabetic probiotic strains, previous studies were focused on lowering blood glucose and reducing body fat levels [9–11]. However, as a probiotic strain, its effect in improving IBS symptoms should be confirmed and its optimal dose should be identified. Therefore, this preliminary study was performed to find the effective dose and evaluate the effect of *Lb. gasseri* BNR17 on IBS symptoms in subjects with IBS.

Materials and methods

Test materials

Lb. gasseri BNR17 was kindly provided by Bioneer Ltd (Daejeon, Korea). Placebo contained only dextrin. Final products had identical shape, texture, and appearance. All test products were labeled and randomized by the study coordinator who was not a participant of this study.

Participants

A total of 78 subjects aged between 20 and 54 years were recruited for this study through advertisement posted on several websites. To be eligible for this study, subjects were required to have a body mass index (BMI) over 23 kg/m^2 and subjective symptoms of IBS. Those who were taking medication and/or dietary supplements within one month prior to screening visit or having inflammatory disease or any other diseases that could affect results of this study were excluded. All subjects were generally healthy based on their medical history and laboratory tests.

Study design

This study was a double-blind, randomized, placebo-controlled, parallel study. They were instructed to avoid foods, medicines, and other dietary supplements that might affect the efficacy of the test material. After 7 days of run-in period, subjects were randomly assigned into four groups (placebo; BNR-L, 2×10^8 CFU/day; BNR-M, 2×10^9 CFU/day; BNR-H, $2 \times 5 \times 10^9$ CFU/day). They were asked to consume test materials or placebo for four weeks. Venous blood was collected to check safety markers. A questionnaire was used to investigate symptom severity of IBS. Fecal samples were collected at baseline and the final visit. They were kept frozen at − 54 °C for PCR analysis. Study protocol was approved by Institutional Review Board (IRB) of Ewha Womans University (No. 2011-12-11). It was carried out in accordance with the Helsinki declaration and registered at WHO International Clinical Trial Registry Platform (www.who.int/ictrp) with the following identification number: KCT0000368.

Questionnaires

At baseline and the final visit, subjects were asked to answer questions about the frequency of their abdominal pain, bloating, and feeling of incomplete evacuation. The severity of symptoms was assessed on a five-point Likert visual analogue scale (1 = not at all; 5 = extremely severe that affected daily life). Symptom scale was adapted from the previously validated 5-point scale [12–14].

Fecal preparation and extraction of DNA

In order to separate fecal *Lb*. species, 1 g of thawed fecal sample was diluted 100 times with PBS. Then 100 µL of diluted sample was seeded onto LBS agar plate (Difco; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and incubated at 37 °C with 5% CO_2 for 24–48 h. After incubation, cells were counted to evaluate total *Lb*. species and colonies were incubated in MRS broth (Difco) at 37 °C with 5% CO_2 environment for 24 h. Incubated MRS broth was centrifuged (4 °C, 3000 rpm for 15 min) and the cell pellet was suspended in 6.7% sucrose solution pre-warmed at 37 °C for 15 min. DNA was extracted from cell pellet using Accuprep Genomic DNA Extraction Kit (Bioneer Ltd).

Polymerase chain reaction (PCR)

PCR was performed using Accupower Hot Start PCR premix (Bioneer Ltd) and *Lb. gasseri* specific primers [15] Lgas_F (5′-AGCGACCGAGAAGAGAGAGA-3′) and Lgas_R (5′-TGCTATCGCTTCAAGTGCTT-3′) using MY GenieTM 96 Gradient Thermal Block (Bioneer Ltd). PCR amplification was performed under the following conditions: denaturation at 95 °C for 10 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 65 °C for 2 min, and extension at 74 °C for 2 min, followed by a final extension step at 74 °C for 5 min.

Gel electrophoresis and gene sequencing

To confirm *Lb. gasseri* BNR17 species, PCR products were subjected to agarose gel electrophoresis. Briefly, 2 g of agarose, 100 mL TBE buffer, and 5 µL EtBr were used to pour the gel. After 30 min, 2 µL DNA size marker (100 bp DNA Ladder) and PCR products mixed with 2 µL 6× Agarose Gel Loading Buffer were loaded to the gel. Gel electrophoresis was performed for 30–40 min. Samples that showed bands at the same position of *Lb. gasseri* BNR 17 (positive control) were purified using Accuprep PCR Purification Kit (Bioneer Corporation, Daejeon, Korea) and subjected to DNA sequencing to confirm homology (≥ 98%) with *Lb. gasseri* BNR17.

Statistical analysis

A Shapiro–Wilk W test was used to assess the normality of each variable. Differences among groups were tested with Fisher's exact test for categorical variables and ANOVA for continuous variables. Difference within group was

tested with paired *t* test. $P < 0.05$ was considered statistically significant. SAS program package version 9.3 (SAS Institute, Cary, NC, USA) was used for all statistical analyses.

Results and discussion

A total of 78 subjects were screened for eligibility and 55 subjects were enrolled. Thirteen subjects were lost during the study period. Finally, 42 subjects were analyzed. There were no significant differences in baseline characteristics among these participants (Table 1). Changes in IBS symptoms scores are shown in Fig. 1. Because IBS symptoms scores are subjective measuring markers, changes in placebo group have been reported in numerous papers [16, 17]. In the present study, severity scores for bloating and feeling incomplete evacuation were significantly changed in BNR-L and BNR-M groups as well as the placebo group compared to baseline values. In BNR-H group, all severity scores were significantly changed ($P < 0.05$). For abdominal pain scores, changes in values from baseline to 4-week in BNR-H group were significantly ($P = 0.020$) lower when compared to those in the placebo group. There was no placebo effect in abdominal pain scores. Probiotic properties should be determined at strain level [18]. Although numerous papers have published beneficial effects of *Lb. gasseri* on health [19–21], those strains are different from our stain isolated from breast milk. Therefore, probiotic properties and optimal intake level of this strain should be confirmed using human subjects. In the present study, *Lb. gasseri* BNR17 was confirmed to have probiotic properties by reducing IBS symptom severity scores, especially it had effect in relieving abdominal pain. Its optimal dose was found to be $2 \times 5 \times 10^9$ CFU/day.

In order to confirm gut colonization of *Lb. gasseri* BNR17, fecal *Lb.* species were quantified through in vitro cultivation and *Lb. gasseri* BNR17 was detected by PCR followed by gel electrophoresis and gene sequencing (Table 2). When changes were compared among treated groups, BNR-H group showed the highest increase in fecal *Lb.* species ($P = 0.015$). After intervention for four weeks, *Lb. gasseri* BNR17 was found in all subjects who consumed *Lb. gasseri* BNR17. Their difference was statistically significant ($P < 0.0001$). For probiotics, colonization through digestive tract is the foremost important characteristic [22]. *Lb. acidophilus* NCFM has been tested for colonization. It is found in feces of 65% of participants after supplementation, suggesting satisfactory compliance [23]. In BNR-L, BNR-M, and BMR-H subjects who participated in this trial, 62, 90, and 75% showed positive responses to *Lb. gasseri* BNR17, respectively. Adhesion capacity of our strain was found to be superior to *Lb. acidophilus* NCFM. This shows that our strain has high potential to be developed as a probiotic strain.

In summary, our results confirmed probiotic properties of *Lb. gasseri* BNR17 in terms of relieving IBS symptoms and colonization. In addition, its optimal intake level was found to be $2 \times 5 \times 10^9$ CFU/day. However, this was a preliminary trial without detecting biochemical or metabolomics changes in feces. In addition, only IBS symptoms were measured. In order to provide pronounce evidence on its beneficial effects, more objective measures should be determined such as transit time, gut microbial changes and metabolites in feces. In the next main trial, biochemical or

Table 1 Baseline characteristics of subjects

	Placebo (n = 12)	BNR-L (n = 9)	BNR-M (n = 11)	BNR-H (n = 10)	*P* value[a]
Age (years)	26.7 ± 2.3	35.6 ± 3.3	24.9 ± 1.7	28.6 ± 2.4	0.103
Female/male (n)	6/6	6/3	2/9	3/7	0.132
Weight (kg)	75.43 ± 2.73	71.11 ± 2.18	75.35 ± 3.51	75.7 ± 3.32	0.679
Body mass index (kg/m^2)	25.5 ± 0.6	25.0 ± 0.7	24.3 ± 0.8	24.9 ± 0.5	0.059
Systolic blood pressure (mmHg)	129.0 ± 4.5	120.7 ± 3.3	125.2 ± 3.3	125.2 ± 4.7	0.674
Diastolic blood pressure (mmHg)	84.2 ± 2.0	81.1 ± 2.3	84.5 ± 2.7	82.0 ± 2.3	0.659
Fasting blood glucose (mg/dL)	101.29 ± 2.36	100.92 ± 4.42	101.40 ± 2.01	97.15 ± 2.64	0.706
Total cholesterol (mg/dL)	183.64 ± 6.63	204.46 ± 8.61	182.00 ± 8.23	173.38 ± 9.38	0.080
HDL- Cholesterol (mg/dL)	55.71 ± 2.40	52.39 ± 2.58	58.13 ± 4.30	50.31 ± 3.05	0.503
LDL- Cholesterol (mg/dL)	105.21 ± 6.32	124.00 ± 7.51	103.87 ± 7.06	98.15 ± 6.64	0.070
Triacylglyceride (mg/dL)	101.79 ± 14.07	142.23 ± 27.54	103.00 ± 11.74	140.15 ± 23.50	0.284

Mean ± SE (all variables)

[a]ANOVA test was used for continuous variables. Fisher's exact test was used for categorical variables

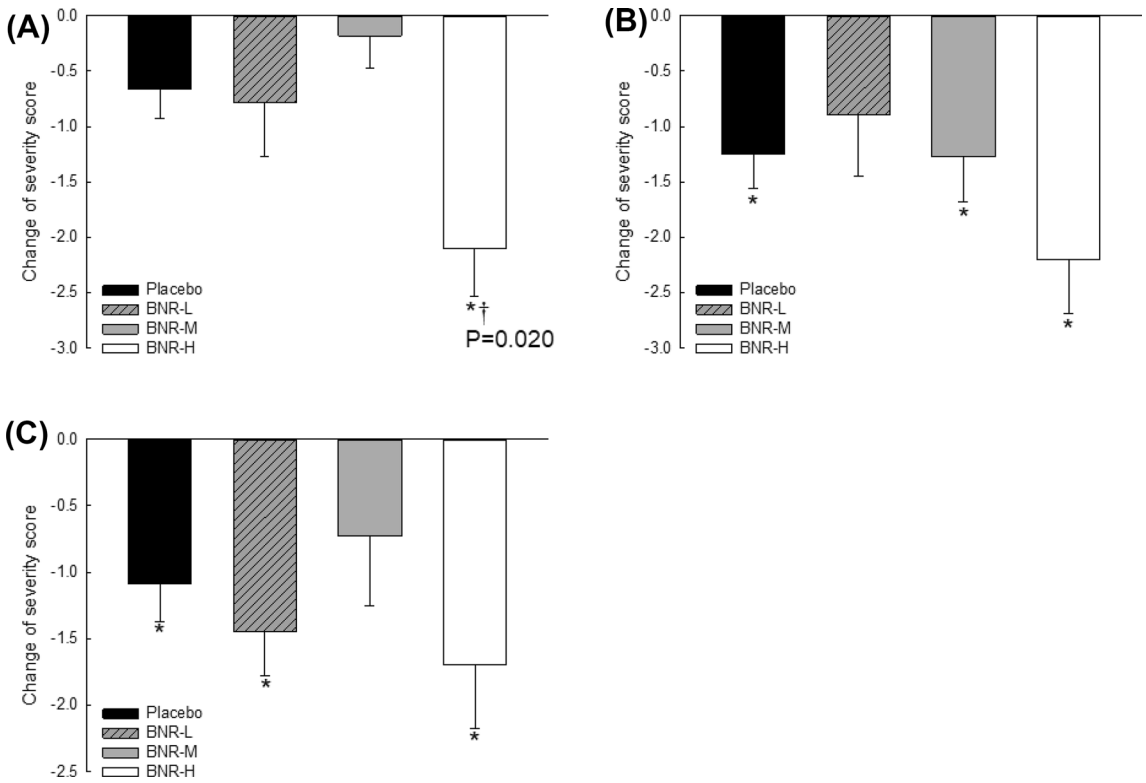

Fig. 1 Changes in severity score for symptoms. (**A**) Abdominal pain; (**B**) Bloating; (**C**) Feeling of incomplete evacuation. Data are presented as mean ± standard error (SE). $^{*}P < 0.05$ versus baseline values by paired *t* test. $^{\dagger}P < 0.05$ versus placebo group by ANOVA with Dunnet's multiple comparison test as post hoc analysis

Table 2 Number of fecal Lactobacillus species and detection of *Lactobacillus gasseri* BNR17 in fecal samples by PCR

		Placebo (n = 12)	BNR-L (n = 9)	BNR-M (n = 11)	BNR-H (n = 10)	*P* value[3]
Lactobacillus species						
Cell count[1] (Log10 CFU/g)	Week 0	4.07 ± 0.59	3.97 ± 0.66	4.14 ± 0.32	2.45 ± 0.69	0.141
	Week 4	4.42 ± 0.38	5.25 ± 0.36	4.73 ± 0.23	5.13 ± 0.39	0.310
	Changes	0.35 ± 0.62^{ab}	1.28 ± 0.41^{bc}	0.58 ± 0.29^{ab}	2.68 ± 0.66^{c}	0.015
	P value[2]	0.585	0.014	0.073	0.003	
Positive/tested (n)	Week 0	10/12	8/9	11/11	8/10	0.093
	Week 4	12/12	9/9	11/11	10/10	–
Lactobacillus gasseri BNR17						
Positive/tested (n)	Week 0	0/12	2/9	0/11	1/10	0.083
	Week 4	0/12	7/9	10/11	9/10	<0.0001

[1]Mean ± SE (all variables)

[2]Paired *t* test was used for comparisons within each group

[3]ANOVA was used for comparisons of continuous variables among groups. Fisher's exact test was used for comparisons of categorical variable among groups

metabolomic markers related to IBS should be studied using the present strain. Additionally, changes in population of gut microbiomes should be identified.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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