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Research article

A probiotic blend improves defecation, mental health, and productivity in healthy Japanese volunteers under stressful situations

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HIGHLIGHTS

- Our probiotics reduced the effects of diarrhea on daily activities in healthy adults.
- The probiotics also improved mental health under stress.
- A butyric acid-producing bacterium in the gut may be related to these benefits.
- The probiotics may be widely applicable in adults with IBS-like diarrhea.

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ABSTRACT

We investigated whether a blend of probiotics (KABP-021, KABP-022, and KABP-023) improved diarrhea-related problems in healthy Japanese adults who routinely lived under stressful conditions. Twenty-six females and 34 males were divided randomly into the probiotic and placebo groups in this double-blind, placebo-controlled, parallel-group comparison study. All participants ingested 1 capsule of probiotics or placebo per day for 4 weeks. Intervention with probiotics significantly reduced diarrhea-related problems assessed by the Izumo scale compared with placebo treatment ($P < 0.001$). In the Short Form-8 questionnaire, probiotic intervention improved mental component scores ($P = 0.002$), role emotional scores ($P = 0.002$), and mental health scores ($P < 0.001$). Treatment with probiotics also reduced the effects of diarrhea on daily activities ($P < 0.001$) and overall working habits ($P = 0.010$), including missing work (absenteeism) and impaired productivity (presenteeism), as assessed by the Work Productivity and Activity Impairment Questionnaire: General Health. Furthermore, there was a correlation between improved scores for diarrhea on the Izumo scale and increased abundance of *Faecalibacterium*, a butyric acid-producing bacterium, in the gut in the probiotic group ($P = 0.047$), whereas no such a correlation or trend was found in the placebo group. Our strategy of supplementation for 4 weeks with a specific blend of probiotics reduced diarrhea-related symptoms and may improve the mental health and daily activities of healthy individuals under stress.

1. Introduction

Healthy individuals suffering from stress-induced abdominal symptoms often do not receive optimal medical treatments and/or therapies because they are regarded as healthy. However, patients with irritable bowel syndrome (IBS) can receive appropriate treatment under the supervision of a physician. Many healthy individuals with sensitive inconvenience of defecation and reduced quality of life (QOL) have no

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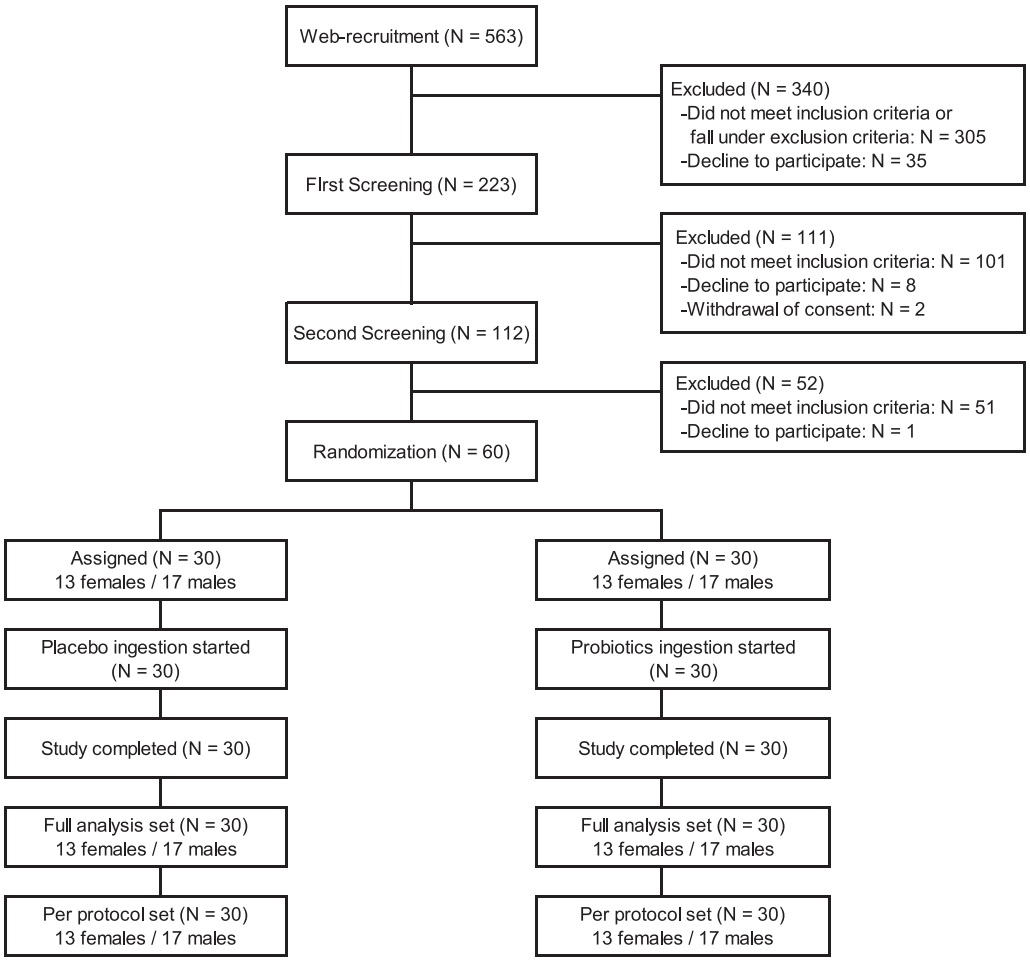

Figure 1. Flowchart of the study.

other choice but to treat themselves by trying over-the-counter drugs, traditional therapies, and specific diets based on self-assessment. These efforts may ease some of their symptoms temporally; therefore, alternative, more sustainable solutions at an early stage are urgently required. With a worldwide prevalence of approximately 4%, IBS is one of the most common functional gastrointestinal disorders (recently renamed as disorders of the gut-brain axis) [1], and many more individuals worldwide are thought to suffer from undiagnosed IBS.

Indeed, a recent internet survey using Rome III diagnostic criteria demonstrated that the prevalence of IBS in Japan was 13.1% among those aged 20 years or older. Of 12 million participants, 21.9%/13.7% (female/male) were in their 20s, 19.0%/13.4% were in their 30s, 14.9%/10.3% were in their 40s, 11.4%/8.9% were in their 50s, and 10.4%/7.0% were 60 years or older [2]. In addition, there may be an added sensitive population with various symptoms related to increased stress levels owing to highly competitive work environments or a fast-paced modern lifestyle. Consistent with this, within the healthy population, there are individuals who experience mild, nonpathological IBS-like symptoms, referred to as "IBS-like healthy people" [3]. Modern society, particularly in advanced countries, has become increasingly stressful; therefore, IBS-like healthy people with stress-induced abdominal symptoms are likely to have a reduced QOL, and their contributions to social activity and productivity may be impaired.

Currently, no medical treatments are available for this healthy IBS-like population. As described above, an imbalance in the microbiome or microbiota may cause or exacerbate chronic low-grade mucosal inflammation, alterations in gut epithelial and immune functions, and visceral hypersensitivity, in a healthy IBS-like population. Recently, new therapeutic strategies with the possibility to improve in intestinal microbiota have been identified. These include a low fermentable oligo-, di-, monosaccharide, and polyols (FODMAP) diet [4] as well as antibiotics [5] and probiotics. Probiotics, defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [6], have the potential to influence the intestinal microbiota and physiology. A recent meta-analysis of randomized controlled trials clearly demonstrated that probiotic supplementation is an effective therapy that improves the overall symptoms and QOL in patients with IBS [7]. Some probiotics have also been shown to be effective in healthy individuals with IBS-like symptoms [8, 9, 10]. Each strain has various function, therefore, multistrain probiotic supplementation may be more beneficial than monostrain supplementation, although more data are needed to support this hypothesis [11].

In this study, we used a blend of three probiotic strains and investigated its efficacy in healthy volunteers reporting problems with defecation, particularly diarrhea, under stressful situations.

2. Methods

2.1. Study design

This was a randomized, double-blind, placebo-controlled, parallel-group comparison study performed at a single clinical center associated with the Tokyo Sky-Tree Station Medical Clinic, Tokyo, Japan.

Table 1. Excerpt baseline data for physical parameters and primary and secondary outcomes.

	Placebo (N = 30)	Probiotics (N = 30)	*P* value
Physical parameters			
Age (years)	47.4 ± 11.5	46.3 ± 8.0	0.669
Height (cm)	164.8 ± 8.6	164.4 ± 9.3	0.844
Body weight (kg)	58.8 ± 10.2	59.0 ± 10.8	0.926
Blood pressure (mmHg)			
Systolic	122.4 ± 10.4	115.2 ± 11.7	0.014
Diastolic	74.6 ± 9.0	71.5 ± 9.4	0.198
Blood biochemical parameters (pg/mL)			
IL-1β	9.32 ± 23.51	3.44 ± 5.79	0.413
IL-6	15.59 ± 32.19	5.91 ± 7.00	0.561
IL-10	49.29 ± 188.39	4.08 ± 4.52	0.458
IL-12p70	15.88 ± 30.93	5.60 ± 5.26	0.119
Defecation			
Izumo scale (degree)			
Sum of Q13–Q15 (for diarrhea)	9.00 (8.25, 11.00)	9.00 (8.00, 11.00)	0.916
Bristol Stool Form Scale (degree of each time for 14 days)	5.23 (4.88, 5.56)	5.14 (5.00, 5.54)	0.795
Stool frequency (sum times for 14 days)	28.0 (21.5, 31.0)	28.5 (20.0, 39.0)	0.617
Abdominal pain (time per day)	2.23 (1.50, 2.75)	2.06 (1.73, 2.49)	0.976
Quality of life			
SF-8 (Frequency)			
Physical component score	50.87 ± 4.31	50.60 ± 4.48	0.811
Mental component score	42.51 ± 5.21	43.66 ± 5.41	0.404
WPAI-GH (%)			
Activity impairment due to health	47.67 ± 17.36	47.00 ± 15.79	0.535
Overall work impairment due to health[1]	42.72 ± 21.81	38.60 ± 19.22	0.510

Values are means ± standard deviations or medians and (first and third interquartiles). *P* values were derived from comparisons between the placebo and probiotic groups. [1], Numbers of participants were 24 and 27 in the placebo and probiotic groups, respectively. SF-8, Short Form-8 questionnaire; WPAI-GH, Work Productivity and Activity Impairment Questionnaire-General Health.

2.2. Participants

Healthy volunteers who met the following inclusion criteria were recruited: Japanese females and males ages ≥20 to <65 years at the time of informed consent, who routinely felt stress and suffered from diarrhea with abdominal pain and/or discomfort, but who were judged not to have functional gastrointestinal disorders (disorders of the gut-brain axis), including inflammatory bowel disease (IBD) and IBS, after review by a physician. Even if some participants were taking foods included with other *Lactobacillus* bacteria such as yogurt and pickled vegetables at preregistration, we did not exclude them. Because if our probiotics alleviated symptoms such as diarrhea, even if the participants consumed these bacteria, which are known to have positive effects on intestinal health, on a daily basis, we believe that our treatment improved overall health. The participants were requested to continue taking the same bacteria during participation. Participants who met the following exclusion criteria were excluded from the study: heavy drinkers (equivalent to ≥66 g ethanol intake per day); those under pharmacotherapy or clinical treatment for serious disease(s); undertaking exercise or diet therapy under instructions of a physician; those who had a risk of developing an allergy to the test food; those with a history of addiction to drugs or alcohol; those under treatment for mental disorders (such as depression) and/or sleep disorders, or with a history of mental disorders;

Table 2. Izumo scale score.

	Placebo (N = 30)	Probiotics (N = 30)	*P* value[1]
Q1: Are you bothered by acid reflux?			
Baseline	0 (0, 1.00)	0 (0, 1.00)	0.244
2 weeks	0 (0, 0)	0 (0, 1.00)#	0.283
4 weeks	0 (0, 0.75)	0 (0, 1.00)	0.388
Q2: Are you bothered by heartburn centered in the anterior chest?			
Baseline	0 (0, 0)	0 (0, 0)	0.588
2 weeks	0 (0, 0)	0 (0, 0)	0.131
4 weeks	0 (0, 0)	0 (0, 0)	0.690
Q3: Are you bothered by throat discomfort?			
Baseline	0 (0, 1.00)	0 (0, 1.00)	0.180
2 weeks	0 (0, 0)#	0 (0, 0)	0.943
4 weeks	0 (0, 0)##	0 (0, 0)	0.898
Q4: Are you bothered by epigastric pain?			
Baseline	0 (0, 2.00)	1.00 (0, 1.00)	0.842
2 weeks	0 (0, 1.00)	0 (0, 1.00)	0.550
4 weeks	0 (0, 1.00)	0 (0, 1.00)	0.752
Q5: Are you bothered by hunger epigastric pain?			
Baseline	1.00 (0, 2.00)	1.00 (0, 1.00)	0.631
2 weeks	0 (0, 1.00)##	0 (0, 1.00)	0.577
4 weeks	0 (0, 1.00)##	0 (0, 1.00)#	0.592
Q6: Are you bothered by an epigastric burning sensation?			
Baseline	0 (0, 0.75)	0 (0, 1.00)	0.729
2 weeks	0 (0, 0)#	0 (0, 0)#	0.690
4 weeks	0 (0, 0)#	0 (0, 0)#	0.429
Q7: Are you bothered by early satiation?			
Baseline	1.00 (0, 2.00)	1.00 (0, 1.00)	0.962
2 weeks	0 (0, 1.00)#	0 (0, 1.00)##	0.618
4 weeks	0 (0, 1.00)##	0 (0, 1.00)#	0.886
Q8: Are you bothered by post-prandial long-lasting epigastric fullness or nausea?			
Baseline	0 (0, 2.00)	1.00 (0, 1.00)	0.594
2 weeks	0 (0, 1.00)	0 (0, 1.00)	0.478
4 weeks	0 (0, 0.75)##	0 (0, 1.00)#	0.302
Q9: Are you bothered by epigastric bloating?			
Baseline	1.00 (0, 2.00)	1.00 (0, 2.00)	0.406
2 weeks	0 (0, 1.00)###	0.50 (0, 1.00)	0.409
4 weeks	0 (0, 1.00)###	0.50 (0, 1.00)#	0.483
Q10: Are you bothered by feeling of incomplete defecation?			
Baseline	0 (0, 0)	0 (0, 0)	0.451
2 weeks	0 (0, 0)	0 (0, 0)	0.459
4 weeks	0 (0, 0)	0 (0, 0)	0.378
Q11: Are you bothered by constipation or hard stool?			
Baseline	0 (0, 0)	0 (0, 0)	0.153
2 weeks	0 (0, 0)	0 (0, 0)	1.000
4 weeks	0 (0, 0)	0 (0, 0)	1.000
Q12: Are you bothered by stress-related constipation?			
Baseline	0 (0, 0)	0 (0, 0)	0.078
2 weeks	0 (0, 0)	0 (0, 0)	1.000
4 weeks	0 (0, 0)	0 (0, 0)	1.000
Q13: Are you bothered by fecal urgency?			
Baseline	3.00 (2.00, 3.75)	3.00 (2.00, 3.00)	0.677
2 weeks	2.00 (2.00, 3.00)	2.00 (2.00, 3.00)	0.936
4 weeks	2.00 (1.00, 2.00)##	2.00 (1.00, 2.00)##	0.402
Q14: Are you bothered by diarrhea or soft stool?			
Baseline	4.00 (3.00, 4.00)	3.00 (3.00, 4.00)	0.523
2 weeks	3.00 (2.00, 3.00)###	3.00 (2.00, 3.00)###	0.763
4 weeks	2.00 (2.00, 2.75)###	2.00 (1.00, 2.00)###	0.190
Q15: Are you bothered by stress-related diarrhea?			
Baseline	3.50 (3.00, 4.00)	3.00 (3.00, 4.00)	0.960
2 weeks	3.00 (2.00, 3.00)###	2.00 (2.00, 3.00)###	0.551

(*continued on next page*)

Table 2 (*continued*)

	Placebo (N = 30)	Probiotics (N = 30)	*P* value[1]
4 weeks	2.00 (2.00, 3.00)$^{\#\#\#}$	1.00 (1.00, 2.00)$^{\#\#\#}$	<0.001***
Heartburn (sum of Q1–3)			
Baseline	1.00 (0, 2.00)	0 (0, 2.00)	0.538
2 weeks	0 (0, 1.00)$^{\#\#}$	0 (0, 1.75)$^{\#}$	0.656
4 weeks	0 (0, 1.00)$^{\#\#}$	0 (0, 1.75)	0.718
Stomach pain (sum of Q4–6)			
Baseline	1.50 (0, 4.00)	2.00 (0, 3.75)	0.951
2 weeks	0 (0, 1.75)$^{\#}$	1.00 (0, 2.75)$^{\#}$	0.491
4 weeks	0 (0, 2.00)$^{\#\#}$	0 (0, 2.00)$^{\#}$	0.742
Stomach learning (sum of Q7–9)			
Baseline	2.00 (0, 6.75)	3.00 (0, 4.00)	0.844
2 weeks	1.00 (0, 2.75)$^{\#\#}$	1.50 (0, 3.00)$^{\#}$	0.499
4 weeks	0 (0, 2.75)$^{\#\#\#}$	1.00 (0, 3.00)$^{\#}$	0.417
Constipation (sum of Q10–12)			
Baseline	0 (0, 0)	0 (0, 0)	0.141
2 weeks	0 (0, 0)	0 (0, 0)	0.685
4 weeks	0 (0, 0)	0 (0, 0)	0.378
Diarrhea (sum of Q13–15)			
Baseline	9.00 (8.25, 11.0)	9.00 (8.00, 11.0)	0.916
2 weeks	7.50 (6.00, 8.00)$^{\#\#\#}$	7.00 (5.25, 8.00)$^{\#\#\#}$	0.637
4 weeks	6.00 (5.00, 7.75)$^{\#\#\#}$	5.00 (4.00, 6.00)$^{\#\#\#}$	0.021*

Data are presented as medians and (first and third interquartiles). [1], *P* values in this table were derived from comparisons between the placebo and probiotic groups. $^{*}P < 0.05$, $^{***}P < 0.001$ versus the placebo group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus baseline within the group.

those with irregular working patterns, such as night shift; those with irregular lifestyle rhythms with regard to food and sleep; those with extremely unbalanced eating habits; those under treatment for gastrointestinal disorders that may affect intestinal function or with a history of surgery and/or history of intestinal diseases other than appendicitis; those diagnosed with diseases, such as IBD and IBS, which affected bowel movements or with a history of such diseases; those with severe diseases, such as brain disorders, malignant tumors, immune diseases, diabetes mellitus, hepatic diseases (hepatitis), kidney diseases, cardiac diseases, and severe metabolic diseases (such as thyroid disorders and adrenal disorders) or with a history of these diseases; users of foods, supplements and/or medicines that affected intestinal function (other *Lactobacillus* bacteria foods that are declared before participation and continued to be taken during participation are not applicable); those who participated in another clinical study within 3 months prior to providing informed consent or who planned to participate in another study during this study; those who donated more than 200 mL whole blood or blood components within 1 month prior to informed consent or more than 400 mL whole blood within 3 months prior to consent; those who were pregnant or breast feeding or might be pregnant; those who had difficulty with filling in various survey forms; and those who were judged as inappropriate for inclusion by a physician. Participants were requested to not change their lifestyle or eating and drinking habits during the intervention period after preregistration. They were asked to record answers for the following questions in their lifestyle-related diaries and submit the answers the next morning for 2 weeks before the intervention and during the intervention period: test food intake, physical condition, dietary changes, medical treatment as needed, health/supplement foods, other foods that may affect the study, drinking amount, and exercise. They also recorded data in a defecation diary as described below. This study was the first to use healthy subjects for the tested probiotic blend; thus, we determined that 60 participants were required based on general suggestions by Dr. Julious [12] and Dr. Hertzog [13], and we allocated 30 participants into each of the placebo and probiotic groups, as described in the Study protocol section.

2.3. Intervention with a probiotic blend

The test food (a probiotic product) was given in a capsule containing a combination of three of the following strains of lactic acid bacteria: *Pediococcus acidilactici* KABP-021 (CECT7483), *Lactiplantibacillus plantarum* KABP-022 (CECT7484), and *L. plantarum* KABP-023 (CECT7485) at a concentration of 1×10^9 colony-forming units per strain. This specific prescription has been reported to improve IBS-related QOL and visceral sensitivity and to alleviate symptoms associated with IBS [14]. We obtained these probiotics from AB-Biotics S.A. (Barcelona, Spain). Capsules of the test food were constructed with these probiotics, starch, calcium stearate, hydroxypropyl methylcellulose, and titanium dioxide and were manufactured at Sunsho Phamaceutical Co., Ltd. (Shizuoka, Japan) according to the Japanese food processing standard. The placebo capsules were indistinguishable in form, color, and taste from the capsules containing probiotic bacteria. The placebo capsules were also manufactured by the same company that manufactured the probiotic capsules, and starch was used instead of probiotics. Both were then placed under the control of a contract research organization (Huma R&D Co., Ltd., Tokyo, Japan). All capsules of the placebo and probiotics were stored at a temperature less than or equal to 25 °C, away from sunlight. Each participant was instructed to take 1 capsule after a meal (recommended after each breakfast) for 28 days.

2.4. Study protocol

Sixty participants were assigned to receive placebo or probiotic capsules by the Study Food Allocation Manager in Huma R&D Co., Ltd., using a computer-generated stratified randomization list that considered the participant's sex, age, Izumo scale score, Bristol Stool Form Scale, stool frequency, frequency of abdominal pain, and presence or absence of concomitant intake of other *Lactobacillus* bacteria. The study allocation list was kept by the Allocation Manager, and blinding was maintained for all parties until completion of the study.

2.5. Efficacy and safety assessment

The primary efficacy endpoint was an improvement in the Izumo scale score based on a questionnaire of abdominal symptom-related QOL [15]. These scores were assessed before (baseline) and 2 and 4 weeks after treatment.

The secondary efficacy endpoints were stool frequency, stool form (Bristol Stool Form Scale), abdominal pain/discomfort accompanying an urge to defecate, abdominal pain/discomfort after defection based on a defecation diary, serum concentrations of cytokines (interleukin [IL]-1β, IL-6, IL-10, IL-12p70), Short Form-8 (SF-8; Japanese version) [16], and Work Productivity and Activity Impairment Questionnaire: General Health (WPAI-GH) [17]. Serum concentrations of cytokines as well as SF-8 and WPAI-GH results were assessed at baseline and 4 weeks after treatment. The defecation diary was recorded at baseline and between 0 and 4 weeks after treatment. Differences between the placebo and probiotic groups as well as differences between baseline and each time point within a group were calculated.

For additional secondary efficacy analyses, intestinal microbiome analysis was performed by Cykinso, Inc. (Tokyo, Japan) according to their technical manual [18] and the QIIME II pipeline (version 2020 11), which is required for metagenomic analysis. Briefly, the participants collected stool samples into restrictive sampling tubes (Mykinso), which were provided in advance, at home on the morning of the inspection day. Then, they carried the sample on ice to the clinical center and submitted the sample for analysis. If a participant was unable to collect stool on the morning of the inspection date, they remained in close contact with the CRO to collect stool within a few days after the inspection date and to carry or ship the sample to the clinical center. Another stool sample was collected for intestinal metabolome analysis using the same collection method as described above; these samples were analyzed by Human

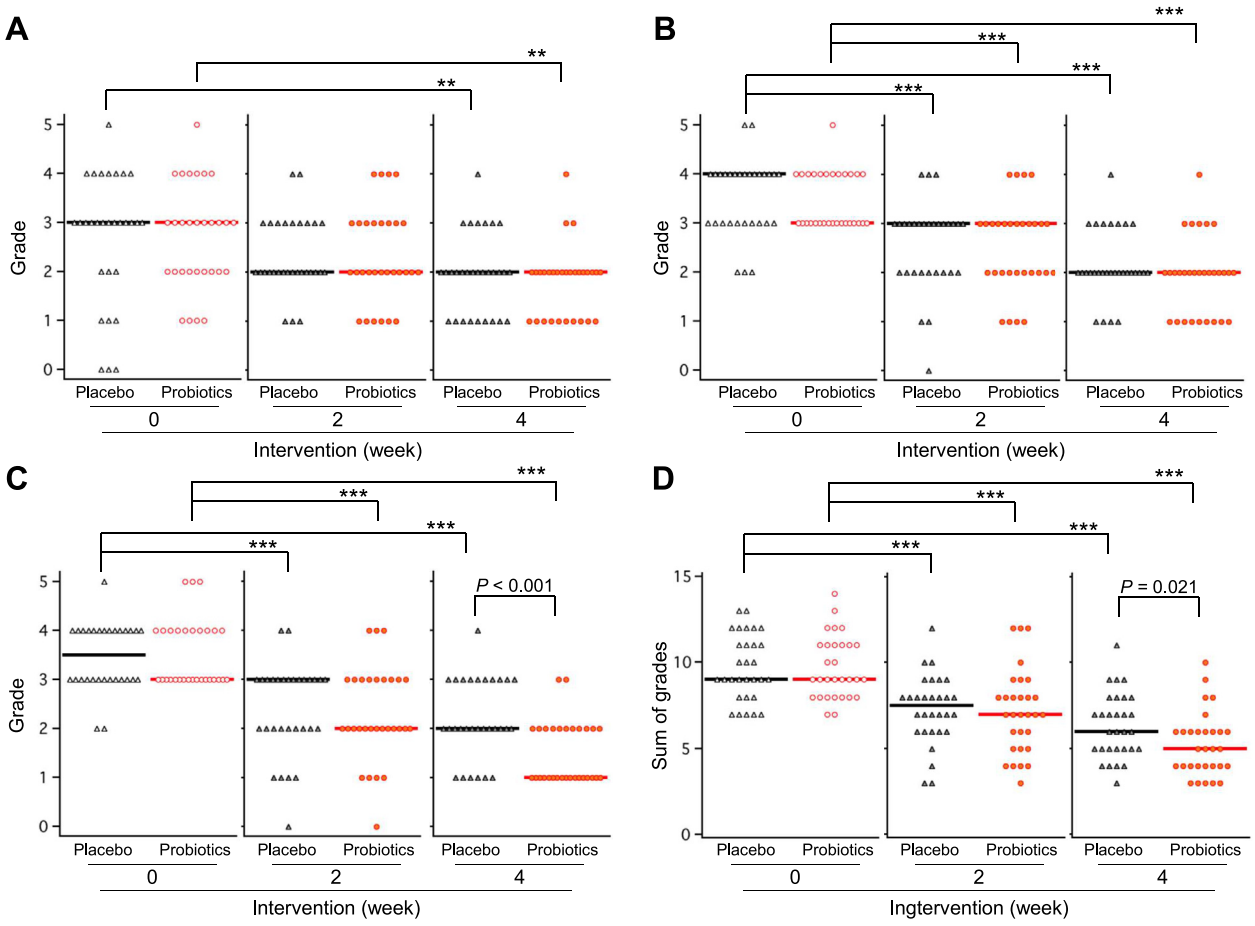

Figure 2. Effect of probiotics on the Izumo scale score for diarrhea. Each symbol and line represent individual Izumo scale scores and the median of the group (N = 30). There were no significant differences in any category at week 0 (baseline, before the intervention) between the placebo and probiotic groups. $^{**}P < 0.01$ and $^{***}P < 0.001$. A: Score for the answer to Question 13 (Are you bothered by fecal urgency?). B: Score for the answer to Question 14 (Are you bothered by diarrhea or soft stools?). C: Score for the answer to Question 15 (Are you bothered by stress-related diarrhea?). D: Sum of the scores for the answers to Questions 13–15 for the Izumo diarrhea score.

Table 3. Summarized data from defecation diaries.

	Placebo (N = 30)	Probiotics (N = 30)	*P* value[1]
Bristol Stool Scale Form (degree of each time for 14 days)			
Baseline	5.23 (4.88, 5.56)	5.14 (5.00, 5.54)	0.795
2 weeks	4.69 (4.49, 5.03)$^{\#\#\#}$	4.65 (4.44, 4.83)$^{\#\#\#}$	0.501
4 weeks	4.64 (4.38, 4.94)$^{\#\#\#}$	4.60 (4.35, 4.84)$^{\#\#\#}$	0.395
Stool frequency (sum times for 14 days)			
Baseline	28.0 (21.5, 31.0)	28.5 (20.0, 39.0)	0.617
2 weeks	24.5 (18.5, 28.0)$^{\#}$	24.5 (18.25, 31.0)$^{\#\#\#}$	0.977
4 weeks	25.0 (18.0, 29.5)$^{\#}$	25.0 (16.0, 29.75)$^{\#\#\#}$	0.770
Abdominal pain accompanying urge to defecate			
Baseline	2.23 (1.50, 2.75)	2.06 (1.73, 2.49)	0.976
2 weeks	1.73 (1.28, 2.17)$^{\#\#\#}$	1.63 (1.49, 1.84)$^{\#\#\#}$	0.807
4 weeks	1.51 (1.12, 2.03)$^{\#\#\#}$	1.46 (1.15, 1.74)$^{\#\#\#}$	0.722
Abdominal discomfort accompanying urge to defecate			
Baseline	2.22 (1.75, 2.84)	2.22 (1.67, 2.52)	0.652
2 weeks	1.81 (1.33, 2.13)$^{\#\#\#}$	1.78 (1.37, 2.12)$^{\#\#\#}$	0.717
4 weeks	1.54 (1.17, 2.00)$^{\#\#\#}$	1.51 (1.11, 1.81)$^{\#\#\#}$	0.378

Data are shown as medians and (first and third interquartiles). [1], *P* values in this table were derived from comparisons between the placebo and probiotic groups. $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ versus baseline within the group. There were no significant differences in any category between the placebo and probiotic groups.

Metabolome Technologies, Inc. (Yamagata, Japan) according to their technical manuals [19, 20].

Furthermore, we investigated the effects of the probiotics on the smells of defecation and flatulence as a preliminary test. Participants subjectively evaluated the smell after every defecation or flatulence event and recorded the results in their defecation diary every day after starting the intervention. The intensity of the smell was quantified as grade 0–5 as follows: 0, no event; 1, no odor; 2, weak odor; 3, moderate odor; 4, severe odor; 5, extremely bad odor. We aggregated weekly averages for each participant and evaluated changes in smells.

For safety evaluation, the following measurements were performed: height, body weight, systolic and diastolic blood pressure; blood biochemical parameters, including triglycerides, total cholesterol (Cho), low-density lipoprotein (LDL)-Cho, high-density lipoprotein (HDL)-Cho, blood urea nitrogen, total bilirubin, total protein, albumin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, γ-glutamyl transpeptidase, creatinine, uric acid, fasted blood glucose, and hemoglobin A1c; hematological parameters, including white blood cells, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelet count; and urine parameters, including pH, specific gravity, protein, glucose, urobilinogen, occult blood, ketones, and bilirubin. Biochemical parameters in blood and urine samples were measured at the clinical center according to the standard procedures recommended by the Japanese Ministry of Health, Labor and Welfare at the time of health examination. Adverse

Table 4. Preliminary evaluation of senses after defecation and smells of stool and flatulence.

	Placebo (N = 30)	Probiotics (N = 30)	*P* value[1]
Abdominal pain after defecation			
1 week	1.26 (1.02, 1.86)	1.44 (1.10, 1.80)	0.846
2 weeks	1.15 (1.00, 1.81)	1.37 (1.02, 1.70)	0.810
3 weeks	1.26 (1.00, 1.76)	1.22 (1.00, 1.51)$^{\$}$	0.722
4 weeks	1.07 (1.00, 1.71)$^{\$}$	1.09 (1.00, 1.56)$^{\$\$}$	0.849
Abdominal discomfort after defecation			
1 week	1.48 (1.17, 1.98)	1.49 (1.28, 1.85)	0.806
2 weeks	1.35 (1.13, 1.87)	1.41 (1.13, 1.79)	0.812
3 weeks	1.28 (1.13, 1.69)	1.31 (1.00, 1.52)$^{\$\$}$	0.403
4 weeks	1.29 (1.00, 1.67)	1.27 (1.00, 1.54)$^{\$\$\$}$	0.803
Smell of stool			
1 week	2.91 (2.34, 3.25)	2.37 (2.00, 2.90)	0.006**
2 weeks	2.65 (2.27, 3.00)$^{\$\$}$	2.17 (2.00, 2.52)$^{\$\$}$	0.002**
3 weeks	2.71 (2.34, 3.00)	2.00 (1.86, 2.28)$^{\$\$\$}$	<0.001***
4 weeks	2.80 (2.02, 3.00)	2.00 (1.92, 2.48)$^{\$}$	0.006**
Smell of flatulence			
1 week	2.43 (1.75, 3.00)	2.00 (1.36, 2.43)	0.103
2 weeks	2.43 (2.00, 2.96)	2.00 (1.75, 2.68)	0.094
3 weeks	2.36 (1.89, 2.86)	2.07 (1.61, 2.64)	0.134
4 weeks	2.29 (2.00, 2.96)	2.00 (1.57, 2.68)	0.041*

Data are aggregated weekly averages (medians and (first and third interquartiles)) evaluated for the degree each time for 7 days. The intensities were quantified as grades 0–5 as follows: 0, no event; 1, no pain/discomfort/odor; 2, weak; 3, moderate; 4, severe; 5, extremely bad. [1], *P* values in this table were derived from comparisons between the placebo and probiotic groups. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus the same time in the placebo group. $^{\$}P < 0.05$, $^{\$\$}P < 0.01$, $^{\$\$\$}P < 0.001$ versus 1 week after intervention within the group.

events were assessed by the physician based on the results of participant communication, blood biochemical and hematologic analyses, and urinalysis. The content of the daily diary for each participant was also used to evaluate Compliance, such as intake of the test food, presence/absence of medical treatment and its contents, and lifestyle-related changes.

2.6. Ethics

The study protocol was approved by the Ethics Committees of Nihonbashi Egawa Clinic, Tokyo, Japan (July 10, 2020; approval no. RD09001TS04). All volunteers provided written informed consent to participate. The study was performed in accordance with the Declaration of Helsinki (adopted in 1964 and revised in October 2013), the Ethical Guidelines for Medical and Health Research Involving Human Subjects (Notification No. 3 issued by the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare in 2014), and the Act on the Protection of Personal Information (Act No. 57 issued on May 30, 2003). This study was registered at UMIN-CTR (UMIN000041470).

2.7. Statistical analysis

Statistical analysis was performed on the full analysis set population. We used the SPSS Statistics 27R software package by IBM. Mann-Whitney *U*-tests (intergroup comparisons) and Wilcoxon signed rank tests (intragroup comparisons) were used for evaluation of grades, such as the Izumo scale, Bristol Stool Form Scale, SF-8, WPAI-GH, and urine biochemical parameters. Student's unpaired *t*-tests or Welch's *t*-tests (intergroup comparisons) and paired *t*-tests (intragroup comparisons) were used for analysis of parameters of physical and vital signs, blood biochemical parameters, pH and specific gravity of urine, metabolites in stool samples, and the continuous values of their properties. Fisher's exact tests were used to evaluate adverse events. Pearson's product-moment correlation coefficients were used for correlations of values that changed (e.g., diarrhea symptoms as the Izumo diarrhea score, which was the sum of the Izumo scale Q13 to Q15 and the relative abundance of different bacteria) from baseline to 4 weeks after the intervention. Two-sided *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Participants and compliance

This study was carried out from October 2020 to March 2021. As shown in Figure 1, 60 participants were enrolled after two-stage screening to exclude those who did not meet the inclusion criteria, met the exclusion criteria, declined to participate, or withdrew their informed consent. All participants, 30 subjects in each group, completed the study without deviating from the criteria set for the study, and thus, this population was used for efficacy and safety analyses. Supplementation with placebo or probiotics was completed at a rate of 100%. No participants changed their lifestyle during the intervention period according to judgements by medical staff, and there were no cases of compliance violations.

3.2. Baseline characteristics of participants

There were no significant differences in any baseline characteristics, excluding systolic blood pressure, between the placebo and probiotic groups (Table 1). The systolic blood pressure of participants in each group was within the standard range for Japanese individuals, and all participants were judged as appropriate to participate in the study by the investigator.

3.3. Primary endpoint

There were no significant differences in enterogastric symptoms or constipation (Q1 to Q12 from the Izumo scale) between the placebo and probiotic groups at baseline and 4 weeks of intervention (Table 2). Regarding diarrhea symptoms, there were no significant differences in Q13 ("Are you bothered by fecal urgency?") or Q14 ("Are you bothered by diarrhea or soft stools?") between the placebo and probiotic groups (Figure 2A and 2B). However, probiotic intervention for 4 weeks caused a significant reduction in the score for Q15 ("Are you bothered by stress-related diarrhea?") compared with placebo ($P < 0.001$; Figure 2C). Moreover, the total diarrhea score of the Izumo scale (sum of Q13–Q15) was significantly reduced by probiotic treatment compared with placebo ($P = 0.021$; Figure 2D).

3.4. Secondary endpoints

Based on the defecation diary, there were no significant differences in stool frequency, stool form, abdominal pain/discomfort accompanying urge to defecate, and abdominal pain/discomfort after defecation between the groups (Tables 3 and 4). However, probiotic treatment significantly reduced the smell of stool at 1 week and beyond during intervention compared with placebo ($P = 0.006$ at 1 week; $P = 0.002$ at 2 weeks; $P < 0.001$ at 3 weeks; and $P = 0.006$ at 4 weeks; Table 4). Probiotic treatment also reduced the smell of flatulence after 4 weeks of intervention ($P = 0.041$), although the scores were not significantly different after 1 and 2 weeks of intervention.

Participants receiving the probiotic intervention displayed a significant decrease in pro-inflammatory IL-6 ($P = 0.036$) and an increase in anti-inflammatory IL-10 ($P < 0.001$) compared with those at baseline; however, no between-group differences were detected compared with placebo (Table 5). Similarly, IL-12p70 was also increased from baseline

Table 5. Physical and biochemical parameters.

		Placebo (N = 30)	Probiotics (N = 30)	*P* value[1]
Physical parameters				
Body weight (kg)	Baseline	58.8±10.2	59.0±10.8	0.926
	4 weeks	59.2±10.4	59.5±11.1	0.901
Blood pressure (mmHg)				
Systolic	Baseline	122.4±10.4	115.2±11.7	0.014*
	4 weeks	122.8±12.5	115.1±15.3	0.038*
Diastolic	Baseline	74.6±9.0	71.5±9.4	0.198
	4 weeks	74.2±10.3	70.6±12.3	0.231
Blood biochemical parameters				
IL-1β (pg/mL)	Baseline	9.32±23.51	3.44±5.79	0.413
	4 weeks	13.98±43.67	4.62±6.36	0.526
IL-6 (pg/mL)	Baseline	15.59±32.19	5.91±7.00	0.561
	4 weeks	15.20±33.15	5.39±10.80$^{\#}$	0.698
IL-10 (pg/mL)	Baseline	49.29±188.39	4.08±4.52	0.458
	4 weeks	29.90±80.00	7.18±8.21$^{\#\#\#}$	0.456
IL-12p70 (pg/mL)	Baseline	15.88±30.93	5.60±5.26	0.119
	4 weeks	17.63±25.75$^{\#}$	8.54±8.53$^{\#\#}$	0.099
Trigriceride (mg/dL)	Baseline	91.6±61.8	106.2±108.6	0.524
	4 weeks	81.5±56.5	107.0±123.3	0.308
Total-Cho (mg/dL)	Baseline	210.3±30.1	228.7±33.5	0.029*
	4 weeks	210.6±28.5	222.1±36.6	0.177
HDL-Cho (mg/dL)	Baseline	67.9±14.6	68.0±15.9	0.966
	4 weeks	70.2±16.8	68.5±17.8	0.710
LDL-Cho (mg/dL)	Baseline	119.9±29.1	134.4±29.6	0.061
	4 weeks	118.2±27.7	125.8±26.8	0.286
Blood urea nitrogen (mg/dL)	Baseline	12.7±4.6	13.5±4.3	0.437
	4 weeks	11.9±3.2	13.4±3.9	0.105
Total bilirubin (mg/dL)	Baseline	0.90±0.27	0.90±0.45	0.944
	4 weeks	0.81±0.23 $^{\#}$	0.76±0.35 $^{\#}$	0.463
Total Protein (g/dL)	Baseline	7.42±0.46	7.42±0.41	1.000
	4 weeks	7.24±0.41$^{\#}$	7.19±0.34$^{\#\#}$	0.608
Albumin (g/dL)	Baseline	4.66±0.39	4.59±0.25	0.432
	4 weeks	4.52±0.33$^{\#\#}$	4.47±0.25$^{\#}$	0.479
Alkaline phosphatase (U/L)	Baseline	193.1±52.6	174.5±42.1	0.135
	4 weeks	195.7±56.2	178.9±44.6	0.205
Aspartate aminotransferase (U/L)	Baseline	23.1±10.1	22.1±9.2	0.689
	4 weeks	22.5±5.5	21.8±6.6	0.655
Alanine aminotransferase (U/L)	Baseline	21.0±13.0	22.1±18.1	0.794
	4 weeks	20.3±7.2	21.6±12.0	0.630
Lactate dehydrogenase (U/L)	Baseline	180.5±32.5	180.8±28.2	0.973
	4 weeks	176.0±29.3	178.6±25.6	0.711
γ-glutamyl transferase (U/L)	Baseline	32.0±23.7	28.0±28.4	0.552
	4 weeks	33.9±25.4	27.4±26.6	0.332
Creatine (mg/dL)	Baseline	0.73±0.14	0.75±0.11	0.548
	4 weeks	0.71±0.15	0.74±0.12	0.419
Uric acid (mg/dL)	Baseline	5.15±1.45	5.15±1.34	0.985
	4 weeks	5.37±1.50	5.02±1.35	0.336
Fasted blood glucose (mg/dL)	Baseline	85.97±8.64	86.03±9.52	0.977
	4 weeks	90.10±14.22	90.10±8.62 $^{\#\#\#}$	1.000
Hemoglobin A1c (%)	Baseline	5.26±0.23	5.30±0.27	0.507
	4 weeks	5.22±0.23	5.29±0.26	0.279
Hematologic parameters				
White blood cells (/mL)	Baseline	5633±1104	4960±829	0.009*
	4 weeks	5120±1404$^{\#}$	4700±1049	0.194

(*continued on next page*)

Table 5 (*continued*)

			Placebo (N = 30)	Probiotics (N = 30)	*P* value[1]
Red blood cells (× 10^4/mL)	Baseline		458.5±39.0	465.5±42.4	0.508
	4 weeks		454.9±37.6	456.4±47.7 #	0.892
Hemoglobin (g/dL)	Baseline		14.20±1.05	14.29±1.35	0.790
	4 weeks		14.09±1.15	13.96±1.85	0.757
Hematocrit (%)	Baseline		42.82±2.90	43.52±3.86	0.425
	4 weeks		42.15±3.09	42.27±5.13#	0.910
Mean corpuscular volume (fL)	Baseline		93.62±4.68	96.60±3.97	0.983
	4 weeks		92.86±4.86	92.55±5.25	0.815
Mean corpuscular hemoglobin concentration (pg)	Baseline		31.04±1.43	30.73±1.67	0.447
	4 weeks		31.01±1.63	30.55±2.15	0.354
Mean corpuscular hemoglobin concentration (%)	Baseline		33.17±0.87	32.82±0.89	0.132
	4 weeks		33.41±0.61	32.98±0.87	0.033*
Platelet count (× 10^4/mL)	Baseline		24.8±4.9	26.3±4.1	0.215
	4 weeks		25.4±4.4	27.0±4.9	0.208
Urine parameters					
pH	Baseline		5.73±0.60	5.92±0.71	0.283
	4 weeks		6.12±0.84#	5.88±0.76	0.263
Specific gravity	Baseline		1.019±0.010	1.021±0.007	0.351
	4 weeks		1.017±0.009	1.019±0.008	0.438
Protein (number)	Baseline	(−)	23	24	0.620
		(±)	4	6	
		(1+)	1	0	
		(2+)	2	0	
		(3+)	0	0	
		(4+)	0	0	
	4 weeks	(−)	25	25	1.000
		(±)	4	4	
		(1+)	1	1	
		(2+)	0	0	
		(3+)	0	0	
		(4+)	0	0	
Glucose (number)	Baseline	(−)	30	30	1.000
		(±)	0	0	
		(1+)	0	0	
		(2+)	0	0	
		(3+)	0	0	
		(4+)	0	0	
	4 weeks	(−)	30	30	1.000
		(±)	0	0	
		(1+)	0	0	
		(2+)	0	0	
		(3+)	0	0	
		(4+)	0	0	
Urobilinogen (number)	Baseline	(−)	30	30	1.000
		(±)	0	0	
		(1+)	0	0	
		(2+)	0	0	
		(3+)	0	0	
		(4+)	0	0	
	4 weeks	(−)	30	30	1.000
		(±)	0	0	
		(1+)	0	0	
		(2+)	0	0	
		(3+)	0	0	
		(4+)	0	0	

(*continued on next page*)

Table 5 (*continued*)

			Placebo (N = 30)	Probiotics (N = 30)	*P* value[1]
Occult blood (number)	Baseline	(−)	28	29	0.556
		(±)	0	0	
		(1+)	0	0	
		(2+)	0	0	
		(3+)	2	1	
	4 weeks	(−)	29	25	0.096
		(±)	0	1	
		(1+)	0	0	
		(2+)	0	2	
		(3+)	1	2	
Ketones (number)	Baseline	(−)	28	29	0.556
		(±)	0	0	
		(1+)	0	0	
		(2+)	2	1	
		(3+)	0	0	
	4 weeks	(−)	29	30	0.317
		(±)	0	0	
		(1+)	1	0	
		(2+)	0	0	
		(3+)	0	0	
Bilirubin (number)	Baseline	(−)	30	30	1.000
		(±)	0	0	
		(1+)	0	0	
		(2+)	0	0	
		(3+)	0	0	
		(4+)	0	0	
	4 weeks	(−)	30	30	1.000
		(±)	0	0	
		(1+)	0	0	
		(2+)	0	0	
		(3+)	0	0	
		(4+)	0	0	

Values are means ± standard deviations or numbers of participants. [1], *P* values in this table were derived from comparisons between the placebo and probiotic groups. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the placebo group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus baseline within the group. Cho, cholesterol; IL, interleukin.

($P = 0.004$), although there were no between-group differences compared with placebo.

Regarding SF-8 scores, the physical component score and other scores, excluding mental-related scores, at 4 weeks after the ingestion of probiotics were not affected (Table 6 and Figure 3A). Notably, however, the probiotic group showed significant improvements in the mental component score compared with that in the placebo group ($P = 0.002$; Figure 3B). The probiotic group also showed improved mental health ($P < 0.001$; Figure 3C) and role-emotional scores ($P = 0.002$; Figure 3D) compared with the placebo group.

The WPAI-GH after 4 weeks of intervention was also improved in the probiotic group compared with that in the placebo group (Figure 3E and 3F). The probiotic group showed alleviation of daily activity impairment ($P < 0.001$; Figure 3E) and overall work impairment (missing work [absenteeism], impaired productivity [presenteeism]; $P = 0.010$; Figure 3F) compared with the placebo group. Other scores of the WPAI-GH after 4 weeks of intervention in the probiotic group were not improved significantly compared with those in the placebo group (Table 6).

All participants were able to submit properly collected stool samples for microbiome and metabolome analyses to the clinical center on a predetermined submission date (visiting date for inspection). Microbiome and metabolome analyses demonstrated no clear differences between the placebo and probiotic groups (Raw data: Microbiome, Supplementary Tables 1–4; metabolome, Supplementary Table 5). There were no significant correlations between the gut abundance of butyric acid-producing bacteria and improvement of Izumo diarrhea scores (the sum of Izumo scale Q13–Q15) in both groups (probiotics, Pearson's R regression coefficient = −0.301, $P = 0.106$; placebo, R = 0.040, $P = 0.833$; Figure 4A). Regarding *Faecalibacterium*, a butyric acid-producing bacteria, there was a significant correlation with improvement in the Izumo diarrhea score in the probiotic group (R = −0.366, $P = 0.047$), although the correlation or trend was not detected in the placebo group (R = 0.049, $P = 0.798$; Figure 4B).

3.5. Safety

Regarding vital signs, blood biochemical analysis, hematological analysis, and urinalysis, occasional significant changes from baseline were observed in both groups (Table 5). However, these changes were small, within the normal range, and clinically irrelevant.

Adverse events were mild/moderate and transient, disappearing within a few days in each group (Supplementary Table 6). The observed adverse events were judged as clinically irrelevant and unrelated to the treatment by the investigator.

4. Discussion

In this study, we demonstrated that our probiotic blend reduced stress-induced abdominal symptoms, particularly diarrhea, in healthy participants and may improve QOL as well. These findings were based on subjective evaluations, such as the Izumo scale score and SF-8 score, but were not supported by objective evaluations, such as the Bristol Stool

Table 6. Summarized data for SF-8 surveys and WPAI-GH scores.

	Placebo (N = 30)	Probiotics (N = 30)	*P* value[1]
SF-8 (Frequency)			
Physical functioning			
Baseline	50.78 ± 3.57	50.19 ± 3.94	0.546
4 weeks	49.59 ± 4.62	51.28 ± 3.45	0.113
Role physical (Physical)			
Baseline	50.50 ± 4.68	49.61 ± 4.03	0.433
4 weeks	48.95 ± 5.21	48.95 ± 5.21	1.000
Body pain			
Baseline	46.73 ± 5.23	49.04 ± 4.56	0.073
4 weeks	49.19 ± 6.87	49.90 ± 6.05	0.670
General health			
Baseline	49.28 ± 5.56	49.93 ± 5.34	0.645
4 weeks	51.72 ± 5.09$^{\#}$	51.98 ± 5.99	0.853
Vitality			
Baseline	49.94 ± 4.60	49.60 ± 4.11	0.764
4 weeks	50.76 ± 4.80	50.69 ± 6.22	0.960
Social functioning			
Baseline	46.21 ± 6.32	46.81 ± 5.42	0.694
4 weeks	49.11 ± 5.56$^{\#\#}$	49.36 ± 7.17	0.879
Role emotional (Mental)			
Baseline	44.14 ± 4.44	44.65 ± 4.39	0.659
4 weeks	45.06 ± 5.04	48.56 ± 3.45$^{\#\#\#}$	0.002**
Mental health			
Baseline	43.83. ± 5.01	45.47 ± 5.74	0.242
4 weeks	45.19 ± 4.29$^{\#}$	49.72 ± 3.79$^{\#\#\#}$	<0.001***
Physical component score			
Baseline	50.87 ± 4.31	50.60 ± 4.48	0.811
4 weeks	50.65 ± 5.37	49.73 ± 5.05	0.496
Mental component score			
Baseline	42.51 ± 5.21	43.66 ± 5.41	0.404
4 weeks	44.66 ± 4.83$^{\#}$	48.38 ± 4.38$^{\#\#\#}$	0.002**
WPAI-GH (%)			
Activity impairment due to health			
Baseline	47.67 ± 17.36	47.00 ± 15.79	0.535
4 weeks	47.33 ± 17.60	31.67 ± 17.44$^{\#\#\#}$	<0.001***
Overall work impairment due to health			
Baseline	42.72 ± 21.81	38.60 ± 19.22	0.510
4 weeks	42.50 ± 20.90	28.15 ± 17.77$^{\#\#}$	0.010*
Work time missed due to health[2]			
Baseline	0.31 ± 1.53	0.17 ± 0.64	0.660
4 weeks	0.00 ± 0.00	0.00 ± 0.00	1.000
Impairment while working due to health[2]			
Baseline	42.50 ± 21.92	38.52 ± 19.16	0.467
4 weeks	42.50 ± 20.90	28.15 ± 17.77$^{\#\#}$	0.010*

Data are means ± standard deviations. [1], *P* values in this table were derived from comparisons between the placebo and probiotic groups. [2], These scores are shown for workers; the numbers of participants were 24 and 27 in the placebo and probiotic groups, respectively. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus the placebo group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus baseline within the same group. SF-8, Short Form-8 questionnaire; WPAI-GH, Work Productivity and Activity Impairment Questionnaire-General Health.

Form Scale and plasma concentrations of pro-inflammatory cytokines. Possible reasons for the differences in these evaluations were that the changes in symptoms in these healthy individuals may be expected to be smaller compared with those in patients with IBS and that changes before and after intervention in the placebo group were as large as those in the probiotic group, which may have obscured any differences. Further investigations using more participants, different intervention strategies, and different dosing regimens (e.g., frequency of intake per day and/or daily amount of intake) may provide more insights into the most effective, safest, and most sustainable methods for supporting IBS-like people. In addition, we evaluated our data by standard statistical methods used in non-large sized clinical trials similar to ours or by common statistical methods for studies targeting healthy people. However, we are concerned that the abilities of our probiotics might have been overestimated because these methods do not consider the factors of multiplicity. Therefore, we understand that it is desirable to evaluate data using other statistical methods with consideration of multiplicity in future studies.

Our findings demonstrated that our probiotic blend alleviated diarrhea-related symptoms, as evaluated by Izumo scale scores, and improved SF-8 scores, corresponding to mental health. The strains included in the product (*P. acidilactici* KABP021, *L. plantarum* KABP022, and *L. plantarum* KABP023) have been found to produce metabolites, such as polyphosphates, acetylcholine, or acetic acid, known to exert positive effects on the intestinal mucosa [21]. Indeed, stress has been reported to damage the intestinal mucosa, leading to increased permeability [22, 23]. Moreover, we also found a significant correlation between butyric acid-producing *Fecalibacterium* and improvement in Izumo diarrhea scores in the probiotic group, but not in the placebo group. Consistent with this, *Fecalibacterium* is known to be stimulated by acetic acid [24], and the strains in our probiotic blend have been shown to produce acetic acid, as described above. Thus, we propose that the probiotic intervention directly reduced intestinal permeability and/or supported beneficial bacteria, such as *Fecalibacterium*, in the host microbiota, ultimately leading to stabilization of mental activity, possibly via the vagal autonomic nerve. This hypothesis is partially supported by previous studies suggesting a correlation between improvement of the gut microbiome and the mental activity of patients with IBS as well as healthy individuals [25, 26]. However, future studies should aim to confirm whether this probiotic activity has direct effects on the intestinal mucosa and/or on the gut microbiome.

Probiotic treatment also reduced the smell of stools and flatulence. Unfortunately, we did not investigate these smells before starting the treatment; therefore, we could not evaluate the precise smell-reducing effects of the probiotics; however, the smell scores at 4 weeks after intervention were significantly lower in the probiotic group than in the placebo group. Moreover, according to WPAI-GH scores following ingestion of the probiotic blend, the treatment alleviated personal problems, such as abdominal symptoms, including diarrhea, and mental health issues and reduced anxiety regarding embarrassment related to their condition, thereby improving social activity and productivity.

Although Izumo diarrhea scale scores were improved at 4 weeks after intervention in the placebo group, the magnitude of improvement did not correlate with increases in the amount of butyric acid-producing bacteria, and metabolome analysis demonstrated a significant reduction in the amount of butyric acid in stools at 4 weeks after intervention ($1.1 \times 10^{-3} \pm 2.0 \times 10^{-3}$ at baseline, $3.1 \times 10^{-4} \pm 3.1 \times 10^{-4}$ at 4 weeks; $P = 0.045$). However, as described above, the increase in *Faecalibacterium* in the probiotic group correlated with the degree of improvement, suggesting that an increase in *Faecalibacterium* may have alleviated stress-induced diarrhea. In addition, our metabolome analysis demonstrated that the amount of butyric acid did not increase at 4 weeks after the ingestion of probiotics ($5.8 \times 10^{-4} \pm 1.2 \times 10^{-3}$ at baseline, $6.7 \times 10^{-4} \pm 1.2 \times 10^{-3}$ at 4 weeks; $P = 0.532$) but did not decrease as was observed in the placebo group; therefore, we speculate that butyric acid produced in the gut may have been consumed, leading to reduced inflammation of the intestinal mucosa.

We hypothesized that improvements in multiple outcomes should occur within most participants if the probiotics could truly improve the intestinal environment, mental health, and work efficiency. In other words, if positive outcomes did not overlap within a relevant fraction of participants, the observed positive outcomes could be considered accidental and/or due to the placebo effect. First, we scrutinized improvements before and after intervention for each individual, focusing on evaluations showing significant differences between groups after the 4-

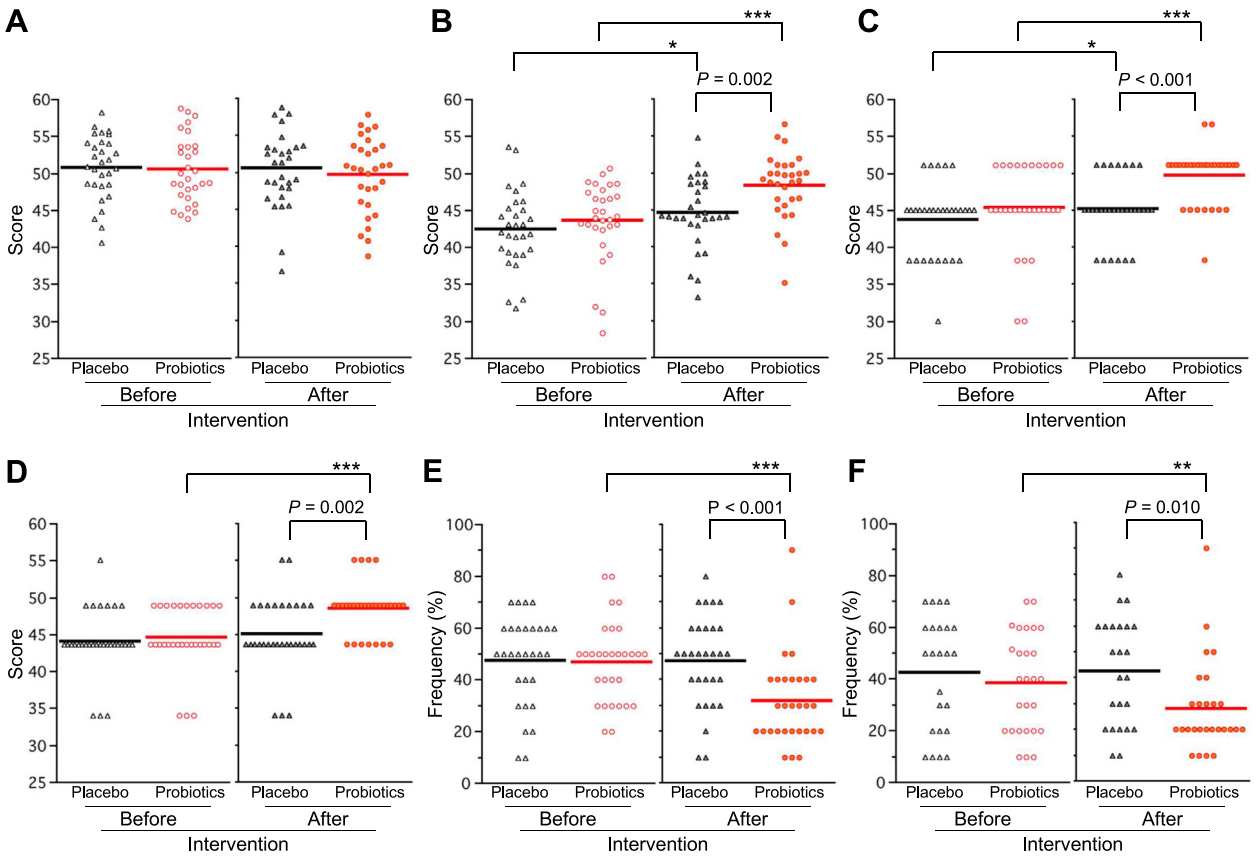

Figure 3. Effect of probiotics on the QOL. Each symbol and line represent individual scores and the mean of the group (A–E: N = 30; F: N = 24 and 27 in the placebo and probiotics groups, respectively). There were no significant differences in the scores of any category between the placebo and probiotic groups at baseline. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. SF-8: Short Form-8 questionnaire survey; WPAI-GH: Work Productivity and Activity Impairment Questionnaire-General Health. A: SF-8, Physical component score (PCS). B: SF-8, Mental component score (MCS). C: SF-8, Mental health (MH) score. D: SF-8, Role emotional (RE) score. 50 of score in A-D represents the mean level for Japanese subjects. E: WPAI-GH, Daily activity impairment. F: WPAI-GH, Overall work impairment.

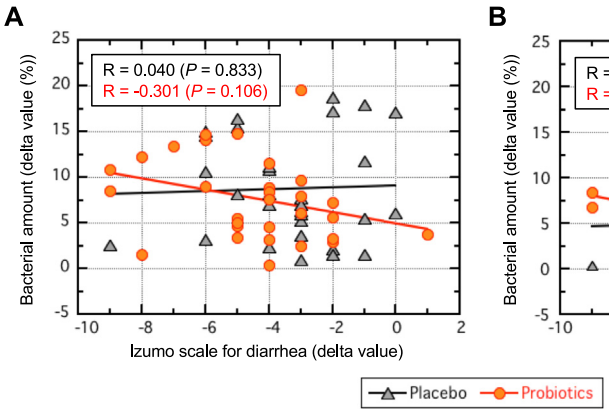

Figure 4. Correlation between treatment-induced changes in the Izumo diarrhea score and the abundances of individual microbiota members. Each symbol represents changes in the abundances of individual gut microbiota and Izumo diarrhea scores (sum of Izumo scale Q13–Q15), and each line shows the regression curve (linear). Black and red colors represent the placebo and probiotic groups, respectively. Delta value = (week 4 value) – (baseline). Pearson's R correlation and corresponding *P* values are shown within each figure. A: Butyric acid-producing bacteria. B: *Faecalibacterium*.

week investigation. Regarding the evaluation for Izumo scale Q15, the numbers of individuals who showed improvements of 2 points or more after 4 weeks of intervention were 24 out of 30 (80%) in the probiotic group and 13 out of 30 (43.3%) in the placebo group. In the overall evaluation of diarrhea (sum of Q13–Q15), the numbers of individuals who showed an improvement of 5 points or more after intervention were 13 out of 30 (43.3%) in the probiotic group and 8 out of 30 (26.7%) in the placebo group. Furthermore, when expanding the results to improvement of 4 points or more for the overall evaluation of diarrhea, 20 out of 30 (66.7%) and 13 out of 30 (43.3%) individuals met this criterion in the probiotic and placebo groups, respectively. These results suggested that more individuals showed improvement in the primary endpoint in the probiotics group than in the placebo group.

Next, we investigated participants who improved in multiple evaluations. The numbers of participants who improved in both the Q15 score and the Izumo diarrhea score (Figure 2C and 2D) were 20 out of 30

(66.7%) in the probiotic group and 11 out of 30 (36.7%) in the placebo group. Moreover, when including the endpoint of the SF-8 score, for which significant differences were confirmed (Figure 3B, 3C and 3D), we identified 20 out of 30 (66.7%) and 8 out of 30 (26.7%) in the probiotic and the placebo groups, respectively. Further consideration of the beneficial effects on WPAI-GH (Figure 3E and 3F), showed that 18 out of 30 (60.0%) and only 2 out of 30 (6.7%) participants in the probiotic and placebo groups, respectively, exhibited positive improvements in all 7 outcomes from the above 3 questionnaires. Even when considering the overlap with the improvement in the Bristol Stool Form Scale, for which significant differences between groups were not clear, 11 out of 30 (36.7%) and 2 out of 30 (6.7%) participants in the probiotic and placebo groups showed significant improvements. These results suggested that many individuals in the probiotic group reported improvements in multiple endpoints, and vice versa in the placebo group. Based on these observations of overlapping on multiple endpoints, we concluded that our probiotic blend may alleviate IBS-like symptoms in healthy individuals under stressful situations, and we believe that these probiotics could support maintenance of the microbiome balance in the gut as well as mental health and behaviors. The high significance of the observed effects on specific questions in the Izumo, SF-8, and WPAI-GH surveys ($P \leq 0.002$) further supported our conclusion that the observed positive effects were not simply due to chance.

In summary, although additional studies are required, the current randomized, placebo-controlled study clearly demonstrated that the strategy of using our probiotic blend could support healthy people who suffer from stress-induced abdominal symptoms, including diarrhea, and improve their QOL, as is required to cope with the increasing stress encountered in today's society.

Declarations

Author contribution statement

Takumi Sato, Jinko Sawashita: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Shinichi Honda, Yuji Tominaga, Yo Miyakoshi: Analyzed and interpreted the data; Wrote the paper.

Takahiro Ueda: Conceived and designed the experiments.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

The clinical trial described in this paper was registered at The Ethics Committees of Nihonbashi Egawa Clinic, Tokyo, Japan under the registration number RD09001TS04.

The clinical trial described in this paper was registered at the UMIN-CTR under the registration number UMIN000041470.

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References

[1] A.D. Sperber, S.I. Bangdiwara, D.A. Drossman, U.C. Ghoshal, M. Simren, J. Tack, et al., Worldwide prevalence and burden of functional gastrointestinal disorders, results of Rome Foundation Global Study, Gastroenterol. 160 (2021) 99–114.

[2] H. Miwa, Prevalence of irritable bowel syndrome in Japan: internet survey using Rome III criteria, Patient Prefer. Adherence 2 (2008) 143–147.

[3] L.R. Krogsgaard, A.L. Engsbro, P. Bytzer, The epidemiology of irritable bowel syndrome in Denmark. A population-based survey in adults ≤50 years of age, Scand. J. Gastroenterol. 48 (2013) 523–529.

[4] L. Böhn, S. Störsrud, T. Liljebo, L. Collin, P. Lindfors, H. Törnblom, M. Simrén, Diet low in FODMAPs reduces symptoms of irritable bowel syndrome as well as traditional dietary advice: a randomized controlled trial, Gastroenterol. 149 (2015) 1399–1407.

[5] M. Pimentel, A. Lembo, W.D. Chey, S. Zakko, Y. Ringel, J. Yu, et al., Rifaximin therapy for patients with irritable syndrome without constipation, N. Engl. J. Med. 364 (2011) 22–32.

[6] Joint FAO/WHO Working Group, Guidelines for the Evaluation of Probiotics in Food. https://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf, 2002. (Accessed 22 September 2021). Accessed.

[7] H.F. Dale, S.H. Rasmussen, Ö.Ö. Asiller, G.A. Lied, Probiotics in irritable bowel syndrome: an up-to-date systematic review, Nutrients 11 (2019) 2048.

[8] D. Eskesen, L. Jespersen, B. Michelsen, P.J. Whorwell, S. Müller-Lissner, C.M. Morberg, Effect of the probiotic strain *Bifidobacterium animalis* subsp. *lactis*, BB-12®, on defecation frequency in healthy subjects with low defecation frequency and abdominal discomfort: a randomized, double-blind, placebo-controlled, parallel-group trial, Br. J. Nutr. 114 (2015) 1638–1646.

[9] N. Fuke, K. Aizawa, H. Suganuma, T. Takagi, Y. Naito, Effect of combined consumption of *Lactobacillus brevis* KB290 and β-carotene on minor diarrhoea-predominant irritable bowel syndrome-like symptoms in healthy subjects: a randomized, double-blind, placebo-controlled, parallel-group trial, Int. J. Food Sci. Nutr. 68 (2017) 973–986.

[10] Y. Sunada, A. Igi, F. Nakamura, T. Kaneko, S. Tanabe, Efficacy and safety of intake of *Bifidobacterium breve* N708-containing food on diarrhea due to stress – randomized double-blind, placebo-controlled, parallel-group comparison study, Jpn. Pharmacol. Ther. 47 (2019) 1883–1900.

[11] D.A. Barraza-Ortiz, N. Pérez-López, V.M. Medina-López, J.I. Minero-Alfaro, F. Zamarripa-Dorsey, N.D.C. Fernández-Martínez, et al., Combination of a probiotic and an antispasmodic increases quality of life and reduces symptoms in patients with irritable bowel syndrome: a pilot study, Dig. Dis. 39 (3) (2021) 294–300.

[12] S.A. Julious, Sample size of 12 per group rule of thumb for a pilot study, Pharmaceut. Stat. 4 (2005) 287–291.

[13] M.A. Hertzog, Considerations in determining sample size for pilot studies, Res. Nurs. Health 31 (2008) 180–191.

[14] V. Lorenzo-Zúñiga, E. Llop, C. Suárez, B. Alvarez, L. Abreu, J. Espadaler, J. Serra, I.31, a new combination of probiotics, improves irritable bowel syndrome-related quality of life, World J. Gastroenterol. 20 (2014) 8709–8716.

[15] E. Kakuta, N. Yamashita, T. Katsube, Y. Kushiyama, H. Suetsugu, K. Furuta, Y. Kinoshita, Abdominal symptom-related QOL in individuals visiting an outpatient clinic and those attending an annual health check, Intern. Med. 50 (2011) 1517–1522.

[16] MRC Epidemiology Unit, University of Cambridge, SF-8 questionnaire, https://epi-meta.mrc-epid.cam.ac.uk/studies/vitamin_d/downloads/SF_8_Questionnaire.pdf. (Accessed 22 September 2021).

[17] European Alliance of Associations for Rheumatology, Work Productivity and Activity Impairment Questionnaire: General Health V2.0 (WPAI:GH). http://oml.eular.org/sysModules/obxOML/docs/id_98/WPAI-GH_English_US_V2.pdf. (Accessed 22 September 2021). Accessed.

[18] S. Watanabe, S. Kameoka, N. Shinozaki, R. Kubo, A. Nishida, M. Kuriyama, A. Takeda, A cross-sectional analysis from the Mykinso Cohort Study: establishing reference ranges for Japanese gut microbial indices, Biosci. Microbiota Food Health 40 (2) (2021) 123–134.

[19] M. Matsumoto, T. Ooga, R. Kibe, Y. Aiba, Y. Koga, Colonic absorption of low-molecula-weight metabolites influenced by the intestinal microbiome: a pilot study, PLoS One 12 (2017) 1–15.

[20] J. Sugama, Y. Moritoh, H. Yashiro, K. Tsuchimori, M. Watanabe, Enteropeptidase inhibition improves obesity by modulating gut microbiota composition and enterobacterial metabolites in diet-induced obese mice, Pharmacol. Res. 163 (2021), 105337.

[21] M. Perez, E. Astó, P. Huedo, C. Alcántara, D. Buj, J. Espadaler, Derived postbiotics of a multi-strain probiotic formula clinically validated for the treatment of irritable bowel syndrome, Faseb. J. 34 (2020) S1–1.

[22] J.P. Karl, L.M. Margolis, E.M. Madslien, N.E. Murphy, J.W. Castellani, Y. Gundersen, et al., Changes in intestinal microbiota composition and metabolism coincide

with increased intestinal permeability in young adults under prolonged physiological stress, Am. J. Physiol. Gastrointest. Liver Physiol. 312 (6) (2017) G559–G571.

[23] T. Vanuytsel, S. van Wanrooy, H. Vanheel, C. Vanormelingen, S. Verschueren, E. Houben, et al., Psychological stress and corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism, Gut 63 (8) (2014) 1293–1299.

[24] A. Heinken, M.T. Khan, G. Paglia, D.A. Rodionov, H.J.M. Harmsen, I. Thiele, Functional metabolic map of *Faecalibacterium prausnitzii*, a beneficial human gut microbe, J. Bacteriol. 196 (2014) 3289–3302.

[25] M.I. Pinto-Sanchez, G.B. Hall, K. Ghajar, A. Nardeli, C. Bolino, J.T. Lau, et al., Probiotic *bifidobacterium longum* NCC3001 reduces depression scores and alters brain activity: a pilot study in patients with irritable bowel syndrome, Gastroenterol 153 (2017) 448–459.

[26] M. Messaoudi, R. Lalonde, N. Violle, H. Javelot, D. Desor, A. Nejdi, et al., Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects, Br. J. Nutr. 105 (2011) 755–64.