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Efficacy and safety of *Bacillus coagulans* LBSC in irritable bowel syndrome

A prospective, interventional, randomized, double-blind, placebo-controlled clinical study [CONSORT Compliant]

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Abstract

Goals: To evaluate safety and efficacy of *Bacillus coagulans* LBSC [DSM17654] in irritable bowel syndrome (IBS) through a prospective, interventional, randomized, double-blind, and placebo-controlled, CONSORT compliant clinical trial.

Background: Bacteriotherapy shows promising impact on alleviating clinical conditions of IBS and associated functional gastrointestinal disorders. *B coagulans* LBSC is a genetically and phenotypically safe probiotic strain used in this study to study its impact on ameliorating IBS symptoms and improving quality of life.

Methods: In this interventional, randomized, double-blind, placebo-controlled clinical study, total 40 subjects (18–65 years) were screened through Rome IV criteria and randomized into 2 groups, that is, interventional and placebo arm (n = 20/arm). Similar dosages were received by both the arm, that is, placebo (vehicle) and interventional arm (*B coagulans* LBSC, 6 billion/d) for a period of 80 days. Study completed with *per protocol* subjects (n = 38) and results were considered to evaluate the primary and secondary endpoints.

Results: Assessment through Digestive Symptom Frequency Questionnaire 5 point Likert scale showed significant improvement in interventional arm compared to placebo on symptoms such as bloating/cramping, abdominal pain, diarrhea, constipation, stomach rumbling, nausea, vomiting, headache, and anxiety. Maximum of "no symptoms" cases and mild to moderate gastrointestinal symptoms along with improved stool consistency were from interventional arm tested following IBS severity scoring system and Bristol stool form scale. Upper gastrointestinal endoscopy revealed no clinical difference of gastrointestinal mucosa between both the arms. *B coagulans* LBSC was well tolerated with no serious adverse events.

Conclusions: *B coagulans* LBSC was safe for human consumption and efficacious in alleviating overall pathophysiological symptoms of IBS and thereby improving inclusive quality of life evaluated.

Abbreviations: AE = adverse events, BSFC = Bristol stool form scale, DSM = Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, EOT = end of treatment, FGID = functional gastrointestinal disorder, FODMAPs = fermentable oligosaccharides, disaccharides, monosaccharides and polyols, IBD = inflammatory bowel disease, IBS = irritable bowel syndrome, IBS-SSS = IBS-Severity Scoring System, IP = investigational product, MD = mean difference, PCoA = principal component analysis, PP = per-protocol, QoL = quality of life, RCT = randomized control trials, SAE = serious adverse events.

Keywords: *B coagulans*, Bristol stool form scale (BSFC), constipation, diarrhea, Digestive Symptom Frequency Questionnaire, irritable bowel syndrome

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Trial registration: This trial was conducted in compliance with the applicable ethical standards Harmonized Tripartite Guidelines for Good Clinical Practice (GCP), the relevant sections of Good Laboratory Practice (GLP), local laws and regulations and the provisions of the Declaration of Helsinki. The EC approvals were obtained from sites. The trial was registered with Clinical Trials Registry - India (CTRI) [Reference No.: CTRI/2018/02/011654 and Dated: 01/02/2018] before patients enrolment begun.

The authors have no conflicts of interest to disclose.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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1. Introduction

Irritable bowel syndrome (IBS) is a chronic gastrointestinal disorder characterized by severe abdominal pain and/or discomfort associated with changes in bowel frequency and/or stool consistency. It is the most commonly diagnosed functional gastrointestinal disorder (FGID) accompanied with recurrent abdominal pain for at least 1 day per week in previous 3 months and associated with pain/discomfort during defecation (Rome IV criteria).[1–3] IBS classes such as diarrhea-predominant (IBS-D), constipation-predominant (IBS-C), mixed bowel habits (IBS-M), and un-subtyped (IBS-U) have been defined based on the stool pattern.[4]

Global prevalence of IBS is estimated to 15% to 45% of general population of all age group.[5] Meta-analyses revealed that IBS is more common among women than men (2:1) and mostly starts in early adulthood.[6] Many scientific groups recognized that compromised gut microbiome, small-bowel bacterial overgrowth (SIBO), altered gut motility, mucosal inflammation, visceral hypersensitivity, and impaired neuro-endocrinal communications could be the underlying etiology for IBS pathogenesis.[4,7]

Current treatments of IBS majorly include dietary [low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs)] and antibiotic interventions.[8–10] A low FODMAP diet is however controversial due to inherent limitations like dietary restrictions, required close monitoring by an expert dietitian, potential nutritional deficiencies, reduction in gut microbiota diversity, lack of predictors of response, dearth of alternative dietary, pharmacological and psychological interventions for IBS.[11] Compared to FODMAPs intervention, antibiotic treatment is more common practice for treating IBS; however, few clinical reports highlighted the significant association of antibiotic use with increased risks of IBS.[12,13] Use of antibiotics promotes *h*orizontal *g*ene *t*ransfer (*hgt*) and confers antibiotic resistance to otherwise sensitive microorganisms.[14] Broad-spectrum antibiotics such as beta-lactam, cephalosporin, and macrolide are known to either eliminate or alter gastrointestinal microbiota; narrowing in microbial diversity which has a magnitude of adverse impacts on IBS.[15,16]

Numerous studies have described the mechanism of IBS onset as a shift from "normal and healthy" gut to "*dysbiosed and unhealthy*" gut where gut microbial community plays a pivotal role.[17–19] Healthy microbiome-modulated intestinal homeostasis is therefore a fundamental therapeutic paradigm where probiotics could offer promising healthcare solution for IBS.[20] Probiotics are live bio-therapeutics[21] which offer promising route for treating various gastrointestinal ailments like diarrhea, indigestion, nutrient malabsorption, SIBO, inflammatory bowel disease (IBD), ulcerative colitis, Crohn's disease, etc, without a risk of spreading antibiotic resistance in microorganisms.

The systematic interventions of lactic acid-producing Bacillus probiotics are well documented in different community-based meta-analysis and ensued significant reduction of IBS symptoms, especially by a monoculture or mixed culture probiotic formulations. The lactic acid-producing Bacillus probiotics, like, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Bifidobacterium longum*, *Bifidobacterium lactis*, *Streptococcus thermophillus*, *Bacillus coagulans* are mainly included in the formulations.[22] Among all, *Bacillus coagulans* is determined as a commonly used heterolactate live biotherapeutic for various gastrointestinal disorders including IBS.[23]

Strains of *B coagulans* are spore-former and widely studied probiotic for production of antimicrobials like coagulin and lactosporin, immune-modulation and modulation of gut microbiome.[24] The naturally encapsulated coating around the cell protects it from various drought conditions like high temperature, desiccation, osmolarity, etc. This highly resilient allochthonous probiotic can survive and proliferate in gastric acid, pepsin, pancreatin, ions, digestive enzymes, bile, and mucin of gastrointestine.[25] The efficacy of probiotic *B coagulans* varies among strains; differs as per dosages used in finished formulations and severity of clinical conditions to exhibit the intended health benefits.[26]

The therapeutic activity of *B coagulans* is well established for various ailments like diarrhea, antibiotic-associated diarrhea, hypercholesterolemia, immune modulation, IBS, IBD, *Clostridium difficile*-induced colitis, *dysbiosis*, *Helicobacter pylori* infection, gingivitis, necrotizing enterocolitis, and other FGID's.[23,24,26,27] Some strains of *B coagulans* have shown clinical efficacy by significantly improving the bowel pattern, diarrheal duration and frequency, abdominal pain, and bloating associated with IBS through randomized control trials (RCTs) studies.[28–34] However, few of these clinical studies have also reported about variable effect of *B coagulans* strains for IBS symptoms instead of complete relive of symptoms subset. Very few reports are available on quality of life (QoL) findings associated with IBS, which directed current study to evaluate the efficacy and safety of *B coagulans* LBSC [DSM17654] in relieving IBS and associated symptoms. This probiotic strain has been previously reported for its genomic safety[35] and clinical efficacy.[24,26] The frequency and severity of gastrointestinal symptoms, stool consistency, and QoL in the intervened (test) and control (placebo) group were assessed as outcome variables.

2. Patients and methods

2.1. Formulation and method of analysis

The investigational product (IP) was the active ingredient, *B coagulans* LBSC mixed with the excipient. The strength of the active ingredient was 2 billion spores per gram per sachet, which was supplied by Advanced Enzyme Technologies Ltd., Thane, India. The placebo contained only excipient, maltodextrin (1.00 g). Both, the investigational and placebo products complied with the specifications (Supplement: Method of Analysis, http://links.lww.com/MD/F368).[26] The packaging and labeling for both the products were same except the coded batch numbers used for differentiation.

2.2. Ethics and informed consent

Trial was registered in the Clinical Trial Registry India (CTRI/2018/02/011654) and conducted with the written ethical approval from Sri Venkateshwara Hospital, Bangalore and People Tree Hospital Clinical Research Center, Bangalore. Approved study protocol was designed in accordance with Declaration of Helsinki (WMA 2000),[36] ICH-harmonized tripartite guideline for good clinical practice (ICH 1996)[37] and Indian Council of Medical Research Guidelines for Biomedical Research on Human subjects (ICMR 2006)[38]; same was followed with no further amendments during trial. Written and oral information was provided to all participating subjects in understandable language. Every subject has given written

informed consent to investigator after understating the objective of this trial, including possible risks and benefits.

2.3. Study design and selection of study subjects

The prospective interventional trial was randomized, double-blinded, parallel, placebo controlled and had a total of 5 visits to the clinical site by the study subjects. Subject selection was based on the defined inclusion and exclusion criteria as follows.

Inclusion Criteria: Subjects were included based on following criteria –

(1) Male and females (18–65 years) with diagnosis of IBS as per Rome IV criteria associated with following symptoms for more than last 3 months; like abdominal discomfort such as mild pain, cramping, bloating, altered bowel habit indicated by frequent diarrhea or constipation and functional dyspepsia;
(2) written informed consent by study participants.

Exclusion Criteria: Subjects those were on antibiotics or laxatives within the preceding 6 weeks, Symptoms of IBD, acute GI tract infection, fever, abdominal mass, signs of bowel obstruction, history of colon cancer or diverticulitis, infection from human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV), celiac disease, scleroderma and gastroparesis and hypothyroidism were excluded from the study (Supplement: Details of Exclusion and Withdrawal Criteria, http://links.lww.com/MD/F368). In addition, subjects having adverse effects or serious adverse effects, pregnancies, disease emergencies, concomitant therapy, and study protocol violation are considered for withdrawal of subjects from the trial.

2.4. Samples, randomization, treatments, and procedures

A total of 40 subjects consisting 20 randomized subjects in each of the tests and the placebo group were enrolled for the current study. The test group – G (n = 20) was on *B coagulans* LBSC with 2 billion colony-forming unit activity (Supplement: Method of Analysis, http://links.lww.com/MD/F368) powder (carrier maltodextrin) thrice a day, whereas, control (placebo) group – H (n = 20) was only on maltodextrin with similar dosing schedule. Treatment duration was up to 80 days and total study duration did not exceed 90 days. The subject randomization (followed by SAS random number generation method), treatment allocation, and procedures are represented in Figure 1. All supportive treatments were, if required, administered to the subjects as deemed necessary by the investigator, but no antibiotics were recommended along with the study drug. No changes or amendments were made to the approved protocol after the trial commenced and no interim data analysis was done during the study period.

2.5. Endpoints: efficacy and safety variables

Primary endpoints were set to study the efficacy outcomes like assessment of change in gastrointestinal symptom's frequency, assessment using Digestive Symptom Frequency Questionnaire (DSFQ) on 5-point Likert scale (0 = never, $1 \leq$ 1 episode/wk; $2 \leq$ 3 episodes/wk; $3 \geq$ 3 episodes/wk; 4 = daily episodes), change in gastrointestinal symptom's severity using IBS severity scoring system (IBS-SSS) and change in stool consistency using Bristol stool form scale. Secondary endpoints were for safety evaluations of *B coagulans* LBSC; measured by evaluating physical examination and vitals, systematic biomarkers, adverse events (AEs) or serious adverse events (SAEs), and Quality of life (QoL) questionnaire. The QoL was assessed based on yes or no responses for pain scale on abdominal pain, diarrhea, constipation, bloating and flatulence, vomiting and nausea, perception of mental well-being, and influence on daily life. Results were processed to a binary numerical response and analyzed through principal component analysis (PCoA).[26] Hematological and hepatic biomarkers were analyzed following the standard medical test protocols. The AE is defined as any medically untoward event detected in clinical study subject after use of the study agents, whether or not caused by the use of the agents. Whereas, SAE is defined as any untoward medical incidence which is life-threatening and results into death or hospitalization, disability or incapacity, and congenital anomaly.

2.6. Sample power and statistical analysis

Data was analyzed with 5% significance level (confidence interval 95%) and maintaining a minimum power of 80% for study using SAS software, version 9.1. The differences within the groups were assessed using *t*-test, whereas, ANOVA and χ^2 test were used for differences between groups. Efficacy analysis was performed for the per-protocol (PP) population. Separate analyses were performed for primary and secondary endpoints. The entire statistical analysis was performed as per the statistical analysis plan.

3. Results

Enrollment of first patient and treatment of the last patient were completed in November 2018 and July 2019, respectively. Trial was initiated with the participation of total 40 "intention to treat" which was screened from a total of 43 (n = 43) patients. With total 2 patients lost to follow up (dropped out); trial was completed with total 38 PP population. Of 2 treatment arms, that is, Test-G and Placebo-H, each arm was allocated with 20 subjects. Total 12 females (F) and 28 males (M) took part in the trial (Test G, 7F:13 M and Placebo H, 5F:15 M). No significant difference was observed in the demographic parameters of both treatment groups (Table 1). Principal investigator and clinical trial team assessed study regulations at each visit along with all the safety and efficacy assays as per the schedule of events (Table 2). Efficacy analyses were performed on PP population, that is, full analysis set of 38 patients. The clinical trial was concluded after the follow-up visit (at Visit 05) of the last enrolled patient and completion of target sample size according to the study procedures.

4. Primary endpoint: efficacy evaluation

4.1. Change in gastrointestinal symptom's frequency assessment

DSFQ on 5-point Likert scale has been used to assess the gastrointestinal symptoms frequency of subjects and to compare the difference between Test-G and Placebo-H arm.[39] Frequency of various gastrointestinal symptoms like bloating/cramping, abdominal pain, diarrhea or constipation, stomach rumbling, nausea, vomiting, headache, and anxiety were assessed for both Test-G and Placebo-H arm at baseline (visit 2) and end of

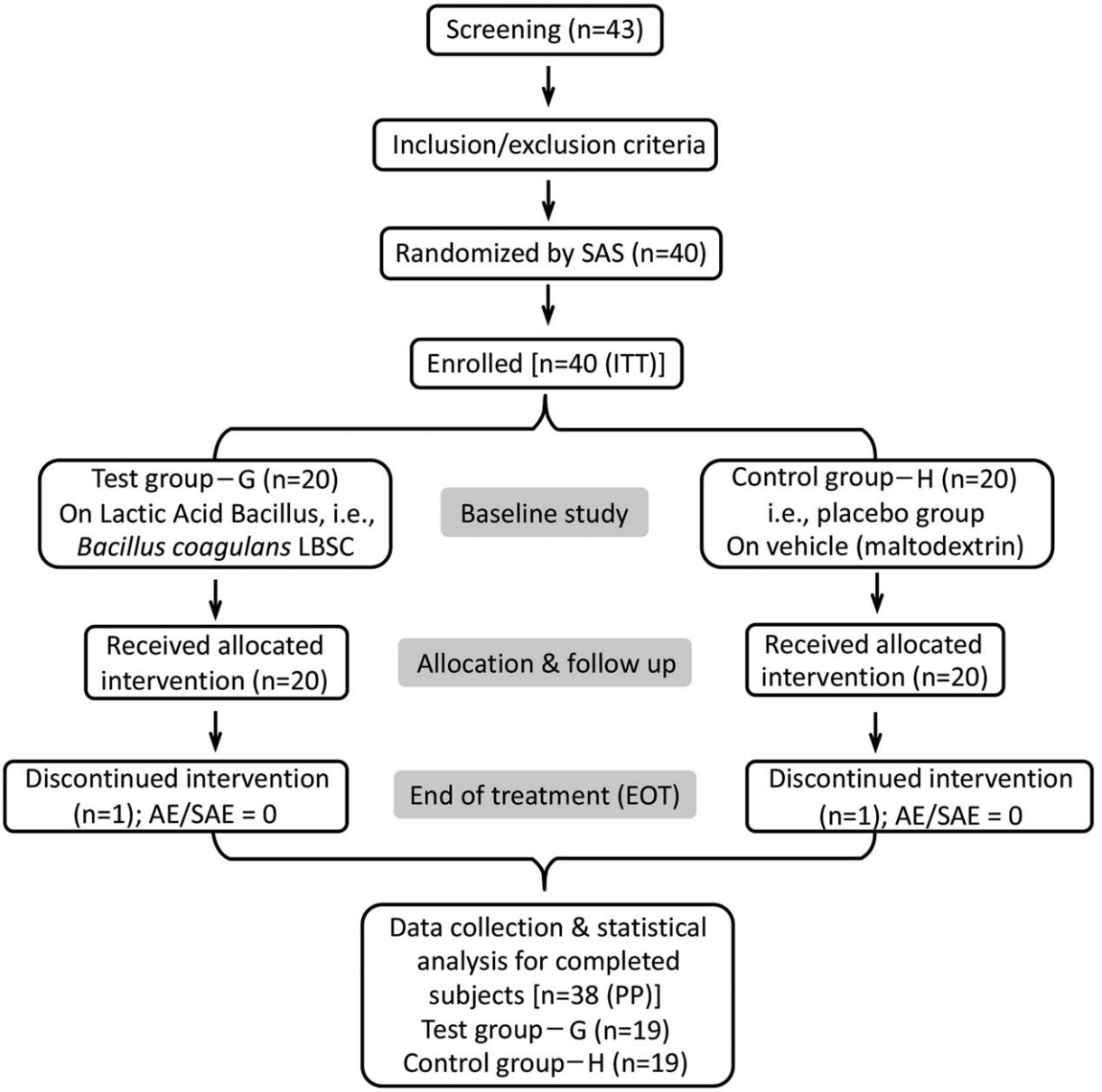

Figure 1. The schematic diagram of current clinical study. The study enrolled "intension to treat" 40 subjects through the inclusion and exclusion criteria screening followed by the SAS random number generation method. Upon discontinuation of one subject from each group, total 38 subjects were considered *per protocol* for evaluation. No subject reported for adverse events or serious adverse events. The study compliance was checked at every follow-up visit alongside study evaluation and assessment by a team of physician-investigator and officers.

treatment (EOT) (visit 4) (Table 2). Boating and cramping symptoms were improved in Test-G [mean difference (MD) −1.21, 95% CI (−1.74, −0.68)] compared to Placebo-H [MD −0.26, 95% CI (−0.79, 0.27)] after *B coagulans* LBSC treatment (IP) and changes [MD −0.95, 95% CI (−1.69, −0.19)] were found statistically significant ($P = .0148$). Relive from abdominal pain was significant ($P < .0001$) in Test-G (*B coagulans* LBSC) [MD −1.74, 95% CI (−2.44, −1.03)] compared to Placebo-H; where no symptoms of improvement were recorded (Table 3). Statistically significant improvement in diarrhea and constipation was noted after *B coagulans* LBSC treatment (Test-G) [MD −1.47, 95% CI (−1.89, −1.05)] compared to Placebo-H [MD −0.53, 95% CI (−0.94, −0.10)] ($P = .0027$).

Results showed that subjects from Test-G arm (*B coagulans* LBSC) were relived from stomach rumbling [MD −1.63, 95% CI (−2.26, −1.01)] than Placebo-H [MD −0.58, 95% CI (−1.20, 0.04)]. Improvement in stomach rumbling [MD −1.05, 95% CI (−1.93, −0.16)] was found statistically significant ($P = .021$) between both groups analyzed through baseline to EOT. Simultaneously, nausea and vomiting conditions were improved after treatment with *B coagulans LBSC* in Test-G, whereas, these symptoms were worsened in Placebo-H. Nausea was reduced in Test-G [MD −0.95, 95% CI (−1.39, −0.49)] than Placebo-H [MD +0.05, 95% CI (−0.39, 0.50)] and the change between group [MD −1.00, 95% CI (−1.63, −0.36)] was significant ($P = .0031$) (Table 3). Vomiting was reduced in Test-G from

Table 1

The demographic details of subjects of two treatment arms participated in the current clinical trial and their descriptive statistics. Values expressed as mean ± SD.

Parameters	Treatment groups		*P* value [95% CI] ($P < .05$)
	Test-G	Placebo-H	
Subjects number (n)	20	20	
Gender [n (%)]			
Male	13 (65.00%)	15 (75.00%)	
Female	7 (35.00%)	5 (25.00%)	
Age (years) [min/max]	36.20 ± 9.81 [21/56]	34.80 ± 11.06 [18/55]	0.674 [−8.092–5.292]
Height (cm) [min/max]	162.38 ± 19.82 [88.97/185.40]	164.84 ± 9.99 [141.00/183.00]	0.623 [−7.587–12.507]
Weight (kg) [min/max]	65.93 ± 10.87 [47.00/90.08]	64.65 ± 9.17 [48.90/81.00]	0.689 [−7.717–5.157]
Body Mass Index (kg m^{-2}) [min/max]	23.37 ± 3.23 [16.05/30.04]	24.03 ± 2.99 [19.30/29.70]	0.506 [−1.332–2.652]
Diet			
Mixed	20 (100.00%)	20 (100.00%)	
Vegetarian	0 (–)	0 (–)	
Smoker/alcohol drinkers	0 (–)	0 (–)	

baseline (0.79) to EOT (0.21) [MD −0.58, 95% CI (−1.01, −0.16)]; while, increased in Placebo-H [MD +0.05, 95% CI (−0.37, 0.47)] with overall significant intragroup difference [MD −0.63, 95% CI (−1.23, −0.03)] ($P = .0386$). Headache was reduced in Test-G, that is, on *B coagulans* LBSC treatment [MD 0.53, 95% CI (-0.98, -0.07)], whereas, increased in Placebo-H [MD +0.74, 95% CI (0.28, 1.19)] with statistically significant MD between both the groups [MD −1.26, 95% CI (−1.90, −0.61)] ($P = .0003$). Additionally, anxiety was reduced after *B coagulans* LBSC treatment (Test-G) compared to Placebo-H. Differences between 2 group means were statistically significant for anxiety score [MD −1.00, 95% CI (−1.82, −0.18)] ($P = .0177$) (Table 3).

4.2. Change in gastrointestinal symptom's severity using IBS-SSS

Severity of gastrointestinal symptoms of IBS subjects was measured following the Rome Foundations IBS-SSS universal questionnaire.[40] Three severity categories were considered, viz, mild, moderate, and severe. At baseline, all subjects from Test-G arm reported mild (10) and moderate (9) level of severity, which was reduced after *B coagulans* LBSC treatment and maximum subjects reported relief from symptoms; no symptoms (12), mild (6) and moderate (1) at EOT (Fig. 2). Reduction in severity in Test-G was found statistically significant between baseline and EOT ($P < .0001$). Whereas, subjects from Placebo-H reported mild (14) and moderate (5) severity at baseline; and maximum

Table 2

Schematic schedule of the clinical trial conducted for irritable bowel syndrome (IBS) patients.

Visits	Visit 01 (Day 0)*	Visit 02 (Day 1)†	Visit 03 (Day 40)‡	Visit 04 (Day 80)§	Visit 05 (Day 90)‖	Visit 06¶
Informed consent	√					
Demography	√					
Inclusion/exclusion criteria	√					
Vital signs	√	√	√	√	√	√
Physical examination	√	√	√	√	√	
Medical/surgical history	√					
Pregnancy test	√					
Rome IV criteria	√					
Laboratory tests	√			√		
Upper GI endoscopy	√			√		
IP Dispensing		√	√			
Issue Dairy card		√	√			
Concomitant medicationschecking		√	√	√	√	√
IBS-SSS Questionnaire and (VAS)		√	√	√	√	
Bristol stool form scale (BSFC)	√				√	
DSFQ	√	√	√	√	√	
Compliance check		√	√	√		
AE/SAE assessment		√	√	√	√	√

VAS = Visual Analogue Scale.

* Visit 01 was the screening day.

† Visit 02 was the baseline day, that is, first day of treatment.

‡ Visit 03 was the fortieth day of treatment.

§ Visit 04 was the eightieth day of treatment, that is, end of treatment (EOT).

‖ Visit 05 was the follow-up visit.

¶ Visit 06 was the unscheduled visit.

Table 3

Comparative DSFQ scores evaluated in IBS subjects based on 5-point *Likert* scale at baseline and EOT from both *B coagulans* LBSC (Test-G) and vehicle (Placebo-H) arm (±SD).

Parameters	Arms	Baseline	EOT	Mean change	*P* value	95% CI
Bloating and cramping	Placebo - H	1.42 ± 0.69	1.16 ± 0.60	−0.26	.0148	−0.79, 0.27
	Test - G	2.21 ± 1.36	1.00 ± 0.82	−1.21		−1.74, −0.68
	Between groups (G-H)			−0.95		−1.69, −0.19
Abdominal pain	Placebo - H	1.84 ± 0.69	1.42 ± 0.61	−0.42	<.0001	−0.92, 0.07
	Test - G	2.84 ± 1.07	0.68 ± 0.95	−2.16		−2.65, −1.66
	Between groups (G-H)			−1.74		−2.44, −1.03
Diarrhea and constipation	Placebo - H	1.79 ± 0.86	1.26 ± 0.73	−0.53	.0027	−0.94, −0.10
	Test - G	2.68 ± 1.06	1.21 ± 0.79	−1.47		−1.89, −1.05
	Between groups (G-H)			−0.95		−1.54, −0.35
Stomach rumbling	Placebo - H	1.58 ± 0.96	1.00 ± 0.67	−0.58	.021	−1.20, 0.04
	Test - G	1.90 ± 1.33	0.26 ± 0.73	−1.63		−2.26, −1.01
	Between groups (G-H)			−1.05		−1.93, −0.16
Nausea	Placebo - H	0.74 ± 0.73	0.79 ± 0.79	0.05	.0031	−0.39, 0.50
	Test - G	1.16 ± 1.34	0.21 ± 0.71	−0.95		−1.39, −0.49
	Between groups (G-H)			−1.00		−1.63, −0.36
Vomiting	Placebo - H	0.26 ± 0.56	0.32 ± 0.48	0.05	.0386	−0.37, 0.47
	Test - G	0.79 ± 0.63	0.21 ± 0.71	−0.58		−1.01, −0.16
	Between groups (G-H)			−0.63		−1.23, −0.03
Headache	Placebo - H	1.37 ± 0.68	1.00 ± 0.67	0.74	.0003	0.28, 1.19
	Test - G	1.90 ± 1.33	0.26 ± 0.93	−0.53		−0.98, −0.07
	Between groups (G-H)			−1.26		−1.90, −0.61
Anxiety	Placebo - H	1.26 ± 0.93	1.00 ± 0.58	−0.26	.0177	−0.84, 0.31
	Test - G	1.58 ± 1.58	0.32 ± 0.95	−1.26		−1.84, −0.69
	Between groups (G-H)			−1.00		−1.82, −0.18

Symptoms considered were bloating and cramping, abdominal pain, diarrhea and constipation, stomach rumbling, nausea, vomiting, headache, and anxiety. Intergroup mean difference [(Test-G)−(Placebo-H)] for all the symptoms was analyzed through ANOVA and 95% confidence interval (CI) estimation.

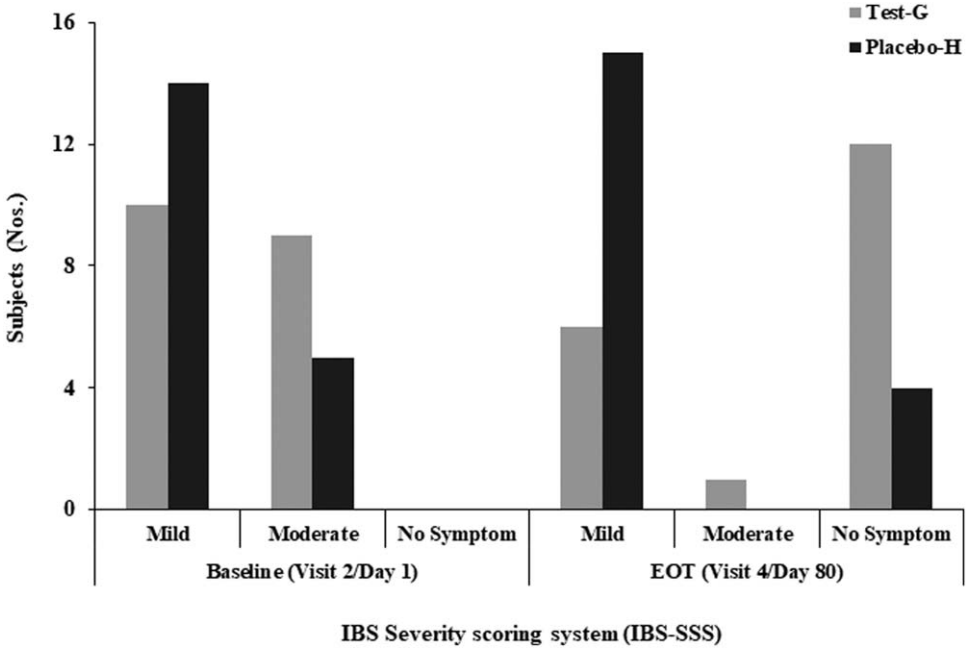

Figure 2. Change in IBS-SSS from baseline (visit 2/d 1) to end of treatment (visit 4/d 80) in Test-G and Placebo-H. The Rome Foundation IBS-SSS universal questionnaire has been considered with 3 severity categories, viz, mild, moderate, and severe as examined by the investigator. IBS-SSS = IBS-Severity Scoring System.

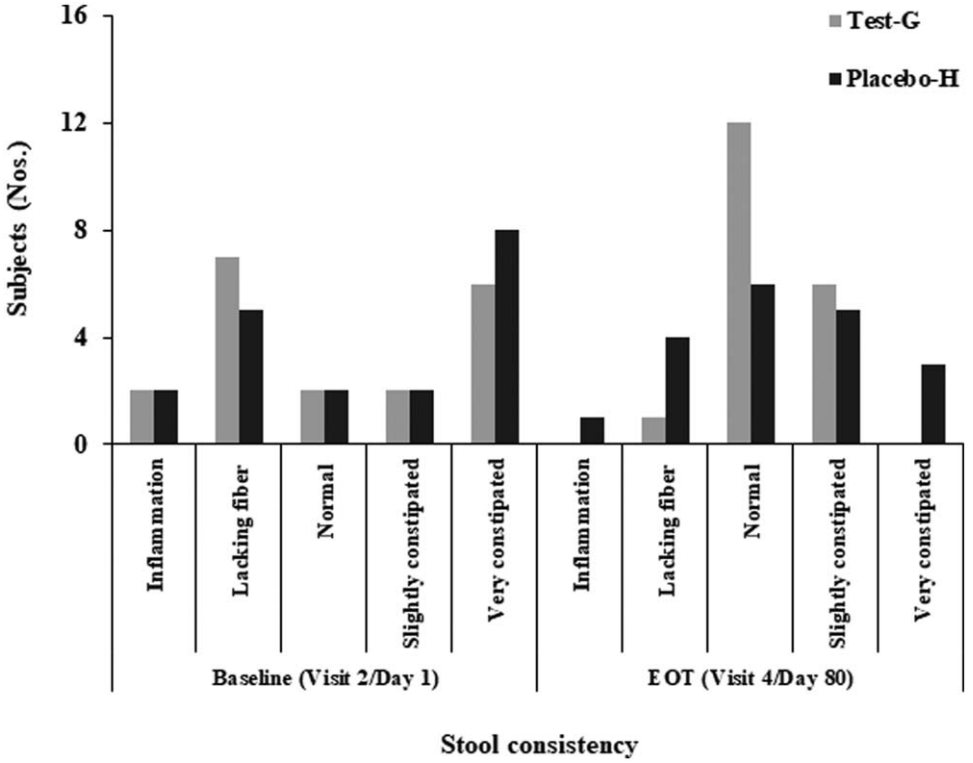

Figure 3. Change in stool consistency from baseline (visit 2/d 1) to end of treatment (visit 4/d 80) in Test-G and Placebo-H. The Bristol stool form scale is used to assess the stool consistency as reported by total number of subjects.

subjects (78.94%) reported with mild severity of IBS at EOT (Fig. 2). Maximum cases "no symptoms" [63.16%, 95% CI (0.41–0.81)] were reported from Test-G, that is, *B coagulans* LBSC treated arm than Placebo-H [21.05%, 95% CI (0.08–0.43)] arm at EOT [ARR, −0.42 (95% CI, −0.64 to −0.11); RR, 3.00 (95%CI, 1.18–7.65); OR, 6.43 (95%CI, 1.52–27.24)].

4.3. Change in stool consistency using Bristol stool form scale

Stool consistency of subjects from both Test-G and Placebo-H had following characteristics at base line as per Bristol form stool scale; inflammation, lacking fiber, normal to slight constipation, and highly constipated. However, stool consistency was significantly improved in interventional arm (Test-G) after *B coagulans* LBSC treatment compared to Placebo-H arm. In Test-G arm, at baseline and EOT, the number of subjects for inflammation was 2 and zero, for lacking fiber 7 and 1, normal stool consistency 2 and 12, slight constipation 2 and 6, for severe constipation 6 and zero, respectively (Fig. 3). None reported for severe constipation in Test-G arm at EOT. A quite similar stool consistency profile in Placebo-H arm reported as follows, 1 IBS patient reported with inflammation, 4 reported for lacking fiber, 6 reported for normal, 5 with slight constipation, and 3 have reported for severe constipation at EOT (Fig. 3). Maximum cases [63.15%, 95% CI (0.41–0.81)] of normal stool consistency were from Test-G compared to Placebo-H [31.57%, 95% CI (0.15–0.54)] at EOT [ARR, −0.32 (95% CI, −0.56 to −0.001); RR, 2.00 (95%CI, 0.95–4.22); OR, 3.71 (95%CI, 0.97–14.23)]. Improvement in stool consistency in Test-G arm was found statistically significant from baseline to EOT ($P = .0002$), whereas, it was insignificant in Placebo-H arm ($P = .1989$).

5. Secondary endpoints: safety evaluation

5.1. Assessment of adverse effect and serious adverse effects and systematic biomarkers

Vital examinations like pulse, respiratory rate, systolic blood pressure, diastolic blood pressure, and body temperature were carried out for all subjects at each visit by the principal investigator (Supplement: Table 1, http://links.lww.com/MD/F368). No significant differences ($P < .05$) were observed in vital parameters in both Test-G and Placebo-H arm [$P^{min} = .059$ ($F^{max} = 4.833$) and $P^{max} = .962$ ($F^{min} = 0.002$)] among all visits and results remained within the normal range. No AE or SAE's were reported related to IP. Biochemical and hematological parameters were studied at baseline and EOT in both Test-G and Placebo-Harm. Biochemical parameters included serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, and creatinine whereas, hematological parameters comprised parameters like total red blood cells and total leucocyte count, eosinophils, basophils, neutrophils, lymphocytes, monocytes, hematocrit, erythrocyte sedimentation rate, and platelet counts (Supplement: Table 2, http://links.lww.com/MD/F368). No statistically significant differences [$P^{min} = .0454$ (95% CI, −2.69 to −0.03) and $P^{max} = .9857$ (95% CI, 5.66–5.56)] were obtained between Test-G and Placebo-H arm both at

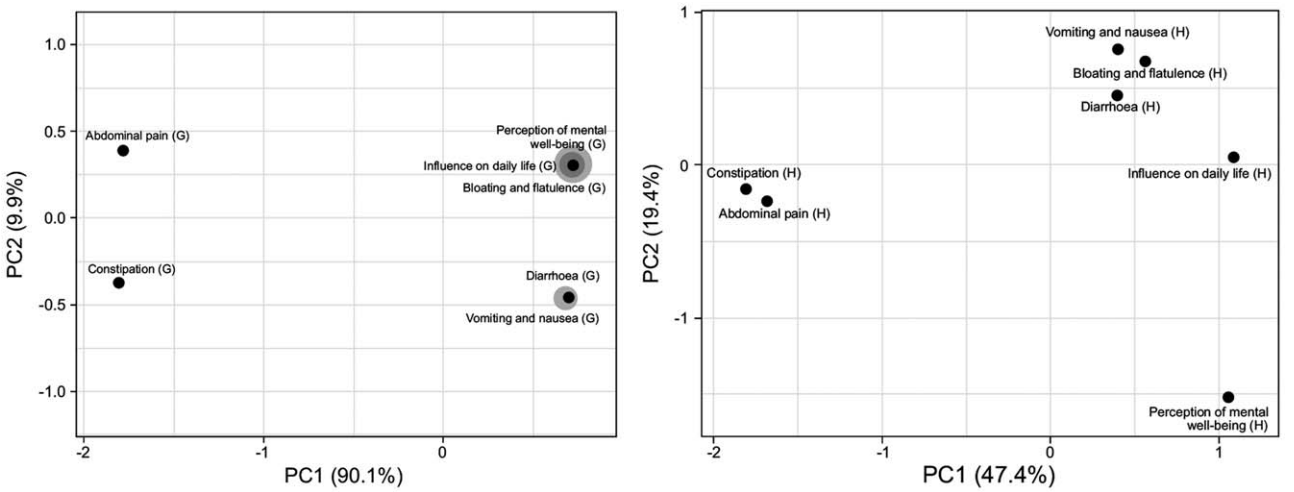

Figure 4. Clustering of multivariate data using principal component analysis analysis of responses for different variables of quality of life from Test-G (G) and Placebo-H (H) arms at end of treatment (visit 04). Variables considered are— abdominal pain, diarrhea, constipation, bloating and flatulence, vomiting and nausea, perception of mental well-being, and influence on daily life.

baseline and EOT for hematological and biochemical parameters. Additionally, hematological and biochemical estimation results were within standard range of reference values. No significant visual changes were observed in the upper GI endoscopy of patients in both Test-G and Placebo-H arm at baseline and EOT.

5.2. Assessment of quality of life by Visual Analogue Scale assessments

Modulation in QoL parameters is visualized through two-dimensional PCoA in Test-G and Placebo-H arm at baseline and EOT. Processed responses clearly indicated improvement in parameters like abdominal pain, diarrhea, constipation, bloating and flatulence, vomiting and nausea, perception of mental well-being and influence on daily life in Test-G arm, that is, treated with *B coagulans* LBSC. However, Placebo-H showed either no or mild improvement or worsened unbearable pain at all pain scale. Vector scaling and singular value decomposition with imputation is used to calculate PCoA. In Test-G, X (PC1) and Y (PC2) – axis explains respectively 90.1% and 9.9% of the total variance; whereas, 47.4% and 19.4% in Placebo-H (Fig. 4).

6. Discussion

Probiotics offer promising therapeutic solution for various health conditions including gastrointestinal illnesses like diarrhea, SIBO, IBS, IBD, ulcerative colitis, Crohn's disease, etc. Microbial *dysbiosis*, that is, imbalance in gastrointestinal microbiome is a known cause for prognosis of such gastrointestinal conditions.[24] Bacteriotherapy with probiotics helps in reprogramming the microbial balance of gut and restore healthy and complex host-microbiota interactions.[41] Clinical efficacies of probiotics are strain dependent and therapeutic effectiveness depends on specific clinical circumstances like digestive and nondigestive disorders.[29,42] Variability in study designs sometimes demonstrated variable probiotic efficacies in gastrointestinal ailments like IBS, IBD, and diarrhea.[21,29,43–46]

This CONSORT compliant, interventional, randomized, placebo-controlled trial (RCT) has evaluated the therapeutic impact of *B coagulans* LBSC on IBS in human subjects. *B coagulans* LBSC [DSM17654; GenBank: CP022701.1 & GenBank: ATW84696.1 (*gyrB*)] is previously reported as a genomically safe probiotic microorganism,[35] efficacious for treating acute diarrhea and abdominal discomforts[26] and can modulate gut microbiome in human subjects.[24]

The intervention of *B coagulans* LBSC (Test-G), compared to placebo-H, showed significant improvement in all gastrointestinal symptoms, notably in abdominal pain (mean change −1.74; $P < .0001$), headache (mean change −1.26; $P = .0003$), and stomach rumbling (mean change −1.05; $P = .0210$). In a previous trial on IBS using a symbiotic composition with *B coagulans*, Rogha et al[31] reported on reduction in abdominal pain and diarrhea but no change in constipation frequency after 12 weeks compared to placebo. An earlier study with *B coagulans* (GBI-30, 6086) also demonstrated relief from abdominal pain and bloating in IBS patients (n = 44) after 8 weeks of intervention.[29] Another study (n = 61) on the same strain (GBI-30, 6086) is reported to reduce average number of bowel movements per day in patients with diarrhea-predominant irritable bowel syndrome (IBS-D) compared to placebo. However, the study was not able to assess any severity scores and QoL due to large variability in baseline scores.[30] In a similar RCT (n = 52), Urgesi et al[47] demonstrated efficacy and the safety of Colinox (a medical device contained combination of simethicone and *B coagulans*) in the treatment of bloating predominant intrusive IBS. Both intragroup and intergroup results showed significant reduction of bloating, discomfort, and pain in Colinox group compared to placebo ($P < .0001$).

Current study demonstrated that *B coagulans* LBSC significantly improved other IBS-associated symptoms like bloating and cramping, diarrhea and constipation, nausea, vomiting, and anxiety from baseline compared to Placebo-H. Similarly, Sudha et al[48] reported significant improvement in stool consistency in children with IBS (n = 141) as well as reduction in abdominal

discomfort, bloating, staining, urgency, incomplete evacuation, and passage of gas after 8 weeks of treatment with *B coagulans* Unique IS-2 ($P \leq .0001$). An analogous study with Unique IS-2 strain on IBS adults (n = 136) also helped in reducing abdominal discomfort/pain intensity and increasing complete spontaneous bowel movements.[34] However, supplementation of *B coagulans* MTCC 5856 (2 billion per g) was reported to improve the clinical conditions of diarrhea predominant irritable bowel syndrome (IBS-D) (n = 36) treated for 90 days. Majeed et al[33] reported improvement in IBS-D clinical conditions included symptoms like bloating, vomiting, diarrhea, abdominal pain, and stool frequency ($P < .01$) compared to placebo group. The data presented in the present study and other published information, in general, establishes that *B coagulans* is efficacious in treating the IBS symptoms.

Comprehensiveness in improvement of DSFQ symptoms are often coupled with IBS-SSS category, which showed maximum cases of "no symptoms" (63.16%) after *B coagulans* LBSC treatment compared to mild and moderate severity observed in Placebo (21.05%) ($P < .0001$). This score 63.16% observed in this study is higher than other *B coagulans* strains as reported in other literature.[34,49] A multispecies probiotic containing 5 strains of lactic acid bacteria and bifidobacteria [*Lactobacillus casei* LMG101 and *Lactobacillus plantarum* CECT4528 (5 billion CFU), *Bifidobacterium animalis* subsp. *lactis* Bi1, B. breve Bl10, *Bifidobacterium breve* Bbr8 (10 billion CFU) was reported to significantly decrease the IBS-SSS as compared with placebo ($P < .001$) along with gastrointestinal symptom rating scale.[50] Similarly, a multistrain probiotic Bio-Kult (14 different bacterial strains) also showed an improvement of 69% IBS-SSS compared to placebo (47%) after 16 weeks.[51] Though a randomized triple-blind trial (n = 340) conducted by Lyra et al[49] which reported on relief of IBS severity in all three groups, viz, placebo, active low-dose (1 billion per g), and active high-dose (10 billion per g) of *Lactobacillus acidophilus* NCFM for 12 weeks. However, no significant difference was observed between placebo and active groups; and respectively, 28.4%, 25.0%, and 26.5% of volunteers considered adequately relieve from IBS symptoms. Noteworthy, alleviations from IBS severity by *B coagulans* LBSC (63.16%) was equally efficacious with *B coagulans* Unique IS2 which showed 60% decrease in severity score after 8 weeks.[34]

In consequence of the improvement in IBS-SSS, maximum patients treated with *B coagulans* LBSC attained normal stool consistency at EOT from baseline as well as in comparison to placebo. This observation is in line with a previous clinical study on *B coagulans* LBSC, where stool consistency was improved in patients with acute diarrhea by right balance in stool water content and regular complete spontaneous bowel movements.[26] Notably, stool consistency ensues due to improvement in stool texture, colors, odor, and bowel frequency which can be correlated with other clinically efficacious strains of *B coagulans* in related gastrointestinal ailments.[33,34,47,52] Further, amelioration of gastrointestinal symptoms along with improved stool frequency and intestinal transit are reported for other than spore forming *Bacillus* probiotics; which predominantly belong to genus *Bifidobacterium* and *Lactobacillus*.[53,54] Mechanisms of such effects were largely unexplored until the compositions of gut microbiota and their role are elucidated in various gastrointestinal *dysbiosis* including IBS. In a double blind RCT (n = 88), Yoon et al[54] have shown *L plantarum* LRCC5193 treatment improved stool consistency in IBS-C; may be due to increased abundance of *L plantarum* in gut which inversely hastened gut transit time and bolus movement. Increasing evidences support the notion of probiotic's favorable actions which is due to reprogramming of gut microbiota, elevation of anti-inflammatory microbial groups, competitive exclusion of pathogens, competitions for nutrients, production of short chain fatty acids, modulation of local epithelial immunity, and augmentation of tight junction proteins.[55,56] In a recent study, *B coagulans* LBSC is reported to modulate gut microbiome of IBS patients comprehended by whole genome metagenome analysis. *B coagulans* LBSC treatment showed positive modulation in gut microbiota, especially upregulation of phyla such as *Actinobacteria* and *Firmicutes*, whereas downregulation of *Bacteroids*, *Proteobacteria*, *Streptophyta,* and *Verrucomicrobia*. Subsequently, it altered various microbiota associated metabolic pathways to create the normalcy of gut microenvironment which can be put together for ameliorative effects of the strain as alternative therapeutic supplementation.[24]

Both Test-G and Placebo-H groups reveal no significant intergroup and intragroup difference in vital signs, hematological, and serum biochemical results. Simultaneously, no study drug-related AEs or SAEs were noted during the trial, which concludes that investigational product is safe to use. Further, a dose of 2 billion CFU of *B coagulans* LBSC for thrice a day was found to be safe and well-tolerated by the study participants for an 80 days study duration and it was with in recommended dose for healthy human (0.1×10^9–36.4×10^9 CFU/person/d) described in various studies.[57] The endoscopy of upper gastrointestinal tract of both the arms appeared normal with no sign of inflammation, which further supports safety of *B coagulans* LBSC.

PCoA plot showed highly modulated Quality of life (QoL) parameters at baseline and at EOT after *B coagulans* LBSC treatment. Contrarily, Placebo-H showed either mild or no improvement at EOT. In an earlier communication, *B coagulans* LBSC has been described as effective in improving QoL parameters in acute diarrhea patients.[26] Strains of *B coagulans* are largely known for modulating QoL in several disease conditions including subtypes of IBS, diarrhea, FGIDs, rheumatoid arthritis, etc in children and adults.[29–33,48,58,59] Mechanism behind the therapeutic health benefits by probiotic *B coagulans* LBSC is mainly the modulation of gut microbiome and associated metabolic normalcy, better digestion and immune homeostasis, and improvement in intestinal health.[24]

In conclusion, probiotic *B coagulans* LBSC [DSM17654] with a dose of 2×10^9 CFU for thrice a day was well tolerated, found safe, and showed significant alleviation in IBS-associated clinical symptoms like bloating/cramping, abdominal pain, diarrhea, constipation, stomach rumbling, nausea, vomiting, headache, and anxiety, compared to placebo group. *B coagulans* LBSC treatment improved stool consistency, decreased the severity, and conferred better QoL to IBS patients. No report of adverse and serious adverse effects, no usage of rescue medicine further confirmed the safety and efficacy of *B coagulans* LBSC; which could be used as a therapeutic supplement in the management of IBS pathophysiology and improving QoL in adults.

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