



Bacillus coagulans Unique IS2 in Constipation: A Double-Blind, Placebo-Controlled Study

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Abstract

Functional constipation has a high prevalence in both adults and children affecting quality of life. Evidence suggests that probiotics can reduce the symptoms of constipation. As the effects of probiotics are strain specific, the efficacy of *Bacillus coagulans* Unique IS2 in the treatment of functional constipation in adults was evaluated. Subjects ($n = 100$) diagnosed with functional constipation were supplemented with either *B. coagulans* Unique IS2 (2 billion CFU) or placebo capsules once daily for 4 weeks. Subjects were evaluated for treatment success (defined as three or more spontaneous stools per week), stool consistency, difficulty of defecation, defecation and abdominal pain. By the end of the fourth week, there was a statistically significant ($p < 0.001$) increase in number of bowel movements in the probiotic treated group as compared to placebo. Ninety eight percent of subjects in the probiotic group achieved normal stool consistency as compared to placebo (74%). Moreover, there was relief from symptoms of incomplete evacuation, painful defecation and abdominal pain associated with constipation in probiotic treated group as compared to placebo. In conclusion, *B. coagulans* Unique IS2 significantly decreased the symptoms of constipation indicating effectiveness of the strain in the treatment of constipation.

Trial registration: CTRI/2017/11/010539.

Keywords Probiotic · *B. coagulans* unique IS2 · Constipation · Bowel movements · Abdominal pain

Introduction

Functional constipation or chronic idiopathic constipation (CIC) is a symptom-based gastrointestinal disorder without an organic origin. It has a prevalence of 14% in adults which represents a huge health care burden [1, 2]. Apart from having a negative

impact on the health-related quality of life (QOL) and imposing an economic burden on the individual, constipation is a worldwide problem that is common across all ages and cultures [3, 4]. It is characterised by infrequent bowel movements, usually fewer than three per week, and symptoms may also include hard stools, a feeling of incomplete evacuation, abdominal discomfort, bloating and distension [5]. The management of functional constipation still remains a challenge, with up to 47% of patients not finding relief from treatments which include bulking agents, osmotic laxatives and stool softeners [6–8].

Probiotics are ‘live microorganisms that, when administered in adequate amounts, confer a health benefit on the host’ [9]. Some of these beneficial effects include regulation of hypertension and lipid levels, diabetes and obesity [10–13]; attenuation of renal calculi [14]; and restoration of digestive health including constipation [15–17]. Probiotic supplementation has been found to be efficacious in decreasing intestinal transit times [18]. Mechanism of action of probiotics in alleviating constipation includes modification of the gastrointestinal microbiota which is altered in constipation [19–21]. Metabolites produced by probiotic may alter gut function, including sensation and motility [21]. Production of lactic acid

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s12602-019-09542-9>) contains supplementary material, which is available to authorized users.

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and short-chain fatty acids reduces luminal pH, which enhances colonic peristalsis and hence shortens gut transit time (GTT) leading to improved bowel movements [2].

The most widely used probiotic strains are the *Lactobacilli* and *Bifidobacteria* [22]. However, *Bacillus* spp. are gaining a lot of attention as they are stable at room temperature [23, 24]. *Bacillus coagulans* Unique IS2 is a spore-forming, shelf-stable probiotic strain with established safety and efficacy in the treatment of diarrhoea, bacterial vaginosis and irritable bowel syndrome (IBS) [25–27]. Being a spore former, *B. coagulans* Unique IS2 is resistant to acidic conditions of the stomach and bile acids. It reaches the intestine without any loss of viability where it produces lactic acid. It has been deposited with the American Type Culture Collection (ATCC; ATCC PTA 11748) and Microbial Type Culture Collection (MTCC; MTCC 5260). As *B. coagulans* Unique IS2 has been found to improve digestive health (viz. diarrhoea and IBS in children and adults), it was of interest to study its efficacy in yet another function of digestive health, i.e. constipation.

Materials and Methods

Study Design

This double-blind, randomised, placebo-controlled, parallel group multicentric study was conducted at two sites, viz., MV Hospital and Research Centre, and KRM Hospital and Research, Lucknow, India (December 2017 to August 2018). This outpatient study was conducted in compliance with the code of conduct for research involving human volunteers as issued by the International Conference on Harmonisation-Good Clinical Practice (ICH-GCP), Indian Council of Medical Research guidelines (ICMR; ethical guidelines for biomedical research on human subjects) and the principles of the Declaration of Helsinki. Informed consent forms were approved by the ethical committees of both the hospitals and the trial was registered prospectively with the clinical trial registry of India (CTRI/2017/11/010539). The study was initiated after obtaining informed consent.

Sample Size

Sample size was calculated to be 50 subjects per arm in order to detect a difference based on the primary endpoint, using a two-tailed test with 90% power, alpha risk of 5% and a drop-out of 20%. Total, enrolled subjects were 100 with 50 in each group.

Study Population

Overall, 101 subjects were screened from which 100 subjects who met the inclusion/exclusion criteria were enrolled in the

study (one subject excluded due to not meeting inclusion criteria). The ITT (intention-to-treat) population consisted of randomised subjects who received one dose of either *B. coagulans* Unique IS2 (2 billion CFU) capsule ($n = 50$) or placebo ($n = 50$) with a post-baseline efficacy assessment. The strength (2 billion CFU) of *B. coagulans* in the capsule was confirmed by plating a serially diluted sample on GYE (glucose yeast extract) agar. The PP (per protocol) population consisted of subjects, both *B. coagulans* Unique IS2 ($n = 50$) and placebo ($n = 50$) having completed the study without any major protocol deviation. All efficacy analyses were performed on ITT population which remained the same as PP population due to no major protocol deviations.

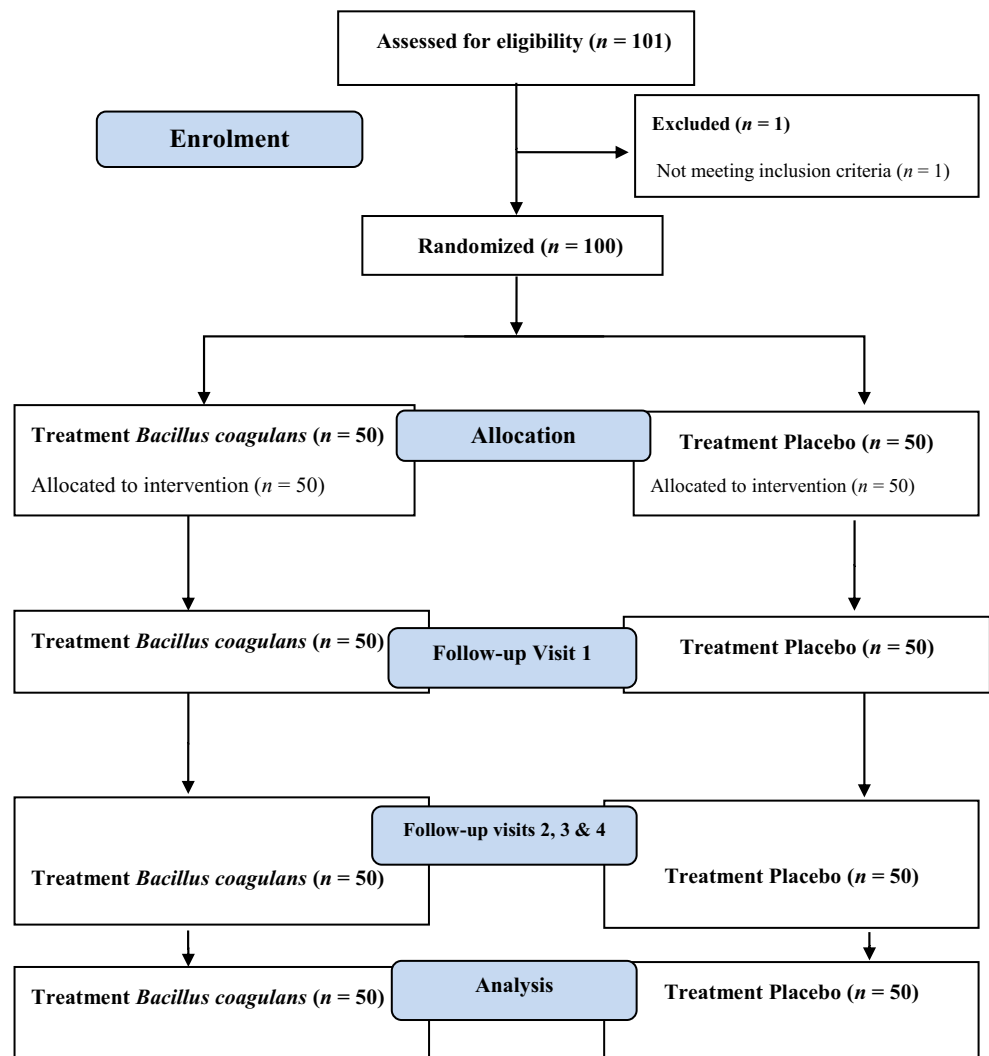
Of the 100 patients, 50 received probiotic, *B. coagulans* Unique IS2 capsules (2×10^9 CFU) and 50 received placebo daily for up to 4 weeks (Fig. 1). The mean age of total patients was 43.92 ± 11.74 years (range, 18–64 years); 59 (59%) patients were males and 41 (41%) were females. The mean age of patients who received *B. coagulans* Unique IS2 was 42.54 ± 12.16 years (range, 18–64 years) and placebo was 45.30 ± 11.33 years (range, 24–64 years). Thirty four (68%) patients were males and 16 (32%) patients were females in *B. coagulans* Unique IS2 group, whereas 25 (50%) patients were males and 25 (50%) were females in placebo (Table 1).

Inclusion criteria: subjects of either sex between 18 and 65 years of age with diagnosis as functional constipation according to Rome Criteria III; should not have/had any major illnesses; willing to give written informed consent and follow study procedures. **Exclusion criteria:** subjects who had undergone major abdominal surgery; documented history of slow colonic transit; presence of any concomitant diseases such as organic GI diseases and/or lactose and gluten intolerance; medical or psychiatric illness; clinical features suggestive of alarming symptoms (rectal bleeding, weight loss etc.); family history of peptic ulcer, colorectal cancer or IBD, abnormal laboratory data or thyroid function; participation in any clinical trial or usage of any investigational product in the past 90 days; known or expected hypersensitivity to any of the active substances or excipients; used any probiotic formulation in the past 30 days; pregnant or lactating; allergic or atopic to any of the ingredients of the study medication.

Randomisation

After having obtained signed, written informed consent, subjects underwent a screening examination. Subjects complying with inclusion/exclusion criteria were enrolled and randomised by block randomisation to one of the two treatment arms. Based on SAS 9.4 randomisation, numbers for two treatment groups were generated. Randomisation was conducted using opaque sealed envelopes that were indistinguishable between groups in order for the investigators also to be blinded to the treatment. Each envelope had the assignment of

Fig. 1 Study flow chart



the patient (probiotic or placebo treatment) with 50 envelopes for each group. The sealed envelopes were provided to the clinical site. The investigators assigned investigational products to patients based on the randomisation numbers. Both groups were characteristically similar pertaining to age, sex and weight of the patient.

Study Follow-up Visits and Treatments

The duration of treatment was for a period of 28 days with a follow up until the end of treatment. *Bacillus coagulans* Unique IS2 or placebo capsules were administered once daily for up to 28 days. As this was an outpatient study, four visits were mandatory and recorded; visit 1 was for screening and treatment initiation (day 1), visit 2 was follow-up 1 (day 8 ± 2), visit 3 was follow-up 2 (day 15 ± 2) and visit 4 was end of study (day 29 ± 2).

Efficacy and Safety Measurement Criteria

Primary efficacy endpoint was treatment success (defined as three or more spontaneous stools per week). The secondary efficacy endpoints were changes from baseline to end of treatment in stool frequency, stool consistency (as assessed by Bristol stool form scale), difficulty in degree of defecation—sensation of incomplete evacuation (CCS scale, constipation scoring system), defecation pain (CCS scale) and abdominal pain (CCS scale). Safety endpoints included the incidences of treatment-emergent adverse events, abnormal vital signs and clinically significant changes from baseline in physical examination.

Statistical Methods

All efficacy analyses were performed on ITT population which remained the same as PP population due to no major

Table 1 Participants' demographic data

	<i>Bacillus coagulans</i> Unique IS2	Placebo	Total
<i>n</i>	50	50	100
Mean age \pm SD (years)	42.54 \pm 12.16	45.30 \pm 11.33	43.92 \pm 11.74
Age range (years)	18–64	24–64	18–64
Sex (M/F)	34 (68%):16 (32%)	25 (50%):25 (50%)	59 (59%):41 (41%)

n, number; SD, standard deviation; M, male; F, female

protocol deviations. Primary endpoint qualitative data was defined as number and percentages, and the data was compared using unpaired *t* test at 5% level of significance between groups. The secondary endpoint data was also interpreted as descriptive data for scores as *n*, mean, median, standard deviation and range (minimum and maximum). Data was analysed using unpaired *t* test at 5% level of significance between groups.

Evaluation of Results

The main criteria in the evaluation of results was to see if there was an improvement in the primary efficacy parameter, i.e. treatment success based on stool frequency (three or more spontaneous stools per week). As the study was a randomised, double-blind study, the differences observed between the two groups (probiotic and placebo) could be ascribed solely to the effect of the treatment as bias was removed. To arrive at the required sample size for the study (50 subjects/arm), sample size calculations were used to arrive at the sample size to identify a significant result in this primary outcome measure.

Results

Primary Efficacy

Treatment Success Based on Stool Frequency (Defined as Three or More Spontaneous Stools per Week)

In patients receiving *B. coagulans* Unique IS2 capsules, the bowel frequency was 0.90 \pm 0.73 (week 1), 1.66 \pm 1.81 (week 2), 4.16 \pm 1.98 (week 3) and 5.98 \pm 1.57 (week 4) spontaneous stools per week. In the placebo group, bowel frequency was 0.94 \pm 0.86 (week 1), 1.62 \pm 1.78 (week 2), 2.34 \pm 1.31 (week 3) and 3.12 \pm 1.18 (week 4) spontaneous stools per week (Fig. 2a, Table S1). At week 3 and week 4, there was a significant improvement ($p < 0.001$) in mean frequency of spontaneous stools per week in patients receiving *B. coagulans* Unique IS2 capsules as compared to placebo.

Secondary Efficacy

Stool Consistency

Stool consistency was assessed by Bristol stool form scale (Table S2) which categorises the stool into seven types on a scale of 1–7 (hard to loose): 1 and 2 indicate constipation (hard stool), 3 and 4 the ideal stool and 5, 6 and 7 indicating loose and watery stool. The stool consistency improved significantly in the probiotic treated group of the functionally constipated subjects from the third week onwards. At week 3, *B. coagulans* Unique IS2 treated group had a mean stool score of 2.28 \pm 0.60, whereas the mean score in placebo group was 2.06 \pm 0.43 ($p = 0.041$). By week 4, the mean stool score in *B. coagulans* Unique IS2 treated group had increased to 3.02 \pm 0.57, whereas in the placebo group, it was 2.65 \pm 0.58 ($p = 0.021$) (Fig. 2b). There was thus an increase in the percentage of patients with normal stool in the *B. coagulans* unique IS2 treated group (Table 2) as compared to placebo group (98% vs. 74%).

Difficulty in Degree of Defecation and Sensation of Incomplete Evacuation (CSS Scale)

The sensation of incomplete evacuation was assessed through constipation scoring system (CSS) scale (Table S3). The CSS score 'completeness: feeling incomplete evacuation' score was a scale of 0–4 with higher CSS scores indicating more severe constipation (0, never; 1, rarely; 2, sometimes; 3, usually; and 4, always). In patients receiving *B. coagulans* Unique IS2 capsules, there was a significant decrease as compared to placebo in the feeling of incomplete evacuation as assessed by constipation scoring system. By visit 3, *B. coagulans* Unique IS2 treated group had a mean score of 1.32 \pm 0.51 as compared to placebo which was 1.62 \pm 0.73 ($p = 0.019$), and by visit 4, in the *B. coagulans* Unique IS2 treated group, the score had further dropped to 0.88 \pm 0.39 as compared to placebo which was 1.04 \pm 0.73 ($p = 0.034$) (Fig. 3a).

Defecation Pain (CSS Scale)

The severity of constipation symptom 'difficulty: painful evacuation effort' was assessed through CSS scale

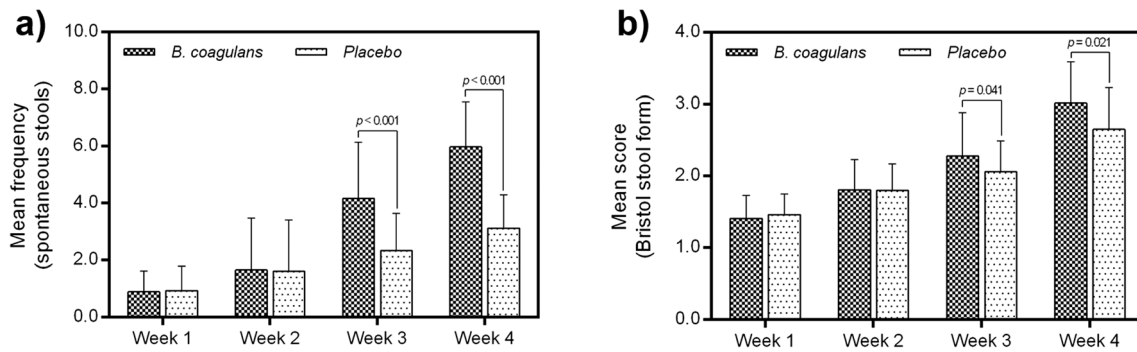


Fig. 2 Effect of *Bacillus coagulans* Unique IS2 and placebo on (a) spontaneous stools per week; (b) consistency of stool

(Table S4). The CSS ‘difficulty: painful evacuation effort’ score was a scale of 0–4 with higher CSS scores indicating more severe pain (0, never; 1, rarely; 2, sometimes; 3, usually; and 4, always). By visit 3, *B. coagulans* Unique IS2 treated group had a mean score of 1.16 ± 0.58 as compared to placebo which was 1.50 ± 0.84 ($p = 0.021$), and by visit 4, in *B. coagulans* Unique IS2 treated group, the score had further dropped to 0.66 ± 0.52 as compared to placebo which was 0.98 ± 0.62 ($p = 0.006$) (Fig. 3b).

Abdominal Pain (CSS Scale)

The severity of constipation symptom ‘pain: abdominal pain’ was assessed through CSS scale (Table S5). The CSS ‘pain: abdominal pain’ score was a scale of 0–4 with higher CSS scores indicating more severe pain (0, never; 1, rarely; 2, sometimes; 3, usually; and 4, always). By visit 3, *B. coagulans* Unique IS2 treated group had a mean score of 0.94 ± 0.68 as compared to placebo which was 1.1 ± 0.84 ($p = 0.038$) and by visit 4, in *B. coagulans* Unique IS2 treated group, the score had further dropped to 0.38 ± 0.49 as compared to placebo which was 0.8 ± 0.81 ($p = 0.002$) (Fig. 4).

Adverse Events

No serious adverse events were observed during the trial. There were three patients from the placebo group who had reported mild adverse events during the study. One patient had mild fever (pyrexia) and two patients had reported headache from placebo group which the investigator had considered as not related to study drug. The vital parameters in both groups remained within normal and acceptable clinical range throughout the study duration (Table S6).

Discussion

In the present study, adults with functional constipation who received *B. coagulans* Unique IS2 capsules had improved bowel movements as compared to group fed with placebo. Probiotic capsules have an advantage over other forms of treatment in that they are generally safe and do not have any side effects [28]. There have been a few studies with other strains of *B. coagulans* in constipation. A clinical study with a limited number of subjects (20 healthy adults) on the effects

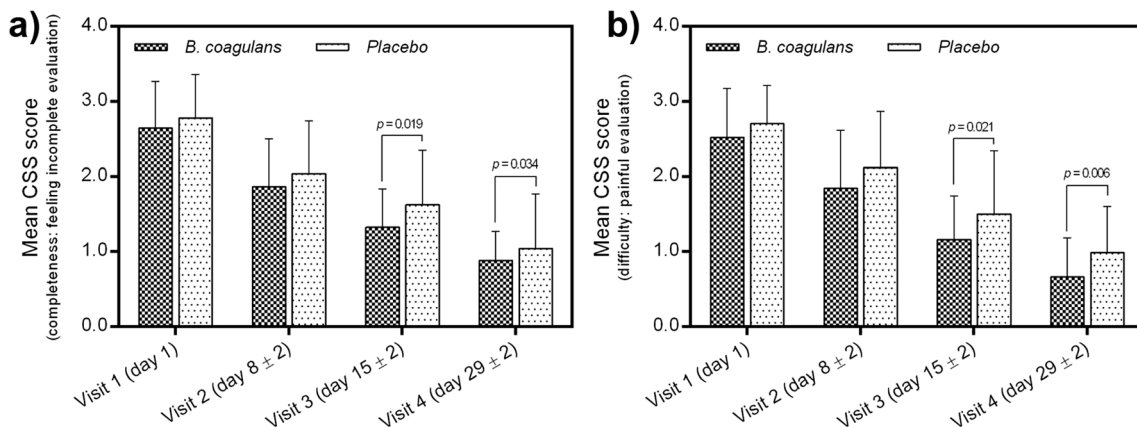


Fig. 3 Effect of *Bacillus coagulans* Unique IS2 and placebo on (a) completeness: feeling incomplete evacuation; (b) difficulty: painful evacuation effort evaluated by constipation scoring system (CSS)

Table 2 Percentage of patients with normal stools

Treatment	Parameters	Baseline	Day 15 ± 2	End of study
<i>Bacillus coagulans</i> Unique IS2 (n = 50)	Constipated stool	50 (100%)	38 (76%)	1 (2%)
	Normal stool	0	12 (24%)	49 (98%)
Placebo (n = 50)	Constipated stool	50 (100%)	42 (84%)	13 (26%)
	Normal stool	0	8 (16%)	37 (74%)

n, number

of *B. coagulans* SANK 70258 (1×10^8 CFU/day for 2 weeks) on faecal properties and defecation frequency revealed that the ingestion of *B. coagulans* SANK 70258 in persons whose defecation frequency was relatively low led to an improvement of faecal shape, change of faecal colour from dark brown to yellowish brown, decrease of faecal odour and faecal pH, and an increase in defecation frequency [29]. The number of subjects, however, was too small to conclude on efficacy.

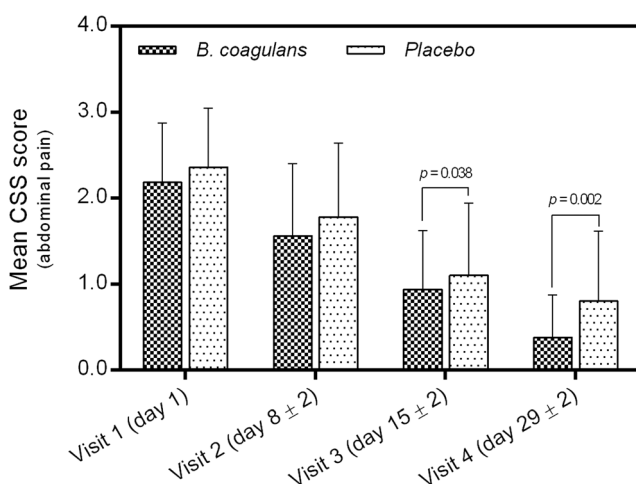
In another study, the effect of a Lilac LAB (*B. coagulans* lilac-01 and okara (soy pulp) powder) on bowel movements/faecal properties was studied through a double-blind placebo-controlled randomised trial on healthy Japanese volunteers with tendency for constipation ($n = 297$) [30]. The subjects in the test group ingested 2 g/day okara powder and *B. coagulans* Lilac LAB (1×10^8 CFU) once a day for 2 weeks. The placebo group was given okra powder only. In the test group of functionally constipated subjects, the changes in the average scores of self-reported faecal size, sensation of incomplete evacuation and defecation frequency were significantly improved compared to the placebo group ($p < 0.05$); faecal colour and odour also tended to improve ($p = 0.07$). The faecal size also tended to improve compared to the placebo group ($p = 0.06$ and $p = 0.07$, respectively) [30]. Lilac LAB was effective in improving bowel movements and faecal properties in functionally constipated persons. No clear-cut evidence on the efficacy of *B. coagulans* in constipation has been obtained as in the clinical studies conducted so far; either the

sample size was too small or it was used in combination with a prebiotic [29, 30].

Members of the genera *Lactobacillus* and *Bifidobacterium* are well-investigated probiotics for functional constipation [31]. The administration of *L. casei* Shirota in patients ($n = 35$) with chronic constipation for 5 weeks resulted in significant ($p < 0.003$) improvement in defecation frequency and stool consistency as compared to placebo [32]. In another study, 2-week supplementation of either 10^{10} CFU/100 ml of *Bifidobacterium animalis* subsp. *lactis* GCL2505 or a milk product without bacteria as a placebo in patients ($n = 17$) with constipation significantly increased defecation frequency and the amount of stool [33]. Recently, Yoon et al. [34] showed that *Streptococcus thermophilus* MG510 and *Lactobacillus plantarum* LRCC5193 significantly improved stool consistency in patients with chronic constipation. A recent study, however, showed no significant effects of *B. lactis* DN-173010 and *Lactobacillus casei rhamnosus* Lcr35 on functional constipation when compared with placebo [35, 36].

The effects of probiotics are strain specific [37] and hence it was of importance to study the efficacy of *B. coagulans* Unique IS2 in the treatment of constipation. It is very important that the safety of the probiotic strain is established before it is recommended for human consumption [38]. *Bacillus coagulans* Unique IS2 is very well characterised with established probiotic properties [39], whole-genome sequencing and safety studies in rats (acute and repeat dose toxicity) have further established its safety [40, 41].

In the present investigation, we have shown that *B. coagulans* Unique IS2 (2 billion CFU) significantly improved the number of bowel movements per week and therefore helped ease constipation. There was also a significant improvement in the stool consistency and feeling of incomplete evacuation as compared to the placebo group with a decrease in abdominal pain and defecation pain from the third week onwards.

**Fig. 4** Effect of *Bacillus coagulans* Unique IS2 and placebo on abdominal pain evaluated by constipation scoring system (CSS)

Conclusion

The present study suggests that *B. coagulans* Unique IS2, a clinically proven and safe probiotic, can be used in the treatment of constipation.

Compliance with Ethical Standards This study was conducted in compliance with the code of conduct for research involving human volunteers as issued by the International Conference on Harmonisation–Good Clinical Practice (ICH-GCP), Indian Council of Medical Research guidelines (ICMR; ethical guidelines for biomedical research on human subjects) and the principles of the Declaration of Helsinki. Informed consent forms were approved by the ethical committees of study sites and the trial was registered prospectively with the clinical trial registry of India (CTRI/2017/11/010539). The study was initiated after obtaining informed consent.

Competing Interests R.S.M., J.N. and J.J.A. are employed by Unique Biotech Ltd. which is a manufacturer of probiotics. They wish to state that the study was conducted independently with no intervention on their part during the duration of the study.

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