

***Lactobacillus acidophilus* CL1285, *Lactobacillus casei* LBC80R and *Lactobacillus rhamnosus* CLR2 improve quality-of-life and IBS symptoms: a double-blind, randomised, placebo-controlled study**

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Abstract

A combination of *Lactobacillus acidophilus* CL1285, *Lactobacillus casei* LBC80R and *Lactobacillus rhamnosus* CLR2 was compared to placebo for relief of symptoms of irritable bowel syndrome (IBS). A total of 113 subjects at 3 clinical sites were randomised in a 2:1 ratio and followed for 12 weeks. Subjects ingested either 2 capsules of active study product, containing 50×10^9 cfu of live organisms, or 2 placebo capsules daily. Endpoints included improvement in abdominal pain, days of pain, distention, stool consistency and frequency, quality of life (QOL), and adequate relief (AR) of IBS symptoms. IBS subtypes constipation (IBS-C), diarrhoea (IBS-D), and mixed (IBS-M) were evaluated separately; the effect of gender was also examined. For all efficacy endpoints improvement of 30% or more vs placebo was considered clinically significant. With the exception of pain intensity and AR, the endpoints demonstrated a therapeutic advantage of active over placebo for IBS symptoms in at least some subject subgroups. The IBS-D and female subgroups showed the largest and most consistent effects. Stool frequency and consistency were evaluated in the IBS-C and IBS-D subgroups, and improvement of active vs placebo was noted in both. QOL improvement was seen overall and in specific domains. Adverse events (AEs) were limited to 7 subjects; all were of mild or moderate intensity except one, severe cramping. Four AEs in the same subject in the placebo group were judged to be related to study product; these resolved by the end of study. There were no serious AEs.

Keywords: irritable bowel syndrome, probiotics, quality of life, constipation, diarrhoea

1. Introduction

Irritable bowel syndrome (IBS) is a chronic, relapsing gastrointestinal disorder that affects 5-20% of the American population. A number of risk factors for IBS have been identified, including female sex, psychological problems, stress, food intolerance, and bacterial overgrowth of the small intestine (Aagaard *et al.*, 2013). The cardinal symptoms of IBS include abdominal pain, bloating, and changes in bowel habits (Aagaard *et al.*, 2013). The pathophysiology is defined and no intestinal structural abnormalities accompany the syndrome. The quality of life (QOL) of individuals with IBS is severely impaired,

with major impacts on the health care system and visits to primary care physicians and gastroenterologists (Coffin *et al.*, 2004). In fact, IBS is the most frequent diagnosis in gastroenterology practices and one of the most frequent diagnoses in primary care practices (Peery *et al.*, 2012). Based on specific symptomatology, patients with IBS can be sub-classified into three major groups: constipation-predominant (IBS-C), diarrhoea-predominant (IBS-D), and mixed bowel patterns (IBS-M), each with an approximately equal distribution. These IBS symptoms are troubling to patients, result in lower QOL, and interfere with social interactions (Coffin *et al.*, 2004).

The ultimate treatment goal for IBS is to provide relief for the multiple symptoms of this condition by using a single, well-tolerated agent. Drug therapies may alleviate some of the symptoms linked with this condition, but none are curative. Therefore, the prospect of long-term treatment efficacy is limited given the current treatment options. There is a clear need for IBS relief procedures that are safe, efficacious, and cost effective (Foxy-Orenstein, 2006).

Probiotics are live micro-organisms that provide health benefits for the host when administered in adequate dosages. In recent years, probiotics have been commonly used to alleviate symptoms in a variety of gastrointestinal disorders. Since dysbiosis may be part of the multifactorial aetiology of IBS, a variety of probiotics have been tested in clinical trials to determine their efficiency and the results have been included in several meta-analyses and review articles (Ford *et al.*, 2014b; Hoveyda *et al.*, 2009; McFarland and Dublin, 2008; Ortiz-Lucas *et al.*, 2013; Whelan and Myers, 2010; Yoon *et al.*, 2015). No firm conclusions could be drawn as to the efficacy of strain-specific probiotics for alleviating the symptoms of IBS. Strong placebo effects, psychological factors, and gender effects make the interpretation of study findings difficult (Ford and Moayyedi, 2010; Lyra *et al.*, 2016; Moayyedi *et al.*, 2010).

The objectives of this clinical trial were to evaluate the effectiveness of a proprietary probiotic product, *Lactobacillus acidophilus* CL1285 + *Lactobacillus casei* LBC80R + *Lactobacillus rhamnosus* CLR2 for relief of specific IBS-related symptoms, improvement in QOL, effect on stool consistency and frequency, and attainment of adequate relief (AR) in otherwise healthy adults with IBS-C, IBS-D and IBS-M subtypes.

2. Materials and methods

Experimental design, study implementation, and data collection

This prospective, double-blind, randomised, placebo-controlled study was registered in Clinicaltrials.gov on March 1st, 2012 as ClinicalTrials.gov identifier: NCT01545037. The protocol was approved by an independent IRB, IntegReview. All participating subjects signed an informed consent. Subjects aged 18 years or older were recruited at 3 clinical study sites located in California, USA.

Subjects ingested 2 capsules active or placebo product with breakfast each day. Each active capsule contained a minimum of 50×10^9 cfu (*L. acidophilus* CL1285, *L. casei* LBC80R and *L. rhamnosus* CLR2) with respective proportions of 1-5%, 80-90%, and 5-15%, plus inert ingredients. The placebo capsules contained the inert ingredients only.

Subjects were required to have met the Rome III criteria for IBS (Shih and Kwan, 2007). The Rome III criteria include presence of recurrent abdominal pain or discomfort at least 3 days/month in the last 3 months, associated with 2 or more of the following: improvement with defecation, onset associated with a change in frequency of stool, and onset associated with a change in form (appearance) of stool. Symptom onset must be at least 6 months prior to diagnosis.

Subjects were required to complete a 7 day placebo run-in period to demonstrate compliance with intake of investigational product (IP) and completion of daily diaries documenting IP consumption, stool frequency, stool consistency as defined by the Bristol Stool Chart (BSC), pain severity, and concomitant medications. Successful completion of the run-in period also required presence of abdominal pain on at least 2 days, associated with at least 2 of the following: improvement with defecation, onset associated with a change in frequency of stool, and onset associated with a change in the form or appearance of the stool. Potential subjects with diagnosed gastrointestinal disease other than IBS, prior abdominal surgery or systemic disease with the potential to confound study results or compromise safety, life expectancy less than 6 months, pregnancy or breastfeeding, lactose intolerance, immunodeficiency, eating disorder, recent use of antibiotics, allergy to the study product, or daily consumption of probiotics, fermented milk, or yogurt were excluded. Following successful completion of the run-in period, 113 subjects were randomised in a 2:1 ratio to active study product or placebo.

Subjects returned to the study site at 6 week intervals for a total of 12 study weeks. At each visit, subjects completed two questionnaires, the IBS-SSS (Symptom Severity Scale) and the IBS-QOL (which includes an overall score and assessment of QOL in eight validated domains: dysphoria, interference with activity, body image, health worry, food avoidance, social reaction, sexual, and relationship) (http://depts.washington.edu/seaqol/docs/IBS-QOL_Info.pdf). Subjects were questioned at each visit as to whether they had had adequate relief of their IBS symptoms. Subjects continued to record stool consistency and frequency, symptom severity, IP consumption, and concomitant medications in diaries, which were collected at each visit and reviewed for legibility and completeness. Returned IP was counted to evaluate compliance, and new IP was issued at Visit 3. Subjects were questioned about any adverse events (AEs) noted in the diary to determine onset and recovery dates and severity. Reported AEs were subsequently classified as to relationship to IP (related, possibly related, unlikely to be related, not related) by the investigator.

Study endpoints

Study endpoints included change in abdominal pain score, distention score, days with pain, score improvements on the IBS-SSS and IBS-QOL (including the QOL domains), and AR. Changes in stool frequency and stool consistency over the study period were examined within IBS subtypes and within subgroups of IBS subtype and gender. Safety endpoints were the incidence, severity, and relationship of IP to reported adverse events.

Study populations

A modified intent-to-treat (mITT) population was defined as subjects who were randomised and received at least one dose of IP; this population was used for the efficacy analysis and the safety analysis.

Data management

Data were collected on hard-copy source documents at the study sites and entered into a web-based relational database. On-site monitoring of 100% of clinical data fields against the source document was completed by clinical research associates; queries were generated as needed for resolution by site clinical teams. After all the data had been entered and all queries resolved, the database was hard-locked for analysis. Data files were then extracted by the study biostatistician and the subject ID numbers were matched with their treatment assignments to unblind the study.

Statistical analysis

The number of subjects screened, number randomised, number withdrawn early, and number completed were tabulated by treatment group. The mITT population as a whole was analysed for symptom endpoints and QOL endpoints, along with subpopulations of IBS subtype and gender. Changes in stool consistency and frequency were analysed for the IBS-C and IBS-D subtypes and by gender within subtype. Descriptive statistics were computed for baseline and demographic characteristics and tabulated by treatment group. Descriptive statistics included means, standard deviations, medians, ranges, and percentages, as dictated by the form of each variable. Inferential methods were not applied to baseline characteristics. Compliance was calculated as percent of intended IP used, determined by returned bottle counts and subject diaries at weeks 6 and 12, and compared across groups. Compliance was also defined as intake of 70% or more of intended IP, and analysed using the chi-square test.

Change in scores between Visit 2 and Visit 4 in the two treatment groups for the IBS-SSS, the IBS-QOL overall and domains, pain severity, days with pain in the last 10

days, distention severity, satisfaction with bowel habit, and interference of IBS with life in general were analysed. Stool consistency scores were assigned by subjects using the BSC and recorded in their subject diaries on a daily basis, and daily stool frequency was determined from the number of stools entered in the diary. Changes in median stool consistency and stool frequency during the 7 day run-in period vs the last 7 days on study were compared. Stool consistency scores were expressed as median BSC scores per week, while stool frequency was expressed as median number of stools per day.

Data analysis revealed that the efficacy endpoints had to be evaluated within subtypes of IBS and for each gender separately, and many of the subgroup sample sizes were small. A large placebo effect was noted for many endpoints. We therefore elected to control the placebo effect by comparing change in the active vs placebo groups; the mean improvement from Visit 2 to Visit 4 was calculated for each treatment group, and the placebo value was then subtracted from the active value, divided by the placebo value, and multiplied by 100. For example, a mean change in pain severity of 15.0 in the active group vs a mean change of 10.0 in the placebo group was reported as 50% improvement of active over placebo. This approach was used for comparing changes in the IBS-SSS, IBS-QOL and domains, pain severity, days with pain, distention severity, satisfaction with bowel habit, and interference of IBS with life in general. The same method was used to compare changes in stool consistency and frequency.

Analysis of change in stool consistency and frequency was carried out in subjects in the IBS-C and IBS-D subtypes and for male and female subjects within those subtypes. Within each subtype, 'improvement' percentage was defined as the percentage change in the desirable direction for that subtype. Thus, the results tables report 'improvement' as a positive change for both subtypes, but the definition is different: for the IBS-C subtype an increase in mean BSC score (corresponding to softening of stools) and an increase in stool frequency were positive scores indicating improvement for that endpoint. For IBS-D subjects, a decrease in mean BSC score (indicating firmer stools) and a decrease in stool frequency were reported as improvement using positive numbers.

At the time the protocol was written, AR was a common primary endpoint in IBS trials, and was adopted as an endpoint for this trial. The endpoint IBS-AR had been shown to be a clinically and statistically relevant benefit in therapeutic IBS trials with alosetron (Camilleri *et al.*, 1999), cilansetron, and tegaserod (Kellow *et al.*, 2003; Tack *et al.*, 2005). The AR consists of a single question: 'Over the past week, have you had adequate relief of your IBS symptoms?'

Safety was evaluated by calculating rates of subjects with adverse events in the active and placebo groups, and comparing them descriptively. Specific categories of adverse events were tabulated descriptively. Comparisons of subjects with specific adverse events were descriptive.

3. Results

A total of 113 subjects were enrolled, of which 86 subjects (76.1%) completed study. Completion rates were 73.0% in the placebo group and 77.6% in the active group. Reasons for early discontinuation included loss to follow-up (10.6%), withdrawal of consent (7.1%), and other/unknown (6.1%). No subjects withdrew due to an adverse event.

Demographics and baseline subject characteristics

The distribution of demographic and baseline characteristics of the mITT population are presented in Table 1. The placebo and active groups were comparable in age, gender, and race.

Table 1. Demographics and baseline subject characteristics at screening visit, by treatment group, mITT population.

	Placebo	Active
Age (years)		
Mean	39.9	40.6
Standard deviation	14.0	13.4
n	37	76
Sex (#, %)		
Male	16 (43.2%)	29 (38.2%)
Female	21 (56.8%)	47 (61.8%)
n	37	76
Race/ethnicity (#, %)		
Caucasian, non-Hispanic	14 (37.8%)	31 (40.8%)
Asian	2 (5.4%)	3 (3.9%)
Hispanic	7 (18.9%)	15 (19.7%)
Native American	1 (2.7%)	0
African-American or Black	13 (35.1%)	25 (32.9%)
Other	0	2 (2.6%)

Distribution of irritable bowel syndrome subtypes

The 113 patients were classified by the investigators at each site as IBS-C, IBS-D, or IBS-M based on their symptoms and history at study entry. The distribution of subjects in the three subtypes varied by clinical site, as shown in Table 2.

Compliance

Subjects in the placebo group consumed $87.0 \pm 17.8\%$ of intended dose, while in the active group consumption was $77.3 \pm 19.9\%$. Based on the protocol, consumption of at least 70% of intended IP, 84.4% of subjects in the placebo group and 87.3% of subjects in the active group were defined as compliant.

IBS symptom severity scale

The IBS-SSS consists of questions on severity of abdominal pain, number of days with pain in the last 10 days, severity of abdominal distention, satisfaction with bowel habit, and extent to which IBS interferes with the subject's life in general. All these except days of pain were scored on a Likert scale with a range of 0-100. When the overall score was computed, no mean improvement of 30% or more favouring the active groups was demonstrated.

In no subgroup of patients did the change in severity of abdominal pain reach 30% for the active vs the placebo arm. However, clinical improvement was seen in many subgroups for the individual symptoms making up the IBS-SSS. Table 3 indicates that the highest percentage of improvement in the score of the IBS-SSS questions was seen in the IBS-D subtype, particularly in females, in whom improvement percentages varied from 50 to 144% in favour of the active treatment. Males in the diarrhoea subtype showed a smaller improvement in 'satisfaction with bowel habit' (43%), and 'interference with activity' (39%). Advantage in the IBS-C subtype was shown in 'days with pain' in females (42%), and in 'satisfaction with bowel habit' in both males and females (30 and 33%, respectively).

Table 2. Number and percentage of subjects in each irritable bowel syndrome (IBS) subtype¹ by investigational site, mITT population.

Site	IBS-C	IBS-D	IBS-M	Total
Garden Grove	12 (75.0%)	1 (6.3%)	3 (18.6%)	16 (100.0%)
San Francisco	12 (23.5%)	22 (43.1%)	18 (34.6%)	52 (100.0%)
Westlake	16 (35.6%)	29 (64.4%)	0	45 (100.0%)
Total	40 (35.7%)	52 (46.4%)	21 (18.6%)	113 (100.0%)

¹ IBS subtypes: -D = diarrhoea; -C = constipation; -M = mixed type.

Table 3. Summary of individual IBS-SSS questions that showed mean differences of 30% or more in favour of active treatment, mITT population.

Endpoint Symptoms	Group ¹	Improvement in score, V2 to V4 (mean ± standard deviation (n))		Improvement active vs placebo (%) ²
		Placebo	Active	
Days/pain	IBS-M ♀♂	1.00±1.00 (3)	2.25±4.17 (8)	125%
	IBS-C ♀	2.14±2.97 (7)	3.03±3.74 (14)	42%
Distention severity	IBS-D ♀♂	11.31±21.27 (13)	21.33±23.82 (27)	89%
	IBS-D ♀	16.56±15.54 (9)	24.83±24.65 (18)	50%
Satisfaction with bowel habit	IBS-C ♀♂	23.27±20.37 (11)	30.71±24.12 (21)	32%
	IBS-C ♀	24.71±25.34 (7)	32.21±24.20 (14)	30%
	IBS-C ♂	20.75±9.25 (4)	27.71±25.57 (7)	33%
	IBS-D ♀♂	23.00±19.77 (13)	37.82±30.95 (27)	64%
	IBS-D ♀	21.44±29.50 (9)	37.72±35.81 (18)	76%
	IBS-D ♂	26.50±31.10 (4)	38.00±19.58 (9)	43%
	All females	24.61±25.86 (18)	35.92±29.66 (40)	46%
	Interfering with life	IBS-D ♀♂	16.00±21.67 (11)	32.81±26.91 (26)
	IBS-D ♀	14.38±20.89 (8)	35.18±30.58 (17)	144%
	IBS-D ♂	20.33±27.97 (3)	28.33±18.91 (9)	39%
	IBS-M ♀♂	13.00±33.20 (4)	22.80±23.64 (10)	75%
	All females	16.88±25.85 (17)	26.49±30.73 (40)	57%

¹ Irritable bowel syndrome (IBS) subtype groups: -D = diarrhoea; -C = constipation; -M = mixed type.

² In many of the subgroups the percentage by which active treatment outperformed placebo on individual questions was considerably above our defined threshold of 30%.

IBS quality of life overall scores

Overall scores on the IBS-QOL were examined for the total population, within IBS-C and IBS-D subtypes, and genders, and by gender within subtype. 85 subjects were evaluable for change in overall IBS-QOL score. The percentage improvement in the active vs placebo groups (Table 4) was comparable to the results obtained in IBS-SSS, with positive responses concentrated in the IBS-D subtype and

in females. In males of the IBS-D subtype a lower degree of improvement (38%) for the active was seen.

IBS quality of life domain scores

A therapeutic effect of active IP over placebo was demonstrated in female subjects for overall QOL scores (Table 4) and in each of the eight domains (Table 5). The effect in the female subgroup was observed in both the IBS-C and IBS-D subtypes. In the male IBS-D subgroup a

Table 4. Summary of improvement in overall quality of life (QOL) score that showed mean differences of 30% or more in favour of active treatment, mITT population.

Group	Subgroup (n) ¹	Improvement in score, V2 to V4 (mean ± standard deviation (n))		Improvement, active vs placebo (%)
		Placebo	Active	
Females and males	All (85)	18.44±23.15 (27)	24.01±22.39 (58)	30%
	IBS-D (37)	15.44±13.10 (11)	25.40±23.47 (26)	65%
Females	All (55)	11.03±18.86 (17)	21.40±20.72 (38)	94%
	IBS-C (20)	10.29±27.84 (7)	20.70±18.72 (13)	101%
	IBS-D (25)	12.78±11.61 (8)	22.40±24.78 (17)	75%
Males	IBS-D (12)	22.55±16.70 (3)	31.05±20.94 (9)	38%

¹ Irritable bowel syndrome (IBS) subtype groups: -D = diarrhoea; -C = constipation; -M = mixed type.

Table 5. Summary for eight irritable bowel syndrome-quality of life domain scores, mITT population^{1,2}.

Domain	Subtypes and genders									
	All subjects	IBS-C	IBS-D	IBS-M	Female	Male	IBS-C female	IBS-C male	IBS-D female	IBS-D male
Dysphoria	33.6	32.2	48.0	3.1	62.8	6.2	139.9	-10.8	39.5	66.7
Interference with activity	42.9	27.8	78.1	8.9	208.4	-9.4	1,704.9	-28.4	84.2	59.4
Body image	18.5	-17.7	95.5	-8.6	67.0	-9.4	18.4	-22.2	166.7	27.8
Health worry	45.2	19.1	112.2	46.6	80.3	19.1	32.8	11.1	131.8	19.1
Food avoidance	3.5	-29.9	73.5	37.2	82.5	-33.0	-25.2	-33.7	641.9	-31.4
Social reaction	36.4	65.3	29.5	-15.1	112.6	-7.3	2,331.5	-9.3	25.0	33.6
Sexual	35.7	35.0	13.4	583.9	88.8	1.9	59.3	-9.3	16.5	0
Relationship	29.1	2.5	68.4	51.0	69.9	4.2	91.6	-21.8	41.7	133.1

¹ Irritable bowel syndrome (IBS) subtype groups: -D = diarrhoea; -C = constipation; -M = mixed type.

² A negative number indicates that improvement was greater in the placebo group than in the active group.

therapeutic effect was seen for overall QOL score and in four domains.

Adequate relief

For the study population as a whole there was no difference between the two study groups with respect to AR of IBS symptoms at Visits 2, 3, and 4. A strong placebo effect was noted. We additionally analysed data from each of the IBS subtypes to discover whether there were any differences in AR of IBS within the subtypes at any study visit. No differences were found between the two study groups in any of the three IBS subtypes. Analysis of subgroups of males and females, and subgroups by gender within each of the 3 IBS subtypes, yielded similar results.

Stool consistency

In the analysis of stool consistency, a positive change ('improvement') indicates increased BSC score in IBS-C

and decreased BSC score in IBS-D. Table 6 shows percent change, active vs placebo, for subgroups with active changes of 30% or more over placebo.

Median stool consistency improved for both the placebo and active treatment groups. Median changes in the placebo group were typically about one BSC scale point, with a range from 0.88 to 1.50, and about 1.75 BSC scale points in the active group, with a range from 1.17 to 1.88. Percent changes echoed those seen in endpoints presented earlier: males and females in the Active IBS-D subtype gave the largest response compared to placebo. For males in the IBS-C subtype there was an advantage of active over placebo, but this was not seen for the IBS-C group overall, nor for females with IBS-C. The largest differences between the treatment groups were seen in the IBS-D subtype, in both males and females. The male subgroup and the subgroup of males with IBS-C also showed improvement in stool consistency vs placebo.

Table 6. Subgroups that showed mean differences of 30% or more for improvement in stool consistency (Bristol Stool Chart), mITT population.

IBS subtypes and genders ¹	Improvement in score, V2 to V4 (Mean ± standard deviation (n))		Improvement, active vs placebo (%)
	Placebo	Active	
IBS-C ♂	1.06±1.01 (4)	1.81±1.16 (8)	71%
IBS-D ♀♂	0.93±1.37 (7)	1.78±1.42 (20)	91%
IBS-D ♀	0.95±1.68 (5)	1.88±1.58 (13)	98%
IBS-D ♂	0.88± 0.18 (2)	1.57±1.13 (7)	78%

¹ Irritable bowel syndrome (IBS) subtype groups: -D = diarrhoea; -C = constipation.

Stool frequency

In the analysis of stool frequency, a positive change ('improvement') indicates increased frequency in IBS-C and decreased frequency in IBS-D. In both the placebo and active groups, stool frequency improved in both the IBS-C and IBS-D subtypes, with subjects in the IBS-C subtype having more frequent stools during their last week on study than during the run-in period, while subjects in the IBS-D subtype reported a decrease in stool frequency over that period. Table 7 shows the IBS subtypes and subgroups in which active outperformed placebo for stool frequency improvement by 30% or more.

Site-specific effects

The Garden Grove clinical site had a particularly interesting subgroup of subjects: among the 16 subjects treated at Garden Grove, 12 were females with severe chronic constipation refractory to treatment. In this subgroup, mean daily stool frequency (an important endpoint for IBS-C; USDHHS, FDA, CDER, 2012) increased on the average 0.25 stools/day in the placebo group and 0.75 stools per day in the active subgroup, a 200% percentage increase for active vs placebo. It is also noteworthy that this clinical site the subjects randomised to active treatment had fewer mean stools per week at baseline than subjects in the placebo

group (0.38 vs 0.75 stools/day), making the greater stool increase in the active group.

Safety

A total of 7 subjects reported one or more AEs while on study; 3 of these subjects were in the placebo group and 4 were in the active group. A total of 14 AEs were reported by the 7 subjects; all were mild or moderate in severity except for severe cramping, reported by one subject in the active group. Four events were judged by the investigator to be probably related to study product: dry mouth with increased thirst, increased respiration, nausea, and fatigue. These events were all reported by one subject in the placebo group; the dry mouth and increased thirst persisted throughout the study period but resolved the day before the subject's last study visit. No AEs were judged to be definitely related to study product, and there were no serious AEs.

4. Discussion

Discovery of an effective treatment for IBS has been the goal of drug and probiotic studies in recent years. While the subject populations of many probiotic studies have been small, several meta-analyses have been published, and certain probiotic species and strains have been shown to be more effective than others in mitigation of IBS symptoms

Table 7. Subgroups that showed mean differences of 30% or more for improvement in stool frequency per day, mITT population.

Subtype ¹	Improvement in score, V2 to V4 (mean ± standard deviation (n))		Improvement, active vs placebo (%)
	Placebo	Active	
IBS-C ♀♂	0.27±0.65 (11)	0.77±0.81 (22)	185%
IBS-C ♀	0.29±0.49 (7)	0.57±0.76 (14)	97%
IBS-C ♂	0.25±0.96 (4)	1.13±0.83 (8)	352%
IBS-D ♀♂	0.57±2.28 (7)	1.45± 1.76 (20)	154%
IBS-D ♀	1.20± (5)	1.62±2.02 (13)	35%
IBS-D ♂	-1.00± no SD (2)	1.14± 1.21 (7)	214%

¹ Irritable bowel syndrome (IBS) subtype groups: -D = diarrhoea; -C = constipation.

Table 8. Summary of improvement in daily stool frequency by visit and treatment group, garden grove site with irritable bowel syndrome subtype constipation, mITT population.

Stools/day	Visit 2		Visit 4		Improvement (visit 2 to visit 4)		Improvement, active vs placebo (%)
	Placebo	Active	Placebo	Active	Placebo	Active	
Mean	0.75	0.38	1	1.13	0.25	0.75	200%
Standard deviation	0.5	0.74	0	0.64	0.5	0.89	
n	4	8	4	8	4	8	

(McFarland and Dublin, 2008; Ortiz-Lucas *et al.*, 2013). The study of IBS is complicated by the fact that patients frequently demonstrate a psychological profile of anxiety and depression (Ford *et al.*, 2014a); these psychological effects may be exacerbated by the absence of effective treatment and public perception of IBS as a non-serious condition. The effects of race, ethnicity, diet and culture must also be considered in the assessment of treatment (Fava *et al.*, 2013; Hughes, 2012).

In this study the effect of combined *L. acidophilus* CL1285, *L. casei* LBC80R and *L. rhamnosus* CLR2 was evaluated on symptoms of IBS and quality of life in 3 IBS subtypes: IBS-C, IBS-D, and IBS-M. As has been reported in many studies, the placebo product used in this study produced improvements of IBS symptoms, reaching the level of a therapeutic effect such as described in the FDA guidance (USDHHS, FDA, CDER, 2012). In line with this guidance, an improvement of 30% of the active product over placebo was defined as a significant increased therapeutic value for the study endpoints change in QOL (overall and domains) and changes in abdominal pain, abdominal distention, days with pain, satisfaction with bowel habit, and interference with life in general. The strains in the active product were effective vs placebo in simultaneously relieving clinical symptoms of IBS-C and IBS-D to varying degrees in both males and females with the exception of abdominal pain, in which the active product did not show greater therapeutic effect than placebo. Stool frequency was improved in both subtypes; stool consistency, as measured by the BSC, improved in male and female subjects with IBS-D and in male subjects in the IBS-C subtype. These endpoints are currently those recommended by regulatory agencies in United States and Europe to demonstrate efficacy in drug trials involving patients with IBS-C and IBS-D. These results show that these symptomatic benefits mirrored parallel trends in the IBS-QOL measure developed specifically for IBS (Drossman *et al.*, 2000).

Female subjects, particularly of the IBS-D subtype, had a good response to the active product in terms of stool frequency and consistency, and were the most responsive in terms of improvement in symptoms and QOL. While male response was also good in terms of stool frequency and consistency, the response to active product over placebo was less striking than in the female subgroup. The symptomatic response observed in both IBS-C and IBS-D subtypes suggests that our three *Lactobacillus* sp. strains are effective in relieving symptoms and improving QOL in this indication.

Probiotic products containing lactobacilli and bifidobacteria, have been preferentially used to improve IBS (Niv *et al.*, 2005; O'Mahony *et al.*, 2005). Positive results have been noted with some *Lactobacillus* sp. strains, for example by Ducrotté *et al.* (2012), who reported resolution of all IBS-

dominant symptoms, including abdominal pain, in 214 patients treated for 4 weeks with *Lactobacillus plantarum* 299V. Halpern *et al.* noted a significant reduction in an IBS symptoms index with a capsule containing 5×10^9 heat-killed *L. acidophilus* (Halpern *et al.*, 1996). Other *Lactobacillus* strains, such as *L. salivarius* UCC4331 did not show any therapeutic gain over placebo in 75 patients (O'Mahony *et al.*, 2005), nor did *Lactobacillus reuteri* ATCC55730 (Niv *et al.*, 2005), suggesting that some strains of *Lactobacillus* sp. may be more effective than others in this indication. While some published studies have included laboratory assessments of changes in microbiota, these were beyond the scope of the current study (Aagaard *et al.*, 2013; Somberg, 2012).

The safety profile of the product used in this study has been documented in previous clinical trials (Beausoleil *et al.*, 2007; Gao *et al.*, 2010; Sampalis *et al.*, 2010) and a quality improvement study (Maziade *et al.*, 2015). The mechanism of action of the study product has been demonstrated in some intestinal pathology, but was not investigated in the present study (Auclair *et al.*, 2015). Interestingly, the therapeutic gains observed with our three *Lactobacillus* strains over placebo and observed in other probiotic studies (Mezzasalma *et al.*, 2016; Yoon *et al.*, 2015) surpass those seen in drug studies, which are not free of significant adverse events (Cremonini *et al.*, 2003; Kellow *et al.*, 2003; Tack *et al.*, 2005). Approved drugs have shown worse safety profiles than probiotic regimens, which have demonstrated an advantageous safety profile.

It is of interest to note that few studies have evaluated the effects of probiotics on QOL, and of those that did, many did not find a significant improvement (Halpern *et al.*, 1996; Kellow *et al.*, 2003; Kim *et al.*, 2003; Moayyedi *et al.*, 2010; Niv *et al.*, 2005). A few studies showed improvement in some domains (Guglielmetti *et al.*, 2011; Kajander *et al.*, 2008; Lorenzo-Zúñiga *et al.*, 2014; O'Mahony *et al.*, 2005), but to our knowledge no effect on the 'interference with activity' domain has been previously documented. O'Mahony *et al.* (2005) found lower IBS-QOL scores for *L. salivarius* and *Bifidobacterium infantis* for most domains.

This study provides evidence of therapeutic effects in specific IBS subtypes and subgroups which were seen consistently for different endpoints: stool frequency and consistency, quality of life, improvement in distention severity, days with pain, and satisfaction with bowel habit. Our findings are in agreement with other studies conducted with probiotics and medications, and further studies investigating changes in the intestinal microbiota in IBS associated with our probiotic treatment are needed (Somberg, 2012).

A low incidence of AEs has been observed in previous studies conducted with the study product. This protocol was

based on previous clinical studies conducted in Canada and United States, with the optimal dosage of the product. The study product has also been previously evaluated in adults for antibiotic-associated diarrhoea and *Clostridium difficile* prevention, demonstrating a large reduction in diarrhoea risk during a *C. difficile* outbreak in China (Gao *et al.*, 2010). In the last decade of clinical research involving the study product, there have been no serious adverse events (SAEs) related to the study product in any of the clinical trials (Beausoleil *et al.*, 2007; Gao *et al.*, 2010; Maziade *et al.*, 2015; Sampalis *et al.*, 2010).

There were a number of limitations to the study design. First, our assumption that all three types of IBS would respond similarly and could be analysed together was not supported by the data; there were major differences in response based not only on IBS subtype, but on gender as well. The analysis was thus carried out on small numbers of subjects within these subgroups, and statistical significance could not be expected. Second, the small number of subjects in the IBS-M subgroup greatly limited conclusions about this subtype. Third, the large placebo effect, characteristic of IBS studies, made it difficult to interpret the results. Fourth, this study did not address the mechanisms of action of the probiotic and its interface with the microbiome, which has become a point of interest.

5. Conclusions

The probiotic combination used in this study produced results which varied between genders and subtypes, but its impact on stool consistency and frequency, quality of life, and IBS symptoms in both genders, without severe adverse events, presents a promising therapeutic option for subjects suffering from IBS.

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Conflict of interest

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