Received: 27 July 2023 | Revised: 7 December 2023 | Accepted: 18 January 2024

DOI: 10.1111/nmo.14751

ORIGINAL ARTICLE

Neurogastroenterology & Motility NGM WILEY

A double-blind, randomized, placebo-controlled study assessing the impact of probiotic supplementation on the symptoms of irritable bowel syndrome in females

B. H. Mullish[1] | D. R. Michael[2] | M. Dabcheva[3] | T. S. Webberley[2] | N. Coates[2] | D. A. John[2] | D. Wang[4] | Y. Luo[5] | S. F. Plummer[2] | J. R. Marchesi[1]

[1]Division of Digestive Diseases, Department of Metabolism, Digestion and Reproduction, Faculty of Medicine, Imperial College London, London, UK

[2]Cultech Limited, Port Talbot, UK

[3]Clinical Research Unit, MC Comac Medical, Sofia, Bulgaria

[4]Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK

[5]Mailman School of Public Health, Columbia University Medical Centre, New York, New York, USA

Correspondence
B. H. Mullish, Division of Digestive Diseases, Department of Metabolism, Digestion and Reproduction, Faculty of Medicine, Imperial College London, London, UK.
Email: b.mullish@imperial.ac.uk

Funding information
Cultech Ltd

Abstract

Background: A previous exploratory study demonstrated the ability of the Lab4 probiotic to alleviate the symptoms of IBS, and *post hoc* data analysis indicated greatest improvements in the female subgroup. The aim of this study is to confirm the impact of this multistrain probiotic on IBS symptom severity in females.

Methods: An 8-week, single-center, randomized, double-blinded, placebo-controlled, superiority study in 70 females with Rome IV-diagnosed irritable bowel syndrome (IBS) receiving the Lab4 probiotic (25 billion colony-forming units) daily or a matched placebo. Changes from baseline in the IBS-symptom severity score (IBS-SSS), daily bowel habits, anxiety, depression, IBS-related control, and avoidance behavior, executive function, and the fecal microbiota composition were assessed. The study was prospectively registered: ISRCTN 14866272 (registration date 20/07/22).

Key Results: At the end of the study, there were significant between-group reductions in IBS-SSS (−85.0, $p < 0.0001$), anxiety and depression scores (−1.9, $p = 0.0002$ and −2.4, $p < 0.0001$, respectively), and the IBS-related control and avoidance behavior score (−7.5, $p = 0.0002$), all favoring the probiotic group. A higher proportion of the participants in the probiotic group had normal stool form ($p = 0.0106$) and/or fewer defecations with loose stool form ($p = 0.0311$). There was little impact on the overall diversity of the fecal microbiota but there were significant differences in *Roseburia*, *Holdemanella*, *Blautia*, *Agathobacter*, *Ruminococcus*, *Prevotella*, *Bacteroides*, and *Anaerostipes* between the probiotic and placebo groups at the end of the study.

Conclusions & Inferences: Daily supplementation with this probiotic may represent an option to be considered in the management of IBS.

KEYWORDS
bowel habit, IBS, microbiota, probiotic

1 | INTRODUCTION

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by abdominal pain, bloating, and altered bowel habits and is associated with psychological comorbidities such as anxiety and depression.[1] The prevalence of IBS in the USA, Canada, and the UK is around 4.5% according to the Rome IV guidelines and there is a notably higher incidence in females compared to men.[2] The multifactorial nature of IBS impacts upon the quality of life of people with IBS which imparts a substantial economic burden on healthcare systems at an estimated annual cost to the National Health Service (NHS) in the UK of £1–2 billion.[3]

The precise etiology of IBS remains elusive, but it is becoming increasingly evident that a combination of factors such as visceral hypersensitivity, loss of gut barrier function, gut motility disturbances, and low-grade inflammation are likely to contribute.[4] It is recognized that the gut microbiota plays a role during health and disease and differences in microbial composition, particularly lower abundances of lactobacilli and bifidobacteria, have been observed in the gut microbiome of people with IBS compared to healthy people.[5–7] These differences have focused attention on the potential of microbiome manipulation to be included in the management of the symptoms of IBS, with one such major approach of interest being the use of probiotic supplementation to contribute to the alleviation of the symptoms of IBS.[8–10]

Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host".[11] There is accumulating evidence supporting the ability of probiotic organisms to reduce IBS severity score and improve both bowel habits and quality of life[9,10] but there are inconsistencies in the outcomes due to different organisms and doses, underpowered studies,[12] placebo effects[13] and the variable nature of IBS symptomology.[14] This has contributed to the lack of consensus on the benefits of probiotic supplementation in sufferers of IBS. Current clinical guidelines for the management of IBS do not recommend the use of probiotics.[12]

In our previous exploratory probiotic study we demonstrated that a combination of lactobacilli and bifidobacteria could alleviate gastrointestinal discomfort and improve bowel habits in subjects with Rome III diagnosed IBS[15] suggesting a promising role for the probiotic in the management of IBS. A *post hoc* analysis of the study data identified the greatest improvement in 18–40-year-old females and selected that population for the current placebo-controlled, randomized, double-blind, intervention study. The aims of the study were to (i) confirm the ability of the probiotic to alleviate gastrointestinal symptom severity and improve bowel habits, (ii) investigate the impact on anxiety, depression, quality of life and executive function, and (iii) assess the fecal microbiota for changes in composition.

Key points

- A previous post hoc analysis had suggested irritable bowel syndrome (IBS) symptom benefit, especially in females, in association with the use of the Lab4 probiotic. The aim of this present study was to explore the potential therapeutic role of Lab4 in a randomized, double-blind, placebo-controlled trial setting.
- In 70 females with Rome IV-diagnosed IBS, randomized 1:1 to Lab4P probiotic or placebo for 8 weeks, there were significant between-group changes favoring the Lab4 group in several clinically relevant domains, including a reduction in IBS-symptom severity score, and anxiety and depression scores. The proportion of females reaching a reduction in IBS-SSS ≥ 50 points also favored the Lab4 group.
- Lab4-treated females had little overall change to gut microbiota diversity, but did have changes in a number of stool bacterial taxa.
- Lab4P was well-tolerated, with no serious adverse events of note associated with its use.

2 | METHODS

2.1 | Study approval

The study was conducted by Comac Medical (Sofia, Bulgaria) in accordance with the ethical principles of the Declaration of Helsinki. Ethical approval was granted by the Ethical Committee of Comac Medical (Reference: #245/13.07.2022). The study protocol was registered with the International Standard Randomized Controlled Trial Number (ISRCTN) registry on July 20, 2022: ISRCTN14866272.

2.2 | Study design

A single-center, double-blind, randomized, placebo-controlled superiority study with the equal allocation of participants between two parallel groups. The sample size calculation was based on the changes after 8 weeks of intervention in IBS-Symptom Severity Score (IBS-SSS) observed among 18- to 40-year-old females from a previous IBS probiotic study (Figure S1).[15] Twenty-seven participants per group were required to detect an 80-point reduction in IBS-SSS (standard deviation of 98) using a Type I error of 0.05 and a power of 85%. Groups of 35 were included to account for potential drop-outs.

2.3 | Recruitment, eligibility and consent

Females (aged 18–40) diagnosed with IBS (according to the Rome IV criteria, any subtype) were identified by General Practitioner (GP) based on medical records, informed about the study by phone in July 2022, and offered the opportunity to take part in the study by attending the Comac Medical trials facility in August 2022. The inclusion criteria were: no other diagnosed gastrointestinal disorders/conditions nor had any recent abdominal surgery, normal/corrected-to-normal vision without color blindness (in order to complete the executive function task), willing to maintain a normal diet and lifestyle throughout the study, and willing to provide fecal and blood samples. Exclusion criteria were: diagnosed diabetes, arrhythmia, ventricular extrasystole, atrioventricular block, cardiovascular disease, or severe systemic disease, for example, cancer, dementia, advanced organ failure, immunodeficiency or undergoing immunosuppressive therapy, pregnancy or planning pregnancy, any unexplained loss of weight in recent months, taken probiotics regularly during the 30-days prior to the study, or taken oral antibiotics during the 90-days prior to the study. At enrolment, participant medical records were re-checked and participants provided informed consent before undertaking any study-related activities. Participants were compensated for their participation.

2.4 | Randomization

The eligible participants were allocated to one of the two study arms in a 1:1 ratio according to a computer-generated random sequence (block size of four) that was generated using SAS PROC PLAN (SAS v9.4). The allocation sequence was not available to any member of the research team until all databases had been completed and locked. Tamper-proof sealed envelopes containing the participant allocation sequence were held at the trial site.

2.5 | Study product

The probiotic (Lab4) comprised white capsules containing a total of 25 billion colony-forming units (cfu) of *Lactobacillus acidophilus* CUL60 (National Collection of Industrial, Food and Marine Bacteria [NCIMB] 30,157), *Lactobacillus acidophilus* CUL21 (NCIMB 30156), *Bifidobacterium bifidum* CUL20 (NCIMB 30153) and *Bifidobacterium animalis* subsp. *lactis* CUL34 (NCIMB 30172) and microcrystalline cellulose. The placebo capsules contained microcrystalline cellulose and were identical in appearance, size, and weight to the active product. The active and placebo products were prepared by Cultech Ltd, Port Talbot, UK, and were packaged into induction-sealed high-density polyethylene pots.

2.6 | Intervention

One capsule was taken daily for 56 days. Participants were instructed to take the capsules with food, avoid hot drinks at the time of ingestion, and store the intervention in a refrigerator. Participants returned any unused capsules for compliance monitoring.

2.7 | Outcomes

The primary study outcome was a change in IBS-SSS. Secondary outcomes were changes in bowel habit, quality of life (anxiety and depression, avoidance and control behaviors, and general well-being), cognitive health (executive function), plasma biomarkers (plasma interleukin-6 concentration), and the composition of the fecal microbiota. The schedule of participant visits to the trial center and data and sample collection is shown in Figure 1A. Participants received paper copies of all study questionnaires to take home on the day 0 visit. Participants completed baseline (day 0) and endpoint (day 56) questionnaires and the executive function test at the trial center.

2.8 | Stool sampling and collection

At the start and end of the study, the participants provided stool samples using the Fe-Col® Fecal Sample Collection kits (Alpha Laboratories, Hampshire, UK) in accordance with the manufacturer's instructions and stored them in anaerobic Genbags (Sigma Aldrich, UK) under refrigeration for up to 48 h prior to transfer to the trial center for storage at −80°C pending analysis.

2.8.1 | Fecal calprotectin analysis

Baseline stools were sampled using Bühlmann Calex® Caps (Alpha laboratories, Hampshire, UK) and assayed using the Bühlmann fCal® ELISA kit (EK-CAL version A2; Alpha Laboratories, Hampshire, UK) according to the manufacturer's instructions. The manufacturer reported cut-off value for the detection of active gastrointestinal inflammation is >160 μg/g.

2.9 | Anthropometric measurements

On day 0 and day 56, body weights were measured using a calibrated column scale (Seca 709, Hamburg, Germany) after the removal of shoes and jackets. The blood pressure of seated participants after 5 min rest was measured using a calibrated blood pressure monitor (Omron, Kyoto, Japan). Shoes were removed before height measurement. Efforts were made to standardized the time of day at which measurements were taken for each participant.

2.10 | IBS symptom severity scores

This was measured on days 0, 14, 28, 42, and 56 using the IBS-SSS questionnaire[16] (Figure S2). The IBS-SSS was based on abdominal

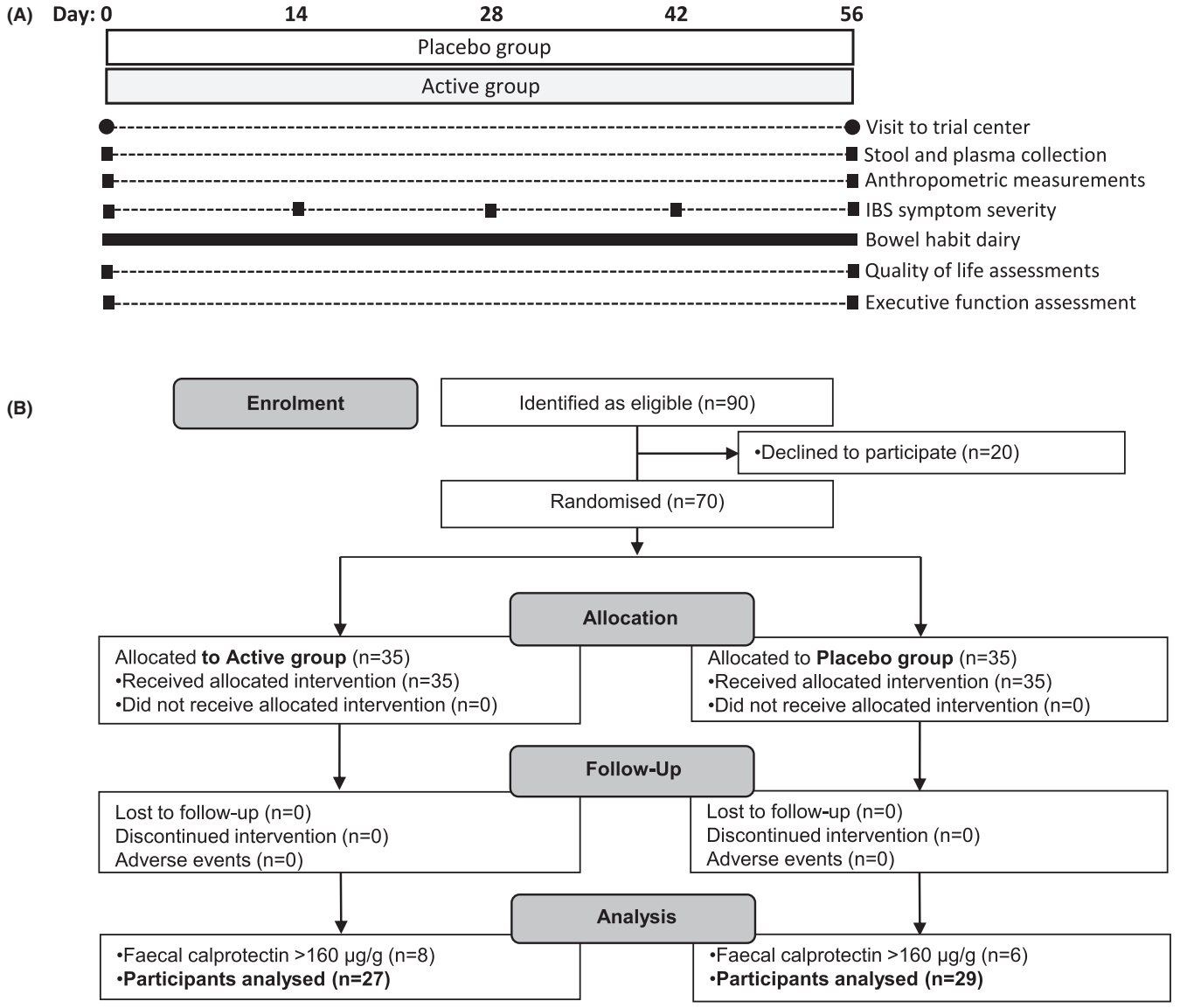

FIGURE 1 (A) Study design and scheme of data/sample collection and (B) flow diagram of the study recruitment.

pain, bloating severity, dissatisfaction with bowel habits, impact on daily life, and days with pain giving a maximum score of 500. Scores of <75 were considered to indicate no IBS symptoms whereas 75–174 indicated mild IBS, 175–299 indicated moderate IBS, and >300 indicated severe IBS. "IBS-SSS responders" were defined as those participants achieving a ≥50-point reduction by the end of the study.[17]

2.11 | Plasma interleukin-6 concentration

Twelve-hour fasted bloods were collected on days 0 and 56 by venepuncture into EDTA vacutainers and the plasma was immediately separated by centrifugation (2000 ***g***, 10 min), aliquoted, and stored at −80°C until required. Plasma levels of interleukin (IL)-6 were quantified using the human IL-6 immunoassay (ab46042; Abcam, Cambridge, UK) in accordance with the manufacturer's instructions.

2.12 | Bowel habits

Monitored using an adapted version of a recognized daily bowel habit diary[18] (Figure S3). Participants recorded (i) the date of attempted defecation, (ii) whether a stool was passed, (iii) whether there was an urgent need to defecate, (iv) whether there was straining to start the defecation, (v) whether there was a feeling of incomplete evacuation, (vi) the Bristol Stool Form Score (BSFS) rating, (vii) whether the stool was provided as a sample and (viii) whether the defecation occurred at the time of antibiotic usage. BSFS scores were grouped into three categories: 1 and 2 indicated hard stool form, 3–5 indicated normal stool form, and 6 and 7 indicated loose stool form.

2.13 | Quality of life

a. *Anxiety and depression*. This was measured on days 0 and 56 using the Hospital Anxiety and Depression Scale (HADS) questionnaire[19] comprising 7 questions related to anxiety (HADS-A) and 7 questions relating to depression (HADS-D, see Figure S4). Each item is scored on a Likert scale between 0 and 3 giving a maximum score of 21 per subscale.
b. *Avoidance and control behavior*. This was measured using the IBS behavioral responses questionnaire (IBS-BRQ)[20] on days 0 and 56. The questionnaire comprises 26 items each scored on a Likert scale from 1 (never) to 7 (always) to give a maximum score of 182 (Figure S5).
c. *General well-being*. This was measured on days 0 and 56 using a modified version of a questionnaire by Grossenbacher et al.[21] assessing general well-being, state of health, state of mood, state of energy, and sleep quality using a visual analog scale of 0 (very poor) to 10 (Very good).

2.14 | Executive function

This was measured on days 0 and 56 using an adaptation of the Stroop Color Word Test (SCWT). Participants were presented with a series of written colors (orange, pink, blue, green, or yellow text) that were congruent or incongruent with text color (orange, pink, blue, green or yellow) and asked to correctly identify the text color (by pressing a button mapped to that color) while ignoring the written word for as many words as possible within 1 min. Outcome measurements were the total number of answers, latency to correct answer, and accuracy (percentage of correct answers). The sequence of word/color combinations was the same for each participant but differed on days 0 and 56. The test was performed on Apple iPads (6th Generation, (Apple, California USA)) using Trialflare software (Seastorm Ltd, Cardiff, UK).

2.15 | Fecal microbiome analysis

DNA was extracted from stool samples using the QIAamp® Fast DNA Stool Mini Kit (Qiagen, Germany). Samples were homogenized in Matrix Lysing D tubes (MPBIO) and a FastPrep®-24 bead beater (MPBIO, United States). Eluted genomic DNA was quantified using a Qubit® (Thermo Fisher Scientific, United States) and stored at −20°C. Extracted DNA was sent to Novogene (Cambridge, UK) for amplification of the V3–V4 region of bacterial 16S rRNA gene using the universal primer set 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACN NGGGTATCTAAT-3′). After 16S rRNA gene library preparation and generation, libraries were sequenced using the Illumina NovaSeq platform to generate 250 bp paired-end raw reads. Paired-end raw reads were filtered, trimmed, forward and reverse reads merged and chimaeras removed using DADA2[22] to obtain amplicon sequence variants (ASVs) which were aligned against the SILVA database v138 to assign taxonomy.[23]

2.16 | Data analysis

Data analysis was performed on those participants that (i) had baseline fecal calprotectin concentrations of <160 µg/g which was selected as representing a cut-off value to differentiate IBS from other inflammatory bowel conditions and (ii) did not report oral antibiotic usage during the study (antibiotics can have a profound effect on the gut microbiota leading to gastrointestinal discomfort and changes in motility[24]). Eight participants from the placebo group and six from the probiotic group had high fecal calprotectin results (Figure S6) and no oral antibiotics usage was reported during the intervention period.

2.17 | Statistical analysis

Statistical analysis of IBS-SSS, HADS, IBS-BRQ, and anthropometric data was based on changes from baseline and was performed using a linear mixed model (LMM) that included baseline measurement of an endpoint, treatment, time, and interaction between treatment and time as fixed effects, baseline values as a covariate, and the subject as the random effect from which the treatment effect in terms of adjusted least square mean difference at each time point with 95% confidence intervals and *p*-values were calculated (SAS® version 9.4; SAS Institute Inc., Cary, NC, USA). Missing data points were inputted using the last observation carried forward (LOCF). The number of "IBS-SSS responders" per study group was compared using Fisher's exact test (GraphPad Prism, Version 9.5.0).

Bowel habit data were analyzed using a generalized linear model (GLM) that included treatment as the only predictor from which the treatment effect in terms of mean difference and incidence rate ratio with 95% CI and *p*-values were calculated (SAS® version 9.4; SAS Institute Inc., Cary, NC, USA). The SCWT data were analyzed by analysis of variance (ANOVA) with repeat measurements that included baseline measurement of an endpoint, treatment, time, and interaction between treatment and time as fixed effects and the subject as the random effect from which the treatment effect in terms of mean difference with 95% and two-sided *p*-values were calculated (GraphPad Prism, Version 9.5.0). For all analyses, values of $p < 0.05$ were considered significant.

2.18 | Statistical analysis of fecal microbiota

The R package Phyloseq[25] was used for data importation and diversity analyses and results were plotted with ggplot2.[26] Wilcoxon rank sum test was performed to compare Shannon's diversity index between interventions and time points. Spatial differences between the groups were observed with a non-metric multidimensional scaling (NMDS) plot based on Bray–Curtis dissimilarity matrix. The R package Vegan was used to perform permutational

analysis of variance (PERMANOVA) computed with 999 permutations using the Adonis function and assess the homogeneity of dispersion.[27] Differential abundance of Amplicon Sequence Variant (ASV) between interventions was analyzed using DESeq2.[28]

3 | RESULTS

A flow diagram of enrolment, allocation, follow-up, and analysis is shown in Figure 1B. Ninety females were contacted by GPs and 20 declined to participate and 70 were inducted into the study between the August 01, 2022 and August 09, 2022. The intervention period took place between August 02, 2022 and October 07, 2022; there were no drop-outs from either arm of the study. The probiotic and placebo were well tolerated with no reported serious adverse effects related to the intervention. Compliance with the intervention (calculated from the number of returned capsules) and completion of the questionnaires exceeded 98% in both arms of the study. Rates of recruitment, drop-outs, adverse events, and compliance are consistent with other probiotic intervention studies conducted by Comac Medical.[29,30] Tamper-proof sealed envelopes containing participant allocations (for emergency unblinding) remained intact over the duration of the study confirming that blinding had been preserved.

When retrospectively analyzed, eight of the women in the active group and six in the placebo group had baseline fecal calprotectin levels exceeding the 160 μg/g cut-off value and were excluded from data analysis. The baseline characteristics of the remaining participants are presented in Table 1 The values for age, BMI, blood

TABLE 1 Baseline characteristics of the studied population.

	Total	Active	Placebo
Demographics and anthropometry			
N° of participants	56	27	29
Female (%)	100	100	100
Age (years), mean (SD)	31.90 (5.84)	31.30 (5.60)	32.62 (6.34)
Height (m), mean (SD)	1.67 (0.06)	1.67 (0.07)	1.66 (0.06)
Weight (kg), mean (SD)	66.57 (12.82)	65.39 (14.19)	66.89 (13.29)
BMI (kg/m^2), mean (SD)	23.83 (4.05)	23.26 (4.46)	24.27 (4.13)
SBP (mmHg), mean (SD)	116.16 (9.08)	116.26 (10.65)	115.41 (8.49)
DBP (mmHg), mean (SD)	71.19 (4.37)	71.74 (5.07)	71.10 (3.42)
Fecal calprotectin concentration (μg/g)	54.43 (37.29)	53.26 (34.26)	55.52 (40.48)
IBS-SSS, score (SD)	262.88 (57.20)	249.68 (59.00)	275.17 (53.57)
Abdominal pain severity	62.86 (14.49)	60.37 (14.54)	65.17 (14.30)
Days with abdominal pain (%)	29.85 (11.88)	30.42 (13.23)	29.31 (10.69)
Bowel habit dissatisfaction	63.39 (14.31)	62.96 (13.25)	63.79 (15.45)
Bloating severity	51.25 (25.09)	44.44 (28.19)	57.59 (20.29)
IBS impact on everyday life	55.54 (16.94)	51.48 (17.25)	59.31 (16.02)
IBS-SSS classification, *n* (%)			
Mild	4 (7.1)	4 (14.8)	0 (0.0)
Moderate	37 (66.1)	17 (63.0)	20 (69.0)
Severe	15 (26.8)	6 (22.2)	9 (31.0)
Predominant bowel habit, *n* (%)			
Mixed bowel habit	31 (55.4)	16 (59.3)	15 (51.7)
Constipation	5 (8.9)	2 (7.4)	3 (10.3)
Diarrhea	20 (35.7)	9 (33.3)	11 (37.9)
Unclassified	0 (0)	0 (0)	0 (0)
IBS-related medication use prior to study, *n* (%)	45 (80.3)	19 (70.4)	26 (89.7)
HADS: Anxiety score, mean (SD)	8.59 (3.95)	7.41 (3.08)	10.52 (3.80)
HADS: Depression score, mean (SD)	7.36 (3.61)	6.85 (2.51)	8.55 (3.70)
IBS-BRQ score, mean (SD)	119.40 (24.96)	112.11 (24.94)	128.24 (24.14)

Note: The data represent the mean ± standard deviation (SD) of the stated number of participants (*n*) in each group.

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HADS, Hospital Anxiety and Depression Scale; IBS-BRQ, IBS Behavioral Responses Questionnaire; IBS-SSS, IBS-Symptom Severity Score; SBP, systolic blood pressure.

pressure, and distribution between IBS subtypes were similar between groups but there were more participants with higher values for IBS-SSS, HADS, and IBS-BRQ in the placebo group than in the probiotic group.

3.1 | The impact of daily supplementation on the symptoms of IBS

3.1.1 | Changes in IBS symptom severity score

Changes from baseline (day 0) in IBS-SSS are shown in Figure 2A (detailed data in Table S1). The between-group differences in severity score favoring the probiotic increased from 14.5% on day 28 (−36.10 points, $p=0.0018$) to a >80-point difference on day 56 (−84.95 points, 33.8% between-group difference, $p<0.0001$) associated with consistent active group symptom severity reduction throughout the study but little change in the placebo group. The final between group IBS-SSS differences were for: abdominal pain severity 31.7% (−19.16 points, $p<0.0001$, Figure 2B), days with abdominal pain, 44.6% (−13.59 points, $p<0.0001$, Figure 2C), bloating severity, 31.4% (Figure 2D), dissatisfaction with bowel habit 39% ($p<0.0001$, Figure 2E), and IBS impact on everyday life, 29.8% difference (−15.67 points, $p<0.0001$, Figure 2F).

The number of participants reaching a ≥50-point reduction in IBS-SSS (responders) was significantly higher in the active group at days 42 ($p<0.0001$) and 56 ($p<0.0001$, Table 2). In the active group, there was a gradual increase throughout the study; at day 14, 1 participant, day 28, 8, up to 15 participants at day 42 and reaching 17 at day 56. In contrast, the responders in the placebo were low and sporadic; 0, 4, 1, and 1 participants on days 14, 28, 42, and 56, respectively. There were no changes in plasma IL-6 levels in either group (Figure S7).

3.2 | The impact of daily supplementation on bowel habits

The daily diaries were used to monitor bowel habits and in both groups defecation rates were ~1/day and the mean BSFS was ~4 (Table 3). The proportion of participants with normal stool form (BSFS 3–5) in the probiotic group was significantly higher than in the placebo (78.04% vs. 66.54%, respectively, $p=0.0106$) whereas there were less loose stools (BSFS 6–7) in the active group (15.72% vs. 24.39% for active and placebo, $p=0.0311$); there were no significant between-group differences in hard stools (BSFS 1–2). There were trends towards fewer "incomplete" defecations (active group: 16.84%, placebo: 25.69%, $p=0.0676$) and "failures" (7.69% in active vs. 14.37% in placebo, $p=0.0525$, Table 3).

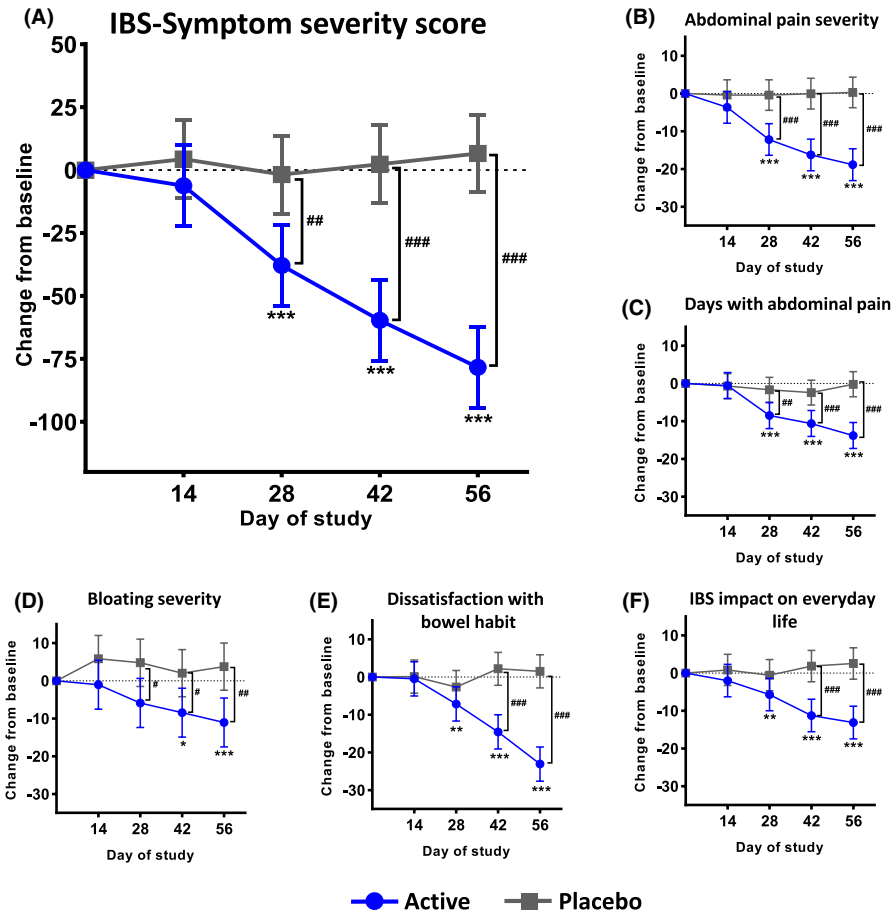

FIGURE 2 Changes from baseline in (A) IBS-Symptom severity score, (B) abdominal pain severity, (C) days with abdominal pain, (D) bloating severity, (E) dissatisfaction with bowel habit and (F) IBS impact on everyday life in the active ($n=27$) and placebo ($n=29$) groups over the duration of the study. Data are presented least square mean change with 95% CI. Values of p were determined by LMM where $^{*}p\leq0.05$, $^{**}p\leq0.01$, and $^{***}p\leq0.001$ for within-group comparisons (vs. baseline) and $^{\#}p\leq0.05$, $^{\#\#}p\leq0.01$ and $^{\#\#\#}p\leq0.001$ for between-group comparisons (active vs. placebo).

3.3 | The impact of daily supplementation on anxiety, depression, and IBS-related behavior

Anxiety was significantly reduced between groups (difference of −1.85, $p=0.0002$) favoring the probiotic group at the end of the study (Table 4A) with a 31.4% (−2.33 reduction, $p<0.0001$) decrease from baseline in the active group.

Depression did not change within the placebo group, but there was an improvement in the probiotic group with a 33.9% (−2.36, $p<0.0001$) final between-group difference (Table 4B); the active group decreased from baseline (−2.20, 32.1% reduction, $p<0.0001$).

TABLE 2 Changes in the number of IBS-SSS responders over the duration of the study.

	Active ($n=27$)	Placebo ($n=29$)
Day 14		
Responders[a], *n* (%)	1 (3.70)	0 (0)
Non-responders[b], *n* (%)	26 (96.30)	29 (100)
p-Value	0.4821	
Day 28		
Responders[a], *n* (%)	8 (29.63)	4 (13.79)
Non-responders[b], *n* (%)	19 (70.37)	25 (86.21)
p-Value	0.1988	
Day 42		
Responders[a], *n* (%)	15 (55.56)	1 (3.45)
Non-responders[b], *n* (%)	12 (44.44)	28 (96.55)
p-Value	<0.0001	
Day 56		
Responders[a], *n* (%)	17 (62.96)	1 (3.45)
Non-responders[b], *n* (%)	10 (37.04)	28 (96.55)
p-Value	<0.0001	

[a]≥50-point reduction.
[b]<50-point reduction.

Avoidance and control behavior was assessed using the IBS-BRQ completed on days 0 and 56 (Table 4C) and there was a significant between-group difference (−7.49, 6.9% reduction, $p=0.0002$) at the end of the study due to a significant improvement (−9.00, 8% reduction, $p<0.0001$) in the active group.

There were no major changes in *general well-being* (Table S2) or *executive function* (Table S3).

3.4 | The impact of daily supplementation on anthropometry

Increases from baseline in bodyweight and BMI (~1%) occurred in the active group (0.84 kg, $p=0.0111$ and 0.30 kg/m^2, $p<0.0001$, respectively, Table 5) but not the placebo group resulting in a between-group difference of 0.70 kg ($p=0.0111$) in bodyweight and 0.25 kg/m^2 ($p=0.0165$) in BMI by the end of the study.

3.5 | The impact of daily supplementation on the composition of the fecal microbiota

At the end of the study, no between-group differences were observed in alpha diversity (Shannon index, Figure 3A). Non-metric multidimensional scaling (NMDS) showed significant spatial separation between baseline and the end of the study in both the placebo and active groups ($p=0.0360$ and $p=0.02307$, respectively) but no between-group differences (Figure 3B). No differences in the homogeneity of dispersion were observed between time points or groups. Differential abundance analysis identified a number of bacterial taxa that were significantly different between the placebo and active groups at the endpoint but not at the baseline (Figure 3C). *Roseburia*, *Holdemanella*, two ASVs assigned to *Blautia*, and *Agathobacter* were enriched in the active group, whereas ASVs from *Ruminococcus*, *Roseburia*, *Prevotella*, *Blautia*, *Bacteroides*, and *Anaerostipes* were more abundant in the placebo group. Relative

TABLE 3 Participant bowel habits during the study.

			Between-group difference (A vs. P) in LSM		
	Active ($n=27$)	Placebo ($n=29$)	95% CI	%	*p*-Value
Number of defecations per day, mean (SD)	1.11 (0.31)	1.02 (0.38)	0.10 (−0.09, 0.28)	9.4	0.3066
Bristol stool form score, mean (SD)	4.13 (0.65)	4.35 (0.85)	−0.22 (−0.62, 0.19)	−5.0	0.2909
Proportion of defecations, % (SD), with					
Hard stool form (BSFS 1 or 2)	6.24 (14.53)	9.07 (17.78)	−2.83 (−11.57, 5.91)	−31.22	0.5186
Normal stool form (BSFS 3, 4 or 5)	78.04 (14.75)	66.54 (17.51)	11.50 (2.79, 20.21)	17.28	0.0106
Loose stool form (BSFS 6 or 7)	15.72 (11.75)	24.39 (16.88)	−8.67 (−16.51, −0.82)	−35.54	0.0311
The feeling of incomplete evacuation	16.84 (15.59)	25.69 (19.52)	−8.84 (−18.35, 0.66)	−34.43	0.0676
Failure to pass a stool	7.69 (10.71)	14.37 (14.15)	−6.69 (−13.45, 0.07)	−46.53	0.0525
An urgent need	26.11 (11.76)	30.63 (17.02)	−4.52 (−12.41, 3.37)	−14.76	0.2559
Straining	11.87 (13.31)	17.67 (22.19)	−5.80 (−15.69, 4.10)	−32.81	0.2454

Abbreviations: BSFS, bristol stool form score; CI, confidence interval; LSM, least square mean; SD, standard deviation.

TABLE 4 Changes in anxiety, depression, and control and avoidance behavior at the end of the study (Day 56).

	Active ($n=27$)	Placebo ($n=29$)
A. HADS anxiety score		
Change from day 0, LSM (95% CI; p-value)	−2.33 (−2.96, −1.70; $p<0.0001$)	−0.48 (−1.09, 0.12; $p=0.1151$)
Difference between groups in LSM (95% CI; p-value)	−1.85 (−2.76, −0.94; $p=0.0002$)	
B. HADS depression score		
Change from day 0, LSM (95% CI; p-value)	−2.20 (−2.92, −1.49; $p<0.0001$)	0.15 (−0.54, 0.85; $p=0.6548$)
Difference between groups in LSM (95% CI; p-value)	−2.36 (−3.37, −1.35; $p<0.0001$)	
C. IBS-BRQ score		
Change from day 0, LSM (95% CI; p-value)	−9.00 (−11.64, −6.37; $p<0.0001$)	−1.51 (−4.05, 1.03; $p=0.2373$)
Difference between groups in LSM (95% CI; p-value)	−7.49 (−11.25, −3.74; $p=0.0002$)	

Abbreviations: CI, confidence interval; LSM, least square mean.

TABLE 5 Changes in anthropometry at the end of the study (Day 56).

	Active ($n=27$)	Placebo ($n=29$)
Body weight (kg)		
Change from day 0, LSM (95% CI; p-value)	0.84 (0.46, 1.23; $p<0.0001$)	0.14 (−0.23, 0.51; $p=0.4496$)
Difference between groups in LSM (95% CI; p-value)	0.70 (0.17, 1.23; $p=0.0111$)	
BMI (kg/m^2)		
Change from day 0, LSM (95% CI; p-value)	0.30 (0.16, 0.45; $p<0.0001$)	0.06 (−0.08, 0.20; $p=0.3988$)
Difference between groups in LSM (95% CI; p-value)	0.25 (0.05, 0.44; $p=0.0165$)	
Systolic blood pressure (mmHg)		
Change from day 0, LSM (95% CI; p-value)	1.20 (−0.25, 2.66; $p=0.1038$)	0.12 (−1.29, 1.53; $p=0.8634$)
Difference between groups in LSM (95% CI; p-value)	1.08 (−0.95, 3.11; $p=0.2892$)	
Diastolic blood pressure (mmHg)		
Change from day 0, LSM (95% CI; p-value)	−0.27 (−1.30, 0.75; $p=0.9649$)	−0.23 (−1.22, 0.76; $p=0.6421$)
Difference between groups in LSM (95% CI; p-value)	−0.04 (−1.47, 1.38; $p=0.9542$)	

Abbreviations: CI, confidence interval; LSM, least square mean.

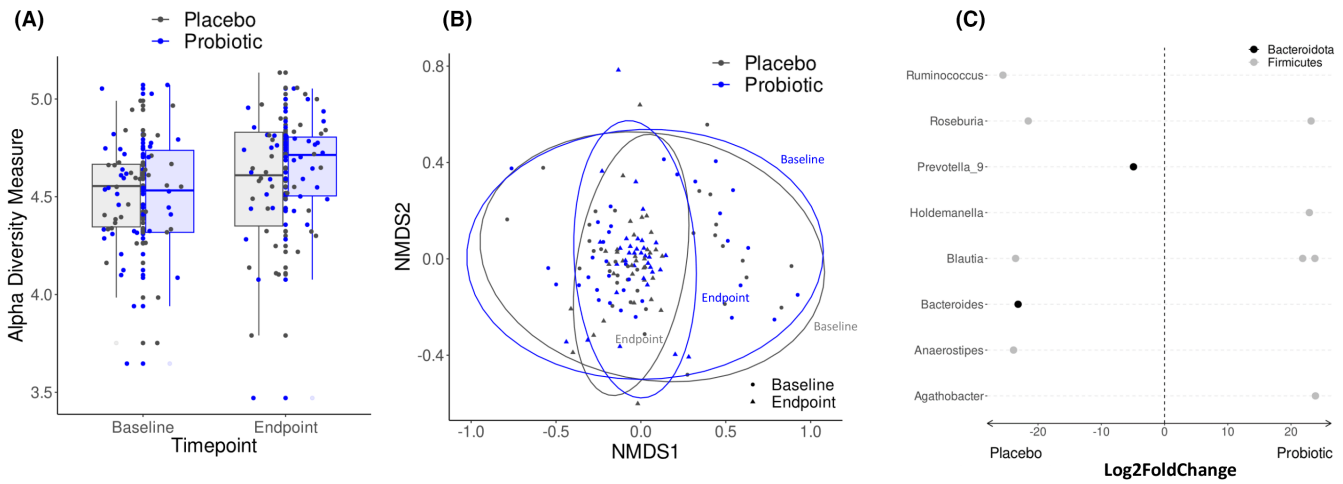

FIGURE 3 Changes in the composition of the fecal microbiota. (A) Shannon alpha diversity measures and (B) non-metric multidimensional scaling (NMDS) plot based on the Bray–Curtis dissimilarity matrix according of the active and placebo groups throughout the study. (C) Bacterial genera that were similar in abundance between treatment groups at baseline but were differentially abundant between treatment groups at the endpoint using DESeq2 normalization for data after logarithmic transformation. Each dot represents a unique ASV.

abundance estimates of the differentially abundant bacterial taxa are shown in Table S4.

4 | DISCUSSION

In this eight-week study there was a significant reduction in IBS-SSS alongside improvements in bowel habits and reductions in levels of anxiety, depression, and IBS-related behaviors in Rome IV-diagnosed IBS females receiving the Lab4 probiotic.

In a previous Lab4 probiotic exploratory IBS study, Williams et al reported a 50-point between-group difference in IBS-SSS favoring the Lab4 group[15] and for a subgroup from that study comprising females aged from 18 to 40, the difference was nearer 80-points (Figure S1). In the current study with females, the between-group difference in IBS-SSS at the end of the study was 85 points supporting the reductions seen. Reductions of ≥50-points are considered to be clinically meaningful[16] and by the end of the current study, 63% of the Lab4 group had achieved a ≥50-points severity reduction whereas this was only 4% for the placebo group. In the probiotic group, the IBS-SSS reduction was associated with fewer days with abdominal pain, reduced pain and bloating severity, and improvements in bowel habit dissatisfaction and general well-being. A study with healthy adults receiving Lab4 also reported reductions in abdominal pain and bloating and improvements in bowel habits.[18] Zhang et al showed in a network analysis of IBS randomized control trials that single-strain probiotics of *Lactobacillus acidophilus* alleviated gastrointestinal symptoms (particularly bloating) and improved quality of life.[10] Similarly, *Bifidobacterium lactis* DN-173010[31] and *B. bifidum* MIMBb75[32] have been shown to reduce IBS-SSS and multistrain probiotics have also been found to improve IBS symptomolgy.[9]

In the study the placebo effect was minimal which contrasts with the vast majority of other IBS studies.[13] Many factors contribute to the placebo effect including psychological responses to receiving an intervention (expectation of improvement), low study population sizes, subjectivity of participant-reported outcomes,[13] over-interaction with physicians and/or multiple participant visits to the trial centre.[33,34] In the current Lab4 study, there was limited contact with GPs (one phone conversation) and only two visits to the trial centers which may have reduced the placebo effect although the exact cause(s) are unknown.

According to Rome IV guidelines, IBS is classified into 4 subtypes—constipation dominant (IBS-C), diarrhea dominant (IBS-D), mixed bowel habit (IBS-M), or unclassified (IBS-U)—determined on the basis of stool type. Our study population composition was predominantly IBS-M followed by IBS-D then IBS-C and with no IBS-U. Participants on the probiotic reported 35% fewer loose stools during the intervention period which indicates a potential anti-diarrhoeal effect and in a Lab4 study with school children (aged 3–10) there was a 44% reduction in the incidence of watery/loose stools.[35] In contrast, in the current IBS study, there were fewer "incomplete" defecations (feeling of incomplete evacuation or failure to defecate) in the probiotic group compared to the placebo group which could be considered to be an "anti-constipation" outcome and similar outcomes have been observed in a Lab4 study with healthy adults.[18] These data suggest the "normalization" of stool form and this was evidenced by the greater incidence of stools with normal form in the Lab4 group. The low numbers for IBS-D and IBS-C prevented meaningful subgroup analyses, but other probiotic studies have shown anti-diarrhoeal and anti-constipation effects in these subtypes.[36,37]

There are known to be psychological and cognitive burdens associated with IBS[38] with anxiety/depression affecting over a third of those with IBS[39] and cognitive impairments have also been observed.[40,41] Probiotic bacteria are gaining recognition for their positive impact on mental health and cognition via interaction between the gut-brain axis[42] and probiotics have shown anti-anxiety and/or anti-depressive responses in people with IBS.[43,44] There were reductions in both HADS anxiety and depression scores in the probiotic group but there was no impact on cognition during the SCWT (assessing executive function[45]). It is unclear if the changes were due to symptom alleviation and/or involved the modulation of the gut-brain axis interaction. The Lab4 probiotic has been shown to improve memory in healthy Wistar rats,[46] preserve cognitive capabilities in an Alzheimer's disease mouse model,[47] and improve mood scores in adults[30] which support the improvements observed. In other studies, probiotics have been shown to alleviate IBS symptoms without an impact on anxiety and depression.[48,49]

The IBS symptoms can affect the quality of life for people with IBS resulting in the adoption of controlling and avoidance behaviors such as food anxieties or the avoidance of social situations.[20] The IBS-BRQ questionnaire[20] indicated reductions in control and avoidance behaviors in the probiotic group alongside the improvements in general life observed in the probiotic group of this study (Figure 2F) and as has been seen other IBS probiotic intervention studies (reviewed in Ref. 50).

The vast majority of individuals with IBS do not present with any demonstrable intestinal abnormalities or inflammation but elevations in circulating pro-inflammatory cytokines, such as IL-6, have been observed.[51] Healthy adults taking the Lab4 probiotic had improvements in gastrointestinal symptoms and bowel habits that were associated with reduced circulating levels of plasma IL-6[18] and another Lab4 ex vivo study showed reduced IL-6 secretion by peripheral blood mononuclear cells.[52] In the current study, the baseline plasma IL-6 levels were within the normal range[53] and remained unchanged.

It has been found that the diversity of the IBS gut microbiota differs from that of healthy subjects[5,6,54–56] particularly in females.[57] Comparison of the baseline alpha diversity of the IBS cohort from this study with that of age, gender and/or geographically matched non-IBS populations from previous studies with the Lab4 probiotic[18,30] showed the alpha diversity of the IBS population was significantly lower (Figure S8). There was a trend towards higher diversity in the active group at the end of the study, but not the

placebo. Both groups presented significant changes in beta diversity between baseline and endpoint, which may be related to an instability/imbalance in the microbiota of these participants but this needs investigation. As has been mentioned, IBS can have an impact upon the diversity of the on the gut microbiota[58,59] and so these changes could be related to the nature of IBS interactions within the gut, but more research is needed in this area to gain greater clarity.

Increased abundances of *Agathobacter* and *Holdemanella* were observed in the probiotic group and *Agathobacter* is a butyrate-producing organism found in healthy individuals and has been observed to be increased in the presence of probiotic bacteria.[60,61] Enriched abundances of *Holdemanella* have been associated with decreased IBS-SSS and fatigue in people with IBS.[62] *Prevotella*, *Bacteroides*, *Anaerostipes*, and *Ruminococcus* were enriched in the placebo group. *Prevotella* and *Ruminococcus* are positively associated with severity of IBS, particularly IBS-D.[63,64] *Anaerostipes* is a butyrate-producing bacteria associated with the alleviation of symptoms during IBS.[65] The role of *Bacteroides* is unclear with both low[66] and high abundance[67] reported during IBS. *Bacteroides* spp. are known to produce acetate, with high levels of this SCFA have been shown to be associated with the severity of IBS symptoms.[68]

The study has a number of strengths; (i) it is a second study demonstrating the benefits of Lab4 probiotic supplementation in an IBS cohort; (ii) the small placebo effect; and (iii) our data analysis was focused on participants who had calprotectin levels below 160 μg/g to ensure we were not including those with suspected inflammatory status. Limitations of the study are (i) the generalisability of the findings is limited due to the female only 18–40-year-old cohort in a single geographical location and (ii) baseline anxiety and depression scores in the probiotic group were lower than the clinical threshold of 8 highlighting the need for further work in an IBS cohort with HADS >8 in order to assess the anti-anxiety and anti-depressive qualities of supplementation.

In summary, this randomized, double-blind, placebo-controlled study in women with IBS confirmed the ability of the Lab4 probiotic to play a beneficial role in the management of IBS via improvements in gastrointestinal status and bowel habits, anxiety, and depression and indications of an impact upon the composition and functioning of the gut microbiota. The mechanisms of action driving these benefits have not been identified and further studies are needed.

AUTHOR CONTRIBUTIONS

BHM, SFP, and JRM were responsible for the design of the study. BHM, DRM, DAJ, and NC prepared the manuscript. MD managed the administration of the study. DRM, TSW, DAJ, and NC performed data processing and analysis. DW performed statistical analysis with support from DRM, DAJ, and LY. All the authors contributed to the review of the manuscript.

ACKNOWLEDGMENTS

This study was funded by Cultech Ltd, Port Talbot, UK. We would like to acknowledge Josh Kerry-Smith, Julio Illanes, Dr Jack Bate, Sophie Thomas and Eleri Hulme of Cultech Ltd (Port Talbot, UK) for technical assistance during the study. Professor Bernard Corfe (Newcastle University, UK) and Dr Elizabeth Williams (University of Sheffield, UK) for critical reading of the manuscript.

FUNDING INFORMATION

BHM is the recipient of an NIHR Academic Clinical Lectureship (CL-2019-21-002). The Division of Digestive Diseases at Imperial College London receives financial and infrastructure support from the NIHR Imperial Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London.

CONFLICT OF INTEREST STATEMENT

SFP, DRM, DAJ, NC, and TSW are/were employees of Cultech Ltd and had no role in recruitment or data collection but contributed to the design of the study, data analysis and interpretation and/or writing, reviewing, and approval of the final manuscript. MD is an employee of Comac Medical. Cultech provided the study intervention. BHM, JRM, and DW are/have been involved in other collaborative projects with Cultech Ltd. JRM and DW have previously received consultancy fees from Cultech Ltd.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Sequence data generated during the current study have been submitted to the European Molecular Biology Laboratory (EMBL) nucleotide sequence database under accession number PRJEB63199.

ORCID

B. H. Mullish https://orcid.org/0000-0001-6300-3100

REFERENCES

1. Chey WD, Kurlander J, Eswaran S. Irritable bowel syndrome: a clinical review. *Jama*. 2015;313(9):949-958.
2. Palsson OS, Whitehead W, Tornblom H, Sperber AD, Simren M. Prevalence of Rome IV functional bowel disorders among adults in the United States, Canada, and the United Kingdom. *Gastroenterology*. 2020;158(5):1262-1273 e1263.
3. Goodoory VC, Ng CE, Black CJ, Ford AC. Direct healthcare costs of Rome IV or Rome III-defined irritable bowel syndrome in the United Kingdom. *Aliment Pharmacol Ther*. 2022;56(1):110-120.
4. Enck P, Aziz Q, Barbara G, et al. Irritable bowel syndrome. *Nat Rev Dis Primers*. 2016;2:16014.
5. Liu H-N, Wu H, Chen Y-Z, Chen Y-J, Shen X-Z, Liu T-T. Altered molecular signature of intestinal microbiota in irritable bowel syndrome patients compared with healthy controls: a systematic review and meta-analysis. *Dig Liver Dis*. 2017;49(4):331-337.
6. Wang L, Alammar N, Singh R, et al. Gut microbial dysbiosis in the irritable bowel syndrome: a systematic review and meta-analysis of case-control studies. *J Acad Nutr Diet*. 2020;120(4):565-586.
7. Pittayanon R, Lau JT, Yuan Y, et al. Gut microbiota in patients with irritable bowel syndrome—a systematic review. *Gastroenterology*. 2019;157(1):97-108.
8. Satish Kumar L, Pugalenthi LS, Ahmad M, Reddy S, Barkhane Z, Elmadi J. Probiotics in irritable bowel syndrome: a review of their therapeutic role. *Cureus*. 2022;14(4):e24240.

9. Niu HL, Xiao JY. The efficacy and safety of probiotics in patients with irritable bowel syndrome: evidence based on 35 randomized controlled trials. *Int J Surg*. 2020;75:116-127.
10. Zhang T, Zhang C, Zhang J, Sun F, Duan L. Efficacy of probiotics for irritable bowel syndrome: a systematic review and network meta-analysis. *Front Cell Infect Microbiol*. 2022;12:859967.
11. Hill C, Guarner F, Reid G, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014;11(8):506-514.
12. Lacy BE, Pimentel M, Brenner DM, et al. ACG clinical guideline: management of irritable bowel syndrome. *Am J Gastroenterol*. 2021;116(1):17-44.
13. Enck P, Klosterhalfen S. Placebo responses and placebo effects in functional gastrointestinal disorders. *Front Psych*. 2020;11:797.
14. Saha L. Irritable bowel syndrome: pathogenesis, diagnosis, treatment, and evidence-based medicine. *World J Gastroenterol*. 2014;20(22):6759-6773.
15. Williams EA, Stimpson J, Wang D, et al. Clinical trial: a multistrain probiotic preparation significantly reduces symptoms of irritable bowel syndrome in a double-blind placebo-controlled study. *Aliment Pharmacol Ther*. 2009;29(1):97-103.
16. Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. *Aliment Pharmacol Ther*. 1997;11(2):395-402.
17. Everitt HA, Landau S, O'Reilly G, et al. Cognitive behavioural therapy for irritable bowel syndrome: 24-month follow-up of participants in the ACTIB randomised trial. *Lancet Gastroenterol Hepatol*. 2019;4(11):863-872.
18. Mullish BH, Michael DR, Webberley TS, et al. The gastrointestinal status of healthy adults: a post hoc assessment of the impact of three distinct probiotics. *Benef Microbes*. 2023;1-14.
19. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand*. 1983;67(6):361-370.
20. Reme SE, Darnley S, Kennedy T, Chalder T. The development of the irritable bowel syndrome-behavioral responses questionnaire. *J Psychosom Res*. 2010;69(3):319-325.
21. Grossenbacher F, Gashi A, Besseling-van der Vaart I. Use of the multispecies probiotic Winclove 500/BactoSan pro FOS leads to less gastrointestinal complaints in adults—an observational in vivo pilot study. *Adv Microbiol*. 2016;6(14):975-985.
22. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: high-resolution sample inference from illumina amplicon data. *Nat Methods*. 2016;13(7):581-583.
23. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res*. 2013;41:D590-D596.
24. Mohsen S, Dickinson JA, Somayaji R. Update on the adverse effects of antimicrobial therapies in community practice. *Can Fam Physician*. 2020;66(9):651-659.
25. McMurdie PJ, Holmes S. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*. 2013;8(4):e61217.
26. Wickham H. *Package 'ggplot2': elegant graphics for data analysis*. Vol 10. Springer-Verlag; 2016:970-978.
27. Oksanen J, Blanchet FG, Kindt R, et al. vegan: community ecology package. 2013. Accessed June 4, 2023. https://cran.r-project.org/web/packages/vegan/index.html
28. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15(12):550.
29. Michael D, Davies T, Jack A, et al. Daily supplementation with the Lab4P probiotic consortium induces significant weight loss in overweight adults. *Sci Rep*. 2021;11(1):5.
30. Michael DR, Jack AA, Masetti G, et al. A randomised controlled study shows supplementation of overweight and obese adults with lactobacilli and bifidobacteria reduces bodyweight and improves well-being. *Sci Rep*. 2020;10(1):4183.
31. Agrawal A, Houghton LA, Morris J, et al. Clinical trial: the effects of a fermented milk product containing *Bifidobacterium lactis* DN-173 010 on abdominal distension and gastrointestinal transit in irritable bowel syndrome with constipation. *Aliment Pharmacol Ther*. 2009;29(1):104-114.
32. Guglielmetti S, Mora D, Gschwender M, Popp K. Randomised clinical trial: *Bifidobacterium bifidum* MIMBb75 significantly alleviates irritable bowel syndrome and improves quality of life – a double-blind, placebo-controlled study. *Aliment Pharmacol Ther*. 2011;33(10):1123-1132.
33. Kaptchuk TJ, Kelley JM, Conboy LA, et al. Components of placebo effect: randomised controlled trial in patients with irritable bowel syndrome. *BMJ*. 2008;336(7651):999-1003.
34. Dorn SD, Kaptchuk TJ, Park JB, et al. A meta-analysis of the placebo response in complementary and alternative medicine trials of irritable bowel syndrome. *Neurogastroenterol Motil*. 2007;19(8):630-637.
35. Garaiova I, Paduchova Z, Nagyova Z, et al. Probiotics with vitamin C for the prevention of upper respiratory tract symptoms in children aged 3–10 years: randomised controlled trial. *Benef Microbes*. 2021;12:1-10.
36. Mezzasalma V, Manfrini E, Ferri E, et al. A randomized, double-blind, placebo-controlled trial: the efficacy of multispecies probiotic supplementation in alleviating symptoms of irritable bowel syndrome associated with constipation. *Biomed Res Int*. 2016;2016:4740907.
37. Ki Cha B, Mun Jung S, Hwan Choi C, et al. The effect of a multispecies probiotic mixture on the symptoms and fecal microbiota in diarrhea-dominant irritable bowel syndrome: a randomized, double-blind, placebo-controlled trial. *J Clin Gastroenterol*. 2012;46(3):220-227.
38. Fadgyas-Stanculete M, Buga AM, Popa-Wagner A, Dumitrascu DL. The relationship between irritable bowel syndrome and psychiatric disorders: from molecular changes to clinical manifestations. *J Mol Psychiatry*. 2014;2(1):4.
39. Hu Z, Li M, Yao L, et al. The level and prevalence of depression and anxiety among patients with different subtypes of irritable bowel syndrome: a network meta-analysis. *BMC Gastroenterol*. 2021;21(1):23.
40. Wong KM, Mak ADP, Yuen SY, et al. Nature and specificity of altered cognitive functioning in IBS. *Neurogastroenterol Motil*. 2019;31(11):e13696.
41. Lam NC-Y, Yeung H-Y, Li W-K, et al. Cognitive impairment in irritable bowel syndrome (IBS): a systematic review. *Brain Res*. 2019;1719:274-284.
42. Gwioździk W, Helisz P, Grajek M, Krupa-Kotara K. Psychobiotics as an intervention in the treatment of irritable bowel syndrome: a systematic review. *Appl Microbiol*. 2023;3(2):465-475.
43. Pinto-Sanchez MI, Hall GB, Ghajar K, et al. Probiotic Bifidobacterium longum NCC3001 reduces depression scores and alters brain activity: a pilot study in patients with irritable bowel syndrome. *Gastroenterology*. 2017;153(2):448-459.e448.
44. Groeger D, Murphy EF, Tan HTT, Larsen IS, O'Neill I, Quigley EMM. Interactions between symptoms and psychological status in irritable bowel syndrome: an exploratory study of the impact of a probiotic combination. *Neurogastroenterol Motil*. 2023;35(1):e14477.
45. Scarpina F, Tagini S. The Stroop color and word test. *Front Psychol*. 2017;8:557.
46. O'Hagan C, Li JV, Marchesi JR, Plummer S, Garaiova I, Good MA. Long-term multi-species lactobacillus and Bifidobacterium dietary supplement enhances memory and changes regional brain metabolites in middle-aged rats. *Neurobiol Learn Mem*. 2017;144:36-47.

47. Webberley TS, Bevan RJ, Kerry-Smith J, et al. Assessment of Lab4P probiotic effects on cognition in 3xTg-AD Alzheimer's disease model mice and the SH-SY5Y neuronal cell line. *Int J Mol Sci*. 2023;24(5):4683. https://doi.org/10.3390/ijms24054683.
48. Whorwell PJ, Altringer L, Morel J, et al. Efficacy of an encapsulated probiotic Bifidobacterium infantis 35624 in women with irritable bowel syndrome. *Am J Gastroenterol*. 2006;101(7):1581-1590.
49. Wong RK, Yang C, Song GH, Wong J, Ho KY. Melatonin regulation as a possible mechanism for probiotic (VSL#3) in irritable bowel syndrome: a randomized double-blinded placebo study. *Dig Dis Sci*. 2015;60(1):186-194.
50. Morvan L, de Sequeira C, Kaeber M, Cekin SE, Enck P, Mack I. The effect of probiotics on quality of life, depression and anxiety in patients with irritable bowel syndrome: a systematic review and meta-analysis. *J Clin Med*. 2021;10(16):3497. https://doi.org/10.3390/jcm10163497
51. Bashashati M, Moradi M, Sarosiek I. Interleukin-6 in irritable bowel syndrome: a systematic review and meta-analysis of IL-6 (-G174C) and circulating IL-6 levels. *Cytokine*. 2017;99:132-138.
52. Hepburn NJ, Garaiova I, Williams EA, Michael DR, Plummer S. Probiotic supplement consumption alters cytokine production from peripheral blood mononuclear cells: a preliminary study using healthy individuals. *Benef Microbes*. 2013;4(4):313-317.
53. Said EA, Al-Reesi I, Al-Shizawi N, et al. Defining IL-6 levels in healthy individuals: a meta-analysis. *J Med Virol*. 2021;93(6):3915-3924.
54. Jeffery IB, O'Toole PW, Öhman L, et al. An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. *Gut*. 2012;61(7):997-1006.
55. Carroll IM, Ringel-Kulka T, Siddle JP, Ringel Y. Alterations in composition and diversity of the intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. *Neurogastroenterol Motil*. 2012;24(6):521-530, e248.
56. Durbán A, Abellán JJ, Jiménez-Hernández N, et al. Structural alterations of faecal and mucosa-associated bacterial communities in irritable bowel syndrome. *Environ Microbiol Rep*. 2012;4(2):242-247.
57. Pecyna P, Gabryel M, Mankowska-Wierzbicka D, et al. Gender influences gut microbiota among patients with irritable bowel syndrome. *Int J Mol Sci*. 2023;24(13):10424. https://doi.org/10.3390/ijms241310424
58. Menees S, Chey W. The gut microbiome and irritable bowel syndrome. *F1000Research*. 2018;7:1-10.
59. Lee BJ, Bak YT. Irritable bowel syndrome, gut microbiota and probiotics. *J Neurogastroenterol Motil*. 2011;17(3):252-266.
60. Hiseni P, Rudi K, Wilson RC, Hegge FT, Snipen L. HumGut: a comprehensive human gut prokaryotic genomes collection filtered by metagenome data. *Microbiome*. 2021;9(1):165.
61. Zhang M, Zheng Y, Sun Z, et al. Change in the gut microbiome and immunity by *Lacticaseibacillus rhamnosus* Probio-M9. *Microbiol Spectr*. 2023;11(2):e0360922.
62. El-Salhy M. Intestinal bacteria associated with irritable bowel syndrome and chronic fatigue. *Neurogastroenterol Motil*. 2023;35:e14621.
63. Tap J, Derrien M, Törnblom H, et al. Identification of an intestinal microbiota signature associated with severity of irritable bowel syndrome. *Gastroenterology*. 2017;152(1):111-123.e118.
64. Lyra A, Rinttilä T, Nikkilä J, et al. Diarrhoea-predominant irritable bowel syndrome distinguishable by 16S rRNA gene phylotype quantification. *World J Gastroenterol*. 2009;15(47):5936-5945.
65. Liu Y, Yu X, Yu L, et al. *Lactobacillus plantarum* CCFM8610 alleviates irritable bowel syndrome and prevents gut microbiota dysbiosis: a randomized, double-blind, placebo-controlled, pilot clinical trial. *Engineering*. 2021;7(3):376-385.
66. Xiao L, Liu Q, Luo M, Xiong L. Gut microbiota-derived metabolites in irritable bowel syndrome. *Front Cell Infect Microbiol*. 2021;11:11.
67. Kevin V, Stephen M, Nitin K, et al. Two microbiota subtypes identified in irritable bowel syndrome with distinct responses to the low FODMAP diet. *Gut*. 2022;71(9):1821-1830.
68. Jiang W, Wu J, Zhu S, Xin L, Yu C, Shen Z. The role of short chain fatty acids in irritable bowel syndrome. *J Neurogastroenterol Motil*. 2022;28(4):540-548.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Mullish BH, Michael DR, Dabcheva M, et al. A double-blind, randomized, placebo-controlled study assessing the impact of probiotic supplementation on the symptoms of irritable bowel syndrome in females. *Neurogastroenterology & Motility*. 2024;00:e14751. doi:10.1111/nmo.14751