Digestive Diseases

Dig Dis , DOI: 10.1159/000528954

Received: November 29, 2021

Accepted: December 27, 2022

Published online: January 11, 2023

"Impact of Saccharomyces boulardii CNCM I-745 on bacterial overgrowth and composition of intestinal microbiota in IBS-D patients: results of a randomized pilot study"

Bustos Fernández LM, Man F, Lasa JS

ISSN: 0257-2753 (Print), eISSN: 1421-9875 (Online)

https://www.karger.com/DDI

Digestive Diseases

Research Article

Title

"**Impact of *Saccharomyces boulardii* CNCM I-745 on bacterial overgrowth and composition of intestinal microbiota in IBS-D patients: results of a randomized pilot study**"

Short title

Saccharomyces boulardii CNCM I-745 in SIBO

Authors

Luis Maria Bustos Fernández[1]; Fernando Man[2]; Juan Sebastian Lasa[3]

Author institutions

1: Centro Médico Dr. Bustos Fernández. Buenos Aires. Argentina

2: Gastro. Buenos Aires. Argentina

3: Centro de Educación Médica e Investigaciones Clínicas "Norberto Quirno" (CEMIC).Gastroenterology Department, Buenos Aires. Argentina

Correspondence:

Dr. Luis M. Bustos Fernández

Centro Médico Dr. Bustos Fernández. Buenos Aires. Argentina

Echeverria 2771. 1428

CABA. ARGENTINA

luisbustosfernandez@gmail.com

Mobile: 005491135810140

Keywords: *Saccharomyces boulardii* CNCM I-745, bacterial overgrowth, intestinal microbiota, irritable bowel syndrome.

TITLE

"**Impact of *Saccharomyces boulardii* CNCM I-745 on bacterial overgrowth and composition of intestinal microbiota in IBS-D patients: results of a randomized, pilot study**"

ABSTRACT

Background

Small intestinal bacterial overgrowth (SIBO) is associated with diarrhea-predominant irritable bowel syndrome (IBS-D). Probiotics like *S. boulardii* CNCM I-745 (*Sb*) may be efficacious in balancing the microbiota.

This randomized open label study assessed the effect of *Sb* in patients with bacterial overgrowth associated with IBS-D and its impact on the intestinal microbiota.

Methods

Patients were randomized to receive for 15 days; *S. boulardii +* dietary advice (*Sb*+DA) or dietary advice (DA) only. SIBO was assessed by the lactulose hydrogen breath test (LHBT). Symptoms were assessed with the IBS Symptom Severity Scale (IBS-SSS) and stool consistency with the Bristol Stool Form Scale. Microbiota and mycobiota were analyzed by 16S rDNA and ITS2.

Results

54 patients were included, among them 48 (27 *Sb*+DA, 21 DA) were evaluated. Decrease of hydrogen excretion was slightly higher in *Sb*+DA group, 41% vs 29% in DA group, and IBS-SSS total score were reduced by -134 and -93, respectively

The proportion of patients with diarrhea was lower in the *Sb*+DA group than in the DA group (25.9% compared to 47.6%)

Bacterial and fungal microbiota showed that *S. boulardii* CNCM I-745 treatment was associated with several modifications. Interestingly, *F. prausnitzii* was more abundant in Sb-treated patients with marked clinical improvement. The safety of *Sb* was excellent.

Conclusions

In patients with SIBO, *S. boulardii* CNCM I-745 associated with dietary advice reduced bacterial overgrowth and improved digestive symptoms while restoring the intestinal microbiota. The increased abundance of *F. prausnitzii* coupled with symptom improvement merits further research.

Key words

Small intestinal bacterial overgrowth; *Saccharomyces boulardii* CNCM I-745; Irritable bowel syndrome IBS; Intestinal microbiota; *Faecalibacterium prausnitzii.*

INTRODUCTION

The role of intestinal microbiota, in the pathogenesis of the Irritable bowel syndrome (IBS) is attracting growing scientific attention. Evidence linking IBS to a dysbiotic microbiota has been known for some time [1,2,3,4]. IBS is a chronic gastrointestinal condition of unknown aetiology, characterized by the presence of abdominal discomfort or pain and altered bowel habits, in the absence of clinical 'alarm' signs, such as anaemia or significant weight loss [5,6]. The pathophysiological basis of the symptoms is still incompletely understood, but it features disturbances of motor and sensory function [7,8], subclinical inflammatory changes [9], altered microbiome [10], associated psychosocial disorders, and genetics [11] .

Small intestinal bacterial overgrowth (SIBO) is a disorder characterized by an abnormal increase of the bacterial population in the small intestine. The term was coined by Baker and Humel in 1939 [12]. The prevalence of SIBO in IBS varies depending on the method and criteria used for diagnosis. [13,14]

The gold standard for diagnosis of SIBO was initially the culture of jejunal aspirate. Much simpler and more available, is the exhaled breath test (EBT), using a non-absorbable substrate, such as lactulose, or an absorbable one, such as glucose[15]. There is a consensus to consider hydrogen values above 20 ppm in the first 90-100 minutes positive for SIBO [16,17]. The area under the curve for hydrogen, a means of prospectively quantifying the sum of hydrogen measurements obtained on the time curve, allows us to assess the patient's total fermentation level.

There is currently increasing interest in the modulation of gastro-intestinal microbiota using probiotics to reduce inflammation and improve the symptoms of IBS. In a systematic review and meta-analysis assessing 43 RCT on the use of probiotics in IBS [18] , the authors concluded that probiotics had beneficial effects on the rates of abdominal pain, flatulence and bloating, and the RR of IBS symptoms persisting with probiotics vs. placebo was 0.79 (95% CI 0.70-0.89). Attempts to modulate the intestinal microbiota in patients with SIBO and IBS are based on restoring the altered eubiosis. Non-absorbable antibiotics have been used, such as rifaximin, which was found to be effective in the initial treatment and of SIBO [19]. The possibility of treating dysbiosis in SIBO using probiotics offers a broad field of research on highly prevalent condition such as IBS.

Saccharomyces boulardii CNCM I-745 (*S. boulardii*) is a probiotic yeast with proven therapeutic effect in various pathologies including IBS. In particular, it has shown efficacy in the prevention and treatment of diarrhea caused by enteropathogens or by antibiotics [20].

The aim of this study was to compare the efficacy and safety of *Sb* plus dietary advice versus dietary advice alone on SIBO positive in Diarrhea-predominant Irritable Bowel Syndrome (IBS-D) patients and to describe their impact on intestinal microbiota and mycobiota composition and clinical improvement.

MATERIAL METHODS

Study design, study participants and randomization and group assignment

The Clinical Evaluation of Small Intestinal Bacterial Overgrowth (also known as "CEIBO" study) was a randomized open label exploratory study. The study protocol was reviewed and approved (on 21JAN2016) by the Ethics Committee *Comite de Etica en Investigation Clinica* (CEIC), *Ciudad Autonoma de Buenos Aires*, Argentina. All patients gave written informed consent before study onset. The protocol was presented to ClinicalTrials.gov and the identification number was NCT04627337.

Adult patients (18 to 70 years old) attending two referral gastroenterology centers in Buenos Aires, Argentina, with irritable bowel syndrome type D (IBS-D) (Rome III criteria) were included. Patients were not included if they had any sign or symptom indicative of severe or organic digestive condition that could confound IBS symptoms, or if receiving drugs known to alter microbiota.

Patients attended the study center at Inclusion Visit (baseline: Day 0) and End-of-study visit. (Day 15). During the Inclusion Visit, patients underwent a lactulose hydrogen breath test (LHBT) to verify the presence of bacterial overgrowth. Patients with a positive LHBT (see below) were randomly assigned (randomization list) to one of two study groups and received either *S boulardii* CNCM I-745 (BIOCODEX , Gentilly, France) 250 mg b.i.d. (morning and evening) plus dietary advice (*Sb*+DA group), or dietary advice alone (DA group). In both cases the dietary advice consisted in a low fermentable diet adapted to local habits and an oral explanation by a health professional.

Lactulose hydrogen breath test (LHBT)

At the Inclusion Visit, as well as at the End-of-study Visit, the presence of bacterial overgrowth was examined by a LHBT, a test of bacterial fermentation, which measures hydrogen concentration within the expelled breath.

Patients ingested 10 g lactulose, then breath samples were collected every 20 minutes over 180 min and analyzed to determine hydrogen concentration (expressed in parts per million, ppm). An excreted hydrogen concentration/time area under the curve (AUC) was then calculated.

A positive SIBO test corresponded to rise in breath hydrogen at least 20 ppm above basal levels within 100 minutes after ingestion of lactulose

IBS Symptom Severity Scale (IBS-SSS) and Bristol Stool Scale

The IBS Symptom Severity Scale (IBS-SSS) is a validated questionnaire [21] that evaluates the intensity of IBS symptoms with a 10-day recall period: abdominal pain, distension, stool frequency and consistency, and general impact on life. The IBS-SSS calculates the sum of these 5 items (each scored on a visual analogue scale from 0 to 100) and the score values range from 0 to 500 (maximum severity). An IBS-SSS questionnaire was filled-in by each randomized patient at both visits (Inclusion and EOS visits).

A Bristol Stool Form Scale is a validated measure of stool consistency [22,23]. It was filled-in by each randomized patient at both visits.

Microbiota analysis

For each patient, two stool samples were collected, on day 0 (including all enrolled patients; the samples of the LHBT-negative patients were analyzed for epidemiologic purposes) and on day 15 (End-of-study Visit), either at the investigation sites or at home, using a ready-to-use kit (OmniGene Gut®, DNA Genotek). Samples were stored at -20°C.

Fecal genomic DNA was extracted, purified and analyzed by Genoscreen (Lille, France) in compliance with standard operating procedures of the International Human Microbiome Standards (IHMS) project. Bacterial and fungal composition analysis was performed by sequencing of the V3-V4 region of 16S rDNA and ITS2, respectively. Briefly, a Metabiote® pipeline was used for amplicon library preparation with primers targeting the (fungal) ITS2 gene or the (bacterial) V3-V4 region of the 16S rRNA gene. The sequencing of the corresponding products was performed with paired-end Illumina MiSeq 2 × 250 bp (Illumina, San Diego, CA, USA). After quality control a total of 4115309 reads (34294±3402 reads/sample) and 1548725 reads (12906±3052 reads/sample) were obtained for the 16S region and the ITS2, respectively. These reads were used to generate “fastq” files.

All metagenomic data were processed using the bioinformatics pipeline QIIME v1. Each 16s and ITS2 sequence was assigned to an operational taxonomic unit (OTU) using respectively “SILVA 128” and “UNITE Otus 1211” as database reference, with a threshold at 97% of identity. The 16S Shannon Index bacterial OTU alpha diversities and ITS2 fungal OTU alpha diversity, as well as the Bray-Curtis Distance beta diversity were calculated and represented with “*alphararefaction.py*” and “*betadiversitythroughplots.py*” respectively.

Additionally, qPCR analysis was performed to quantify the abundance of *Faecalibacterium prausnitzii*, in the samples, targeting the 16S rRNA gene. The qPCR validations and quantifications from fecal DNA were performed by Genoscreen. Efficiency was between 90 and 100% as determined using serial dilutions of fecal DNA. The primers and probe for *F. prausnitzii* were: Forward primer: TGT AAA CTC CTG TTG TTG AGG AAG ATA; Reverse primer: GCG CTC CCT TTA CAC CCA; Probe: FAM-CAA GGA AGT GAC GGC TA ACTA CGT GCC AG-TAMRA.

Endpoints and statistical methods

Clinical endpoints

As an exploratory study, the sample size was calculated based on a hypothetical 30% difference of the primary endpoint between *Sb*+DA and DA groups in the absence of relevant existing data. At least 25 patients in each treatment group were necessary to show a statistically significant difference with a 1st-species risk (alpha error) of 5% and a 2nd-species risk (beta error) 20% (corresponding to a power of 80%).

The primary objective was to evaluate the changes in bacterial overgrowth by the amount of exhaled H_2, derived from the area under the curve of the LBHT (LBHT-AUC), after 15-days of observing *Sb*+DA treatment *versus* dietary advice only (DA). The primary end point was the change from baseline of the LBHT-AUC compared between the groups *Sb*+DA and DA. To be considered relevant, change of AUC had to be associated with clinical symptom modulation.

A covariance analysis (ANCOVA) of the primary end point was done. ANCOVA is a parametric method reducing the within-group error of variance by considering the confounding variables baseline LHBT-AUC; IBS abdominal pain score at Day 0; IBS-SSS score at Day 0.

Secondary objectives included evaluation of symptom severity, evaluation of intestinal transit, safety of *S. boulardii* CNCM I-745 and microbiota changes.
The change in SIBO (main secondary criterion) was analyzed as the number and percentage of patients with abnormal LHBT values at day 15 and compared between treatment groups using a chi square test.

Microbiota endpoints

The statistical analysis and graphical representations for sequence analysis were performed with the R tool software (R Foundation for Statistical Computing, v 3.5.0)). Side by side comparisons were done using the Mann Whitney Wilcoxon non-parametric test. ANOSIM was used to compare diversity of the samples.

RESULTS

Baseline characteristics

Seventy-one (71) patients were screened and underwent LHBT. Fifty-four patients (76%) were positive and were randomized to either *Sb*+DA or DA only. In the DA group, two patients were lost to follow-up, and 3 others discontinued prematurely. In the *Sb*+DA group, one patient discontinued the intervention for the occurrence of an adverse event (sinusitis) judged unrelated to the study medication. In total, 48 evaluable patients completed the study (Figure 1)

Baseline characteristics of these LHBT-positive 48 patients are summarized in Table 1 and were as expected in this patient population, notably with elevated IBS-SSS scores (around 300 in both groups).

Effects of S. boulardii CNCM I-745 on SIBO

At baseline, there was no significant difference in mean amount of exhaled H_2 following lactulose intake (LHBT AUC) between the two groups 6433.8 ppm H2/time (SD 3626.7) in the *Sb*+DA group; 7196.5 ppm H2/time (SD 4147.8) in the DA group (p=0.5). A high inter-patient variability of the parameter was observed: the overall range of AUC values was 3078 to 15786 ppm H2/time.

At end of study visit, the mean (SD) change from baseline in LHBT AUC was -2587.3 (3858.1) ppm H2/time in the *Sb*+DA group compared to -1467.4 (5499.4) ppm H2/time in the DA group.

The decrease in the mean change from baseline in LHBT AUC observed at D15 in both treatment groups tended to be more marked in the *Sb*+DA group. The treatment in the *Sb*+DA group leads to a significantly larger decrease from baseline in AUC by ~1800 ppm (95% CI of (3595, -53.1)) compared to the DA group (p=0.044) (Table 2; Figure 2A).

Effects on IBS symptoms severity

The proportion of patients with SIBO was 100% at baseline in both groups and switched to 59.3% in the *Sb*+DA group and 71.4% in the DA group. The difference between groups was not statistically significant (p=0.38).

At day 15, the IBS-SSS decreased to 179.9 +/-80.6 (change from baseline: -134.2) in the *Sb*+DA group and to 202.9 +/-68.6 (change from baseline: -92.9) in the DA group. Both decreases were above the expected clinically minimal difference of 50 points. The most significant reduction was for abdominal pain: the proportion of patients with this symptom decreased from 97.9% at baseline to 56.0% in the *Sb*+DA group and from 100% to 85.7% in the DA group.

The proportion of patients with diarrhea at day 15 was lower in the *Sb*+DA group (25.9%) than in the DA group (47.6%). The proportion of patients with normal stool consistency was higher in the *Sb*+DA group (70.4%) than in the DA group (47.6%) (p=0.14). The reduction of the LHBT-AUC in patients with improved IBS-SSS was more pronounced in the *Sb*+DA group than in the DA group (Figure 2B). The LHBT-AUC increased in 3 patients with no improvement of the IBS-SSS score. When correlated to intestinal transit, the decrease from baseline of the LHBT-AUC was more important in the *Sb*+DA group than in the DA group for patient with diarrhea or normal transit at day 15. (Figure 2C)

Effects of *S. boulardii* CNCM I-745 on the bacterial microbiota

Microbiological analyses on the basis of bacterial 16S rDNA (and fungal ITS2) sequences demonstrated a good quality of the samples: Alpha diversity analysis confirmed that the depth of the sequencing was sufficient to be representative of the microbial diversity present in each sample (data not shown). There was no significant difference between the beta-diversity of OTUs in samples collected at Day 0 vs Day 15 in both groups.

The 16s rDNA sequence analysis (Figure 3 A to H) of the samples collected at Day 0 from SIBO patients of both groups showed that the intestinal microbiota was dominated by the phyla *Firmicutes* (~73.18%), *Bacteroidetes* (~13.21%), *Proteobacteria* (~7.65%) and *Actinobacteria* (~5.37%). Abundance of each other phyla was below 1%, in comparison with the database reference "SILVA 128" using the bioinformatics pipeline QIIME. At the genus level; *Alistipes* from the *Bacteroidetes* phylum was the most abundant, with 12.53% of abundance.

In the *Sb*+DA group, the Coriobacteriia class decreased with an average fold change (AFC) - 67% (p-value = 0.010; p-value adjust with FDR = ns) , the *Deltaproteobacteria* class decreased with an AFC of -77.6% (*p*-value = 0.0021; *p*-value adjust with FDR = 0.006), and of the *Hungatella* genus decreased with an AFC of -74.9% (*p*-value = 0.038; *p*-value adjust with FDR = ns). (Figure 3 C to E)

On Day 15, *F. prausnitzii* was detected in 47 of 48 samples of $1.8x10^4$ copy/ng of DNA ($8x10^3$ to $3x10^4$ copy/ng DNA), No difference of the abundance of *F. prausnitzii* was found between the D0 and D15 samples (Data not shown).

Interestingly, a refined analysis in subgroups of patients according to their clinical improvement demonstrated that *Sb*+DA intervention significantly increased number of *F. prausnitzii* (+120%, *p*-value = 0.049; *p*-value adjust with FDR = NS) inpatients with normalized transit at Day 15, when compared to whose still presenting diarrheic stools. Also, an increased number of *F. prausnitzii* (+400%, *p*-value = 0.045; *p*-value adjust with FDR = NS) was found after *Sb*+DA treatment in patients with the best improvement (defined by the resolution of the bacterial overgrowth and with an IBS-improved symptomatology when compared to those who became SIBO-negative but without IBS improvement). An increased number of *F. prausnitzii* (+76.5%, *p*-value = 0.021; *p*-value adjust with FDR = NS) was found in samples from *Sb*+DA patients with improvement of their abdominal pain scores when compared to patients without improvement of their abdominal pain. (Figure 3 F to H)

Effects of *S. boulardii* CNCM I-745 on fungal microbiota

The ITS2 DNA sequences analysis (Figure 4 A to D) of all samples collected at Day 0 from SIBO patients of both groupsindicated that the fungal intestinal microbiota was dominated by the phyla *Ascomycota* (91.4%), *Basidiomycota* (2%) and fungi that could not be identified when the bioinformatics pipeline Qiime searched for sequences identities in the "Unite Otus 1211" database (4.3%).

At genus level, *Saccharomyces* and *Debaryomyces* were the most abundant with 70.1% and 11.2% of abundance respectively. *Candida, Penicillium, Nakaseomyces* (a genus closely related to *Candida*) and *Filobasidium* were relatively well represented in feces of SIBO patients (3.5%, 2.7%, 1.9% and 0.9% respectively).

Sb+DA significantly increased the proportion of *Saccharomyces* (AFC: + 27.31%, *p*-value = 0.00028; *p*-value adjust with FDR = 0.00056) and other upper ranked taxa (*Saccharomycetaceae* AFC + 8.19%; *Saccharomycetales* AFC +

4.54%; *Saccharomycetes* AFC + 4.54%; *Ascomycota* AFC + 2.83%). Whereas, a decrease of *S. boulardii* was found in the DA group (AFC: -7.44%, *p*-value = 0.633; *p*-value adjust with FDR = ns).

A major finding was that *Sb*+DA increased the proportion of the *Filobasidiales* order, the family *Filobasidiaceae*, and the genus *Filobasidium* belonging to that order (AFC: +1011.67%, *p*-value = 0.00748; *p*-value adjust with FDR = 0.045). These fungi were detectable in 19.23% of the *Sb*+DA samples on Day 0, and in 57.69% of the *Sb*+DA samples on Day 15. There was no such increase in the DA group with 33.33% of patients at Day 0 and 42.86% of patients at D15(*p*-value = 0.604; *p*-value adjust with FDR = ns). (Figure 4)

Safety

Five patients in each group reported at least one adverse event. Most frequent adverse events were pharyngitis (2 patients in the DA group) and headache (2 patients in the *Sb*+DA group). Digestive adverse events occurred in 2 patients in each group.
One AE (sinusitis in the *Sb*+DA group) led to study treatment discontinuation; this AE was judged unrelated to study medication and was ongoing at the end of the study. One patient (DA group) reported 1 serious AE (papillophlebitis); the patient recovered within 5 days. This AE was also judged unrelated to study intervention.

DISCUSSION

Our study shows that *S. boulardii* CNCM I-745 has beneficial effects in patients with IBS and SIBO. Indeed, in addition to improving clinical symptoms associated with SIBO, *S. boulardii* CNCM I-745 added to dietary advice reduced the incidence of diarrhea compared to dietary advice alone. Our findings are consistent with previous data which showed that *S. boulardii* CNCM I-745 prevents or treats diarrhea from different etiologies [24,25], induces an overall clinical improvement among patients with SIBO [26] and exerts a relief in global symptoms in diarrhea-predominant IBS patients [27]; this is further observed in a meta-analysis on efficacy and safety of *S. boulardii* in adults [20].

Our findings should be considered cautiously due to some limitations: this was designed as an exploratory study with the aim to provide preliminary data on the impact of *S. boulardii* treatment on bacterial overgrowth and microbiome composition in IBS-D patients and some outcomes did not reach statistical significance due to the limited sample size.

Despite the well-known low-to-moderate sensitivity and sensibility of LHBT [28],we found a trend towards a lower hydrogen excretion among SIBO-positive patients who received treatment with *S. boulardii*. Indeed, hydrogen excretion diminished after *S. boulardii* plus diet treatment. This modification in terms of lactulose breath test results was associated with an overall symptom improvement, and the reduction of LHBT-AUC was more pronounced in the *Sb+DA* group (14.7 AUC points per 1 IBS-SSS point) compared to the DA group (40.6 points) (p=0.0082), suggesting that the treatment effect might be bigger in patients with severe IBS symptoms at baseline.

A lower proportion of patients had diarrhea after treatment completion, in the *Sb*+DA group. *S. boulardii* efficacy in the treatment of acute diarrhoea has been extensively studied and this probiotic drug proved to be efficacious [25,26]. However, its efficacy in the context of a chronic condition such as IBS has been less studied. It is worth noting that the *S. boulardii* treatment duration in our study was relatively short, a feature that has been observed in other clinical studies assessing *S. boulardii* efficacy in the setting of IBS [27,29]. Although more evidence is clearly needed, our study showed that *S. boulardii* could be used as a long-term maintenance therapy to alleviate symptoms in patients with IBS, a feature that makes maintenance therapy with probiotics such as *S. boulardii* a promising alternative.

Previous data has shown that *S. boulardii* CNCM I-745 supports the restauration of intestinal microbiota after diarrheic dysbiosis [30].

In this study, at the bacteria level, Firmicutes, Bacteroidetes and Actinobacteria levels were found to be in the range of generally described proportions in healthy intestinal microbiota. However, a high ratio of Proteobacteria (5-10%) was detected in the patients, indicative of dysbiosis (the proportion of Proteobacteria in healthy samples is generally around 1%) [31]. The reduction of Proteobacteria in *Sb* patients with clinical signs improvement might reflect a positive effect of *S. boulardii* CNCM I-745. Interestingly *F. prausnitzii* level was higher in patients with

marked clinical improvement in the *Sb* group .Validated method and established thresholds showed that *F.prausnitzii* is associated with healthy condition [32] and even helpful in transplantation to improve a dysbiotic community structure [33]. It is known that *F.prausnitzii* exerts an anti-inflammatory effect. Various current findings support the hypothesis of an inflammatory-immunological etiopathogenesis in IBS [34]. Thus, one of the major findings of this explorative study is the increase of *F. prausnitzii* level in clinically improved patients treated by *S. boulardii* CNCM I-745.

CONCLUSIONS

Although further investigations are required, this exploratory study demonstrates positive effects of *S. boulardii* CNCM I-745 on both clinical improvement of the gastrointestinal symptoms and a reduction in the bacterial overgrowth.

The symptoms improvement seems to be associated with modifications of the intestinal microbiota. Further studies are required to explore the increase in the abundance *of F. prausnitzii* in patients treated with *S. boulardii***.**

STATEMENT OF ETHICS

This research was conducted according to the principles of the Declaration of Helsinki and local and international laws. The study protocol was reviewed and approved (on 21JAN2016) by the Ethics Committee Comite de Etica en Investigation Clinica (CEIC), Ciudad Autonoma de Buenos Aires, Argentina. All patients gave written informed consent before study onset. The protocol was presented to ClinicalTrials.gov and the identification number was NCT04627337.

Conflict of Interest Statement

Authors received research funding from Biocodex

Funding Sources

Partial financial support was received from Biocodex for this work.

Author Contributions

Luis Maria Bustos Fernandez

Study investigator and/or managed the patients.

Analysis and interpretation of data.

Conception and design of the study and the collection, analysis, and interpretation of data.

Critically reviewed the manuscript and approved the final draft for submission.

Fernando Man

Study investigator and/or managed the patients.

Analysis and interpretation of data.

Conception and design of the study and the collection, analysis, and interpretation of data.

Critically reviewed the manuscript and approved the final draft for submission.

Juan Sebastian Lasa

Analysis and interpretation of data.

Conception and design of the study and the collection, analysis, and interpretation of data.

Critically reviewed the manuscript and approved the final draft for submission.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the study database. All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

BIBLIOGRAPHY

1. Jeffery IB, O'Toole PW, Öhman L, Claesson MJ, Deane J, Quigley EMM et al. An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. Gut 2012; 61(7):997–1006.

2. Kassinen A, Krogius-Kurikka L, Mäkivuokko H, Rinttilä T, Paulin L, Corander J et al. The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. Gastroenterology 2007; 133(1):24–33.

3. 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):521-30, e248.

4. Chassard C, Dapoigny M, Scott KP, Crouzet L, Del'homme C, Marquet P et al. Functional dysbiosis within the gut microbiota of patients with constipated-irritable bowel syndrome. Aliment Pharmacol Ther 2012; 35(7):828–38.

5. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. Gastroenterology 2006; 130(5):1480–91.

6. Mearin F, Lacy BE, Chang L, Chey WD, Lembo AJ, Simren M et al. Bowel Disorders. Gastroenterology 2016.

7. Braak B, Klooker TK, Wouters MM, Welting O, van der Loos CM, Stanisor OI et al. Mucosal immune cell numbers and visceral sensitivity in patients with irritable bowel syndrome: Is there any relationship? Am J Gastroenterol 2012; 107(5):715–26.

8. Ludidi S, Conchillo JM, Keszthelyi D, van Avesaat M, Kruimel JW, Jonkers DM et al. Rectal hypersensitivity as hallmark for irritable bowel syndrome: Defining the optimal cutoff. Neurogastroenterol Motil 2012; 24(8):729-33, e345-6.

9. Ford AC, Talley NJ. Mucosal inflammation as a potential etiological factor in irritable bowel syndrome: A systematic review. Japan; 2011 Apr.

10. Simrén M, Barbara G, Flint HJ, Spiegel BMR, Spiller RC, Vanner S et al. Intestinal microbiota in functional bowel disorders: A Rome foundation report. Gut 2013; 62(1):159–76.

11. Hetterich L, Stengel A. Psychotherapeutic Interventions in Irritable Bowel Syndrome. Front Psychiatry 2020; 11:286.

12. Barker WH, Hummel L. Macrocytic anemia in association with intestinal strictures and anastomosis. Bull Johns Hopkins Hosp 1939; 46:215.

13. Ford AC, Spiegel BMR, Talley NJ, Moayyedi P. Small intestinal bacterial overgrowth in irritable bowel syndrome: Systematic review and meta-analysis. United States; 2009 Dec.

14. Man F, Bolino C, Bustos Fernandez L. Prevalence of Abnormal Hydrogen Excretion in Non-Constipated Irritable Bowel Syndrome Patients. J Gastroenterol Hepatol 2013; (28) Suppl 3, 124.

15. Walters B, Vanner SJ. Detection of bacterial overgrowth in IBS using the lactulose H2 breath test: Comparison with 14C-D-xylose and healthy controls. Am J Gastroenterol 2005; 100(7):1566–70.

16. Shah ED, Basseri RJ, Chong K, Pimentel M. Abnormal breath testing in IBS: A meta-analysis. United States; 2010 Sep.

17. Rezaie A, Buresi M, Lembo A, Lin H, McCallum R, Rao S et al. Hydrogen and Methane-Based Breath Testing in Gastrointestinal Disorders: The North American Consensus. Am J Gastroenterol 2017; 112(5):775–84.

18. Pimentel M, Lembo A, Chey WD, Zakko S, Ringel Y, Yu J et al. Rifaximin therapy for patients with irritable bowel syndrome without constipation. N Engl J Med 2011; 364(1):22–32.

19. Ford AC, Quigley EMM, Lacy BE, Lembo AJ, Saito YA, Schiller LR et al. Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: Systematic review and meta-analysis. United States; 2014 Oct.

20. McFarland LV. Systematic review and meta-analysis of Saccharomyces boulardii in adult patients; 2010 May 14.

21. 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.

22. Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. Scand J Gastroenterol 1997; 32(9):920–4.

23. Blake MR, Raker JM, Whelan K. Validity and reliability of the Bristol Stool Form Scale in healthy adults and patients with diarrhoea-predominant irritable bowel syndrome. Aliment Pharmacol Ther 2016; 44(7):693–703.

24. Szajewska H, Kołodziej M. Systematic review with meta-analysis: Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea. England; 2015 Oct.

25. Szajewska H, Kołodziej M, Zalewski BM. Systematic review with meta-analysis: Saccharomyces boulardii for treating acute gastroenteritis in children-a 2020 update. England; 2020 Apr.

26. García-Collinot G, Madrigal-Santillán EO, Martínez-Bencomo MA, Carranza-Muleiro RA, Jara LJ, Vera-Lastra O et al. Effectiveness of Saccharomyces boulardii and Metronidazole for Small Intestinal Bacterial Overgrowth in Systemic Sclerosis. Dig Dis Sci 2020; 65(4):1134–43.

27. Abbas Z, Yakoob J, Jafri W, Ahmad Z, Azam Z, Usman MW et al. Cytokine and clinical response to Saccharomyces boulardii therapy in diarrhea-dominant irritable bowel syndrome: A randomized trial. Eur J Gastroenterol Hepatol 2014; 26(6):630–9.

28. Saad RJ, Chey WD. Breath testing for small intestinal bacterial overgrowth: Maximizing test accuracy. Clin Gastroenterol Hepatol 2014; 12(12):1964-72; quiz e119-20.

29. Choi CH, Jo SY, Park HJ, Chang SK, Byeon J-S, Myung S-J. A randomized, double-blind, placebo-controlled multicenter trial of saccharomyces boulardii in irritable bowel syndrome: Effect on quality of life. J Clin Gastroenterol 2011; 45(8):679–83.

30. Moré MI, Swidsinski A. Saccharomyces boulardii CNCM I-745 supports regeneration of the intestinal microbiota after diarrheic dysbiosis - a review. Clin Exp Gastroenterol 2015; 8:237–55.

31. Shin N-R, Whon TW, Bae J-W. Proteobacteria: Microbial signature of dysbiosis in gut microbiota. Trends Biotechnol 2015; 33(9):496–503.

32. Lopez-Siles M, Enrich-Capó N, Aldeguer X, Sabat-Mir M, Duncan SH, Garcia-Gil LJ et al. Alterations in the Abundance and Co-occurrence of Akkermansia muciniphila and Faecalibacterium prausnitzii in the Colonic Mucosa of Inflammatory Bowel Disease Subjects. Front Cell Infect Microbiol 2018; 8:281.

33. Ganesan K, Chung SK, Vanamala J, Xu B. Causal Relationship between Diet-Induced Gut Microbiota Changes and Diabetes: A Novel Strategy to Transplant Faecalibacterium prausnitzii in Preventing Diabetes. Int J Mol Sci 2018; 19(12).

34. Carco C, Young W, Gearry RB, Talley NJ, McNabb WC, Roy NC. Increasing Evidence That Irritable Bowel Syndrome and Functional Gastrointestinal Disorders Have a Microbial Pathogenesis. Front Cell Infect Microbiol 2020; 10:468.

FIGURES LEGENDS

Figure 1 Flow Diagram of the study patients. CONSORT diagram of study patient distribution.

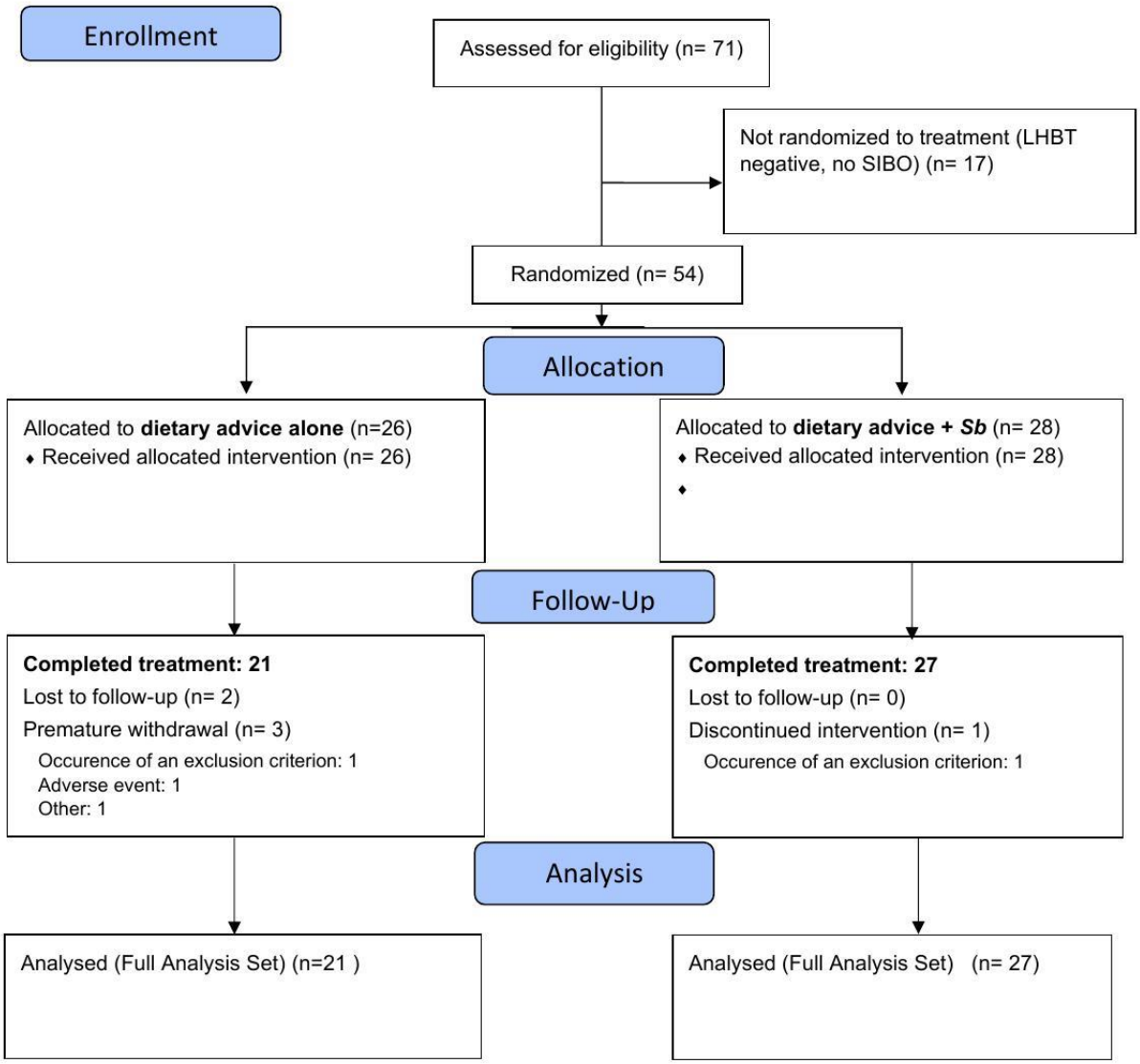
LHBT, Lactulose-hydrogen breath test; SIBO, small intestinal bacterial overgrowth; S, Saccharomyces boulardii CNCM I-745

Figure 2 Clinical changes at end of study. Figure 2A Illustration of changes from baseline of LHBT AUC (primary evaluation criterion) expressed as mean +/- SD. p=0,044 (ANCOVA) for the difference between groups. Figure 2B Change from baseline of the IBS-SSS. Figure 2C Distribution of patients according to transit status (normal, diarrhea, constipation). LHBT: Lactulose Hydrogen Breath Test; AUC: Area Under the Curve; IBS-SSS, Irritable bowel syndrome – Symptom severity score.

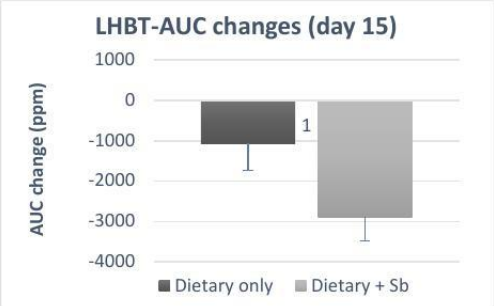
Figure 3 Overview on the bacterial microbiota composition and effect of S boulardii on F prausnitzii. Overview of the bacterial microbiota composition of the 48 selected patients at start and end of the study. A) Bacterial composition at phylum level. Each column stands for a subgroup. Each color bar represents the relative abundance of a given phylum. B) Bacterial composition at genus level, only the 10 more abundant genera are represented. C) Relative abundance of the Coriobacteriia class at D0 and D15 in the treatment groups. D) Relative abundance of the Deltaproteobacteria class at D0 and D15 in the treatment groups. E) Relative abundance of the genus Hungatella at D0 and D15 in the treatment groups. p values are indicated. * : $p<0.05$; ** : $p<0.01$. F) Number of genome copies of Fecalibacterium prausnitzii by ng of DNA in feces of Sb treated or not patients with SIBO negative and IBS improvement or not. *:$p<0.05$. G) Number of genome copies of F. prausnitzii by ng of DNA in feces of Sb treated or not patients with normal or diarrheic stools. *:$p<0.05$. H) Number of genome copies of F. prausnitzii by ng of DNA in feces of Sb treated or not patients with abdominal pain improvement or not. *:$p<0.05$

Figure 4: Overview of the fungal microbiota composition. Overview of the fungal microbiota composition of the 48 selected patients at start and end of the study. A) Fungal composition at phylum level. Each column stands for a subgroup. Each color bar represents the relative abundance of a given phylum. B) Fungal composition at genus level, only the 10 more abundant genera are represented. C-D) Relative abundance of the Filobasidiales and Saccharomyces genera, respectively, at D0 and D15 in the control and Sb-treated groups.

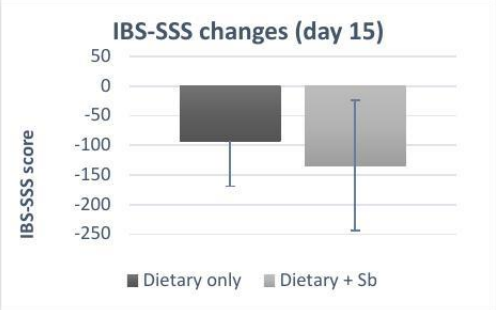
*: $p<0.05$; ** : $p<0.01$; *** : $p<0.001$


Enrollment
Assessed for eligibility (n= 71)
Not randomized to treatment (LHBT negative, no SIBO) (n= 17)
Randomized (n= 54)
Allocation
Allocated to **dietary advice alone** (n=26)
♦ Received allocated intervention (n= 26)
Allocated to **dietary advice + *Sb*** (n= 28)
♦ Received allocated intervention (n= 28)
♦
Follow-Up
Completed treatment: 21
Lost to follow-up (n= 2)
Premature withdrawal (n= 3)
Occurence of an exclusion criterion: 1
Adverse event: 1
Other: 1
Completed treatment: 27
Lost to follow-up (n= 0)
Discontinued intervention (n= 1)
Occurence of an exclusion criterion: 1
Analysis
Analysed (Full Analysis Set) (n=21)
Analysed (Full Analysis Set) (n= 27)

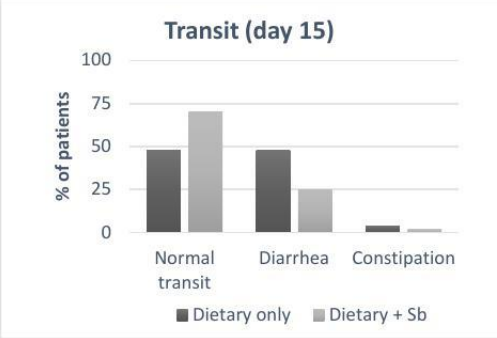
2A

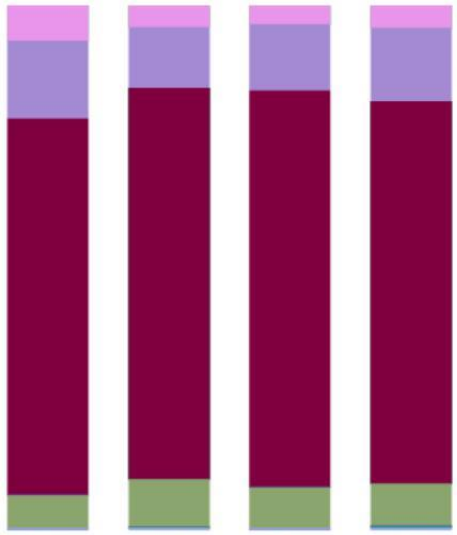

LHBT-AUC changes (day 15)
AUC change (ppm)
1000
0
-1000
-2000
-3000
-4000
1
Dietary only
Dietary + Sb

2B


IBS-SSS changes (day 15)
IBS-SSS score
50
0
-50
-100
-150
-200
-250
Dietary only
Dietary + Sb

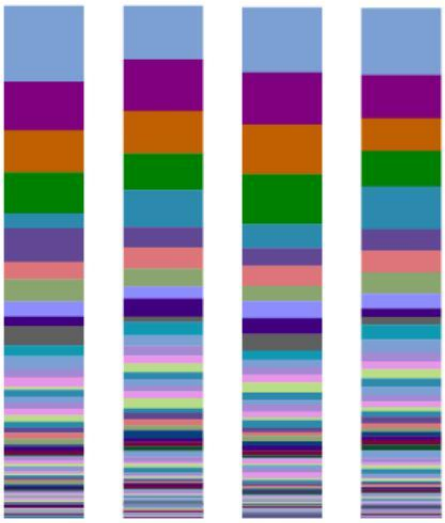
2C


Transit (day 15)
% of patients
100
75
50
25
0
Normal transit
Diarrhea
Constipation
Dietary only
Dietary + Sb



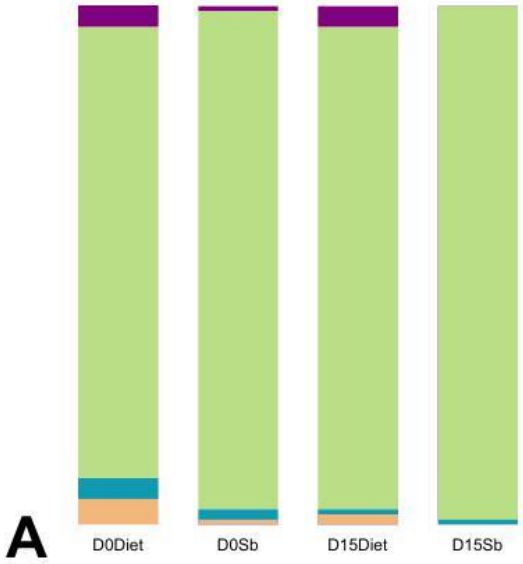
A

Taxonomy	D0Diet	D0Sb	D15Diet	D15Sb
Euryarchaeota	0.0%	0.0%	0.0%	0.0%
Acidobacteria	0.0%	0.0%	0.0%	0.0%
Actinobacteria	6.7%	4.1%	3.5%	4.2%
Bacteroidetes	14.9%	11.6%	12.7%	14.0%
Cyanobacteria	0.0%	0.0%	0.0%	0.0%
Elusimicrobia	0.0%	0.0%	0.0%	0.0%
Firmicutes	71.7%	74.7%	75.6%	72.9%
Fusobacteria	0.2%	0.0%	0.0%	0.0%
Lentisphaerae	0.0%	0.0%	0.0%	0.0%
Proteobacteria	6.2%	9.1%	7.6%	8.0%
Saccharibacteria	0.0%	0.0%	0.0%	0.0%
Spirochaetae	0.0%	0.0%	0.0%	0.0%
Synergistetes	0.0%	0.1%	0.0%	0.3%
Tenericutes	0.0%	0.0%	0.0%	0.0%
Verrucomicrobia	0.1%	0.0%	0.2%	0.1%
Bacteria;Other	0.0%	0.0%	0.0%	0.0%
Other	0.3%	0.3%	0.3%	0.4%

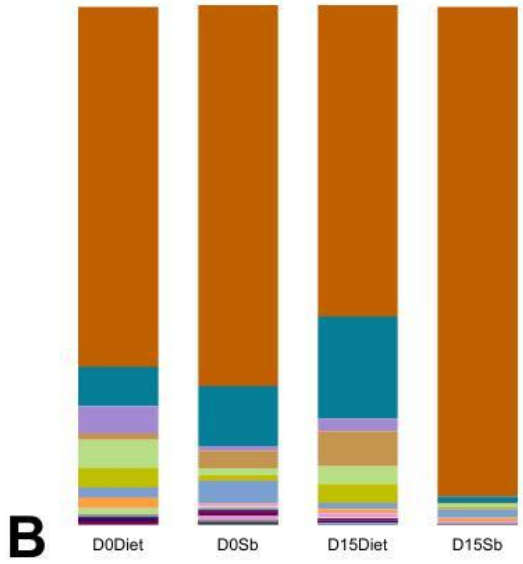


B

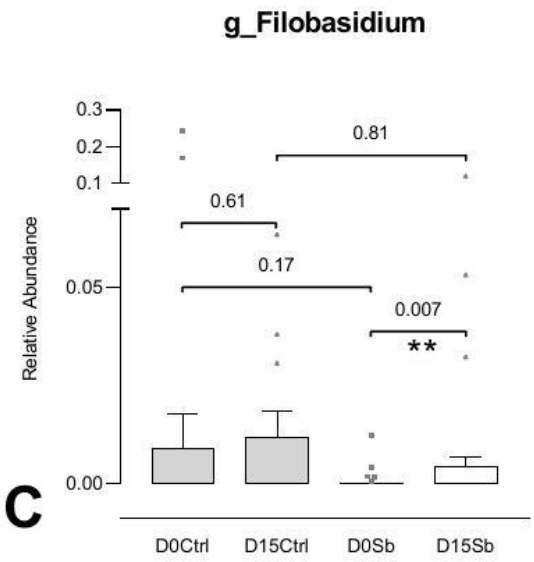
Taxonomy	D0Diet	D0Sb	D15Diet	D15Sb
Alistipes	14.7%	10.4%	12.6%	13.0%
[Eubacterium] rectale group	9.4%	10.0%	10.1%	8.4%
Lachnospiraceae;other	8.2%	8.2%	9.6%	6.3%
Roseburia	7.9%	7.1%	9.7%	6.9%
Dialister	2.9%	7.2%	4.7%	8.3%
Bifidobacterium	6.5%	3.9%	3.4%	4.1%
Ruminococcaeae;uncultured	3.4%	4.1%	3.9%	4.2%
Ruminococcaceae UCG-002	4.3%	3.5%	2.9%	4.2%
Ruminococcaceae;Other	2.9%	2.3%	3.3%	2.8%
Parasuterella	1.8%	3.5%	3.1%	1.7%
Ruminococcus	3.8%	1.1%	3.2%	1.5%
Suterella	2.1%	2.6%	1.7%	2.9%

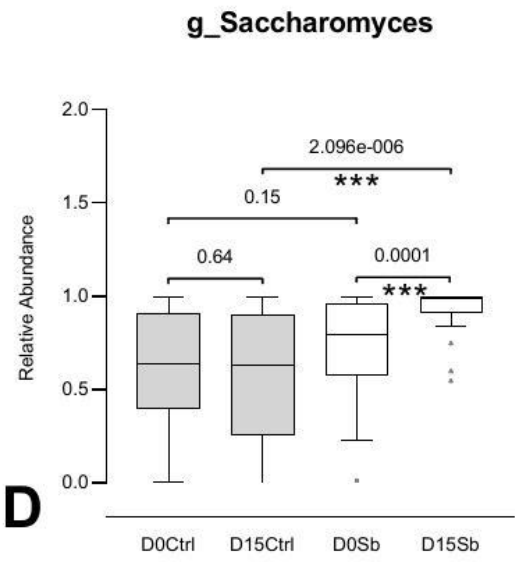


Taxonomy	D0Diet	D0Sb	D15Diet	D15Sb
No blast hit;Other	3.8%	1.1%	3.6%	0.4%
Ascomycota	87.1%	96.1%	92.6%	98.7%
Basidiomycota	3.9%	1.9%	1.1%	0.8%
Chytridiomycota	0.0%	0.0%	0.3%	0.0%
Zygomycota	0.0%	0.0%	0.0%	0.0%
Fungi;unidentified	5.3%	0.9%	2.4%	0.1%



Taxonomy	D0Diet	D0Sb	D15Diet	D15Sb
Saccharomyces	69.4%	73.3%	60.0%	94.4%
Debaryomyces	7.6%	11.6%	19.7%	1.2%
Fungi;unidentified	5.3%	0.9%	2.4%	0.1%
Candida	1.2%	3.4%	6.8%	0.1%
Penicillium	5.4%	1.3%	3.4%	0.6%
Other	3.8%	1.1%	3.6%	0.4%
Nakaseomyces	1.9%	4.3%	1.2%	1.7%
Filobasidium	2.0%	0.1%	0.8%	0.7%
Kluyveromyces	0.1%	0.5%	0.8%	0.4%
Fungi;unidentified	1.2%	0.3%	0.2%	0.0%





Patients' initial characteristics Mean (SD) or N or %)	**DA (N=21)**	**DA+Sb (N=27)**	**Total (N=48)**
Age (years)	39.9 (13.9)	47.5 (15.3)	44.2 (15.1)
Sex			
Male	11 (52.4%)	11 (40.7%)	22 (45.8%)
Female	10 (47.6%)	16 (59.3%)	26 (54.2%)
Height (cm)	165.6 (9.5)	165.2 (8.7)	165.4 (8.9)
Weight (kg)	65.6 (14.8)	68.8 (12.4)	67.4 (13.4)
Body Mass Index (kg/m²)	23.76 (4.17)	25.19 (4.31)	24.57 (4.27)
IBS according to Rome III	21 (100%)	27 (100%)	
MEDICAL HISTORY			
GASTROINTESTINAL DISORDERS			
Abdominal discomfort	2 (9.5%)	1 (3.7%)	3 (6.3%)
Abdominal distension	4 (19.0%)	1 (3.7%)	5 (10.4%)
Abdominal pain	2 (9.5%)	3 (11.1%)	5 (10.4%)
Diarrhoea	4 (19.0%)	3 (11.1%)	7 (14.6%)
DISEASE CHARACTERISTICS			
LHBT ppm H2/time	7196.5 (4147.8)	6433.8 (3626.7)	
IBS-SSS score	300.3 (59.26)	305.6 (77.89)	

Abbreviation: DA, dietary alone; LHBT: Lactulose Hydrogen Breath Test; IBS-SSS, Irritable bowel syndrome – Symptom severity score

Table 1 Patients' baseline characteristics. Patient clincial characteristics (demography, disease characteristics and medical history) at inclusion. Only most frequent (>10%) or relevant medical history is displayed; IBS-SSS : score ranges from 0 (no symptoms) to 500 (maximum severity). Transit was estimated according to the Bristol stool scale.

Table 2A				
LHBT AUC (ppm H2/time) of hydrogen excretion		**DA (N=21)**	**DA+Sb (N=27)**	**p-value**
Baseline (D0)	**N**	**21**	**27**	
	Mean (SD)	7196.5 (4147.8)	6433.8 (3626.7)	
	Median	5490.0	5490.0	
	Q1 ; Q3	3996.0 ; 8892.0	3834.0 ; 7578.0	
	Min; Max	3078 ; 15000	3168 ; 15786	
	Missing	0	0	
EOS (D15)	**N**	**21**	**27**	
	Mean (SD)	5729.0 (3846.3)	3846.4 (2103.8)	
	Median	5904.0	3600.0	
	Q1 ; Q3	2268.0 ; 7722.0	2106.0 ; 5598.0	
	Min; Max	18 ; 15000	522 ; 8694	
	Missing	0	0	
Change from baseline	**N**	**21**	**27**	**0.4928**
	Mean (SD)	-1467.4 (5499.4)	-2587.3 (3858.1)	
	Median	-1242.0	-1458.0	
	Q1 ; Q3	-4122.0 ; 2106.0	-4578.0 ; 180.0	
	Min; Max	-13820 ; 9510	-12750 ; 2754	
	Missing	0	0	

Table 2B				
LHBT AUC (ppm H2/time) change from baseline		**No treatment (N=21)**	**Sb treatment (N=27)**	**p-value**
	Estimate (SE)	-1071 ± 658	-2895 ± 580	
	95 CI	[-2396, 254]	[-4064, -1727]	
	Difference Sb – No treatment	-1824 ± 879		**0.044**
	95 CI of the difference	[-3595, -53.1]		
	Missing	0	0	

Abbreviation : DA, dietary alone; LHBT: Lactulose Hydrogen Breath Test; AUC: Area Under the Curve; Q1, Q3: 1st and 3rd interquartile interval; SE: standard error; 95 CI: confidence interval at 95%.

Table 2 Primary efficacy endpoint: AUC changes of the Lactulose Hydrogen Breath Test. Analysis of the primary efficacy analysis: change from baseline of AUC of the LHBT. Table 2A: primary analysis, using a non-parametric test Wilcoxon-Mann Whitney. Mean values at baseline (day 0), end of study (day 15) and difference day15 – day0 are displayed. Table 2B: post-hoc analysis, using a-parametric test ANCOVA