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Effect of multispecies probiotic on gut microbiota composition in individuals with intestinal constipation: A double-blind, placebo-controlled randomized trial

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Objective: The aim of this study was to evaluate the effect of a multispecies probiotic on gut microbiota composition and constipation symptoms.
Methods: A randomized, double-blind, placebo-controlled clinical trial was conducted with 35 individuals with constipation for 30 days. The individuals were randomized into two groups: the control capsule (CC) and the probiotic capsule (PC) groups. Constipation symptoms were evaluated by the ROME IV criteria and by evacuation diaries. Fecal microbiota was analyzed by 16 S rRNA gene sequencing.
Results: The majority of participants were women (85.7%). There was a significant reduction in the percent of participants who had incomplete defecation ($P = 0.034$), blockage sensation ($P = 0.025$), and rarely present liquid stools without the aid of laxatives ($P = 0.046$) only within the PC group (but no significant difference between groups). There was a significant increase in the relative abundance percentage of *Blautia faecis* and *Ruminococcus torques* in the CC group ($P = 0.003$ and $P = 0.011$, respectively), although there was no significant change in the PC group ($P = 0.794$ and $P = 0.958$, respectively), with a significant difference between groups ($P = 0.029$ and P 0.013, respectively), suggesting that probiotic treatment prevented the increase of percent relative abundance of these two species.
Conclusion: These results suggest that multispecies probiotics in capsule form may modulate gut microbiota by reducing the bacteria that are commonly increased in patients with constipation, contributing to the balance of microbiota and, consequently, to the well-being of the individual. Future studies with larger numbers of patients are required.

Introduction

The gastrointestinal (GI) tract forms a complex ecosystem with resident gut microbiota, which performs numerous important functions for intestinal health, such as fermentation of nondigestible compounds, production of short-chain fatty acids, modulation of the intestinal immune system, and regulation of intestinal motility [1].

In situations such as intestinal constipation, the gut microbiota may be out of balance, with a higher abundance of potentially pathogenic bacteria with commensal characteristics as *Pseudomonas aeruginosa* and *Escherichia coli*, in detriment of *Bifidobacteria* and *Lactobacillus* strains. Thus, symptoms of intestinal constipation have been associated with intestinal dysbiosis [2]. This fact highlights the importance of gut microbiota modulation, especially when classic manifestations of constipation are present.

Modulation of gut microbiota can be achieved by the intake of probiotics. Probiotics are living microorganisms that, when ingested in sufficient amounts, can provide some benefits to the host's health [3]. Recent evidence suggests a benefit of using this product in patients with constipation, improving symptoms and contributing to the patient's quality of life [4]. In a meta-analysis, Dimidi et al. [5] demonstrated that the use of probiotics led to a

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significant increase in weekly evacuation frequency in patients with constipation. However, caution is required in the interpretation of the data as the effects of probiotics are strain-dependent and vary according to host initial gut microbiota composition [5]. This strain-dependent effect has been demonstrated by Wang et al. [6]. The authors supplemented mice with three different variations of *B. adolescentis* (CCFM 626, 667, 669) and found that variations 667 and 669 presented better effects on constipation as there was a greater adhesion of these bacteria to the intestinal cells and, consequently, a greater effect on gut microbiota. Additionally, the evaluation of the co-administration of different strains have not been thoroughly investigated yet, and therefore there remains a lack of evidence on the real efficacy of this type of product in intestinal health.

In addition to the strain-dependent effect, it is important to emphasize that there are few studies that investigated the effect of probiotic in healthy individuals with intestinal constipation. Most of the published studies on gut microbiota have considered the effect of products containing probiotics only on constipation outcomes [7–9], with few actually investigating the effect on gut microbiota composition—the latter often adopting targeted or semi-targeted approaches [10,11]. Untargeted metagenomic techniques, such as 16S sequencing, offer a more comprehensive approach to understanding how gut microbiota might be influenced by probiotics.

Thus, this study aimed to evaluate the effect of a multispecies probiotic product on the microbiota composition in individuals with constipation.

Materials and methods

Experimental design

A randomized, double-blind, placebo-controlled clinical trial with 48 individuals with constipation was conducted. Individuals were recruited between September and October 2017 from the Federal University of Goiás, Brazil, by inviting students and employees who were interested in participating and who fit the established eligibility criteria. Additionally, the study recruitment was advertised through leaflets and via the university's official website as well as via social media, such as Facebook and Instagram, with digital posts published online containing an invitation with basic contact details for further information.

All procedures involving human participants were approved by the Ethics Committee of the University of Goiás. Written informed consent was obtained from all participants. The study was registered at ensaiosclinicos.gov.br.

A questionnaire was applied to assess inclusion and exclusion criteria and to identify eligible volunteers. The following inclusion criteria were used: between 19 and 70 y of age, with a constipation diagnosis according to ROME IV diagnostic criteria for constipation. Individuals were excluded if they presented a diagnosis of diseases of the GI tract or complications from surgeries due to these diseases, had hepatic or renal dysfunction, or took either antibiotics or vitamins, minerals, fortified foods, herbal products, or other supplements aimed to reduce the digestive symptoms in the previous 4 wk. Individuals who consumed food containing probiotics and prebiotics or food/nutraceuticals with anti-inflammatory activity before or during the intervention also were excluded.

The individuals were randomized into two groups according to sex, age, and body mass index (BMI) using a Software R 2.15.3 for Windows. The first group was the control capsule group (CC). This group received maltodextrin (75 mg) in capsules for 30 d. The second group, the probiotic capsule group (PC), received a mix of probiotics, containing 5×10^9 CFU of *Lactobacillus acidophilus* (NCFM), *L. casei* (Lc-11), *Lactococcus lactis* (Li-23), *Bifidobacterium bifidum* (BB-06), and *B. lactis* (HN019) in capsules for 30 d.

Participants were instructed to ingest one capsule daily, at least 30 min after the last meal of the day, with water at room temperature. They were also instructed to maintain routine and lifestyle habits and to avoid ingestion of products containing pre- and probiotics. The capsules and the encoded bottles provided had the same appearance and color and were distributed by a researcher not involved in the project, according to randomization protocol. Therefore volunteers and researchers/staff were blinded. The blinding code was provided to the investigators after statistical analysis was complete. Compliance was assessed by the returning of the bottles with the remaining capsules and individuals who consumed <80% of the capsules were excluded from the study.

Food intake and physical activity

Food intake and physical activity were assessed during the intervention. These two parameters were used to check whether changes in markers were actually obtained by the treatment, hence the instructions to maintain habitual lifestyle provided.

To evaluate habitual diet, a 3-d food record was used. Six records were collected for each individual, three of which referred to the first week of the study and the other three to the last week of the study.

Physical activity was evaluated by International Questionnaire of Physical Activity, which was validated for the Brazilian population. Metabolic equivalent of task (MET) values were calculated according to the following formulas:

1. MET – walking min/wk: 3.3 × walking minutes × walking days
2. MET– moderate activity min/wk: 4 × moderate activity minutes × moderate activity days
3. MET – vigorous activity min/wk: 8 × vigorous activity minutes × vigorous activity days
4. MET – total minutes of physical activity/wk: sum of MET – walking minutes + moderate activity + vigorous activity

Energy expenditure (kcal MET/wk) was calculated considering the min/wk for each activity estimated in METS, using the following formula:

MET – minutestotal × weightoftheindividual/60 min.

Constipation symptoms (primary outcome)

Participants were instructed to complete an intestinal diary with daily records for evacuation frequency and consistency of feces, according to the Bristol scale. Participants were also requested to record in their diary any adverse effects and inform the researchers immediately.

GI tract functional health was evaluated at baseline and after 30 d, through a questionnaire containing the criteria stablished by ROME IV: excessive exertion in ≥25% of bowel movements, hardened and/or dry stools in ≥25% of the stools, feeling of incomplete defecation in ≥25% of bowel movements, blockage sensation or anorectal obstruction, manual maneuver, evacuation frequency <3 times/wk, rarely presents liquid stools without the aid of laxatives.

Intestinal microbiota analysis (secondary outcome)

DNA isolation and 16S rRNA gene sequencing

Fecal samples were collected by swabs with stabilizing solution and were submitted to cell lysis and subsequent DNA extraction using the magnetic beads technique with a registered protocol (Neoprospecta Microbiome Technologies, Brazil). Samples of bacterial isolates (*Salmonella enterica* [ATCC 14028], *Listeria monocytogenes* [ATCC 19111], *Staphylococcus aureus* [ATCC 25923], *Bacillus cereus* [ATCC 10876], *Escherichia coli* [ATCC 8739], *Bacillus spizizenii* [ATCC 6633], *Enterococcus faecalis* [ATCC 29212], *Pseudomonas aeruginosa* [ATCC27853], *S. epidermidis* [ATCC 12228], *Acinetobacter baumanii*, and *Klebsiella pneumoniae* [isolated and identified by VITEK2]) were submitted to the same processing with magnetic beads to DNA obtainment. Dilutions were performed on a 10 × scale for DNA, and on a scale of 1 log of CFU for the isolated microorganisms. Additionally, a synthetic DNA molecule was inserted into some samples before DNA extraction and at different concentrations (~600, 6000, 60 000 molecules) to evaluate its recovery profile after sequencing. Bacteria identification was performed using the high-performance sequencing of the V3/V4 regions of the *16S rRNA* gene. Sequencing was performed on the MiSeq equipment (Illumina Inc., San Diego, CA, USA) using the single-end 300-cycle V2 kit, without library normalization. The DNA sequences of the microorganisms were analyzed through a proprietary pipeline (Neoprospecta Microbiome Technologies, Brazil), considering a maximum of 1% accumulated error in the sequencing. For the identification of the microorganism species present in the samples, the obtained DNA sequences were compared with a database containing other DNA sequences already characterized for the species of interest. After the bioinformatics analyses, the results were loaded onto the Neobiome platform for visualization.

Analysis of microbiota composition and statistics and network analyses

After completion of the Illumina MiSeq sequencing, the fastq files were trimmed to low-quality sequences and chimeras using proprietary Neotools software. Then, using the same software, the sequences that passed the quality steps were identified for bacterial taxonomies using the Neoref16S database. For the taxonomic identifications, the blastn software v.2.7.1 [12] was used, using lowest common ancestor algorithm for species definition (99.8% similarity). Taxonomies not meeting this minimum requirement were evaluated for the definition of sex, families, or other taxonomic levels [13]. The taxonomy and the operational

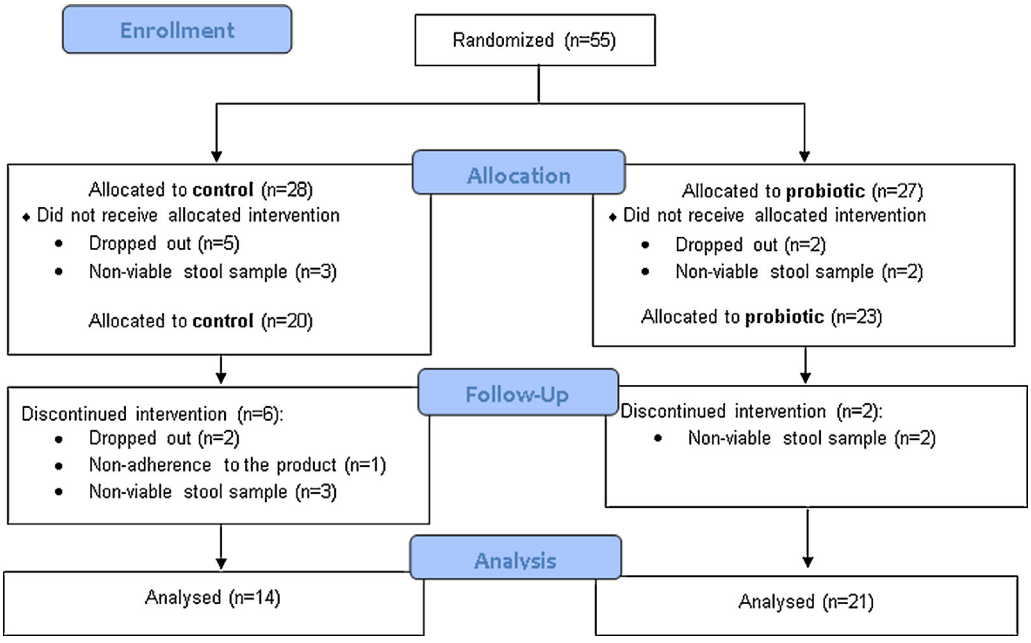

Fig. 1. Flowchart of study volunteers.

taxonomy unit (OTU) table, fasta sequences (clusters only) and metadata table were introduced in the α- and β-diversity analysis pipeline in R version 3.4.4 [14]. The phylogenetic tree was made from the fast sequences of the clusters, which are displayed on the platform of the Neoprospecta microbiological profile. Clustal Omega version 1.2.3 [15], construction of phylogenetic tree the FastTree version 2.1.10 [16] and mid-point rooting with Retree version 3.697 of the Phylip package [17] were used for sequence alignment. For the production of richness (observed OTUs, Chao1, ACE) and diversity (Shannon, Simpson, InvSimpson, Fisher, Evennes) indices, as well as other analyzes of α- (phylogenetic tree of taxonomic ranks) and β-diversity (principal component analysis and heatmap) the Phyloseq version 1.22.3 program package [18] was used. These data were normalized by the median and rarefaction method. Identification of differentially abundant species in the treatments was performed with the DESeq2 version 1.18.1 software package [19]. Walt test and negative binomial model were used for data normalization. Differentially abundant bacteria were considered to have $P < 1\%$ and log2FoldChange >2.

Statistical analysis

The sample calculation initially considered a design effect of 0.98 for the comparison of means of independent groups obtained from data of means and SD of evacuation frequency after intervention with symbiotics [8]; an α of 0.05 and test power (1-B) of 80%, and the result was 18 participants per group (n = 36). Therefore, we aimed to recruit 54 participants (27 for each group) in order to include an additional 50% of the required 36, accounting for possible dropouts. The calculation was performed by G * Power 3.1.9.2 program. Results are presented as mean ± SD. Values were initially assessed for normality by the Shapiro–Wilk test. The mean differences between groups at baseline and at final visit and total change were assessed by independent *t* test or Mann–Whitney test (the corresponding nonparametric test). The mean difference between baseline and final visit for each group was assessed by paired *t* test or Wilcoxon test (the corresponding nonparametric test). The value for α = 0.05 was adopted as critical for rejection of the null hypothesis. Statistical analysis was conducted using SPSS version 25 (IBM, Armonk, NY, USA).

Results

Characterization of the study population

We selected 55 participants according to the eligibility criteria and agreed to participate in the study (CC: 27; PC: 28). From those, 43 attend the baseline visit and delivered viable stools. During the clinical trial, eight participants were excluded from the study: two for no show, one for poor compliance (ingested <80% of the capsules), and five for non-viable stools samples. Thirty-five participants were included in the present analysis (CC: 14; PC: 21; Fig. 1). No difference was observed between groups at baseline (Table 1).

Food intake and physical activity

No significant differences between groups were observed for energy and macronutrient intake nor for level of physical activity during the intervention (at both baseline and post-intervention measurements; all $P > 0.05$; Table 2).

Table 1
Participants characteristics at baseline

Variables	CC (n = 14)	PC (n = 21)	*P*-value*
Sex			
Women, n (%)	12 (85.7)	18 (85.7)	1.000†
Men, n (%)	2 (14.3)	3 (14.3)	
Age (y)	31.00 ± 11.64	25.71 ± 7.22	0.106
BMI (kg/m^2)	24.54 ± 5.00	23.14 ± 3.57	0.374
Income (R$)	11,998.21 ± 31,130.20	3488.75 ± 3177.32	0.326
Years of study, n (%)			
≤11 y	2 (14.3)	4 (19.1)	0.714†
≥12 y	12 (85.7)	17 (80.9)	
Alcoholic beverage intake, n (%)			
Yes	6 (42.8)	9 (42.8)	1.000†
No	8 (57.2)	12 (57.2)	
Alcohol frequency, n (%)			
Do not drink	8 (57.2)	12 (57.1)	0.082†
1–2 times/wk	0	5 (23.8)	
1–3 times/mo	6 (42.8)	4 (19.1)	
Water intake, n (%), L/day			
≤1	7 (50)	8 (38.1)	0.555†
1–2	6 (42.8)	9 (42.8)	
>2	1 (7.2)	4 (19.1)	

CC, control capsule group; PC, placebo capsule group
*Statistical analysis: Independent *t* test unless otherwise stated.
†Statistical analysis: χ^2 test.

Table 2
Energy, macronutrients, and fiber intake and physical activity level of participants at baseline and after 30 d of intervention

Variables	CC (n = 14)				PC (n = 21)				
	T0	T30	Δ	*P*-value*	T0	T30	Δ	*P*-value*	P-value†
Energy (kcal)	1472.98 ± 413.63	1445.76 ± 372.25	−27.22 ± 374.35	0.937	1650.98 ± 413.63	1714.73 ± 687.36	63.75 ± 437.60	0.681	0.815
Carbohydrate (g)	172.68 ± 55.01	269.67 ± 61.92	−3.01 ± 56.70	0.857	182.44 ± 61.34	169.67 ± 61.92	10.54 ± 56.26	0.412	0.516
Protein (g)	66.84 ± 19.59	66.22 ± 25.64	−0.61 ± 21.25	0.937	74.85 ± 31.80	74.44 ± 33.66	−0.40 ± 31.92	0.940	0.613
Lipids (g)	57.20 ± 17.47	55.79 ± 14.59	−1.41 ± 21.41	0.823	64.64 ± 31.40	67.22 ± 28.46	2.58 ± 23.00	0.621	0.629
Fibers (g)	12.22 ± 6.15	10.54 ± 4.94	−1.68 ± 4.17	0.191	15.39 ± 5.72	13.81 ± 6.02	−1.57 ± 5.08	0.181	0.953
Total MET (min)	1822.32 ± 2078.42	2181.80 ± 1290.37	274.69 ± 1780.63	0.588	1333.50 ± 1103.68	1469.37 ± 1768.68	−174.70 ± 1427.19	0.621	0.449
MET (kcal)	2232.59 ± 2812.16	2584.49 ± 1719.97	−234.71 ± 2296.02	0.719	1426.90 ± 1457.77	1612.30 ± 2086.93	−175.92 ± 1425.85	0.618	0.552

CC, control capsule group; PC, placebo capsule group; MET, metabolic equivalent of task; T0, baseline; T30, post-intervention
Δ = T30 − T0
*Statistical differences between T30 and T0 within the same group.
†Statistical differences between Δ values of the control and treatment groups.

Richness and α-diversity indices

There was a significant increase of Shannon, Simpson, and InvSimpson indices only within the PC group ($P = 0.002$; $P = 0.013$; $P = 0.006$, respectively). An increase of Fisher, observed OTUs, and Chao1 and ACE indices occurred within both groups (all $P < 0.001$). No significant differences were observed for these indices between groups ($P > 0.05$; Fig. 2A, B).

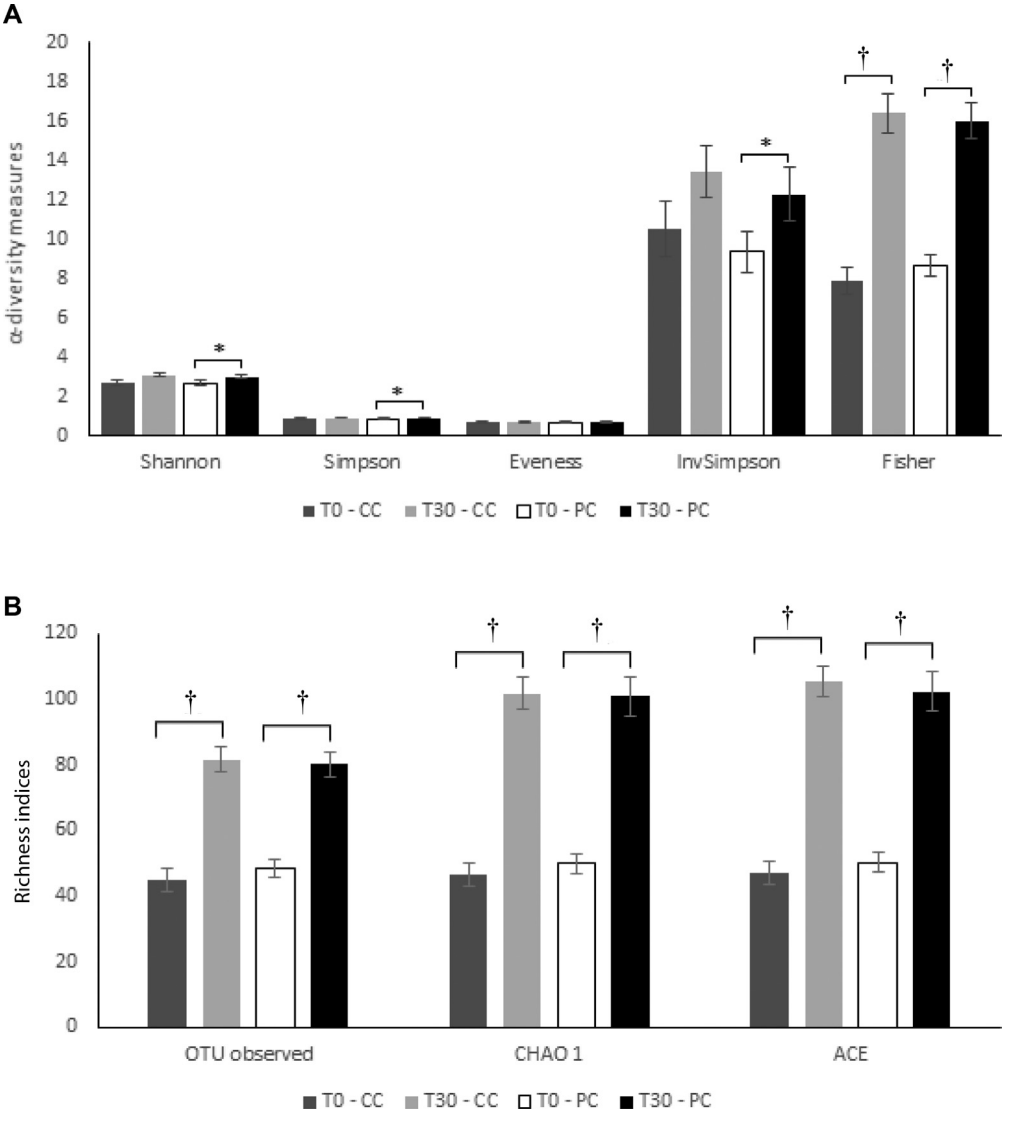

Fig. 2. Effect of probiotic on (**A**) α-diversity indices (Shannon, Simpson, InvSimpson, Fisher, Evennes) and (**B**) richness (observed OTUs, Chao1, ACE) indices. *$P < 0.05$. †$P < 0.001$. CC, control capsule group; OTU, operational taxonomy unit; PC, placebo capsule group.

β-*diversity*

Among the samples, 251 species of bacteria were identified. Of those, 75 presented $P < 1\%$ (corrected by false discovery rate) and 41 differed in absolute frequency among samples, being considered differentially abundant once presented $P < 1\%$ and log2FoldChange >2 by DEseq. Heatmap and phylogenetic tree of species detected by DEseq revealed significant within-group changes in both groups but without statistical difference between groups (Fig. 3A, B). The grouping profile of the samples by principal component analysis did not vary between treatments. The observed variation can be explained by the sum of the two main components, which results in ~19% (Fig. 4).

Relative abundance percentage

Further analyses were conducted to determine differences in percentage of relative abundance between treatment groups for phylum and for 75 species presented $P < 1\%$ (corrected by false discovery rate; data normalized by median).

Bacteria belonging to eight phylum were identified in the samples (Table 3). The most abundant phylum were Bacteroidetes (CC:

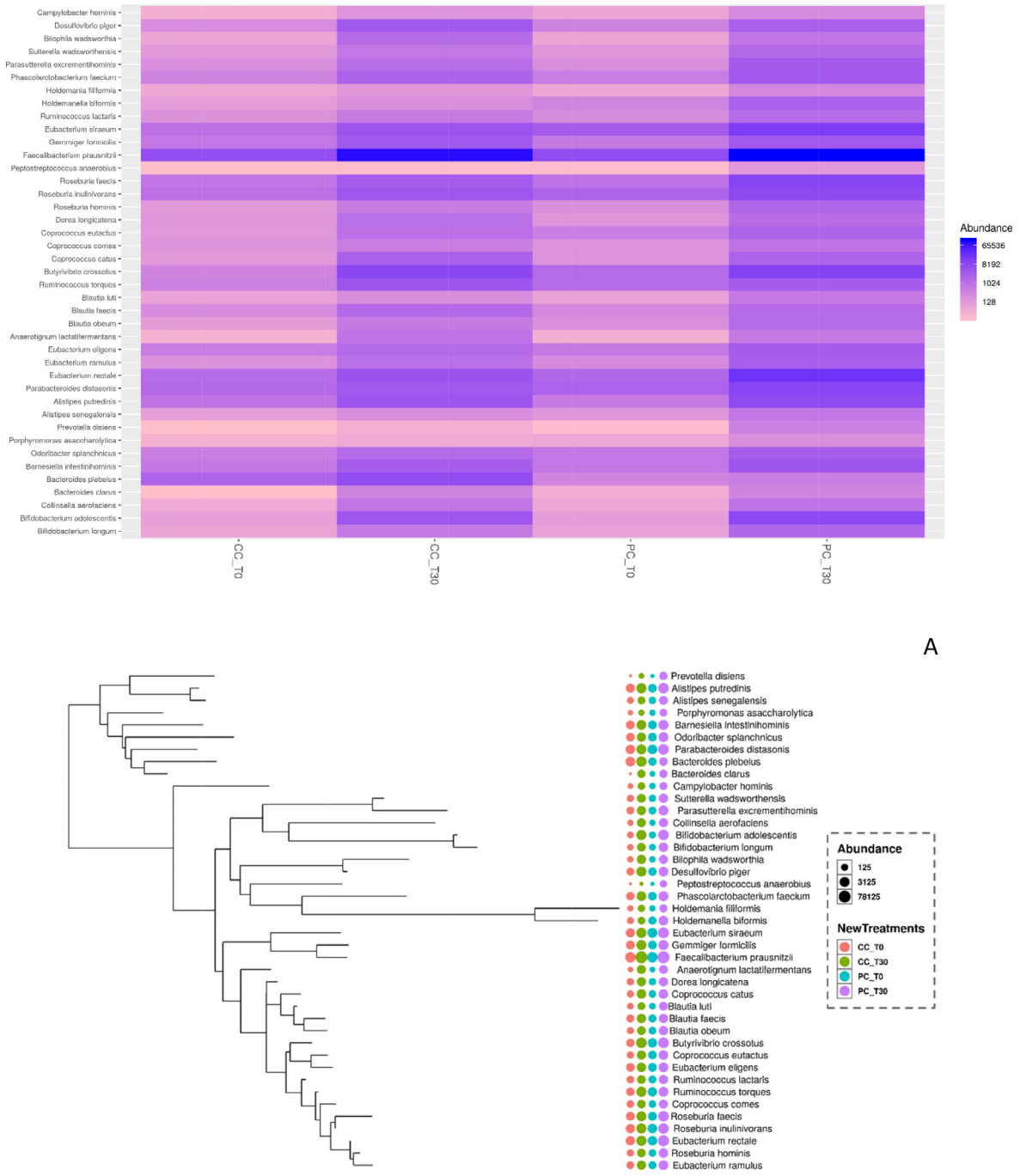

Fig. 3. (**A**) Phylogenetic tree and (**B**) heatmap with frequency of bacterial species identified as differentially abundant by DESeq2 (log2 FoldChange > ±2). CC, control capsule group; PC, placebo capsule group.

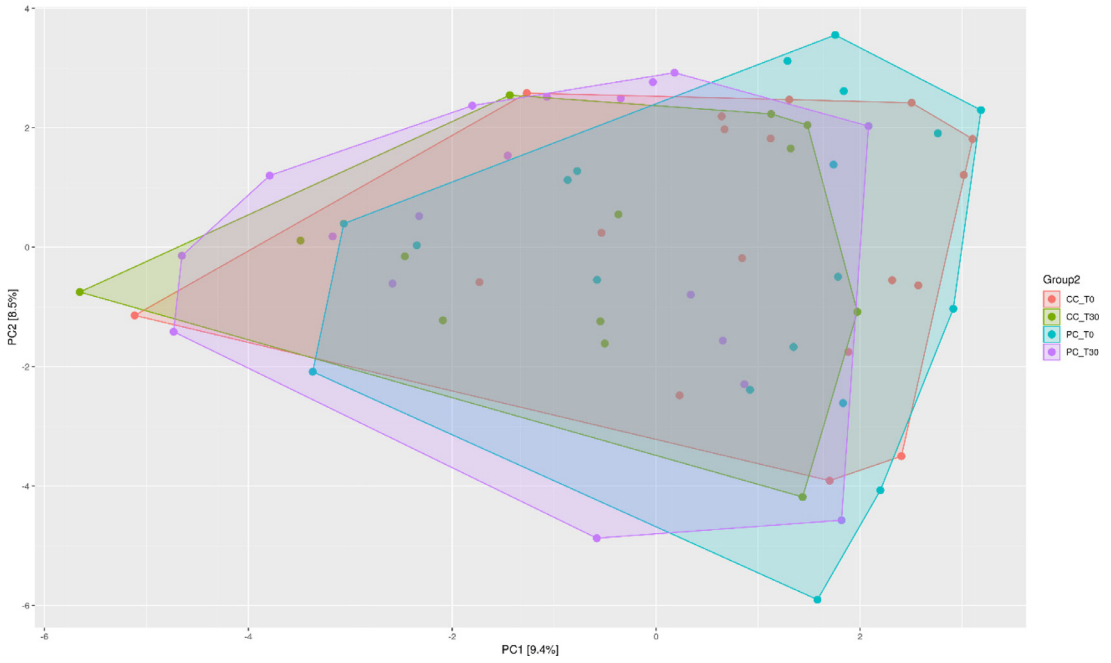

Fig. 4. Clustering profile of samples according to PCA analysis. CC, control capsule group; PC, placebo capsule group; PCA, principle component analysis.

Table 3
Effect of multispecies probiotic on percentage of relative abundance of phylum

Filo	CC (n = 14)				PC (n = 21)				
	T0	T30	Δ	*P*-value*	T0	T30	Δ	*P*-value*	*P*-value†
Bacteroidetes	73.68 ± 9.79	53.21 ± 14.63	−20.47 ± 14.96	<0.001	70.34 ± 1.42	51.41 ± 14.12	−18.93 ± 12.66	<0.001	0.744
Firmicutes	21.68 ± 8.44	40.18 ± 13.98	18.50 ± 15.26	0.001	24.93 ± 10.16	41.39 ± 15.52	16.46 ± 12.44	<0.001	0.667
Fusobacteria	0.38 ± 0.16	0.53 ± 0.79	0.15 ± 0.68	0.925	0.39 ± 0.20	0.35 ± 0.43	−0.04 ± 0.39	0.357	0.840
Proteobacteria	1.92 ± 0.76	3.00 ± 2.45	1.08 ± 2.33	0.106	2.18 ± 1.19	2.60 ± 1.75	0.42 ± 1.78	0.291	0.350
Verrucomicrobia	0.96 ± 1.46	0.45 ± 0.83	−0.50 ± 1.48	0.064	0.78 ± 0.89	1.73 ± 4.99	0.95 ± 4.58	0.876	0.312
Synergistetes	0.09 ± 0.04	0.07± 0.11	−0.02 ± 0.11	0.109	0.08 ± 0.04	0.05 ± 0.07	−0.03 ± 0.08	0.042	0.814
Actinobacteria	1.11 ± 0.38	2.44 ± 2.37	1.32 ± 2.33	0.300	1.17 ± 0.55	2.36 ± 3.17	1.18 ± 3.33	0.590	0.567
Euryarchaeota	0.17 ± 0.16	0.10 ± 0.14	−0.07 ± 0.20	0.064	0.14 ± 0.08	0.11 ± 0.13	−0.02 ± 0.14	0.322	0.934
Firmicutes/Bacteroidetes	0.31 ± 0.16	0.91 ± 0.67	0.60 ± 0.65	0.004	0.39 ± 0.23	0.97 ± 0.67	0.58 ± 0.56	<0.001	0.936

CC, control capsule group; PC, placebo capsule group; MET, metabolic equivalent of task; T0, baseline; T30, post-intervention
Δ = T30 − T0
*Statistical differences between T30 and T0 within the same group.
†Statistical differences between Δ values of the control and treatment groups.

73.68 ± 9.79, PC: 70.34 ± 1.42), Firmicutes (CC: 21.68 ± 8.44, PC: 24.93 ± 10.16), and Proteobacteria (CC: 1.92 ± 0.76, PC: 2.18 ± 1.19). There was no difference between groups for the relative abundance percentage for any phylum. However, there was a significant reduction of phylum Synergistetes within the PC group only ($P = 0.043$).

There was a significant increase within group for the relative abundance percentage of *Blautia faecis* (T0: 0.19 ± 0.14; T30: 0.39 ± 0.25; $P = 0.003$) and *Ruminococcus torques* in CC group (T0: 0.49 ± 0.37; T30: 1.21 ± 1.12; $P = 0.011$), whereas in the PC group, the abundance did not change (*B. faecis*: T0: 0.33 ± 0.67; T30: 0.26 ± 0.22; $P = 0.794$; *R. torques*: T0: 0.68 ± 0.73; T30: 0.65 ± 0.83; $P = 0.958$). There also was a significant difference between groups ($P = 0.029$ and $P = 0.013$, respectively), suggesting that probiotic treatment prevented the increase of percent relative abundance of these two species belonging to Firmicutes phylum (Fig. 5). No difference was observed for the other 73 species (data not shown).

Of the probiotic strains administered, only *Lactococcus lactis* and *Bififobacterium bifido* were identified in feces, but without significant differences between the two intervention groups ($P = 0.400$ and $P = 0.213$, respectively).

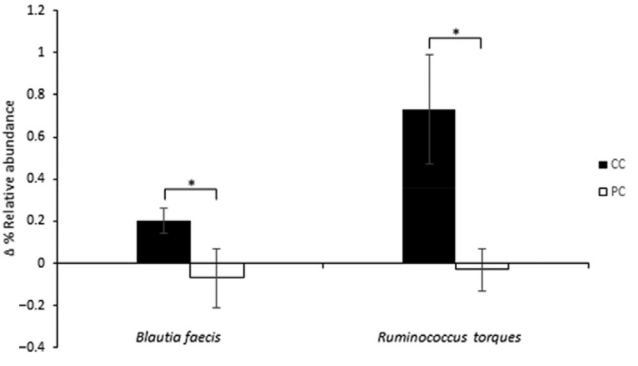

Fig. 5. Change of percent relative abundance of *Blautia faecis* and *Ruminococcus torques*. *$P < 0.05$. CC, control capsule group; PC, placebo capsule group.

Table 4
Effect of multispecies probiotic on constipation symptoms

ROME IV criteria	Control (CC) (n = 14)			Probiotic (PC) (n = 21)			
	T0	T30	*P*-value*	T0	T30	*P*-value*	*P*-value†
Evacuation frequency, n (%), times/wk			0.006			0.013	0.222
1	1 (7.1)	0 (0)		3 (14.3)	0 (0)		
<3	7 (50)	1 (7.1)		5 (23.8)	2 (9.5)		
3–4	5 (35.7)	8 (57.1)		9(42.9)	12 (57.5)		
>4	1 (7.1)	5(35.7)		4(19)	6 (28.6)		
Stool consistency, n (%)							
25% of evacuations Bristol scale 1 and 2	14 [100]	6 (42.9)	0.005	20 (95.2)	14 (66.7)	0.059	0.110
ROME IV criteria, n (%)							
Excessive exertion in ≥25% of bowel movements	12 (85.7)	7 (50)	0.059	16 (72.2)	10 (47.6)	0.058	0.778
Hardened and/or dry stools in ≥25% of stools	13 (92.9)	9 (64.3)	0.102	18 (85.7)	13 (61.9)	0.059	0.803
Feeling of incomplete defecation in ≥25% of bowel movements	10 (71.4)	9 (64.3)	0.564	15 (71.4)	9 (42.9)	0.034	0.342
Blockage sensation or anorectal obstruction	7 (50)	4 (28.6)	0.257	9 (42.9)	4 (19)	0.025	0.960
Manual maneuver	2 (14.3)	1 (7.5)	0.317	5 (23.8)	2 (9.5)	0.180	0.727
Evacuation frequency <3 times/wk	12 (85.7)	8 (57.1)	0.096	15 (71.5)	15 (71.4)	0.405	0.752
Rarely presents liquid stools without aid of laxatives	12 (85.7)	11(78.6)	0.527	20 (85.2)	16 (76.2)	0.046	0.561

T0, baseline; T30, post-intervention* Statistical Analysis: Wilcoxon test † Statistical Analysis: Mann- Whitney test

Constipation symptoms

No significant differences were observed between the two intervention groups regarding evacuation frequency, stool consistency, and Rome IV criteria (Table 4). However, there was a significant reduction within the PC group only in the prevalence of individuals who presented incomplete defecation (P = 0.034), blockage sensation (P = 0.025), and rare occurrence of liquid stools without the aid of laxatives (P = 0.046).

Discussion

The present study demonstrated that a multispecies probiotic can be efficient in preventing an increase of the relative abundance percentage of *B. faecis* and *R. torquis* in healthy individuals with constipation. Similarly, Ferrario et al. [20] showed that probiotic intake leads to a reduction of bacteria belonging to the genus *Blautia* in healthy adults.

B. faecis and *R. torquis* belong to the genus *Blautia*, family Lachnospiraceae, and filo Firmicutes. Zhu et al. [21] observed that bacteria of Lachnospiraceae and Ruminococccaceae families are increased in individuals with constipation and that this increase can be explained mainly by the presence of bacteria of the genus *Blautia, Coprococcus,* and *Ruminococus*.

R. torques is a known mucin degrader [22], which is a glycoprotein produced by goblet cells and contributes to the maintenance of the intestinal barrier. The intestinal mucosa is the first line of intestinal defense against pathogenic or invasive commensal bacteria in the intestinal lumen [23]. Castro-Combs et al. [24] demonstrated that the impairment in the mucin content may be a contributing factor to the development of chronic constipation. Moreover, these bacteria have been associated with irritable bowel syndrome in several studies [25–29].

Thus, considering that shifts in certain bacterial populations could be potentially beneficial in healthy individuals with constipation, particularly if these bacterial populations are associated with disease states, the results mentioned here suggest that the probiotic may have a protective effect on the gut microbiota by the modulation of relative abundance percentage of *B. faecis* and *R. torquis* .

Contrary to our expectations, there was no increase in populations *Bifidobacterium* and *Lactobacillus*. Similar results were found by Zhu et al. [21] and Yu et al. [30]. In contrast, Mezzassalma et al. [31] conducted a randomized, double-blind, three-arm parallel group trial with 150 patients with irritable bowel syndrome associated with constipation divided into three groups (F_1, F_2, and F_3). Each group received a daily oral administration of probiotic mixtures (*L. acidophilus* and *L. reuteri* and another with *L. plantarum* and *L. rhamnosu*) for 60 d for F_1 or F_2 or placebo F_3, respectively. The authors observed an increase in *Lactobacillus* and *Bifidobacterium* in the stool after 60 d of supplementation but did not evaluate the complete stool composition and whether there was a reduction in pathogenic bacteria.

It has been widely described in the literature that species belonging to these families are reduced in individuals with intestinal motility disorders and supplementation would be an effective strategy to increase their population [32,33]. However, colony adhesion and formation do not always occur, although the effects on the gut microbiota may be due to the other mechanisms [34] such as direct antimicrobial activity, producing bacteriocins or even through competitive exclusion [35]. Guo et al. [36] suggested that *L. acidophilus* may secrete bacteriocin. Similarly, it has been shown that antibacterial substances could be produced by bacteria *L. acidophilus* and *L. fermentum*, offering a broad inhibitory range including gram-negative and gram-positive pathogenic strains [37,38].

In the present study, there were no differences between groups for α- and β-diversity indices. Inter-individual variations in the gut microbiota may have masked changes due to probiotic intake. On the other hand, within the PC group only, there was a significant intragroup reduction in the prevalence of individuals who had incomplete defecation (P = 0.034), blockage sensation (P = 0.025), and rarely presented liquid stools without the aid of laxatives (P = 0.046), but without significant differences between groups. Clinical studies have shown that supplementation with probiotics such as *L. casei* [39] and *Bifidobacterium animalis* [40] presents modest or no effect on the symptoms of constipation. In contrast, Eskesen et al. [41] observed that the consumption of *Bifidobacterium animalis* spp Lactis BB-12 improves the GI health of individuals whose symptoms are not sufficiently severe. This variation in the results may occur because the effects of probiotics are known to be strain-dependent and vary according to the subspecies and the host gut microbiota composition [5].

Previous studies have found a significant improvement in other parameters related to the symptoms of constipation with the administration of a variety of probiotic strains for ≤4 wk, which was the basis for deciding the duration of the intervention in this study

[5,42]. However, considering the probiotics types administered in the present study and the microbiota profile of the studied population, it may be that a longer intervention time would have been necessary for the observed changes in gut microbiota to be translated into more significant changes in the intestinal movement.

Moreover, conditions associated with patient-reported symptom severity without reliable physiologic correlates, such as functional GI disorders, are known to have high placebo response rates [43], even in short-term studies [44,45] which may have contributed to not reaching significance between the two intervention groups in the present study.

No significant differences were observed for energy and macronutrient intake and for level of physical activity during the intervention. Therefore, it can be speculated that the effects described here were most likely due to probiotic ingestion and not to lifestyle changes.

The strengths of this study included the evaluation of factors that could influence the composition of the microbiota, such as level of physical activity, alcoholic beverage intake, water intake, and changes in diet as well as a robust and efficient fecal microbiota analysis. However, some limitations of the study were the high dropout rates during the randomization and intervention period, the non-adjustment of the model according to the subtypes of constipation and the intervention time (30 d). A longer treatment time may lead to more prominent changes in the intestinal constipation variables, although 30 d of intervention is commonly observed in previous literature.

Conclusion

A multispecies probiotic in capsule form can be efficient in reducing bacteria that are commonly increased in patients with constipation, contributing to the balance of microbiota and, consequently, to the well-being of the individual.

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