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MICROBIOLOGY

Effects of probiotic supplementation on the gut microbiota composition of adults: a systematic review of randomized clinical trials

ANA PAULA MÖRSCHBÄCHER, EMELIN PAPPEN, JOÃO ANTONIO P. HENRIQUES & CAMILLE E. GRANADA

Abstract: Researchers have associated the therapeutic potential of probiotics with its ability to modulate gut microbiota, which is considered an "invisible organ" of the human body. The present study investigates the effects of probiotic supplementation on the gut microbiota composition of adults. The authors conducted a systematic review of the literature published in six different databases. The search followed PRISMA guidelines and aimed to identify randomized clinical trials on probiotic supplementation. All relevant publications indexed up to May 28, 2021, were retrieved. Then, the authors defined the inclusion and exclusion criteria. Two independent reviewers performed study screening, data extraction, and quality assessment. A total of 2,404 publications were retrieved, and eight studies met the eligibility criteria. The included randomized clinical trials were published between 2015 to 2020. The worldwide studies included adults aged from 18 to 79 years, most of whom were women (66.5%). Only one of the included studies observed significant effects on fecal microbiota composition in the relative abundance of Bacteroidetes and Firmicutes phyla in comparison with the placebo treatment. Overall, this systematic review could not draw consistent conclusions on the effects of probiotic supplementation on the gut microbiota composition of adults.

Key words: Probiotics, *Lactobacillus*, *Bifidobacterium,* gut microbiota, systematic review.

INTRODUCTION

The human gastrointestinal tract is home to more than 100 trillion different microorganisms, including bacteria, fungi, protozoa, and viruses. These microorganisms act in syntrophy, forming a complex and dynamic microbial population, i.e., the gut microbiota (Gill et al. 2006, Honda & Littman 2012). The gastrointestinal tract harbors more than 1,000 different bacterial species (Qin et al. 2010), mainly belonging to *Bacillus* spp., *Bacteroides* spp., *Bifidobacterium* spp., *Clostridium* spp., *Enterococcus* spp., *Lactobacillus* spp., *Prevotella* spp., and *Ruminicoccus* spp. (Arumugam et al. 2011). The four dominant phyla, Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria, account for more than 90% of the human gut microbiota (Hugon et al. 2015). The abundance and diversity of prokaryotic cells vary throughout the gastrointestinal tract, from the mouth to the anus (Arumugam et al. 2011). However, it was reported that Bacteroidetes and Firmicutes are dominant bacteria in the gut of healthy adults (Sheehan et al. 2015, Andoh 2016).

Researchers consider gut microbiota as an "invisible organ" of the human body (Chen et al. 2020, Li et al. 2020), which plays essential functions in maintaining the host healthy (Mohr et al. 2020). These include: controlling epithelial cell proliferation and differentiation (Sekirov et al. 2010); strengthening gut integrity or shaping the

intestinal epithelium (Natividad & Verdu 2013); acting on nutrient uptake, vitamin synthesis, and energy harvesting (Ursell et al. 2012); protecting against pathogens (Bäumler & Sperandio 2016); affecting behavioral and neurological functions (Wahlström et al. 2016, Zheng et al. 2019); and changing drug absorption, toxicity, metabolism, and bioavailability (Li et al. 2020). The intestinal microbiota also assists in metabolic functions and improves the host immune system (Chen et al. 2020). However, factors such as ethnicity, age, birth method, body mass index, antibiotic use, lifestyle, exercise frequency, and feeding habits shape the gut microbiota and influence important differences among people (Rinninella et al. 2019, Mohr et al. 2020).

Imbalance of gut microbiota, or dysbiosis, alters host–microbiota interaction and the host immune system (Nishida et al. 2018, Rinninella et al. 2019). Indeed, studies have associated gut microbiota disturbance with the degree of pathogenesis in human diseases such as acne (Lee et al. 2019), allergy (Pascal et al. 2018), depression (Zalar et al. 2018), type 2 diabetes (Gurung et al. 2020), stress (Foster et al. 2017), gastrointestinal cancer (Elsalem et al. 2020), obesity (Maruvada et al. 2017), autism spectrum disorder (Sivamaruthi et al. 2020), cardiovascular disease (Tang et al. 2017), inflammatory bowel disease (Khan et al. 2019), and Parkinson's disease (Parashar & Udayabanu 2017). A recent study reported that gut microbiota also influences the severity of coronavirus disease (or Covid-19) (Dhar & Mohanty 2020). On the other hand, homeostasis restoration can alleviate symptoms of such diseases or revert/prevent their development (Altamura et al. 2020).

Studies have increasingly highlighted the importance of the gut microbiota in human health and disease development in the last few years. Many researchers address new interventions to modulate this microbiota (Quigley 2019). In this context, the modulation of the gut microbial community is a promising treatment target for many diseases related to gut dysbiosis. Efficient methods to restore microbial balance include the consumption of prebiotics, probiotics, and fecal microbiota transplantation (Hasan & Yang 2019).

Probiotics are living microorganisms that, when administered in adequate amounts, confer health benefits to the host (Hill et al. 2014). Thus, regular consumption of probiotics can modulate immune responses and metabolic processes (Quigley 2019) as well as antioxidant and anti-inflammatory effects, with these microorganisms acting as gut microbial modulators (Neto et al. 2018). Notwithstanding, the mechanisms or metabolic pathways through which probiotic supplementation benefits human health are not yet well established (Neto et al. 2018, Quigley 2019).

This systemic review of randomized clinical trials discusses the effect of multi-strain probiotic supplementation (*Lactobacillus* spp. and *Bifidobacterium* spp.) on the gut microbiota composition of adults. Aiming to evaluate the therapeutic efficiency of these probiotics, the authors analyzed changes on the relative abundance of the main intestinal bacterial phyla (Actinobacteria, Bacteroidetes, Euryarchaeota, Firmicutes, Fusobacteria, Proteobacteria, Synergistetes, and Verrucomicrobia). The review protocol was registered in the PROSPERO database (CRD42020177567).

MATERIALS AND METHODS

Literature search

This systematic review of the current literature followed the PRISMA statement guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Moher et al. 2009). Two authors (APM and EP) independently searched

for articles published until May 28, 2021, in the databases Embase, Lilacs, PubMed, ScienceDirect, SCOPUS, and SpringerLink. These searches aimed at identifying studies that reported the effects of probiotic supplementation on the gut microbiota composition of adults. The keywords used as search strategy were: "probiotics" AND "*Lactobacillus*" AND "*Bifidobacterium*" AND ("gut microbiota" OR "fecal microbiota") AND human AND "clinical trial". There were no restrictions on language or year of publication. The titles and abstracts of the selected articles were assessed. Articles whose titles appeared to meet the inclusion criteria or lacked sufficient information for exclusion were read in full. The reference lists of selected records were also searched to include potentially relevant additional studies.

Inclusion and exclusion criteria

Inclusion criteria were: (1) randomized control trials; (2) use of probiotics as intervention method and placebo as control; (3) strains of *Lactobacillus* spp. and *Bifidobacterium* spp. as probiotic supplementation; (4) report of changes in the gut microbiota composition of adults; and (5) assessment of differences in the relative abundance of prokaryotic cells at phylum level between groups or before–after supplementation. Exclusion criteria were: case reports, reviews, systematic reviews, meta-analyses, duplicate publications, *in vitro* experimental trials, children and adolescent studies, animal trials, prebiotic and symbiotic studies, research assessing probiotic supplementation with a single strain of *Lactobacillus* spp. or *Bifidobacterium* spp., and studies investigating the survival of probiotic strains only. Studies with multiple comparisons between probiotic and placebo groups were considered for evaluation.

Data extraction

Two authors independently collected the data (APM and EM), and discrepancies were solved by reevaluation of the original record by both authors. The authors extracted the data directly from the articles using a standardized form, including the following information from each clinical trial: author, year of publication, original country, study design, study population, sample size (numbers of case and control subjects), gender and mean age of patients, probiotic supplementation (strains, intake form, dosage, and intervention time), and data on microbiota (including intervention effect, the phyla and genera of bacteria detected, and the methodology used for microbiological assessment). The studies included the measurement of the effects of probiotic supplementation on the overall gut microbiota composition of adults, recording major alterations in the relative abundance of the phyla Bacteroidetes and Firmicutes.

Quality assessment

The same two authors who performed data extraction assessed the methodological quality of the clinical trials. For that, the authors used the Jadad score, with a five-point scale evaluating three traits: randomization quality, blinding quality, and reasons for withdrawal/dropout (Jadad et al. 1996). The included studies were of medium or high quality, with low quality studies being excluded from the selection process.

Risk of bias assessment

Two authors (APM and EP) used the Cochrane Collaboration's tool for assessing risk of bias in each randomized trial included (Higgins et al. 2011). The investigation included the following items: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting,

and any other sources of bias. Risk of bias in each trial was classified as high, moderate (unclear), or low. Studies with high risk of bias were excluded from the selection process.

Data analyses

The included studies reported the relative abundance of prokaryotic cells belonging to the phyla Actinobacteria, Bacteroidetes, Euryarchaeota, Firmicutes, Fusobacteria, Proteobacteria, Synergistetes, and Verrucomicrobia in the gut microbiota of adults. The selected clinical trials were identified and examined as a systematic review rather than a meta-analysis due to the high heterogeneity of study designs and methods.

The relative abundance of the previously cited phyla was extracted using WebPlotDigitizer version 4.5 (https://automeris.io/WebPlotDigitizer). This variable was used to identify differences between pre- and after- probiotic or placebo consumption by discriminant analysis (PAST3 software – Hammer et al. 2001).

RESULTS

Study selection

The search in the electronic databases included articles published up to May 28, 2021. From the predesigned search strategy, the authors identified 2,404 relevant publications. The first clean strategy eliminated 126 publications since they were duplicate records. After that, from the analysis of titles and abstracts, 2,147 publications were excluded because they were reviews, case reports, conferences and abstracts, design studies, animal trials, children and adolescent studies, *in vitro* experimental trials, prebiotic and symbiotic studies, or studies on probiotic supplementation with *Bacillus* spp. and/or *Saccharomyces* spp. strains. Then, 132 publications were selected for full-text analysis, of which 124 were excluded for the following reasons: being nonrandomized clinical trials; using a combination of probiotics and prebiotics; not identifying total fecal microbiota; investigating only the survival of the probiotic strain. Finally, eight studies achieved all inclusion criteria and were selected for this systematic review (Table I). Figure 1 summarizes the screening strategy.

Selected studies

The selected articles were published from 2015 to 2020. Table I identifies these articles through the first author's surname and year of publication. All clinical trials were approved by the Ethics Committees. Of these, five were performed according to the Helsinki Declaration. The trials were performed in Brazil (Botelho et al. 2020), Canada (Martoni et al. 2019), China (Liu et al. 2020, Wu et al. 2020), USA (Ford et al. 2020), Italy (Francavilla et al. 2019), Korea (Song et al. 2020), and Spain (Plaza-Díaz et al. 2015).

The studies analyzed people from 18 to 79 years. Seven clinical trials evaluated both male and female participants, and one (Ford et al. 2020) recruited only females. The sick participants under study presented constipation (Martoni et al. 2019, Botelho et al. 2020), bacterial infectious diseases (Wu et al. 2020), celiac disease with irritable bowel syndrome-type symptoms (Francavilla et al. 2019) or needed hemodialysis treatment (Liu et al. 2020). Healthy subjects consisted of elderly women (Ford et al. 2020), obese adults (Song et al. 2020), and healthy volunteers (Plaza-Díaz et al. 2015).

The intervention period ranged from two weeks to six months. All volunteers received multistrain probiotic supplementation in powder or capsule format. The main bacterial species studied were *Bifidobacterium animalis* subsp. *lactis*, *B. bifidum*, *B. breve*, *B. lactis*, *B.*

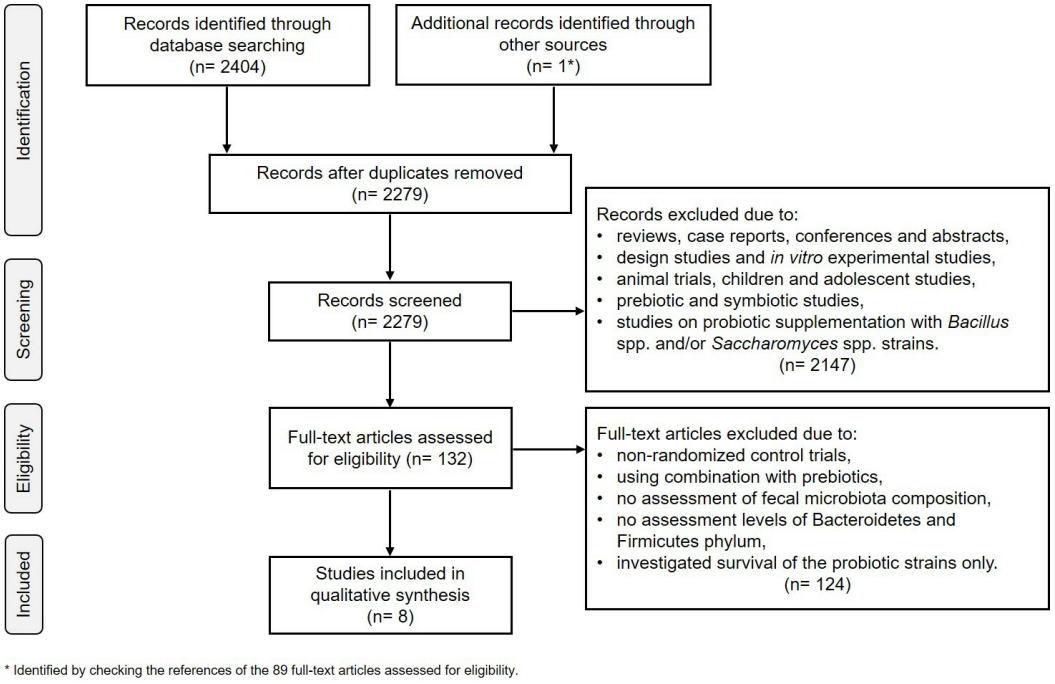

Figure 1. Flow chart of the screening process for inclusion of studies in the systematic review.

longum, *Enterecoccus faecalis*, *Lactobacillus acidophilus*, *L. casei*, *L. plantarum*, *L. rhamnosus*, and *Lactococcus lactis*.

The studies identified alterations in the gut microbiota of adults by high-throughput sequencing methods (*16S rRNA* sequencing) on Illumina MiSeq platforms (n = 6) (Botelho et al. 2020, Ford et al. 2020, Liu et al. 2020, Song et al. 2020, Martoni et al. 2019, Wu et al. 2020) or by 454 pyrosequencing (n = 2) (Francavilla et al. 2019, Plaza-Díaz et al. 2015). The target hypervariable regions of the *16S rRNA* gene were V1-V3 (n=1) (Plaza-Díaz et al. 2015), V3 (n= 1) (Francavilla et al. 2019), V3-V4 (n=3) (Botelho et al. 2020, Liu et al. 2020, Song et al. 2020), and V4 (n=2) (Ford et al. 2020, Martoni et al. 2019).

Quality assessment

Table II shows the results of the methodological quality of the clinical trials. According to the Jadad score (Jadad et al. 1996), six studies (Botelho et al. 2020, Ford et al. 2020, Liu et al. 2020, Song et al. 2020, Francavilla et al. 2019, Martoni et al. 2019) presented high quality, and two (Wu et al. 2020, Plaza-Díaz et al. 2015) presented medium quality. All studies were designed as randomized clinical trials. Wu et al. (2020) used an open-label design, while other studies used a double-blind design. Ford et al. (2020) used a cross-over design, and other studies used a parallel design.

Risk of bias assessment

The analysis of the bias risk of the selected clinical trials followed the methodology described in Cochrane Collaboration's tool (Higgins et al. 2011) (Figure 2). Four studies (50.0%) showed low risk of bias in all evaluated criteria. Two studies (25.0%) had one unclear risk of bias each. One study (12.5%) presented two risks of bias, and another presented one risk of bias and one unclear risk. Random sequence generation was the main risk of bias, followed by selective reporting (reporting bias). All studies reported lost data or patients who did not attend follow-up appointments. Overall, all studies presented low risk of bias. Publication bias assessment

Table I. Main characteristics of the included clinical trials.

Study	Participant characteristics*	Intervention	Design	Microbiology Assessment	Bacteria detected (phylum)
Botelho et al. (2020)	27/55 (86%) 19 – 43 years Brazil	*L. acidophilus* NCFM, *L. casei* Lc-11, *Lc. lactis* Li-23, *B. bifidum* BB-06 and *B. lactis* HN019 (5 x 10^9 CFU) in capsules	Randomized, double-blind, placebo-controlled and parallel (30 days)	*16S rRNA* (regions V3-V4) sequencing on Illumina MiSeq platform	Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, Fusobacteria, Synergistetes, Verrucomicrobia, Euryarchaeota and Firmicutes/Bacteroidetes
Ford et al. (2020)	26/26 (100%) 68 – 79 years EUA	*B. bifidum* HA-132 (1.54 x 10^9 viable cells), *B. breve* HA-129 (4.62 x 10^9 viable cells), *B. longum* HA-135 (4.62 x 10^9 viable cells), *L. acidophilus* HA-122 (4.62 x 10^9 viable cells) and *L. plantarum* HA-119 (4.62 x 10^9 viable cells) in capsules	Randomized, double-blind, placebo-controlled and crossover (18 weeks)	*16S rRNA* (region V4) sequencing on Illumina MiSeq platform	Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Euryarchaeota, Tenericutes, Verrucomicrobia, Cyanobacteria, Synergistetes and Fusobacteria
Liu et al. (2020)	25/50 (47%) 38 – 58 years China	*B. longum* NQ1501 (2.2 x 10^9 CFU), *L. acidophilus* YIT2004 (0.53 x 10^9 CFU) and *E. faecalis* YIT0072 (1.1 x 10^9 CFU) in capsules	Randomized, double-blind, placebo-controlled and parallel (6 months)	*16S rRNA* (regions V3-V4) sequencing on Illumina MiSeq platform	Firmicutes, Bacteroidetes and Bacteroidetes/ Firmicutes
Song et al. (2020)	25/50 (84%) 34 – 54 years Korea	*B. breve* and *L. plantarum* CBT LP3 (15 x 10^9 viable cells) in capsules	Randomized, double-blind, placebo-controlled and parallel (12 weeks)	*16S rRNA* (regions V3-V4) sequencing on Illumina MiSeq platform	Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Fusobacteria and others
Wu et al. (2020)	64/131 (45%) 46 – 80 years China	*B. longum, L. acidophilus and E. faecalis* (1 x 10^9 CFU/g) in capsules	Randomized, open-label, placebo-controlled and prospective (2 weeks)	*16S rRNA* amplicon sequencing	Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Fusobacteria and others
Francavilla et al. (2019)	55/109 (86%) 18 – 62 years Italy	*L. casei* LMG 101/37 P-17504 (5 x 10^9 CFU), *L. plantarum* CECT 4528 (5 x 10^9 CFU), *B. animalis subsp. lactis* Bi1 LMG P-17502 (10 x 10^9 CFU), *B. breve* Bbr8 LMG P-17501 (10 x 10^9 CFU) e *B. breve* Bl10 LMG P-17500 (10 x 10^9 CFU) in power	Randomized, double-blind, placebo-controlled and parallel (6 weeks)	*16S-based* (region V3) pyrosequencing	Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria and Fusobacteria
Martoni et al. (2019)	47/93 (53%) 29 – 57 years Canada	*L. acidophilus*, *B. animalis subsp. lactis*, *B. longum* and *B. bifidum* (1.5 × 10^{10} CFU) in capsules	Randomized, double-blind, placebo-controlled and parallel (4 weeks)	*16S rRNA* (region V4) sequencing on Illumina MiSeq platform	Firmicutes, Actinobacteria, Bacteroides, Euryarcharota, Verrumicrobiota, Proteobacteria and Tenericutes
Plaza-Díaz et al. (2015)	4/9 (44%) 20 – 30 years Spain	*B. breve* CNCM I-4035 and *L. rhamnosus* CNCM I-4036 (9 x 10^9 CFU) in capsules	Randomized, double-blind, placebo-controlled and parallel (30 days)	*16S rRNA* (regions V1-V3) pyrosequencing	Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria and others

*** Participant characteristics regarding the number of individuals in the probiotic group/total number of individuals included in the study. Participant characteristics (% women and age range of participants in years) are based on the total number of individuals included in the study. CFU, colony-forming units. The study by Plaza-Díaz et al. (2015) had two intervention arms not relevant for the present study, where the number of participants are approximately two-thirds of the total number of participants included in the study.**

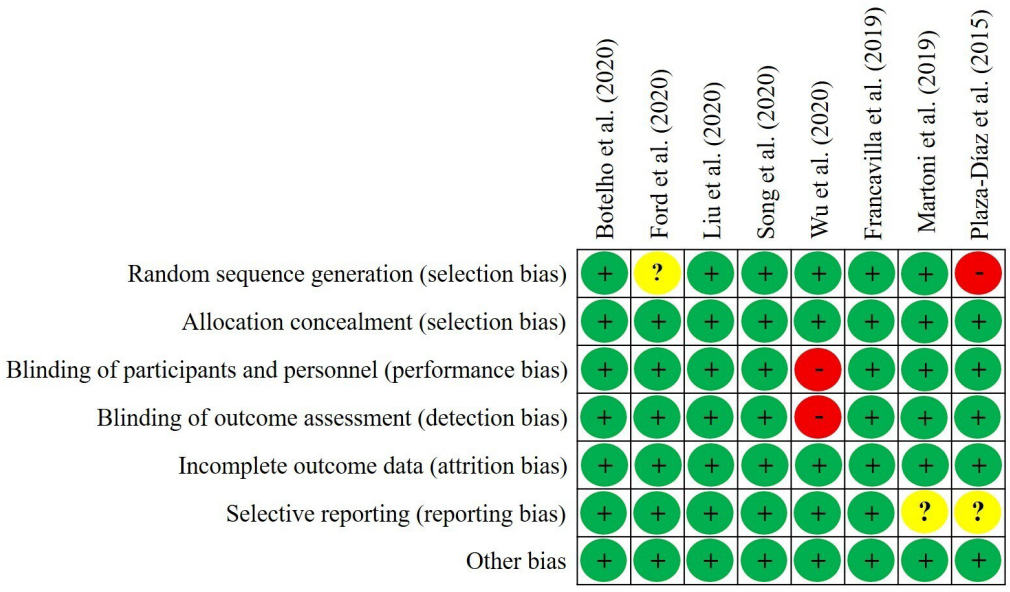

Figure 2. Risk of bias in the included clinical trials.

through funnel plot analysis was not performed since the number of included studies was less than ten (Cooper et al. 2019).

Data from selected studies

Botelho et al. (2020) reported that probiotic supplementation with strains *L. acidophilus* NCFM, *L. casei* Lc-11, *Lc. lactis* Li-23, *B. bifidum* BB-06, and *B. lactis* HN019 increase the α-diversity of the gut microbiome in adults (Shannon, Simpson, and InvSimpson indices). Comparison between probiotic supplementation and placebo treatments showed no significant differences in β-diversity and richness indices (as determined by operational taxonomic units, Chao1, and ACE). The relative abundance of the main phyla identified (Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, Fusobacteria, Synergistetes, Verrucomicrobia, and Euryarchaeota) did not differ either. However, probiotic intervention prevented an increase in the relative abundance of *Blautia faecis* and *Ruminococcus torques* (genus *Blautia*, family *Lachnospiraceae*, and phylum Firmicutes), which commonly increase in adults with constipation, contributing to the imbalance of the gut microbiota.

Ford et al. (2020) showed that probiotic supplementation with strains *B. bifidum* HA-132, *B. breve* HA-129, *B. longum* HA-135, *L. acidophilus* HA-122, and *L. plantarum* HA-119 did not affect α-diversity (Shannon index; comparison between placebo and probiotic supplementation). Principal coordinate analysis using the UniFrac metric showed similar dissimilarity among the treatments. Furthermore, the gut microbiome profile did not differ at three distinct taxonomic levels (phyla, family, and genus). Accordingly, the relative abundance of bacteria belonging to the phyla Bacteroidetes and Firmicutes was similar in the treatments.

Liu et al. (2020) showed that regular probiotic supplementation with strains *B. longum* NQ1501, *L. acidophilus* YIT2004, and *E. faecalis* YIT0072 did not alter bacterial richness and α-diversity (Chao1 and Shannon indices) in the gut microbiome. The authors compared placebo and probiotic supplementation after six months of intervention. However, probiotic supplementation reduced the relative abundance of Firmicutes and increased

Table II. Methodological quality assessment in the included clinical trials.

	Botelho et al. (2020)	Ford et al. (2020)	Liu et al. (2020)	Song et al. (2020)	Wu et al. (2020)	Francavilla et al. (2019)	Martoni et al. (2019)	Plaza-Díaz et al. (2015)
Describe as randomized*	1	1	1	1	1	1	1	1
Randomization method described and appropriate**	1	1	1	1	1	1	1	0
Describe as double-blind*	1	1	1	1	0	1	1	1
Double-blind method described and appropriate**	1	1	1	1	0	1	1	0
Describe of withdrawals*	1	1	1	1	1	1	1	1
Jadad score	5	5	5	5	3	5	5	3

*** A study receives a score of 1 for "yes" and 0 for "no". ** A study receives a score of 0 if no description is given, 1 if the method is described and appropriate, and -1 if the method is described but inappropriate.**

the abundance of Bacteroidetes. Probiotics enhanced the proportion of *Enterococcaceae* (phylum Firmicutes) and reduced that of *Peptostreptococcaceae* and *Ruminococcaceae* (phylum Firmicutes). These three families were remarkable in the use of probiotics versus placebo. Serum albumin, inflammatory markers, endothelial activation markers, SF-36 (short-form patient-reported health survey), and GSRS (Gastrointestinal Symptom Rating Scale) scores did not differ between probiotic and placebo treatments.

Song et al. (2020) also observed that probiotic supplementation (*B. breve* CBTBR3 and *L. plantarum* CBT LP3) did not influence Shannon and Simpson indexes (α-diversity). Nonmetric multidimensional scaling (NMDS) based on the Bray–Curtis distance matrix showed no obvious separation between the probiotic and placebo groups. Furthermore, probiotic supplementation did not change the relative abundance of the top five most abundant phyla (Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, and Fusobacteria) and of the most abundant families (*Alcaligenaceae*, *Bacteroidaceae*, *Bifdobacteriaceae*, *Clostridiaceae*, *Enterobacteriaceae*, *Fusobacteriaceae*, *Lachnospiraceae*, *Paraprevotellaceae*, *Porphyromonadaceae*, *Prevotellaceae*, *Rikenellaceae*, *Rumminococcaceae*, and *Veillonellaceae*) in each group. However, twelve weeks of probiotic intervention ameliorated obesity-related markers such as waist circumference, total fat area, visceral fat, and biochemical markers such as triglyceride, total cholesterol, high-density lipoprotein cholesterol, glucose, and insulin.

Wu et al. (2020) showed that, after two weeks of probiotic supplementation with strains of *B. longum, L. acidophilus*, and *E. faecalis*, the number of operational taxonomic units (OTUs) of the probiotic group was significantly higher, increasing gut microbiota richness (Chao 1 index). The Shannon index of the gut microecological structure was similar among the placebo and probiotic groups (after 7 and 14 days of treatment). Noteworthy, the most abundant phyla did not differ from each other: Bacteroidetes, Firmicutes, Proteobacteria, and Fusobacteria. The phyla Acidobacteria,

Fusobacteria, Cyanobacteria, Deferribacteres, Euryarchaeota, and Synergistetes were present at low proportions. In turn, Bacteroidetes and Firmicutes accounted for more than 90% of all bacterial flora at phylum level. Notwithstanding, probiotic intervention can reduce the incidence of gut dysbiosis and antibiotic-induced diarrhea, and the prophylactic use of BIFICO can stabilize the gut microbiota.

Francavilla et al. (2019) showed that probiotic supplementation (*L. casei* LMG 101/37 P-17504, *L. plantarum* CECT 4528, *B. animalis subsp. lactis* Bi1 LMG P-17502, *B. breve* Bbr8 LMG P-17501, and *B. breve* Bl10 LMG P-17500) did not change total prokaryotic community richness (rarefaction curves and Chao1 estimates) and α-diversity (Shannon index). Moreover, β-diversity did not show clear separation for the gut microbiome composition of placebo and probiotic groups (inferred by principal coordinate analysis using the UniFrac metric). The relative abundance of the phyla Bacteroidetes, Firmicutes, Proteobacteria, and Fusobacteria did not differ, neither did metabolically active bacteria among the treatments. However, the abundance of Actinobacteria OTUs was higher in the probiotic treatment than in the placebo treatment. In addition, IBS-SSS (Irritable Bowel Syndrome - Severity Scoring System) and GSRS scores decreased after probiotic supplementation.

Martoni et al. (2019) showed that the probiotic group presented OTU richness and Shannon index (α-diversity) values similar to baseline values after four weeks of probiotic supplementation with *L. acidophilus*, *B. animalis* subsp. *lactis*, *B. longum*, and *B. bifidum*. In contrast, OTU richness was lower in the placebo group than in the probiotic supplementation group. The relative abundances of the phyla Bacteroidetes, Firmicutes, and Actinobacteria were similar over time in both groups. Patient assessment of constipation symptom score was similar between groups (regarding β-diversity, samples did not cluster or separate according to time point in either group).

The probiotic supplementation (*B. breve* CNCM I-4035 and *L. rhamnosus* CNCM I-4036) performed by Plaza-Díaz et al. (2015) did not change the α-diversity (Shannon indices) of the gut microbiome in comparison with the placebo group. Bacteroidetes and Firmicutes were the most abundant phyla in fecal compositions of the placebo and probiotic groups. However, the relative abundance of these phyla did not differ between groups after 30 days of intervention.

Discriminant analysis was performed with 307 samples from the eight selected studies (Figure 3). The samples were divided into four treatments: pre- and post probiotic/placebo consumption. Joint analysis of all data from the eight selected studies confirms the individual conclusion: the pattern of prokaryotic community did not differ significantly at phylum level. However, all selected studies identify health benefits and encourage probiotic consumption. Thus, the health mechanism activated by gut microbiota remains unclear.

DISCUSSION

This study is an updated comprehensive systematic review on the effects of probiotic supplementation with *Lactobacillus* spp. and *Bifidobacterium* spp. strains on the gut microbiota composition of adults. The final review included eight randomized clinical trials. Only the study performed by Liu et al. (2020) observed changes in gut microbiota composition after intervention with probiotic strains. These authors reported that probiotic supplementation reduced the relative abundance of bacteria belonging to the phylum Firmicutes after six months of treatment with a mixture of *E. faecalis*, *B. longum*, and *L. acidophilus*. The results suggest that these

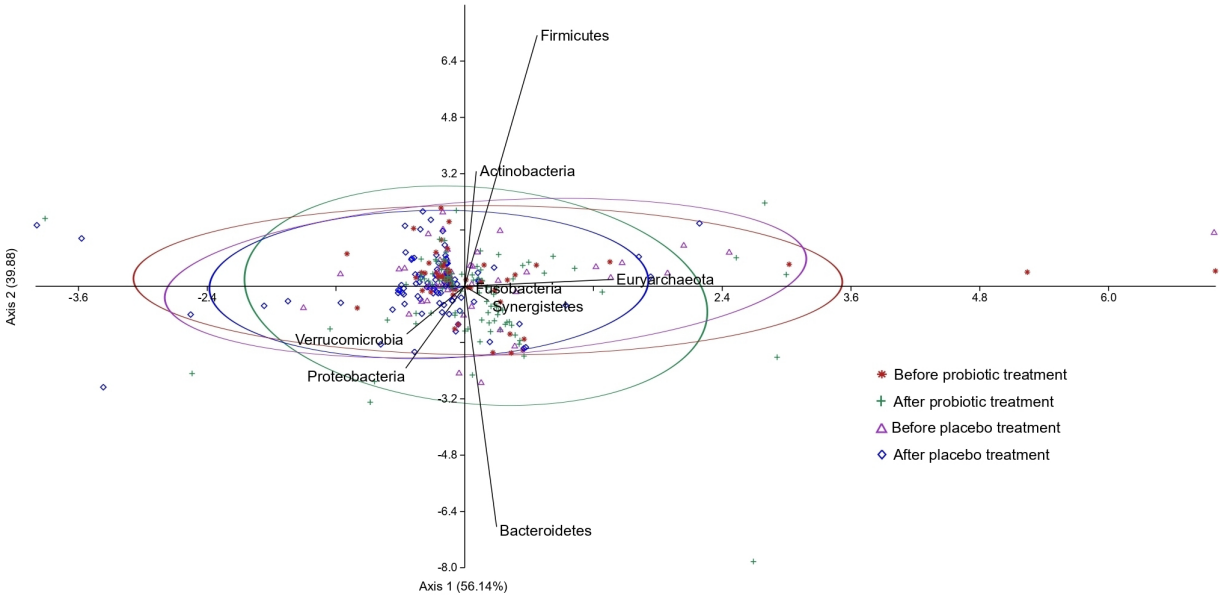

Figure 3. Discriminant Analysis performed at phylum level with 307 samples extracted from the eight selected studies.

bacterial strains act as probiotics and help to restore the intestinal flora balance of uremic patients undergoing hemodialysis, consequently modulating their gut microbiota. Another factor to be considered is the supplementation time used by Liu et al. (2020). These authors used probiotic supplementation for six months. It could explain the data obtained by these authors. In addition, Liu et al. (2020) evaluated patients with chronic kidney disease subjected to a restrictive diet. This diet could also be a determining factor in the data observed.

The clinical trials investigated by this systematic review did not provide consistent evidence to conclude that probiotic supplementation affects microbial community composition in adults (Figure 3). The high heterogeneity in the selected studies can be due to: (I) very restrictive criteria for choosing the studies; (II) trials with small number of participants, with the smallest sample having nine participants; (III) short period of intervention, ranging from 2 to 4 weeks; and (IV) trials with different multi-strain probiotic interventions (see Table I).

Gut microbiota alterations may require a long time, typically eight weeks (Dethlefsen et al. 2008, McFarland 2008), and less than half of the selected studies used an appropriate period of analysis. Studies that failed to detect microbiota alterations could present positive results by increasing the experimental time. In addition, all the clinical trials used different multi-strain probiotic interventions, and only the study of Ford et al. (2020) examined the effects of a diet associated with probiotic supplementation. Efficacy of probiotic supplementation is strain-dependent (Derrien & Vlieg 2015) and varies according to feeding habits and the baseline microbiota structure (Albenberg & Wu 2014, David et al. 2014, Maier et al. 2017). Thus, to observe alterations in the gut microbiome, clinical trials must analyze each probiotic strain alone before evaluating multi-strain formulations. An increase in the number of participants is also essential to visualize these differences.

All participants selected for randomized clinical trials were adults with widely distinct characteristics. Plaza-Díaz et al. (2015) studied only healthy adults. Ford et al. (2020) studied elderly women, who may respond differently to probiotics than young individuals. Botelho et al. (2020) evaluated obese adults. Liu et al. (2020) studied patients with chronic kidney disease. Wu et al. (2020) studied hospitalized patients with bacterial infectious diseases, who probably respond differently to probiotics than healthy adults. Different biological variables have different effects on the gut microbiota (McFarland 2008). Among other factors, age (O'Sullivan et al. 2011, Yatsunenko et al. 2012), geographic location (De Filippo et al. 2010, Clemente et al. 2015), lifestyle (Song et al. 2013), habitual physical activity (Cook et al. 2016), sleep deprivation (Benedict et al. 2016), prolonged physiological stress (Karl et al. 2017), and pregnancy (Koren et al. 2012) interfere directly in the gut microbiome composition and in the potential health effects of probiotic supplementation. In addition to these factors, health status influences the composition of the intestinal microbiota and may be a determining factor for the high heterogeneity observed among the studies.

Kristensen et al. (2016) found results similar to those of the present study. These authors evaluated seven randomized clinical trials which investigate alterations in the composition of healthy human fecal microbiota by high-throughput molecular approaches. Only one study reported significant changes in the overall structure of the fecal bacterial community in terms of β-diversity (compositional dissimilarity) after probiotic intervention in comparison with the placebo group. According to the authors, this demonstrates the lack of consistent evidence to conclude whether there is a positive effect of probiotics on fecal microbiota composition in healthy adults.

McFarland (2014) published a systematic review on the effects of probiotic intervention on human gut microbiota. Their study selected three groups: model A (restoration) assayed patients with healthy and undisturbed microbiota and then assayed postdisruptive event and probiotic therapy; model B (alteration) assayed patients with pre-existing disrupted microbiota and then post probiotic therapy; and model C (no dysbiosis) assayed volunteers with undisturbed microbiota and absence of disruptive event. All groups received probiotic therapy. From the inclusion criteria, the authors selected a total of 63 clinical trials. In these, 83% of the probiotic treatments using model A restored the microbiota, 56% of treatments using model B improved the microbiota, and only 21% of treatments using model C had some effect on the microbiota. The authors mention the need for more studies to conclude which probiotic strains have a beneficial impact on gut microbiota. The search for evidence that links probiotic supplementation efficacy to its ability to modulate the gut microbiome is an important tool to prove the healthy action of probiotics.

Limitations

Systematic literature reviews can present some limitations. In the case of this review, the number of published randomized clinical trials that evaluate the effects of probiotics on the fecal microbiota composition at the phylum level is still limited. Selected studies used supplementation with multi-strain probiotics formulated with both *Lactobacillus* spp. and *Bifidobacterium* spp.. In this sense, studies that used probiotic supplementation with *Bacillus* spp., *Enterococcus* spp., *Lactococcus* spp., or *Saccharomyces* spp. were excluded. Finally, six databases and a wide variety of terms have

been used for the literature review; however, some articles may have not been identified. To minimize the impact of this selection strategy, the references used in selected articles were searched and added to the literature review. Therefore, the need of studies that group and critically revise the effects of probiotic supplementation on the gut microbiota composition reinforces the importance of this review, regardless of quantitative synthesis.

CONCLUSIONS

Overall, this systematic review was not able to identify consistent effects caused by probiotic supplementation on the gut microbiome of adults. However, it was possible to identify that is necessary to improve the time of probiotic supplementation for identify the modulation of gut microbiome, improving the efficacy of this type of therapeutics. Thus, the present study can be a helpful reference for future research because highlight the importance of carefully planning experiments to obtain specific sample characteristics (number, health status, age, diet) that improve the probability of identifying the effects of probiotics in gut microbiome.

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ANA PAULA MÖRSCHBÄCHER
https://orcid.org/0000-0002-4978-805X

EMELIN PAPPEN
https://orcid.org/0000-0002-6109-1706

JOÃO ANTONIO P. HENRIQUES
https://orcid.org/0000-0002-5298-932X

CAMILLE E. GRANADA
https://orcid.org/0000-0002-7493-1348

University of Taquari Valley (Univates), Avelino
Talini Avenue, 171, 95914-014 Lajeado, RS, Brazil

Correspondence to: **Camille Eichelberger Granada**
E-mail: cegranada@univates.br

Author contribution

APM and EP collected literature data, and designed and wrote the manuscript. JAPH and CEG edited and revised the manuscript. All authors checked and approved the manuscript.