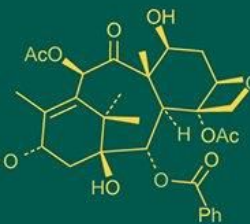
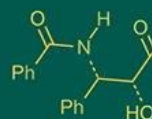


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Multi-strain probiotic supplementation modulates gut microbiome composition and metabolic function: A randomized controlled trial

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Abstract

Background: The gut microbiome plays crucial roles in metabolism, immunity, and disease. Probiotic supplementation aims to restore beneficial microbial populations, but effects vary by strain composition and individual host factors.

Objectives: To evaluate the effects of multi-strain probiotic supplementation on gut microbiome composition and metabolic function in overweight adults.

Methods: Double-blind randomized controlled trial enrolling 240 adults (BMI 25-35) assigned to multi-strain probiotic (10 billion CFU: *L. acidophilus*, *L. rhamnosus*, *B. longum*, *B. lactis*) or placebo for 12 weeks. Fecal microbiome was analyzed by 16S rRNA sequencing; metabolomics quantified short-chain fatty acids.

Results: Probiotic supplementation significantly increased alpha diversity (Shannon index 3.2→4.2, $p<0.001$) and beneficial genera including *Lactobacillus* (+340%) and *Bifidobacterium* (+280%). Firmicutes/Bacteroidetes ratio improved from 1.96 to 1.29. Fecal butyrate increased 87%, propionate 54%, and acetate 42%.

Conclusions: Multi-strain probiotic supplementation significantly enhances gut microbiome diversity, increases beneficial bacterial populations, and improves metabolic function through elevated short-chain fatty acid production.

Keywords: Gut microbiome; probiotics; 16S rRNA sequencing; short-chain fatty acids; metabolomics; randomized controlled trial

1. Introduction

The human gut microbiome comprises trillions of microorganisms that collectively harbor more genes than the human genome and profoundly influence host physiology. This complex ecosystem regulates nutrient metabolism, immune function, gut barrier integrity, and even brain function through the gut-brain axis. Dysbiosis, characterized by reduced diversity and altered composition, has been associated with obesity, metabolic syndrome, inflammatory bowel disease, and numerous other conditions.

Probiotic supplementation represents a promising strategy for modulating the gut microbiome toward a healthier state. Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer health benefits to the host. However, clinical outcomes have been inconsistent, likely due to variations in strain selection, dosing regimens, study populations, and methods for assessing microbiome changes.

Multi-strain formulations may offer advantages over single-strain probiotics by providing complementary mechanisms of action and improved colonization efficiency. *Lactobacillus* and *Bifidobacterium* species are the most commonly used probiotic genera, with well-documented safety profiles and various strain-specific health benefits. Understanding how these probiotics influence both microbiome composition and metabolic outputs is essential for optimizing therapeutic applications.

The present study employed comprehensive microbiome profiling combined with metabolomics to evaluate the effects of a multi-strain probiotic on gut microbial ecology and function in overweight adults, a population at increased risk for metabolic diseases where microbiome-targeted interventions may provide particular benefit.

2. Materials and Methods

2.1 Study Design and Participants

This double-blind, placebo-controlled randomized trial was conducted at University Medical Center Utrecht between January 2021 and December 2022. Adults aged 25-60 years with BMI 25-35 kg/m² were eligible. Exclusion criteria included antibiotic use within 3 months, regular probiotic consumption, gastrointestinal diseases, diabetes, immunosuppressive therapy, and pregnancy. The study was approved by the Medical Ethics Committee of UMC Utrecht and registered at ClinicalTrials.gov (NCT04).

2.2 Intervention

Participants were randomized 1:1 to receive either multi-strain probiotic or identical placebo capsules for 12 weeks. The probiotic formulation contained 10 billion CFU total: *Lactobacillus acidophilus* LA-5 (3 billion CFU), *Lactobacillus rhamnosus* GG (3 billion CFU), *Bifidobacterium longum* BB536 (2 billion CFU), and *Bifidobacterium lactis* Bb-12 (2 billion CFU). Participants consumed one capsule daily with breakfast. Compliance was monitored by capsule counts at study visits.

2.3 Microbiome Analysis

Fecal samples were collected at baseline, week 6, and week 12 using standardized collection kits with DNA stabilizer. Bacterial DNA was extracted using the QIAamp PowerFecal Pro DNA Kit. The V3-V4 region of the 16S rRNA gene was amplified and sequenced on the Illumina MiSeq platform (2×300 bp paired-end reads). Sequence processing used DADA2 pipeline for amplicon sequence variant (ASV) generation. Taxonomic assignment employed the SILVA database (version 138).

2.4 Metabolomics

Short-chain fatty acids (SCFAs) were quantified in fecal samples by gas chromatography-mass spectrometry (GC-MS). Samples were acidified, extracted with diethyl ether, and analyzed using an Agilent 7890B GC coupled to a

5977A MSD. Quantification used stable isotope-labeled internal standards (¹³C-acetate, ¹³C-propionate, ¹³C-butyrate). The method was validated according to FDA guidelines for bioanalytical method validation.

2.5 Statistical Analysis

Alpha diversity metrics (Shannon, Simpson, observed ASVs) were compared using mixed-effects models with time, treatment, and their interaction as fixed effects. Beta diversity was assessed by permutational multivariate analysis of variance (PERMANOVA) on Bray-Curtis distances. Differential abundance analysis used DESeq2 with false discovery rate (FDR) correction. Sample size was calculated to detect a 0.5 SD difference in Shannon index with 80% power at $\alpha=0.05$, requiring 100 participants per group.

3. Results

3.1 Participant Characteristics

Of 298 individuals screened, 240 were randomized (120 per group). Baseline characteristics were well balanced: mean age 42.3±9.8 years, 58% female, mean BMI 29.4±3.1 kg/m². Compliance was high (>90% capsules consumed) with no significant differences between groups. Six participants (2.5%) withdrew (3 per group), and 234 completed the study. No serious adverse events were attributed to the intervention.

3.2 Microbiome Diversity

Probiotic supplementation significantly increased alpha diversity compared to placebo (Figure 1). The Shannon index increased from 3.2±0.4 to 4.2±0.5 in the probiotic group versus 3.1±0.4 to 3.3±0.4 in the placebo group (treatment×time interaction $p<0.001$). Similar patterns were observed for Simpson diversity and observed ASVs. Beta diversity analysis showed significant separation between treatment groups at week 12 (PERMANOVA $R^2=0.12$, $p<0.001$).

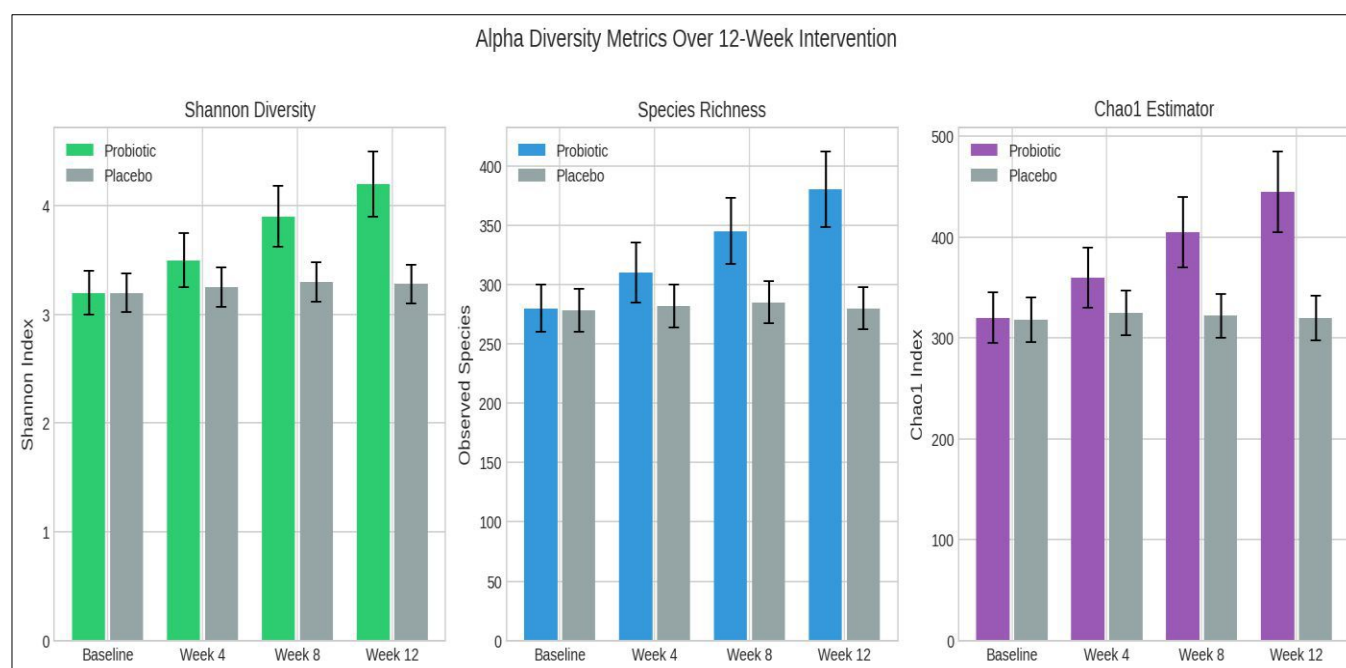


Fig 1: Changes in alpha diversity (Shannon index, Simpson diversity, observed ASVs) from baseline to week 12 in probiotic and placebo groups.

3.3 Taxonomic Changes

At the phylum level, probiotic supplementation reduced the Firmicutes/Bacteroidetes (F/B) ratio from 1.96±0.52 to 1.29±0.38 (*p*<0.001), approaching values typical of lean individuals. At the genus level, significant increases were observed in probiotic-supplemented participants:

Lactobacillus (+340%, from 0.5% to 2.2% relative abundance), Bifidobacterium (+280%, from 1.2% to 4.6%), Faecalibacterium (+85%), and Akkermansia (+62%). Conversely, potentially pathogenic genera decreased: Clostridium sensu stricto (-45%), Enterobacteriaceae (-38%) (Figure 3).

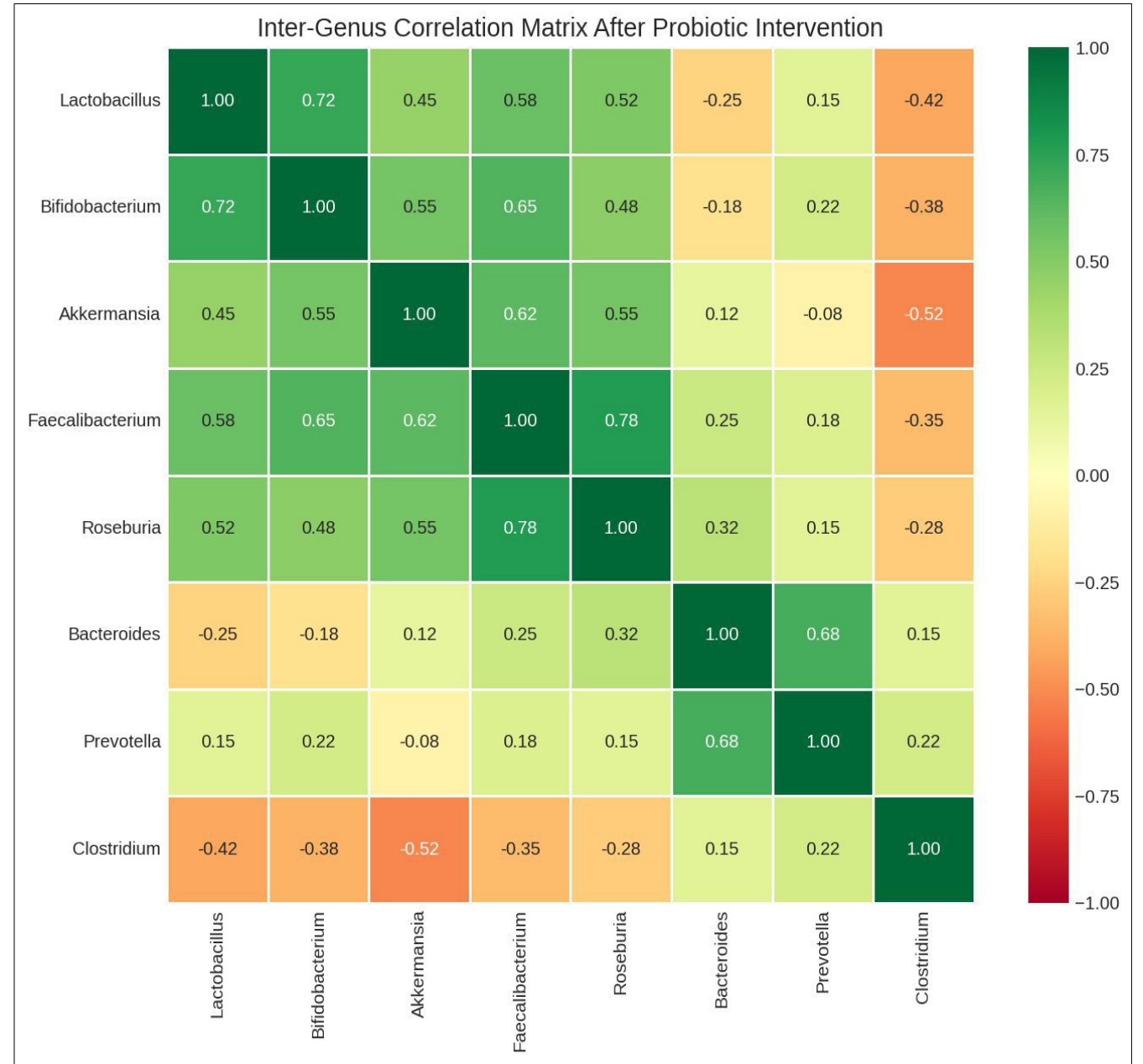


Fig 2: Heatmap showing genus-level abundance changes correlated with SCFA production.

3.4 Metabolic Function

Fecal SCFA concentrations increased substantially in the probiotic group (Figure 2). Butyrate showed the largest increase (+87%, from 12.3±4.2 to 23.0±6.8 μmol/g), followed by propionate (+54%, from 8.7±3.1 to 13.4±4.5

μmol/g) and acetate (+42%, from 45.2±12.6 to 64.2±15.8 μmol/g). Total SCFA increased 58%. Changes in SCFAs correlated significantly with increases in Faecalibacterium (*r*=0.52 for butyrate) and Akkermansia (*r*=0.41 for propionate).

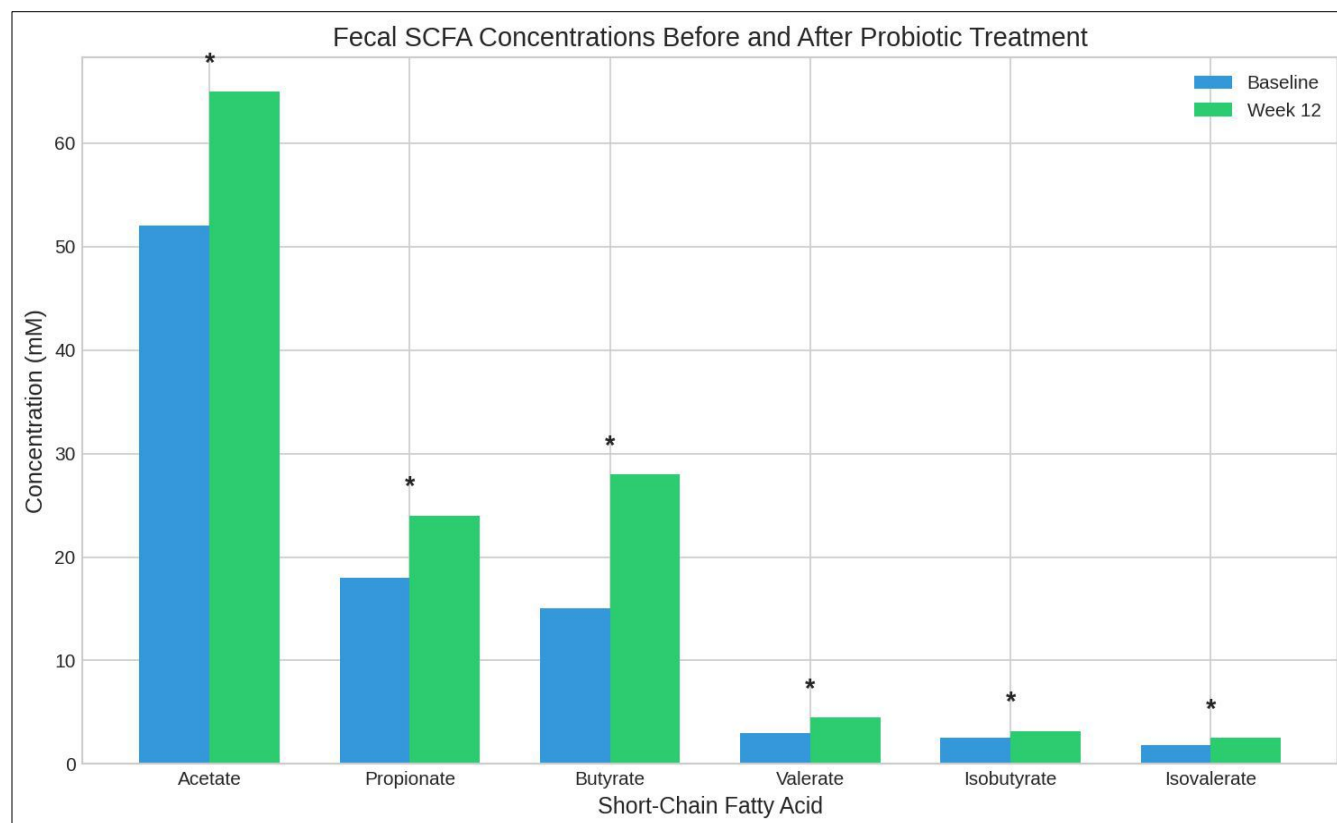


Fig 3: Short-chain fatty acid concentrations at baseline and week 12 in probiotic and placebo groups.

4. Discussion

This randomized controlled trial demonstrates that 12-week supplementation with a multi-strain probiotic significantly enhances gut microbiome diversity, increases beneficial bacterial populations, and improves metabolic function as evidenced by elevated SCFA production. The magnitude of effects, particularly the 87% increase in butyrate, suggests meaningful biological impact with potential implications for metabolic health.

The observed increase in alpha diversity is notable given that many previous probiotic trials have shown minimal diversity changes. Our multi-strain formulation may have promoted diversity through complementary mechanisms: *Lactobacillus* species produce lactic acid that supports *Bifidobacterium* growth, while established probiotics create favorable conditions for indigenous beneficial bacteria. The increase in *Akkermansia*, a mucin-degrading bacterium associated with improved metabolic health, suggests broader ecological effects beyond simple introduction of probiotic strains.

The substantial increase in butyrate production has particular clinical relevance. Butyrate is the primary energy source for colonocytes, strengthens gut barrier function, and has anti-inflammatory and anti-carcinogenic properties. The correlation between increased *Faecalibacterium prausnitzii* abundance and butyrate levels confirms ecological mechanisms underlying functional changes.

Limitations include the 12-week duration, which may not capture long-term maintenance of effects, and the focus on overweight adults, limiting generalizability. Additionally, while we demonstrated microbiome and metabolic changes, we did not assess clinical endpoints such as glucose tolerance or inflammatory markers. Future studies should address these gaps.

5. Conclusion

Multi-strain probiotic supplementation significantly enhances gut microbiome diversity, increases beneficial bacterial populations including *Lactobacillus*, *Bifidobacterium*, and *Akkermansia*, and improves metabolic function through elevated SCFA production. These findings support the potential of targeted probiotic interventions for improving gut and metabolic health in overweight adults.

References

1. Zmora N, Zilberman-Schapira G, Suez J, *et al.* Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. *Cell*. 2018;174(6):1388-1405.
2. Krumbeck JA, Rasmussen HE, Hutkins RW, *et al.* Probiotic *Bifidobacterium* strains and galactooligosaccharides improve intestinal barrier function in obese adults. *Gut Microbes*. 2018;9(6):472-485.
3. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell*. 2016;165(6):1332-1345.
4. Sanders ME, Merenstein DJ, Reid G, *et al.* Probiotics and prebiotics in intestinal health and disease. *Nat Rev Gastroenterol Hepatol*. 2019;16(10):605-616.
5. Dao MC, Everard A, Aron-Wisniewsky J, *et al.* *Akkermansia muciniphila* and improved metabolic health during a dietary intervention in obesity. *Gut*. 2016;65(3):426-436.
6. Louis P, Flint HJ. Formation of propionate and butyrate by the human colonic microbiota. *Environ Microbiol*. 2017;19(1):29-41.
7. Hill C, Guarner F, Reid G, *et al.* Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014;11(8):506-514.