Universal Library of Medical and Health Sciences Research Article

ISSN: 3064-6545 | Volume 3, Issue 4

Open Access | PP: 36-41

DOI: https://doi.org/10.70315/uloap.ulmhs.2025.0304005

Multi-Strain Probiotic Supplement Enhances Gut Microbiota Composition in Rats: Evidence for Functional Food Potential

Tzu-Chin Wu, BSs[1], Hui-Yu Huang, PhD[2], Yu Syuan, Jhang, MS[1], Yu-Jou Chien, PhD[1]*

[1]SummitBio Corporation, 3 F, No. 192, Zhonggong 2nd Road, Xitun Dist., Taichung City 407, Taiwan.

[2]Taipei Medical University College of Pharmacy, No. 250, Wuxing St., Xinyi Dist., Taipei City 110301, Taiwan.

Abstract

Background: *Probiotics, particularly Lactobacillus and Bifidobacterium species, are widely recognized for their beneficial effects on gut microbiota balance and host health. Alterations in gut microbial composition are associated with gastrointestinal disorders and the development of colorectal cancer. Functional probiotic formulations are increasingly investigated for their ability to enhance beneficial bacteria and suppress harmful strains.*

Objective: *This study aimed to evaluate the effects of a compound probiotic powder on gut microbiota composition in Sprague–Dawley rats, with a focus on changes in total anaerobic bacteria, Lactobacillus, Bifidobacterium, Escherichia coli, and Clostridium perfringens populations.*

Methods: *Forty male Sprague–Dawley rats were randomly divided into four groups (n = 10 each): control, low dose (1×), medium dose (2×), and high dose (5×). The probiotic powder was administered by oral gavage for 8 weeks. Fecal samples were collected at baseline, week 4, and week 8, and cecal contents were collected at week 8. Microbial populations were quantified by colony-forming unit (CFU) counts using selective culture media under aerobic and anaerobic conditions, following established methods for gut microbiota enumeration (Matsumoto et al., 2006; Yang & Li, 2010; Yeo & Liong, 2010). Physiological parameters, including body weight, feed intake, water intake, and organ weights, were also assessed.*

Results: *Probiotic supplementation significantly increased total anaerobic bacteria, Lactobacillus, and Bifidobacterium counts in fecal and cecal samples at weeks 4 and 8 compared with the control group ($p < 0.05$). No significant changes were observed in Escherichia coli or Clostridium perfringens populations across groups. Body weight, feed intake, water intake, and organ weights showed no significant differences among groups ($p > 0.05$).*

Conclusion: *The compound probiotic powder promoted the proliferation of beneficial gut bacteria—particularly Lactobacillus and Bifidobacterium—without altering harmful bacterial populations or physiological parameters in rats. These findings support its potential as a functional probiotic supplement for maintaining gut microbiota balance.*

Keywords: *Probiotics/ Gut Microbiota Modulation/ Intestinal health/ Lactobacillus/ Bifidobacterium.*

INTRODUCTION

The gastrointestinal tract harbors a complex community of microorganisms that play a pivotal role in host metabolism, immune regulation, and maintenance of intestinal homeostasis [1]. Perturbations of gut microbiota composition have been associated with gastrointestinal disorders, metabolic syndromes, and impaired immune responses [2]. Probiotics, defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" [3], have been extensively studied for their potential to restore microbial balance and improve gut health [4].

Among probiotic strains, *Lactobacillus* and *Bifidobacterium* species are the most extensively studied due to their demonstrated effects in modulating gut microbiota, inhibiting pathogen colonization, and enhancing mucosal barrier function [5,6]. According to relevant literature, *Lactobacillus acidophilus* LA02 exhibits potential in modulating gut microbiota composition, enhancing intestinal barrier function, and promoting immune responses [7]. Meanwhile, *Lactobacillus casei* LC03 has demonstrated beneficial effects in reducing intestinal inflammation, improving gut motility, and supporting the growth of beneficial bacteria [8].

Citation: Tzu-Chin Wu, Yu-Jou Chien, et al., "Multi-Strain Probiotic Supplement Enhances Gut Microbiota Composition in Rats: Evidence for Functional Food Potential", Universal Library of Medical and Health Sciences, 2025; 3(4): 36-41. DOI: https://doi.org/10.70315/uloap.ulmhs.2025.0304005.

Multiple studies have indicated that both strains, whether used individually or in combination, can effectively improve gut microecology and provide supportive benefits for gastrointestinal disorders such as irritable bowel syndrome, constipation, and diarrhea. Further research into their mechanisms and clinical applications will contribute to the precise development of probiotics in the field of gut health [9].

In particular, *Lactobacillus acidophilus*, *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, and *Bifidobacterium bifidum* have been reported to exert beneficial impacts on gut microbial composition and gastrointestinal function [10–13]. Dietary supplementation with multi-strain probiotic formulations has gained growing attention because the synergistic interactions between strains may enhance colonization and broaden the spectrum of beneficial effects compared to single-strain preparations [14]. Nevertheless, strain-specific efficacy and appropriate dosage remain critical considerations when evaluating functional foods designed for gut health. Preclinical studies in rodent models, such as Sprague–Dawley rats, offer valuable insights into host–microbiota interactions and facilitate the evaluation of probiotic interventions prior to clinical application [13].

In this study, we evaluated the gastrointestinal modulatory effects of a probiotic formulation containing *Lactobacillus acidophilus* LA02, *Lactobacillus paracasei* LPC00, *Lactobacillus casei* LC03, *Lactobacillus rhamnosus* LR05, *Bifidobacterium bifidum* BB01, *Lactobacillus plantarum* LP01, and *Bacillus coagulans* Unique IS-2. Using a controlled eight-week feeding trial in Sprague–Dawley rats, we assessed the impact of different doses on fecal and cecal microbiota composition, with the aim of elucidating its potential as a functional food ingredient for gut health improvement.

MATERIALS AND METHODS

Experimental Animals

The animal use protocol listed below has been reviewed and approved (approval no. LAC2023-0196; 17 Jun 2023) by the institutional Animal Care and Use Committee or Panel (IACUC/IACUP) in Taipei Medical University (Taipei, Taiwan)This protocol adhered to the guidelines contained in the "Current International Laws and Policies", a part of the "Guide for the Care and Use of Laboratory Animals" of The National Academies Press (8th Ed., 2011). We did our best to minimize the total number of gerbils used in this study and to reduce their pain.

Probiotic Formulation

The probiotic powder ("complex probiotic powder package") was provided by SummitBio Corporation (Taiwan). Each 2 g sachet contained indigestible maltodextrin, isomalto-oligosaccharide, corn starch, digestive enzymes, and a consortium of lactic acid bacteria, including *Lactobacillus acidophilus* LA02 (Probiotical®, Italy); *Lactobacillus paracasei* LPC00 (Probiotical®, Italy); *Lactobacillus casei* LC03 (Probiotical®, Italy); *Lactobacillus rhamnosus* LR05 (Probiotical®, Italy); *Bifidobacterium bifidum* BB01 (Probiotical®, Italy); *Lactobacillus plantarum* LP01 (Probiotical®, Italy); and *Bacillus coagulans* Unique IS-2, along with spore-forming lactic acid bacteria. The recommended human daily dose is one 2 g sachet.

Animals and Experimental Groups

The animal grouping and oral probiotic administration schedule were adapted from previous rodent studies investigating probiotic effects on gut microbiota [15]. Forty male Sprague–Dawley rats (5 weeks old, 190–200 g; BioLASCO Taiwan Co., Ltd.) were acclimated for 1 week (23 ± 2 °C, 55 ± 10% humidity, 12 h light/dark cycle) before being randomly divided into four groups (n = 10): Control (vehicle), Low dose (0.207 g/kg/day, 1×), Medium dose (0.413 g/kg/day, 2×), and High dose (1.03 g/kg/day, 5×), based on the human equivalent dose of 2 g/day converted using the body surface area normalization method (conversion factor 6.2), the test substance was dissolved in deionized water before oral gavage. Animals were individually housed, given standard chow (LabDiet® 5001, Purina Mills, USA) and water ad libitum, and administered the test product daily by oral gavage for 8 weeks. Body weight was measured weekly, and feed/water intake was recorded. Fecal samples [16] (1 g) were collected at Weeks 0, 4, and 8 under isoflurane anesthesia after 12 h fasting, while cecal contents (1 g) were obtained at Week 8 following euthanasia for microbiota analysis.

Preparation of Anaerobic Diluent and Selective Media

Anaerobic Diluent

Prepare gelatin 0.2 g + salts solution 50 mL + distilled water 50 mL + resazurin 0.4 mL (25 mg/100 mL) mixed, boiled, cooled, then add cysteine 0.05 g. Under anaerobic conditions, aliquot into 9 mL per tube and sterilize at 121 °C for 15 min.

Medium: Total Anaerobic Bacteria (CFU) Medium: Use Commercial CDC Anaerobe

Blood agar plates (BBL).; *Lactobacillus* (selective medium): MRS agar supplemented with 0.5 % $CaCO_3$ (i.e. MRS + $CaCO_3$) (modified MRS).; *Bifidobacterium* (selective medium): According to the method of Muñoa & Parés [17], BIM-25 (*Bifidobacterium* iodoacetate medium 25) was used as a selective medium for the enumeration of *Bifidobacterium spp.*; *Escherichia coli* (selective medium): Endo agar (Endo Broth 41.5 g + Agar 5 g in 1 L).; *Clostridium perfringens* (selective medium): was enumerated on Tryptose-sulfite-D-cycloserine (TSC) agar prepared with two layers, consisting of a basal agar and an overlay supplemented with D-cycloserine and egg yolk emulsion, following the standard protocol described in FDA BAM [18].

Bacterial Enumeration (Colony Counting)

From each homogenized fecal or cecal sample (1 mL),

serial tenfold dilutions were prepared in anaerobic diluent. For each bacterial group (total anaerobes, *Lactobacillus, Bifidobacterium, E. coli*), three appropriate dilutions were selected, and 50 µL was spread onto corresponding selective agar plates using sterile glass rods. Anaerobic plates (for anaerobes, *Bifidobacterium, Clostridium*) were incubated in anaerobic jars (with anaerobic pack and indicator) at 35–37 °C. Aerobic plates (*E. coli*) were incubated under ambient conditions. After ~24 h incubation, colony counts (CFU) were recorded. *Clostridium perfringens* colonies (characterized by black centers and surrounding halos) were enumerated from TSC plates using overlay methods.

Colony counts were converted to CFU per g of sample using standard formulas. Enumeration methods for *Bifidobacterium* using selective media have been well established (e.g. Muñoa& Pares, 1988; Yeo & Liong, 2010) [17, 19].

Probiotic Strains Used

The probiotic bacterial strains used in this study were: *Lactobacillus acidophilus* LA02 (DSM 21717), *Lactobacillus paracasei* LPC00 (LMG P21380), *Lactobacillus casei* LC03 (DSM 27537), *Lactobacillus rhamnosus*LR05 (DSM 19739), *Bifidobacterium bifidum* BB01 (DSM 22892), *Lactobacillus plantarum* LP01 (LMGP21021), and *Bacillus coagulans* Unique IS-2. The strains were cultured and prepared in-house or by a certified microbial supplier prior to formulation into the powder.

Statistical Analysis

All data were expressed as *Mean ± SD.* Statistical significance was determined using one-way ANOVA, followed by Duncan's multiple range test. Differences were considered statistically significant at $p< 0.05$.

RESULTS

Body weight (A) and organ weights at the endpoint (B) of SD rats is presented in Table 1.Throughout the eight-week feeding period, no physiological abnormalities, illnesses, or deaths were observed among the experimental animals. The changes in body weight of rats in each group during the feeding period are shown in (A), there were no statistically significant differences in body weight among the groups ($p> 0.05$). After eight weeks of feeding, the organ weights at sacrifice are shown in (B). Compared with the control group, the low-, medium-, and high-dose groups showed no significant differences in kidney, liver, or cecum weights ($p> 0.05$).

Table 1. Body weight (A) and organ weights at the endpoint (B) of SD rats

(A)	Body weight (g)			
Period	Control	Low dose	Medium dose	High dose
Week 0	192.1±5.8[a]	190.7±11.9[a]	192.4±4.2[a]	192.4±8.8[a]
Week 1	255.9±9.6[a]	253.8±13.4[a]	256.7±10.1[a]	254.0±17.6[a]
Week 2	312.0±11.2[a]	294.8±23.6[a]	305.4±26.1[a]	310.5±23.5[a]
Week 3	348.6±14.1[a]	337.9±26.4[a]	356.3±24.6[a]	340.2±30.2[a]
Week 4	378.5±19.9[a]	386.6±17.1[a]	392.4±27.6[a]	382.9±31.0[a]
Week 5	388.6±29.1[a]	407.5±25.3[a]	415.9±31.2[a]	400.1±27.0[a]
Week 6	419.1±17.3[a]	426.7±29.6[a]	423.7±35.4[a]	418.3±28.2[a]
Week 7	447.1±16.7[a]	452.2±32.0[a]	459.0±37.0[a]	439.8±31.1[a]
Week 8	469.4±19.8[a]	474.7±34.4[a]	483.3±40.1[a]	462.0±30.5[a]

(A) Body weight changes of rats in each group during feeding

Values are expressed as *Mean ± SD*.

Superscript letters (*a*) indicate no significant difference compared with the control group at the same time point ($p> 0.05$).

(B)	Organ weight (g)			
Organ	Control	Low dose	Medium dose	High dose
Liver weight	18.3±2.8[a]	20.0±1.6[a]	20.1±1.5[a]	19.2±2.8[a]
Kidney weight	3.7±0.5[a]	3.8±0.4[a]	3.8±0.5[a]	3.8±0.3[a]
Cecum weight	6.6±0.4[a]	6.6±0.6[a]	6.6±0.5[a]	6.5±0.6[a]

(B) Kidney, liver, and cecum weights of rats in each group after eight weeks of feeding

Values are expressed as *Mean ± SD*.

Superscript letters (*a*) indicate no significant difference compared with the control group at the same time point ($p> 0.05$).

According to the results of total anaerobic bacterial counts (Table 2), no significant differences (p> 0.05) were observed among the low-, medium-, and high-dose groups compared with the control group at Week 0.AtWeek 4, the fecal total anaerobic bacterial counts in the low-, medium-, and high-dose groups were significantly higher than those in the control group (p< 0.05).Similarly, atWeek 8, the fecal total anaerobic bacterial counts in all treatment groups showed a significant increase compared with the control group (p< 0.05). After eight weeks of feeding, the cecal total anaerobic bacterial counts at sacrifice were also significantly higher in the low-, medium-, and high-dose groups than in the control group (p< 0.05).

Table 2. Total anaerobic bacterial counts (log *CFU*/g)

	Total anaerobic bacterial counts (log CFU/g)			
Item	Control	Low dose	Medium dose	High dose
0 Week fecal	8.20±0.06^a	8.22±0.04^a	8.18±0.02^a	8.20±0.03^a
4 Week fecal	8.25±0.06^a	8.46±0.18^b	8.48±0.19^b	8.43±0.13^b
8 Week fecal	8.23±0.07^a	8.47±0.12^b	8.55±0.10^b	8.50±0.13^b
8 Week cecum	8.20±0.11^a	8.44±0.11^b	8.50±0.17^b	8.43±0.10^b

Values are expressed as *Mean ± SD.*

Superscript letters (*a*, *b*) indicate that the low-dose, medium-dose, and high-dose groups show significant differences compared with the control group at the same time point (p< 0.05).

According to the analysis of *Lactobacillus* counts (Table 3), no significant differences (p> 0.05) were observed in fecal *Lactobacillus* counts among the low-, medium-, and high-dose groups compared with the control group at Week 0. At Week 4, fecal *Lactobacillus* counts in all treatment groups were significantly higher than those in the control group (p< 0.05). Similarly, at Week 8, fecal *Lactobacillus* counts in the low-, medium-, and high-dose groups were significantly increased compared with the control group (p< 0.05). After eight weeks of feeding, the cecal *Lactobacillus* counts at sacrifice were also significantly higher in all treatment groups than in the control group (p< 0.05).

Table 3. Lactobacillus bacterial counts (log *CFU*/g)

	Lactobacillus bacterial counts (log CFU/g)			
Item	Control	Low dose	Medium dose	High dose
0 Week fecal	8.20±0.06^a	8.17±0.05^a	8.20±0.08^a	8.18±0.04^a
4 Week fecal	8.25±0.10^a	8.41±0.14^b	8.42±0.11^b	8.39±0.11^b
8 Week fecal	8.23±0.12^a	8.44±0.13^b	8.43±0.14^b	8.46±0.14^b
8 Week cecum	8.20±0.10^a	8.37±0.12^b	8.40±0.14^b	8.40±0.12^b

Values are expressed as *Mean ± SD*.

Superscript letters (*a*, *b*) indicate that the low-dose, medium-dose, and high-dose groups show significant differences compared with the control group at the same time point (p< 0.05).

According to the analysis of *Bifidobacterium* counts (Table 4), no significant differences (p> 0.05) were observed in fecal *Bifidobacterium* counts among the low-, medium-, and high-dose groups compared with the control group at Week 0. At Week 4, fecal *Bifidobacterium* counts in all treatment groups were significantly increased compared with the control group (p< 0.05). Similarly, at Week 8, fecal *Bifidobacterium* counts in the low-, medium-, and high-dose groups were significantly higher than those in the control group (p < 0.05). After eight weeks of feeding, the cecal *Bifidobacterium* counts at sacrifice were also significantly higher in all treated groups compared with the control group (p < 0.05).

Table 4. Bifidobacterium bacterial counts (log *CFU*/g)

	Bifidobacterium bacterial counts (log *CFU*/g)			
Item	Control	Low dose	Medium dose	High dose
0 Week fecal	7.14±0.05^a	7.12±0.06^a	7.12±0.07^a	7.13±0.06^a
4 Week fecal	7.22±0.06^a	7.35±0.15^b	7.42±0.18^b	7.35±0.09^b
8 Week fecal	7.18±0.10^a	7.36±0.14^b	7.45±0.17^b	7.47±0.10^b
8 Week cecum	7.18±0.09^a	7.32±0.09^b	7.39±0.24^b	1.39 ±0.07^b

Values are expressed as *Mean ± SD*.

Superscript letters (*a*, *b*) indicate that the low-dose, medium-dose, and high-dose groups show significant differences compared with the control group at the same time point ($p< 0.05$).

According to the analysis of *Escherichia coli* counts (Table 5), no significant differences ($p> 0.05$) were observed in fecal *E. coli* counts among the low-, medium-, and high-dose groups compared with the control group at Weeks 0, 4, and 8. Similarly, after eight weeks of feeding, no significant differences ($p> 0.05$) in cecal *E. coli* counts were observed among the treatment groups and the control group at sacrifice.

Table 5. Escherichia coli bacterial counts (log *CFU*/g)

	Escherichia coli bacterial counts (log *CFU*/g)			
Item	Control	Low dose	Medium dose	High dose
0 Week fecal	5.19±0.06[a]	5.20±0.06[a]	5.15±0.05[a]	5.17±0.05[a]
4 Week fecal	5.18±0.06[a]	5.17±0.04[a]	5.16±0.04[a]	5.17±0.01[a]
8 Week fecal	5.20±0.06[a]	5.19±0.06[a]	5.20±0.07[a]	5.18±0.05[a]
8 Week cecum	5.17±0.06[a]	5.18±0.09[a]	5.18±0.08[a]	5.18±0.12[a]

Values are expressed as *Mean ± SD*.

Superscript letters (*a*) indicate that the low-dose, medium-dose, and high-dose groups show significant differences compared with the control group at the same time point ($p< 0.05$).

According to the analysis of *Clostridium perfringens* counts (Table 6), no significant differences ($p> 0.05$) were observed in fecal *C. perfringens* counts among the low-, medium-, and high-dose groups compared with the control group at Weeks 0, 4, and 8. Similarly, after eight weeks of feeding, no significant differences ($p> 0.05$) in cecal *C. perfringens* counts were observed among any of the treatment groups and the control group at sacrifice.

Table 6. Clostridium perfringens bacterial counts (log *CFU*/g)

	*Clostridium perfringens*bacterial counts (log *CFU*/g)			
Item	Control	Low dose	Medium dose	High dose
0 Week fecal	4.14±0.05[a]	4.14±0.05[a]	4.16±0.01[a]	4.15±0.02[a]
4 Week fecal	4.15±0.03[a]	4.14±0.04[a]	4.15±0.07[a]	4.15±0.03[a]
8 Week fecal	4.14±0.03[a]	4.16±0.05[a]	4.15±0.06[a]	4.13±0.03[a]
8 Week cecum	4.16±0.02[a]	4.15±0.04[a]	4.13±0.03[a]	4.15±0.02[a]

Values are expressed as *Mean ± SD*.

Superscript letters (*a*) indicate that the low-dose, medium-dose, and high-dose groups show significant differences compared with the control group at the same time point ($p< 0.05$).

DISCUSSION

In the present study, administration of the multi-strain probiotic powder containing *Lactobacillus acidophilus* LA02, *Lactobacillus paracasei* LPC00, *Lactobacillus casei* LC03, *Lactobacillus rhamnosus* LR05, *Bifidobacterium bifidum* BB01, *Lactobacillus plantarum* LP01, and *Bacillus coagulans* Unique IS-2resulted in statistically significant increases in beneficial microbial populations in the gut of Sprague–Dawley rats over an 8-week feeding period. Specifically, fecal counts of total anaerobic bacteria, *Lactobacillus* and *Bifidobacterium* were significantly elevated in all treatment groups compared with the control group at Weeks 4 and 8 ($p< 0.05$), and cecal counts at sacrifice likewise demonstrated higher levels in treated animals ($p< 0.05$). Conversely, counts of potential opportunistic pathogens including *Escherichia coli* and *Clostridium perfringens* were not significantly altered by treatment ($p> 0.05$). These results suggest that the probiotic intervention selectively enhanced beneficial microflora without promoting overgrowth of undesirable bacterial populations, supporting a gut-microbiota-modulating effect without dysbiotic risk.

Comparison with prior research underscores these findings. For example, Park et al. [20]reported that in aged mice, supplementation with *Lactobacillus fermentum* JDFM216 increased *Lactobacillus* abundance ($p< 0.05$) and enhanced immune parameters, without shifting the overall microbiota composition dramatically. Similarly, De Castilho et al. [21] demonstrated that probiotic administration modulates intestinal microbial networks in rats via metagenomic shifts. Our data extend these results by showing that a multi-strain probiotic can elevate both *Lactobacillus* and *Bifidobacterium* counts in healthy rats over time while maintaining stability in opportunistic pathogen counts. This selective modulation aligns with the hypothesis that probiotic supplementation

can support gut health by boosting commensal taxa without disrupting microbial balance.

In conclusion, the current study demonstrates that daily supplementation with the designated probiotic formulation over eight weeks effectively increases beneficial gut microbes in rats, with no adverse elevation of pathogenic bacterial groups. These findings provide preclinical support for the product's potential as a functional food ingredient targeted at gut health improvement. Future work should investigate functional outcomes such as gut barrier integrity, metabolite production (e.g., short-chain fatty acids) and translation to human studies.

REFERENCES

1. Thursby, E., & Juge, N. (2017). Introduction to the human gut microbiota. *Biochemical Journal*, 474(11), 1823–1836.
2. Rinninella, E., et al. (2019). What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms*, 7(1), 14.
3. Hill, C., et al. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*, 11(8), 506–514.
4. Di Cerbo A, et al. (2015). Mechanisms and therapeutic effectiveness of lactobacilli: a review of the literature. *Journal of Clinical Gastroenterology & Hepatology*. (review).
5. Ouwehand, A. C., et al. (2002). Probiotic and other functional microbes: from markets to mechanisms. *Curr Opin Biotechnol*, 13(5), 483–487.
6. Plaza-Diaz, J., et al. (2019). Mechanisms of action of probiotics. *Advances in Nutrition*, 10(suppl_1), S49–S66.
7. Taverniti, V., Guglielmetti, S., Fiore, W., & Arioli, S. (2023). *Lactobacillus acidophilus* LA02 and its role in gut barrier modulation and immune support. *Microorganisms*, 11(2), 456.
8. Guglielmetti, S., Mora, D., & Arioli, S. (2022). Anti-inflammatory properties of *Lactobacillus casei* LC03 in intestinal models. *Frontiers in Immunology,* 13, 840245.
9. Fiore, W., Arioli, S., & Taverniti, V. (2021). Synergistic effects of *Lactobacillus acidophilus* LA02 and *Lactobacillus casei* LC03 on gut microbiota modulation. *Beneficial Microbes,* 12(5), 389–398.
10. Guarner, F., & Malagelada, J. R. (2003). Gut flora in health and disease. *Lancet*, 361(9356), 512–519.
11. Sánchez, B., et al. (2017). Probiotics, gut microbiota, and their influence on host health and disease. *Molecular Nutrition & Food Research*, 61(1), 1600240.
12. Azad, M. A. K., et al. (2018). Probiotic species in the modulation of gut microbiota: An overview. *Biomed Research International*, 2018, 9478630.
13. Khokhlova, E. V., et al. (2012). Anti-inflammatory properties of intestinal *Bifidobacterium* strains isolated from healthy infants. *Microbiology and Immunology*, 56(1), 27–39.
14. Chapman, C. M. C., et al. (2011). Multi-strain probiotics: functional synergy or strain redundancy? *International Journal of Food Microbiology*, 149(3), 185–197.
15. Yang, J., & Li, Y. (2010). Effect of probiotics on intestinal microflora in rats with experimental colitis. *World Journal of Gastroenterology,* 16(15), 1908–1915.
16. Matsumoto, M., Ohishi, H., & Benno, Y. (2006). Impact of LKM512 yogurt on improvement of intestinal environment of the elderly. *FEMS Immunology & Medical Microbiology,* 46(3), 381–389.
17. Muñoa FJ, Pares R. (1988) Selective medium for isolation and enumeration of *Bifidobacterium spp.Applied and Environmental Microbiology.* 54(7):1715–1718.
18. U.S. Food and Drug Administration. *Bacteriological Analytical Manual (BAM), Chapter 16: Clostridium perfringens*. FDA, Silver Spring, MD.
19. Yeo, S. K., & Liong, M. T. (2010). Effect of prebiotics on viability and growth characteristics of probiotics in soymilk. *Journal of Food Science,* 75(6), M373–M380.
20. Park, M.R., Shin, M., Mun, D. et al.(2020) Probiotic *Lactobacillus fermentum* strain JDFM216 improves cognitive behavior and modulates immune response with gut microbiota. *Sci Rep.,* 10, 21701.
21. Castilho TJC, et al. (2023) Metagenomics of microbiota following probiotic supplementation in rats subjected to intestinal anastomosis. *Surg Open Sci.* Jun 28;14:22-30.