

Brief Report

In Vitro Modulation Processes, Prebiotic vs. Postbiotic, of Microbiota Pattern: A Preliminary Study

Emanuel Vamanu ^{1,2,*}  and Laura Dorina Dinu ^{1,*} ¹ Department of Industrial Biotechnology, Faculty of Biotechnology, University of Agricultural Sciences and Veterinary Medicine, 59, Marasti Blvd., 011464 Bucharest, Romania² Anoom Laboratories SRL, Voluntari, 077190 Ilfov, Romania

* Correspondence: emmanuel.vamanu@gmail.com (E.V.); laura.dinu@biotehnologii.usamv.ro (L.D.D.); Tel.: +40-742-218-240 (E.V.)

Abstract

The human gut microbiota helps maintain metabolic balance, supports immune function, and defends against opportunistic pathogens that can disrupt the microbiota ecosystem. An imbalance or dysbiosis in microbial composition is linked to various diseases, including inflammatory bowel disease, metabolic syndromes, and neurodegenerative disorders. Using microbiota modulation with prebiotics and postbiotics is a practical approach to address these imbalances. Prebiotic compounds are defined as substrates that promote metabolic activity and restore microbial patterns. Postbiotics include short-chain fatty acids (SCFAs), microbial cell lysates, and extracellular compounds. This research aims to investigate how the gut microbiota can be modulated in vitro using the prebiotic ColonX and a postbiotic derived from Kombucha fermentation within a controlled GIS1 in vitro system. These products demonstrate potential for modulation, as they support selective bacterial growth and enhance microbial diversity. Prebiotics help stabilize gut pH, while postbiotics play a crucial role in biofilm formation. Together, they provide an innovative approach to treating dysbiosis and enhancing overall gut health. The findings highlight the importance of utilizing prebiotics and postbiotics to modulate gut microbiota in chronic diseases characterized by dysbiosis. This paper is especially relevant for elderly populations, as gut dysbiosis is common, and microbiota modulation supports healthy aging.

Keywords: mushrooms; black tea; *Lactobacillus*; fingerprint; simulation; gut microbiota; modulation



Academic Editor: Luisa Tesoriere

Received: 6 August 2025

Revised: 17 September 2025

Accepted: 30 September 2025

Published: 10 October 2025

Citation: Vamanu, E.; Dinu, L.D. In Vitro Modulation Processes, Prebiotic vs. Postbiotic, of Microbiota Pattern: A Preliminary Study. *Nutraceuticals* **2025**, *5*, 30. <https://doi.org/10.3390/nutraceuticals5040030>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The human microbiota has a unique and constantly changing fingerprint, and maintaining buffer capacity is vital for human health; however, it is directly affected by daily habits and medical conditions. This adaptable fingerprint is essential for each individual because it influences digestion, controls key biomarkers and the immune system, and protects against disease [1]. Dysbiosis, which refers to an imbalance of microbial fingerprints, has been associated with specific health issues, including inflammatory bowel diseases, type 2 diabetes, and neurodegenerative disorders [2]. Several methods, especially in vitro approaches, for modifying the gut microbiota fingerprint are valuable options for researchers [3].

Prebiotics and postbiotics could be crucial product categories for shaping gut microbiota from natural sources [4]. Prebiotics are non-digestible food supplements that directly and positively influence the host microbiota by selectively encouraging the growth and/or

metabolic activity of beneficial gut bacteria. Common prebiotics include fructooligosaccharides (FOS), galactooligosaccharides (GOS), lactulose, and inulin. They are found in various foods such as garlic, onions, leeks, and artichokes. These compounds act as substrates (carbon sources) for beneficial bacteria, promoting cell multiplication (as seen in *Lactobacillus* species, for example) and metabolic activity [5].

Maintaining a balanced microbiota is crucial for elderly individuals, especially centenarians, because their microbial patterns are often associated with inflammation, metabolic health, and increased longevity [2,3]. Nutraceuticals that can influence the microbiota (such as prebiotics and postbiotics) may play a direct role in maintaining or restoring microbial balance in aging populations [4].

Based on metabolic activity under specific conditions, postbiotics are bioactive compounds produced by probiotic bacteria and all bacterial cell components. These include biomarkers for health conditions, such as SCFAs, microbial cell components, and extracellular polysaccharides essential for human health, depending on the type of postbiotic [6]. Postbiotics can have various health effects, ranging from enhancing gut barrier function to modulating immune responses and inhibiting the growth of different pathogens [7].

In this study, prebiotics (ColonX product) and postbiotics (Postbiotic product) are used to modulate the normal microbiota in vitro as an alternative therapeutic approach. ColonX, a mushroom-derived prebiotic rich in β -glucans, was selected due to its demonstrated ability to restore microbial stability and modulate dysbiosis through the fermentation of polysaccharides. The Kombucha-derived postbiotic was included because it provides a complementary mechanism by delivering organic acids, polyphenols, and extracellular polysaccharides. All of these support microbial resilience and gut barrier integrity. The combined use of these products allows for a comparative evaluation of substrate and metabolite strategies for modulating microbiota composition under controlled in vitro conditions. In vitro studies in the GIS1 system were conducted in a controlled environment to assess the effects of these two functional products (prebiotic and postbiotic) on microbial and metabolomic profiles through a modulation process. The study demonstrated that prebiotic and postbiotic products can selectively promote the growth of beneficial bacteria and alter microbiota patterns. The research also revealed increased microbial diversity and a higher abundance of beneficial bacteria linked to gut health, along with enhanced metabolomic activity.

2. Materials and Methods

2.1. Sample Obtaining

The postbiotic and prebiotic products (ColonX spray-dried powder) were sourced from SC Anoom Biolaboratories SRL in Romania. The prebiotic was produced from dried mushrooms (*Boletus edulis*), as detailed in a previous study [8]. The prebiotic product ColonX was obtained from *Boletus edulis* fruiting bodies through aqueous extraction followed by spray drying with resistant dextrin as a carrier. The final powder was standardized to contain a minimum of 25–30% β -glucans, known for their prebiotic properties.

The postbiotic product was produced through Kombucha fermentation with black tea as the substrate. After at least two weeks of fermentation, the liquid was spray-dried with arabic gum. In both cases, the daily dose was one capsule (250 mg), which was dissolved in sterile water before administration [9]. The postbiotic product was obtained via fermentation with the Kombucha culture for 14–16 days under controlled conditions.

After 14–16 days of Kombucha fermentation, the culture was heat-treated at 70 °C for 30 min to inactivate all microorganisms before spray-drying. The absence of viable cells was confirmed by plating on MRS agar and nutrient agar (no growth after 48 h, 37 °C).

2.2. In Vitro Simulation of Microbiota Following Antibiotic Treatment

Following a previously described method, the in vitro simulation was performed using the GIS1 single-chamber gut simulator [8], with the modification that the working volume for each run was reduced to a maximum of 50 mL. The GIS1 system was designed to reproduce human colonic conditions in vitro under controlled parameters for studying microbiota modulation.

For each simulation, the fermentation parameters were set as follows: incubation temperature of 37 °C, mixing rate of 50 rpm using a laboratory mixer, initial pH of 6.5, microaerobic conditions, and weekly sampling intervals.

To investigate microbiota modulation, three independent fecal microbiota samples—each obtained from a different healthy adult donor and representing a distinct microbial community—were used. A dysbiotic phase was induced by administering amoxicillin and clavulanic acid for seven days, with two daily doses of 500 mg each given at 12 h intervals.

Donors were healthy adults (ages 25–50, mixed sex, omnivorous diet) with no history of gastrointestinal diseases, antibiotic treatments, or probiotic/prebiotic supplementation in the previous six months. Microbiota samples were collected through the ColHumB Collection at the Laboratory of Pharmaceutical Biotechnologies, UASVM Bucharest (www.gissystems.ro). The normal microbiota refers to microbial compositions obtained from such healthy donors, while independent fecal microbiota designates the use of samples from three different donors to ensure biological variability. All fecal samples were processed according to the ethical standards of UASVM Bucharest (ColHumB Registration number: 1418/23.11.2017 [10]).

Microbiota samples, representing microbial profiles, were stored in 20% glycerol at a freezer until used for quantitative PCR (qPCR) analysis.

2.3. Microbiome Analysis by qPCR Following Product Administration

For qPCR analysis, genomic DNA from samples was isolated using the Quick-DNA Miniprep Plus kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. To assess DNA concentration and purity, the samples were quantified with a NanoDrop 8000 (Thermo Fisher Scientific, Waltham, MA, USA). For generating the standard curve and microbial quantification, qPCR reactions were performed using a Rotor-Gene 6000 5plex HRM (Qiagen–Corbett Life Science, Mortlake, Australia) instrument and software as previously described. A mixture of three *Lactobacillus* strains was used, species belonging to the Lactobacillaceae family, and Firmicutes quantification ($R^2 = 0.9918$). In another experiment, a mixture of two *Bifidobacterium* strains was used for *Bifidobacterium* spp. quantification ($R^2 = 0.9995$). For Bacteroidetes phylum quantification ($R^2 = 0.9682$), a strain of *Prevotella* sp. was used, while *E. coli* ATCC 8739 was used for Enterobacteriaceae quantification ($R^2 = 0.9953$) [11–15]. All qPCR reactions (final volume 25 µL each) included 1 µL of template DNA, 12.5 µL of Maxima SYBR Green Mix (Thermo Fisher Scientific, Waltham, MA, USA), and 0.5 µL of each primer. The cycling parameters for the 3-step melting process were 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 30 s at 60 °C, and 45 s at 72 °C, depending on the PCR product size. The specificity of the real-time PCR reactions was confirmed by melting curve analysis. Reactions were carried out in triplicate, and the results were statistically analyzed.

2.4. Other Methods

Antioxidant capacity was assessed using the DPPH radical scavenging assay, following the validated method [16,17]. The total phenolic content was measured by the Folin–Ciocalteu method, with results expressed as milligrams of gallic acid equivalents (GAE) per gram [18].

2.5. Statistical Analysis

Each parameter was measured in triplicate, and the data are presented as mean values with standard deviations (SD). Statistical analyses were performed using IBM SPSS software, version 23. A two-way ANOVA was applied, followed by Dunnett's post hoc test. Significance levels were defined as follows: $p \leq 0.05$ (significant), $p \leq 0.01$ (very significant), $p \leq 0.001$ (highly significant), and $p \leq 0.0001$ (extremely significant), indicated by the letters a through d or *.

3. Results and Discussions

Based on the initial simulation data of pH changes, a key parameter directly related to the microbiota's metabolomic pattern showed a different profile during the first two weeks. The simulation for ColonX indicated that the pH stabilized, ranging from 5.5 to 5.8, as observed in previous studies [19]. The administration of PostBiotic caused unstable pH values. In the first week, the values were high (around 7.1) but decreased daily, similar to what was seen after ColonX administration.

Following qPCR analysis, it was observed that in vitro administration of ColonX caused a significant decrease in Lactobacilli populations (from 9.8 ± 0.2 log genome copies/mL during the M phase to 5.9–6.3 log during the treatment period; Figure 1) and in Firmicutes (from 17.5 ± 0.1 to 9.0–9.8 log genome copies/mL; $p < 0.0001$; Figure 2). Subsequent postbiotic administration helped restore the microbial balance. During the first two weeks, postbiotic treatment maintained Lactobacilli levels at around 9.7–9.8 log and Firmicutes at 14.5–15.3 genome copies/mL (both $p < 0.0001$). In contrast, weeks 3 and 4 showed moderate recoveries ($p < 0.05$ –0.01).

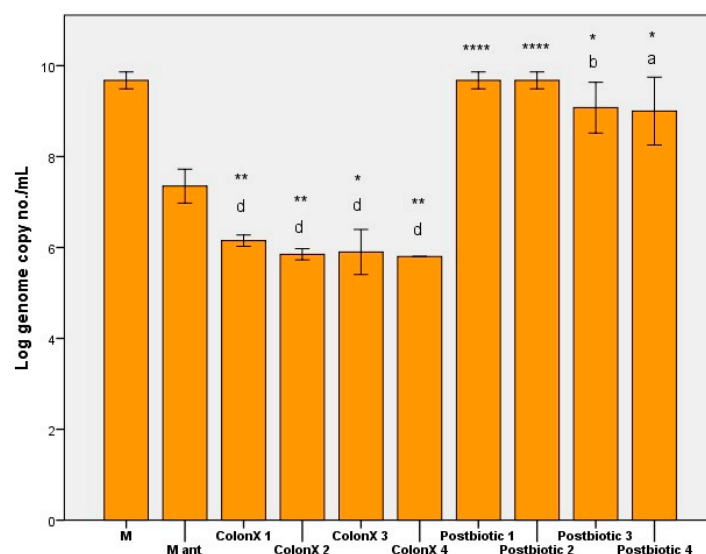


Figure 1. The effect of ColonX and PostBiotics on Species belonging to the Lactobacillaceae family in the microbiota. M—control microbiota without treatments; M ant—control microbiota with antibiotic treatment; ColonX 1, 2, 3, 4—microbiota after 1 to 4 weeks of treatment with ColonX; Postbiotic 1, 2, 3, 4—microbiota after 1 to 4 weeks of treatment with the Postbiotic product.

This evolution can be understood in different ways, but the altered metabolomic pattern might show a similar profile. These initial findings are important because, depending on the disease type, treatments can be customized for each target group. The interaction among pH, prebiotics, and the microbiota signature can help prevent and treat gut dysbiosis, which is essential for managing chronic illnesses. pH plays a crucial role in shaping the gut bacteria signature, and prebiotics help maintain a balance between beneficial and

harmful bacteria (Figures 3 and 4), as demonstrated by the similar pH levels observed in this study.

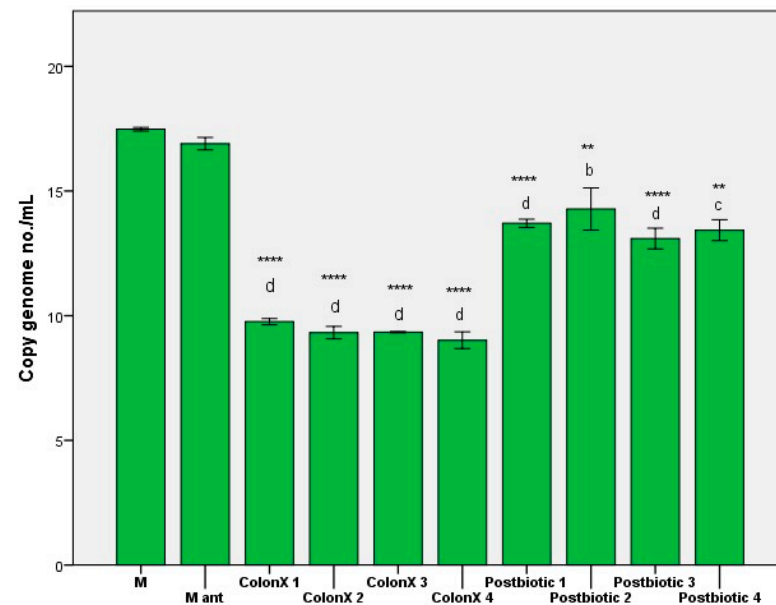


Figure 2. The effect of ColonX and PostBiotics on Firmicutes in the microbiota. M—control microbiota without treatments; M ant—control microbiota with antibiotic treatment; ColonX 1, 2, 3, 4—microbiota after 1 to 4 weeks of treatment with ColonX; Postbiotic 1, 2, 3, 4—microbiota after 1 to 4 weeks of treatment with the Postbiotic product.

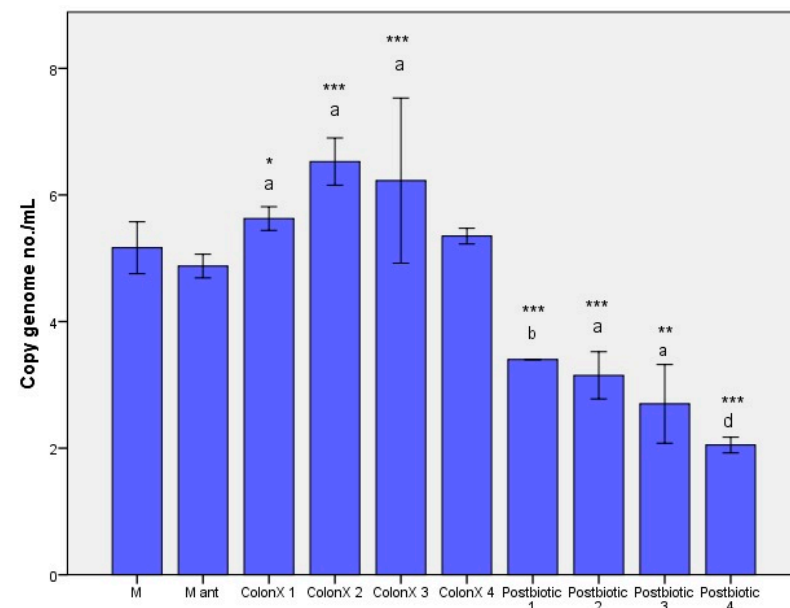


Figure 3. The effect of ColonX and PostBiotics on Bacteroides in the microbiota. M—control microbiota without treatments; M ant—control microbiota with antibiotic treatment; ColonX 1, 2, 3, 4—microbiota after 1 to 4 weeks of treatment with ColonX; Postbiotic 1, 2, 3, 4—microbiota after 1 to 4 weeks of treatment with the Postbiotic product.

Through its fermentative action, ColonX plays a key role in modulating the gut microbiome's composition and metabolic activity, acting as a prebiotic. The fermentation process produces SCFAs that impact host metabolism, support gut health, and help prevent chronic diseases. Organic acids can serve as reliable biomarkers for evaluating prebiotic and postbiotic effects in clinical nutrition studies. The pH stability observed from the first week of use directly indicates the biological health effects on the colon. Additionally,

PostBiotic differed from ColonX; its pH was more alkaline, but the most notable result was the formation of biofilm, which demonstrates a complex interaction with the microbiota. This is due to specific polysaccharides that promote biofilm formation, a vital factor in enhancing colonization capacity in the intestinal environment [20].

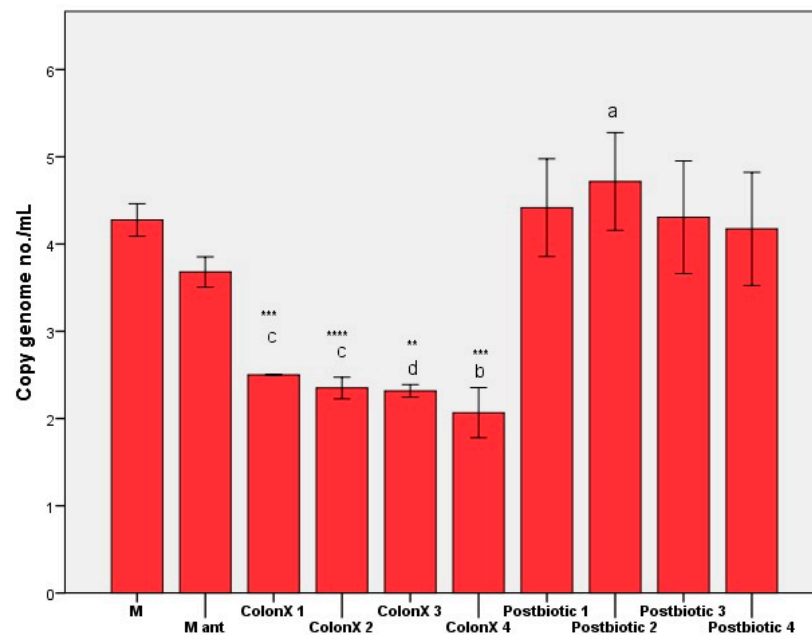


Figure 4. The Effect of ColonX and PostBiotics on *E. coli* in the Microbiota. M—control microbiota without treatments; M ant—control microbiota with antibiotic treatment; ColonX 1, 2, 3, 4—microbiota after 1 to 4 weeks of treatment with ColonX; Postbiotic 1, 2, 3, 4—microbiota after 1 to 4 weeks of treatment with the Postbiotic product.

The formation of biofilm and the stabilization of microbial communities are crucial for most elderly people, especially when the gut microbiota pattern is fragile and less resilient. Promoting a stable microbiota pattern by increasing diversity could help reduce age-related inflammatory and metabolic diseases.

The observed pH stabilization and biofilm formation after Postbiotic administration were attributed to the presence of polyphenolic compounds and antioxidant molecules found in Kombucha-derived products (5.00 ± 0.1 mg gallic acid equivalent/g dried mass). These compounds are known to enhance microbial resilience, regulate activity in the gut environment, and contribute to anti-inflammatory effects. The potential antioxidant activity (measured as $45 \pm 10\%$ DPPH scavenging activity) could support the survival of beneficial bacteria, such as *Bacteroides* and species belonging to the Lactobacillaceae family, under oxidative stress—a condition often worsened in aging individuals with dysbiosis. Although the detailed phytochemical profile of the tested products was not quantified in this study, it may significantly influence microbial fingerprint dynamics. Both direct antimicrobial effects and indirect microbiota-supporting mechanisms played key roles in increasing initial microbial diversity.

As this is a preliminary study, pH and biofilm formation were discussed qualitatively to provide context, consistent with previous reports highlighting their importance for gut microbiota stability [20,21]. Future research will include detailed quantitative analyses and graphical representations. The potential for biofilm formation was based solely on indirect parameters (pH changes, possible presence of extracellular polysaccharides, polyphenols), and not on experimental confirmation in our work.

The pH level in the colon helps regulate the microbiota, which, in turn, supports the improvement of gut and overall health [22]. Prebiotics, the indigestible parts of food, stim-

ulate bacterial growth in the large intestine and can alter the colon's pH, thereby changing the microbial composition and activity. Beneficial gut bacteria, such as *Bifidobacteria* and *Lactobacilli*, increase in number due to a lower pH, often caused by prebiotic fermentation by gut bacteria [23]. SCFAs are utilized as energy sources, enhance gut barrier properties, reduce inflammation, and enhance immune system function [24]. However, some people experience an increase in pH, which allows pathogenic bacteria to grow since they thrive in less acidic environments. In cases of dysbiosis, microbiota patterns are linked to various gastrointestinal and metabolic disorders [25]. Therefore, controlling colonic pH through prebiotics can help promote the growth of beneficial gut microbiota.

Postbiotics have been shown to influence the gut microbiota positively. Being produced during fermentation can lower the pH of the gut environment, thereby inhibiting the growth of harmful bacteria and promoting the development of beneficial ones [26]. According to the study data, postbiotics can strengthen the intestinal barrier by enhancing tight junction integrity and stimulating mucus production, thus preventing pathogen translocation and systemic inflammation [27]. A comparative analysis of the effects of prebiotics and postbiotics on modifying the normal microbiota is essential for optimizing their use in health interventions. Prebiotics provide substrates that selectively feed beneficial microbes, while postbiotics supply direct bioactive compounds capable of producing immediate physiological effects (3.50 ± 0.5 mg gallic acid equivalent/g dried mass and an average of $50 \pm 10\%$ DPPH scavenging activity). Combining these strategies offers synergistic benefits, improving the survival and colonization of beneficial microbes in the gastrointestinal tract [4].

As a future goal, this research shows that specific prebiotics can boost the production of SCFAs by the gut microbiota. Additionally, postbiotics may influence immune responses by interacting with gut-associated lymphoid tissue, thereby enhancing mucosal immunity [28]. Applying these in vitro findings to real-world situations requires further research and the development of various models, such as dynamic systems. The complexity of the human microbiota pattern depends on individual differences, diet, lifestyle, and the effects of prebiotic and postbiotic use. After in vitro studies provide valuable insights, more research is needed to confirm their health benefits in humans [29].

The comparative analysis of preliminary data revealed notable differences in microbiota composition between the administration of ColonX and Postbiotic. ColonX caused a significant increase in *Bacteroides* levels (Figure 3), especially after two weeks, showing a strong ability to promote certain beneficial microbial strains. Conversely, the Postbiotic exhibited moderate yet significant effects, with a clear inhibitory impact on *E. coli* strains (Figure 4). These results highlight the importance of combining ColonX and Postbiotics to cultivate a balanced human microbiota, supporting the growth of beneficial species and controlling potentially dysbiotic states.

After three weeks of ColonX treatment, the *Bacteroides*/Firmicutes ratio approached 1 (~ 0.93), which is significantly higher than the control group's ratio (~ 0.32). This indicates a clear shift toward a more balanced microbiota or even a slight dominance of the *Bacteroides* genus, seen as a positive effect on microbiota composition. The observed values suggest a reduction in Firmicutes dominance, which has been linked in the literature with proinflammatory states and metabolic disorders [30]. Therefore, ColonX appears to be a key modulator of gut microbiota, promoting microbial stability.

The human gut microbiome is crucial for maintaining metabolic health, and it is well known that diet significantly influences its composition. Prebiotics, such as β -glucan from ColonX, which are selectively fermentable ingredients supporting the growth of beneficial gut bacteria, have gained attention for their potential health benefits. Advances in metabolomics have enabled researchers to study how prebiotics affect metabolic pathways,

mainly through their impact on organic acid production. This article aims to clarify the relationship between dietary prebiotics, changes in the metabolome, and organic acids as markers of metabolic health.

The aging process is linked to reduced microbial diversity, increased gut permeability, and a proinflammatory state. Including functional nutraceuticals like ColonX and Kombucha-derived postbiotics in daily diets may offer a non-invasive and accessible way to counteract these changes. Future research should explore how such interventions can be tailored for centenarians with chronic conditions to help reduce symptoms and enhance well-being.

4. Conclusions

In conclusion, both prebiotics and postbiotics present promising options for regulating gut microbiota to improve health. In vitro studies have clarified how they work, showing their potential to prevent and treat various health issues related to dysbiosis. Future research should aim to enhance these approaches, investigate synergistic combinations, and verify their effectiveness through carefully designed clinical trials. Besides their ability to influence microbiota, the polyphenol content and antioxidant capacity of ColonX and postbiotics may also offer additional benefits in managing oxidative stress-related dysbiosis, especially in aging populations. This highlights their potential as functional ingredients in nutritional strategies focused on promoting healthy aging.

These data are crucial for healthy aging, as centenarians often exhibit a unique microbiota profile linked to longevity and chronic diseases. Using targeted prebiotic and/or postbiotic nutraceuticals improves microbial composition and function in older adults, directly supporting gut health and helping reduce conditions associated with microbiota imbalance.

Author Contributions: Supervision, methodology, formal analysis, E.V. and L.D.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: All fecal samples were processed according to the ethical standards of UASVM Bucharest (ColHumB Registration number: 1418/23.11.2017).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

References

1. Moles, L.; Otaegui, D. The Impact of Diet on Microbiota Evolution and Human Health. Is Diet an Adequate Tool for Microbiota Modulation? *Nutrients* **2020**, *12*, 1654. [\[CrossRef\]](#) [\[PubMed\]](#)
2. DeGruttola, A.K.; Low, D.; Mizoguchi, A.; Mizoguchi, E. Current Understanding of Dysbiosis in Disease in Human and Animal Models. *Inflamm. Bowel Dis.* **2016**, *22*, 1137–1150. [\[CrossRef\]](#)
3. Hitch, T.C.A.; Hall, L.J.; Walsh, S.K.; Leventhal, G.E.; Slack, E.; de Wouters, T.; Walter, J.; Clavel, T. Microbiome-based interventions to modulate gut ecology and the immune system. *Mucosal Immunol.* **2022**, *15*, 1095–1113. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Ji, J.; Jin, W.; Liu, S.; Jiao, Z.; Li, X. Probiotics, prebiotics, and postbiotics in health and disease. *MedComm (2020)* **2023**, *4*, e420. [\[CrossRef\]](#)
5. Davani-Davari, D.; Negahdaripour, M.; Karimzadeh, I.; Seifan, M.; Mohkam, M.; Masoumi, S.J.; Berenjian, A.; Ghasemi, Y. Prebiotics: Definition, Types, Sources, Mechanisms, and Clinical Applications. *Foods* **2019**, *8*, 92. [\[CrossRef\]](#)
6. Rafique, N.; Jan, S.Y.; Dar, A.H.; Dash, K.K.; Sarkar, A.; Shams, R.; Pandey, V.K.; Khan, S.A.; Amin, Q.A.; Hussain, S.Z. Promising bioactivities of postbiotics: A comprehensive review. *J. Agric. Food Res.* **2023**, *14*, 100708. [\[CrossRef\]](#)
7. Scott, E.; De Paepe, K.; Van de Wiele, T. Postbiotics and Their Health Modulatory Biomolecules. *Biomolecules* **2022**, *12*, 1640. [\[CrossRef\]](#)

8. Avram, I.; Pelinescu, D.; Gatea, F.; Ionescu, R.; Barcan, A.; Rosca, R.; Zangfirescu, A.; Vamanu, E. *Boletus edulis* Extract—A New Modulator of Dysbiotic Microbiota. *Life* **2023**, *13*, 1481. [\[CrossRef\]](#)
9. Su, J.; Tan, Q.; Tang, Q.; Tong, Z.; Yang, M. Research progress on alternative kombucha substrate transformation and the resulting active components. *Front. Microbiol.* **2023**, *14*, 1254014. [\[CrossRef\]](#)
10. Vamanu, E.; Gatea, F.; Sărbu, I. In Vitro Ecological Response of the Human Gut Microbiome to Bioactive Extracts from Edible Wild Mushrooms. *Molecules* **2018**, *23*, 2128. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Rinttilä, T.; Kassinen, A.; Malinen, E.; Krogus, L.; Palva, A. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *J. Appl. Microbiol.* **2004**, *97*, 1166–1177. [\[CrossRef\]](#)
12. Barbau-Piednoir, E.; Denayer, S.; Botteldoorn, N.; Dierick, K.; De Keersmaecker, S.C.J.; Roosens, N.H. Detection and discrimination of five *E. coli* pathotypes using a combinatory SYBR® Green qPCR screening system. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 3267–3285. [\[CrossRef\]](#)
13. Yang, T.; Santisteban, M.M.; Rodriguez, V.; Li, E.; Ahmari, N.; Carvajal, J.M.; Zadeh, M.; Gong, M.; Qi, Y.; Zubcevic, J.; et al. Gut dysbiosis is linked to hypertension. *Hypertension* **2015**, *65*, 1331–1340. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Dinu, L.-D.; Gatea, F.; Matei, F.; Banciu, C.; Vamanu, E. Gut microbiota modulation by selenium and zinc enrichment postbiotic on dysbiosis associated with hypertension. *Curr. Vasc. Pharmacol.* **2024**, *24*, 1570–1611. [\[CrossRef\]](#)
15. Zheng, J.; Wittouck, S.; Salvetti, E.; Franz, C.M.A.P.; Harris, H.M.B.; Mattarelli, P.; O'Toole, P.W.; Pot, B.; Vandamme, P.; Walter, J.; et al. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *Int. J. Syst. Evol. Microbiol.* **2020**, *70*, 2782–2858. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT—Food Sci. Technol.* **1995**, *28*, 25–30. [\[CrossRef\]](#)
17. Prior, R.L.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290–4302. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Singleton, V.L.; Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158. [\[CrossRef\]](#)
19. Zangfirescu, A.; Avram, I.; Gatea, F.; Roșca, R.; Vamanu, E. In Vitro and In Vivo Antihyperglycemic Effects of New Metabiotics from *Boletus edulis*. *Life* **2024**, *14*, 68. [\[CrossRef\]](#)
20. Limoli, D.H.; Jones, C.J.; Wozniak, D.J. Bacterial Extracellular Polysaccharides in Biofilm Formation and Function. *Microbiol. Spectr.* **2015**, *3*. [\[CrossRef\]](#)
21. Scott, A.J.; Alexander, J.L.; Merrifield, C.A.; Cunningham, D.; Jobin, C.; Brown, R.; Alverdy, J.; O'Keefe, S.J.D.; Gaskins, H.R.; Teixeira, T.F.S.; et al. The influence of diet on the gut microbiome and implications for human health. *Expert. Rev. Gastroenterol. Hepatol.* **2022**, *16*, 1–15.
22. Procházková, N.; Falony, G.; Dragsted, L.O.; Licht, T.R.; Raes, J.; Roager, H.M. Advancing human gut microbiota research by considering gut transit time. *Gut* **2023**, *72*, 180–191. [\[CrossRef\]](#)
23. You, S.; Ma, Y.; Yan, B.; Pei, W.; Wu, Q.; Ding, C.; Huang, C. The promotion mechanism of prebiotics for probiotics: A review. *Front. Nutr.* **2022**, *9*, 1000517. [\[CrossRef\]](#)
24. Silva, Y.P.; Bernardi, A.; Frozza, R.L. The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. *Front. Endocrinol.* **2020**, *11*, 25. [\[CrossRef\]](#)
25. Petracco, G.; Faimann, I.; Reichmann, F. Inflammatory bowel disease and neuropsychiatric disorders: Mechanisms and emerging therapeutics targeting the microbiota-gut-brain axis. *Pharmacol. Ther.* **2025**, *269*, 108831. [\[CrossRef\]](#)
26. Prajapati, N.; Patel, J.; Singh, S.; Yadav, V.K.; Joshi, C.; Patani, A.; Prajapati, D.; Sahoo, D.K.; Patel, A. Postbiotic production: Harnessing the power of microbial metabolites for health applications. *Front. Microbiol.* **2023**, *14*, 1306192. [\[CrossRef\]](#)
27. Gou, H.Z.; Zhang, Y.L.; Ren, L.F.; Li, Z.J.; Zhang, L. How do intestinal probiotics restore the intestinal barrier? *Front. Microbiol.* **2022**, *13*, 929346. [\[CrossRef\]](#)
28. Szydlowska, A.; Sionek, B. Probiotics and Postbiotics as the Functional Food Components Affecting the Immune Response. *Microorganisms* **2022**, *11*, 104. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Al-Habsi, N.; Al-Khalili, M.; Haque, S.A.; Elias, M.; Al Olqi, N.; Al Uraimi, T. Health Benefits of Prebiotics, Probiotics, Synbiotics, and Postbiotics. *Nutrients* **2024**, *16*, 3955. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Magne, F.; Gotteland, M.; Gauthier, L.; Zazueta, A.; Poeso, S.; Navarrete, P.; Balamurugan, R. The Firmicutes/Bacteroidetes Ratio: A Relevant Marker of Gut Dysbiosis in Obese Patients? *Nutrients* **2020**, *12*, 1474. [\[CrossRef\]](#) [\[PubMed\]](#)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.