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Impact of *Lactobacillus acidophilus*—La5 on Composition and Metabolism of the Intestinal Microbiota of Type 2 Diabetics (T2D) and Healthy Individuals Using a Microbiome Model

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Abstract: Type 2 diabetes is characterized by dysbiosis in the gut, which may lead to systemic inflammation. Therefore, the use of probiotics may help to achieve a balanced microbiota and improve glycemic control. The aim of this study was to verify the impact of *Lactobacillus acidophilus*—La5 on the gut microbiome of type 2 diabetes adults using the Human Gut Microbial Ecosystem Simulator (SHIME[®]) and compare this to the microbiome of healthy subjects. Four groups (Control Group: NormoGlycemic; Treatment Group: T2D) were evaluated in SHIME[®] for 6 weeks. After 7 and 14 days of colonic fermentation, the intestinal microbiota (16S rRNA gene sequencing) and metabolites (short-chain fatty acids) were analyzed. La5 altered the composition of the microbiota after 14 days of treatment for both groups, by increasing the abundance of Bacteroidetes and a decrease in Firmicutes in the NormoGlycemic. Treatment with La5 resulted in a shift in the microbial community of NormoGlycemic with increased abundance of *Bacteroides* and *Mitsuokella* and a decrease in *Achromobacter* and *Catabacter*, whereas T2D gut microbiome was enriched with *Faecalibacterium* and reduced in *Bacteroides*. *Megasphaera* spp. stimulated with La5 treatment in NormoGlycemic has already been reported to produce intestinal metabolites and recognized to contribute to increased anti-inflammatory and immune responses. *Faecalibacterium*, on the other hand, can modulate the intestinal epithelium and be a major butyrate product in the microbiota. Finally, this study showed a positive and promising result of La5 treatment in increasing intestinal homeostasis in the microbiota of T2D.

Keywords: microbiota; probiotics; *L. acidophilus*—La5; SHIME; short-chain fatty acids; type 2 diabetes; ammonia ions

1. Introduction

Diabetes is a metabolic disorder characterized by elevated blood glucose levels. The incidence of diabetes is widespread and the International Diabetes Federation (IDF) reports that 463 million people worldwide suffer from diabetes, which is estimated to reach 700 million by the year 2045 [1]. Diabetes has been classified into three classes (type 1, type 2, and gestational diabetes) depending on the underlying pathophysiology, and type 2 diabetes (T2D) accounts for more than 90% of all diabetes [1,2]. Genetic susceptibility

is a critical determinant of T2D, but non-genetic factors, including diet and physical activity, also play a significant role in the development and severity of T2D.

Administration of probiotics has the potential to alter the resident microbiome, and, consequently, collaboration with the prevention and control of the imbalance of gut bacteria is associated with several conditions in humans [3], including obesity [4], inflammation [5], and T2D [6].

However, the use of these beneficial organisms has yielded controversial results, mostly due to inappropriate strains and poor clinical designs [7–9]. Interestingly, studies revealed that organisms of the families *Lactobacillaceae* and *Bifidobacteriaceae* are enriched in the feces of participants with T2D after antidiabetic treatment with a single use of acarbose [10] or metformin [11,12].

Among the probiotic bacteria belonging to the *Lactobacillaceae* family, the *Lactobacillus acidophilus*—La5 strain is present in a wide variety of beverages, dairy products, and supplements, and it offers a wide range of health benefits, improving gut health [13]. *L. acidophilus* has been shown to help regulate the composition of the gut microbiota by promoting the growth of beneficial bacteria and reducing harmful bacteria, which may lead to improved gut health [14].

Experimental animal models have shown that oral administration of *L. acidophilus*—La5 can reduce total and LDL cholesterol levels in hypercholesterolemic rats [15], improve fat induced obesity in mice [16], and modulate cellular processes that can be altered in senescence [17].

Clinical studies using supplementation with *L. acidophilus*—La5 alone or in combination with other probiotic strains revealed that it improved gastrointestinal symptoms and quality of life in patients with irritable bowel syndrome (IBS) [15], reduced the pro-inflammatory profile of PMNs in obese subjects [18], and reduced total cholesterol levels in healthy adults [19,20]. There is also evidence that consumption of *L. acidophilus*—La5 in combination with other probiotic strains improved glycemic control [21] and reduced risk factors for cardiovascular diseases in T2D subjects [22].

Despite these promising results, little is known about the mechanisms underlying the beneficial effects of *L. acidophilus*—La5. A better understanding of the link between the gut microbiota and metabolic disorders, especially T2D, may lead to advances in current treatment approaches, accurate disease monitoring, and development of new therapeutics. Thus, we aimed to evaluate the effect of *L. acidophilus*—La5 on the gut microbiota of healthy adult subjects and adults with type 2 diabetes using the Simulator Human Intestinal Microbial Ecosystem (SHIME®). This simulator allows the study of the human gut microbiota under highly controlled conditions in reactors that mimic different colon regions. Therefore, this model yields reproducible results in shorter periods than clinical studies, employing human intestinal microbiota derived from fecal samples under simulated physiological conditions [23].

2. Materials and Methods

2.1. Study Design

The total experimental period comprised 5 weeks for each group: the normoglycemic and T2D group, and the *L. acidophilus*—La5™ (CHR Hansen Holding A/S, Hørsholm, Denmark) was used as the probiotic strain. Pooled feces from healthy adult volunteers or type 2 diabetic subjects (T2D) were inoculated into the corresponding triplicate compartments of the ascending colon of the Simulator of Human Intestinal Microbial Ecosystem (SHIME®). After stabilization of the microbiota for two weeks, followed by a one-week interval of a control period, *L. acidophilus*—La5 was administered for two weeks. Samples were obtained from triplicate compartments of the ascending colon, and the effect of the probiotic treatment was determined by evaluating the composition of the microbiota through 16S rRNA sequencing analysis and the metabolic activity through ammonium (NH_4^+) and short-chain fatty acids (SCFAs) analyses. The survival of *L. acidophilus*—La5 in the compartment simulating the stomach and duodenum was assessed during the treatment period.

2.2. Samples Collection

Fecal samples were obtained from volunteers recruited in the city of Araraquara (São Paulo, Brazil) after approval by the Institutional Ethics Committee for Research with Humans (Universidade Estadual Paulista/UNESP, Araraquara; CAAE: 30156020.8.0000.5426). The compartments were colonized, with the inclusion criteria for the Normoglycemic and T2D groups being 45–50 years of age, with no food allergies or intolerances; the exclusion criteria were reported use of dietary supplements, medications for gastrointestinal diseases, use of antibiotics within the past 6 months, and use of probiotics or prebiotics within the past 3 months. Both groups were not to use medications for metabolic diseases, except for the treatment of the T2D group. Donor stools were collected by the volunteers and delivered to the research laboratory in 2 h, always stored under refrigeration (4 °C).

2.3. Experimental Protocol

The experimental period in the SHIME[®] reactor lasted 5 weeks. For the microbiota stabilization period, the feed medium (1.0 g/L arabinogalactan (Sigma, St. Louis, MO, USA), 2.0 g/L pectin (Sigma), 1.0 g/L xylan (Roth, Karlsruhe, Germany), 3.0 g/L starch (Êxodo, Hortolândia, Brazil), 0.4 g/L glucose (Synth, Diadema, Brazil), 3.0 g/L yeast extract (Neogen, Lansing, MI, USA), 1.0 g/L peptone (Kasvi, Rome, Italy), 4.0 g/L mucin (Sigma), and 0.5 g/L cysteine (Sigma)) was inserted 240 mL into the system and left to stabilize for 14 days [24]. After these two weeks, for one week, daily, 210 mL of feed medium and 60 mL of pancreatic juice (12.5 g/L NaHCO₃, 3.6 g/L Oxgall, 0.9 g/L pancreatin, Sigma-Aldrich, St. Louis, MO, USA) were inserted in the system (Control period). Thereafter, the treatment was administered twice daily for 14 days. NormoGlycemic Treatment: *L. acidophilus* (La5 10⁸ CFU/g), twice daily for 14 days. T2D Treatment: *L. acidophilus* (La5 10⁸ CFU/g), twice daily for 14 days (Figure 1).

2.4. Metabolic Activity: Ammonium (NH₄⁺) Analysis and Short-Chain Fatty Acids (SCFAs)

Samples from the ascending colon compartment (*n* = 3) were collected after the control period, after 7 days of treatment, and at the end of 14 days of treatment with the probiotic, and stored at −20 °C. The ammonium ions (NH₄⁺) were quantified according to Bianchi et al. [25], using a specific ion meter (Model 710A, Orion) coupled to a selective ammonia ion electrode (Model 95–12, Orion). To analyze SCFAs (acetic, propionic, and butyric acids), 2 mL of colonic fermented samples was centrifuged (14,000 rpm (g) for 5 min), and 1 mL of the supernatant stored for fatty acid analysis. The supernatants were injected into an Agilent gas chromatograph (model HP-6890, Santa Clara, CA, USA), equipped with an Agilent selective mass detector (model HP-5975) using a DB-WAX capillary column (60 m × 0.25 mm × 0.25 µm) under the following conditions: temperature of injector = 220 °C, column = 35 °C, 2 °C/min, 38 °C; 10 °C/min, 75 °C; 35 °C/min, 120 °C (1 min); 10 °C/min, 170 °C (2 min); 40 °C/min, 170 °C (2 min) and detector = 250 °C. Helium was used as a drag gas at a flow rate of 1 mL/min. Analytical curves were constructed using the stock solution of the acids of interest: acetic, propionic, and butyric acids. The samples were analyzed in triplicate per ascending colon replica, before and after treatment. Data are expressed in mmol/g [26].

2.5. Survival of *Lactobacillus acidophilus*—La5 under Simulated Conditions of the Stomach and Duodenum in the SHIME[®]

During the treatment period with the probiotic, samples were collected from the reactors corresponding to the stomach and duodenum to verify the survival of *Lactobacillus acidophilus*—La5[®]. A quantity of 1 mL of the samples from each reactor was suspended in 9 mL of sterile peptone water. Serial dilutions were carried out and plated in MRS agar supplemented with 1.5 g L⁻¹ bile salts (Sigma-Aldrich[®], St. Louis, MO, USA) (Lima et al., 2009). The plates were incubated in anaerobiosis (Probac, Brazil) at 37 °C for 72 h [27].

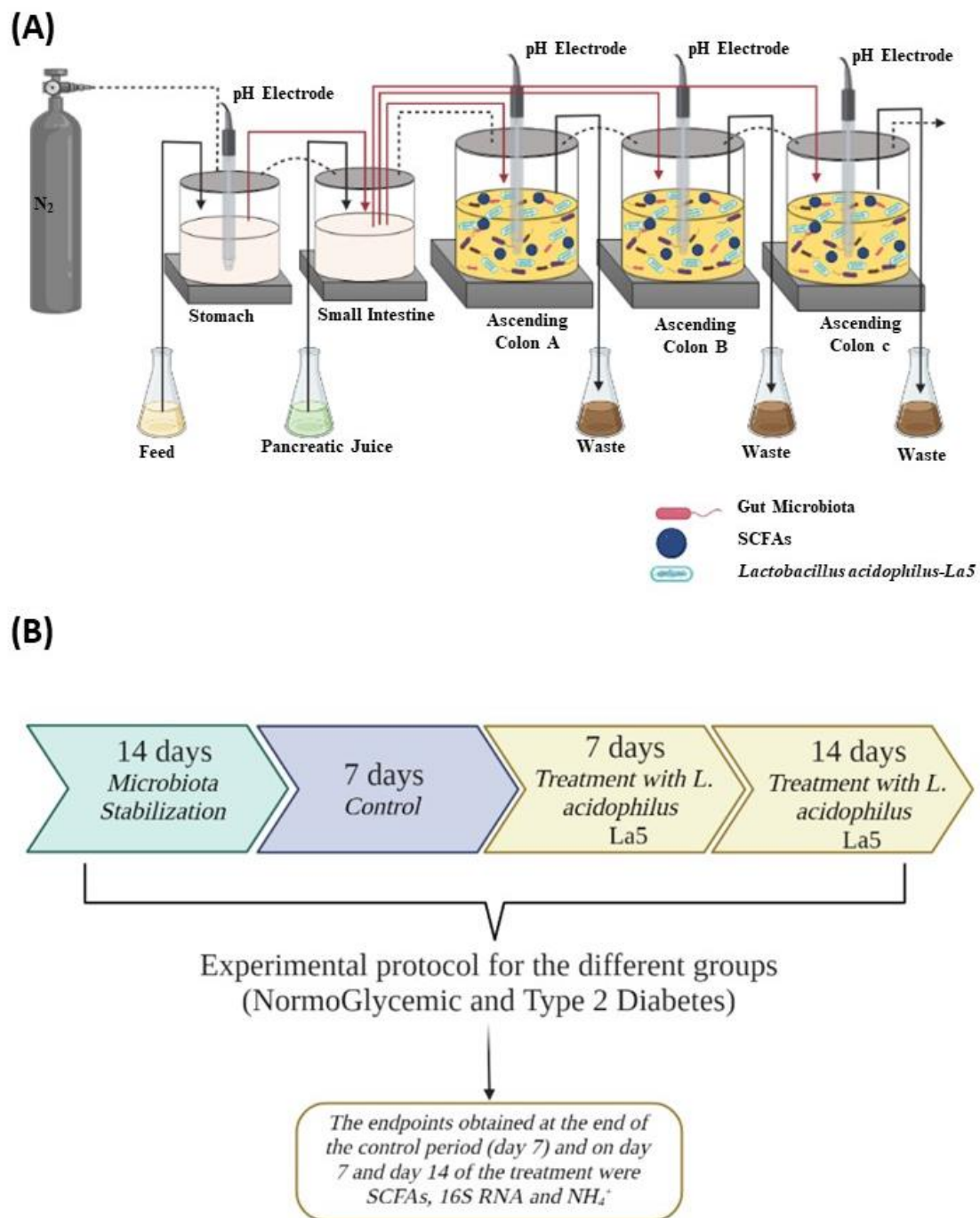


Figure 1. Illustration of the experimental protocol in Type 2 Diabetes and NormoGlycemic groups. (A) Simulator of the Human Intestinal Microbial Ecosystem (SHIME®). (B) Experimental protocol for the different treatments, and the main endpoints.

2.6. Microbiological Analysis Employing 16S rRNA Gene Sequencing

The diversity of the intestinal microbiota was analyzed by next-generation sequencing (Neoprosperta Microbiome Technologies, Florianópolis, Brazil) where specific primers amplified the V3–V4 region of the 16S rRNA, and 341F and 806R of 1 ng of DNA [28,29]. The PCR was carried out in triplicate using a Platinum Taq (Invitrogen, Carlsbad, CA, USA) under the following conditions: 95 °C for 5 min, twenty-five cycles at 95 °C for 45 s, 55 °C for 30 s and 72 °C per 45 s, with a final extension of 72 °C for 2 min for the first PCR (PCR 1). For the second PCR (PCR 2), the conditions were 95 °C for 5 min, ten cycles at 95 °C for 45 s, 66 °C for 30 s and 72 °C for 45 s, with a final extension of 72 °C for 2 min. After the last

PCR, the samples were cleaned with AMPure beads (Beckman coulter) [30]. To measure the taxa present in the samples, a predictor model of the V3 and V4 regions was used (SILVA 138.99% OTUs from the 515F/806R region of sequences). The operational taxonomic units (OTUs) were grouped by cluster readings with 99% similarity. The taxonomy was assigned to OTUs using the SILVA 138 reference database (<https://www.arb-silva.de/>, accessed on 30 May 2023).

2.7. Statistical Analysis

Data are presented as mean $\pm$ standard deviation (SD). Paired *t*-test, one-way analysis of variance (ANOVA), and Tukey's test were used to analyze the results, with $p < 0.05$ considered statistically significant. Statistical analysis was performed using GraphPad Prism software, version 8.0 (La Jolla, CA, USA). 16S rRNA gene sequence analyses were performed in RStudio, version 3.2.4 [31] using the phyloseq package [32] to import sample data and calculate alpha- and beta-diversity metrics. The significance of categorical variables was determined using the non-parametric Wilcoxon test for two category comparisons or the Kruskal–Wallis test when comparing three or more categories. Principal coordinate plots were based on the PERMANOVA test to estimate the *p*-value [33]. The *p*-values were adjusted for multiple comparisons using the FDR algorithm [34,35].

3. Results and Discussion

3.1. Survival of *L. acidophilus*—La5 under Simulated GI Condition

The survival of *L. acidophilus*—La5 under adverse gastrointestinal conditions in vitro was evaluated and specifically established as low pH, presence of pancreatin, and tolerance to bile salts (Table 1). Survival of the probiotic strain decreased significantly throughout the gastric (reduction of 3.64 log CFU/mL) and enteric phases (reduction of 3.80 log CFU/mL) in both treatments (Normoglycemic and Type 2 Diabetic). The effects of probiotics are known to be strain-specific. *L. acidophilus* showed good viability during exposure to gastric juice at pH 2. Generally, growth of lactic acid bacteria is optimal at pH 6–7 (near neutral pH). Some metabolic reactions change when pH is <5 or <4.4 [36]. The probiotic ability to survive adverse conditions in gastrointestinal tract functions is a fundamental key for probiotics to exert their beneficial effects on the colon [35]. The viability of probiotic *L. acidophilus* decreased significantly during exposure to intestinal juice for 60 min; a decrease in the number of free cells may reflect cell death, which may be caused by factors other than the pH of the medium [37].

Table 1. Population (log CFU/mL) and survival rate (%) of *Lactobacillus acidophilus*—La5 under simulated gastrointestinal conditions.

	NormoGlycemic	Type 2 Diabetes
Initial Time	7.98 ^a $\pm$ 0.08	8.34 ^a $\pm$ 0.07
Gastric phase	4.34 ^b $\pm$ 0.03	4.54 ^b $\pm$ 0.06
Enteric phase	4.18 ^b $\pm$ 0.05	4.97 ^b $\pm$ 0.04

Lactobacillus acidophilus—La5. Initial time = 0 h; Gastric phase = 2 h; Enteric phase I = 4 h, simulated in SHIME®.

^{a, b} Different lower-case letters on the same line indicate a statistical difference according to Tukey's post-test ($p < 0.05$). Data are presented as mean $\pm$ SD ($n = 3$).

3.2. Metabolic Activity

Bacterial metabolic activity was evaluated in ascending colonic vessels (ammonium ions and short-chain fatty acids production). A decrease was observed ($p < 0.05$) in ammonium ion production in the first 7 days of La5 treatment for both groups (NormoGlycemic and Type 2 Diabetic), and the values stabilized until the 14th day of treatment (values between 200–255 ppm) (Table 2). In the control period, the microbiota of the NormoGlycemic Group showed a higher ammonia ion production than the Type 2 Diabetics Group, but after the probiotic treatment (7 and 14 days), the values became closer in both groups and maintained the downward trend.

Table 2. Ammonium ion production (mmol/L) by microbiota from healthy subjects and subjects with type 2 diabetes during all experiments in SHIME®. Period of control and treatment with La5 in both groups.

	NormoGlycemic-Group	Type 2 Diabetic Group
Control	529.33 ± 47.64 ^a	491.67 ± 2.52 ^a
La 5–7 Days	255.33 ± 37.81 ^b	222.00 ± 2.65 ^b
La 5–14 Days	246.02 ± 40.70 ^b	201.67 ± 3.22 ^c

^{a, b, c} different letters indicate a statistical difference ($p < 0.01$).

Protein breakdown and/or amino acid metabolism and is also produced by gut bacteria [38]. Then, this ammonia is transported through the circulation to the periportal hepatocytes, where 90% of the ammonia enters the urea cycle and is converted to urea. Furthermore, ammonia is not excreted due to its low water solubility; the remaining 10% is transported to periportal hepatocytes where ammonia is condensed with glutamate to glutamine via glutamine synthase (GS) [39].

According to Scott et al. [40], the release of ammonium ions may be associated with increased metabolic activity of some *Bifidobacterium* species, among others, that participate in deamination processes. The reduction in ammonia in the colon is considered beneficial because these ions in high amounts can alter the morphology as well as the metabolism of intestinal cells, increasing DNA synthesis and promoting tumor development [41]. Hughes et al. [42] demonstrated that ammonia can increase cell permeability in colonocytes, causing various diseases in the host. The presence of ammonia can reduce the uptake and utilization of short-chain fatty acids by colonocytes [43]. In addition, the blood level of ammonia must remain very low because even slightly elevated concentrations (hyperammonemia) are toxic to the central nervous system. The peak ammonia level and the duration of hyperammonemia are the main risk factors for hyperammonemia-related neurological deficits and death [44]. The increased serum ammonia levels found in patients with type 2 DM may be associated with delayed gastrointestinal transit [45] as well as increased DB monoamine oxidase activity [46]. Since the presence of elevated blood ammonia levels was not related to liver dysfunction, this could indicate that other extrahepatic cells, such as RBCs, participate in the control of nitrogen metabolism [47]. Ammonia toxicity can induce cellular damage, as occurs during neurodegeneration in aging, and Alzheimer's disease [48], and in diabetic rats, there was a marked increase in the ammonium-dependent component of L-glutamate transport, causing higher intracellular glutamate concentration [49].

The concentrations of SFCAs are shown in Table 3. In the NormoGlycemic Group after 14 days of La5 treatment, an increase ($p < 0.05$) in acetic and propionic acids production was observed when compared to the control period. Short-chain fatty acids act as important anions in the colon cavity and thus increase sodium and water absorption [50], increase intestinal blood flow [51,52], promote colon epithelial cell proliferation and mucosal growth, provide metabolic energy, and serve as nutrients for the colon mucosa [53].

Table 3. Short-chain fatty acid production (mmol/L) by microbiota from the NormoGlycemic Group and Type 2 Diabetics Group during all experiments in SHIME®. Period of control and treatment with La5 in both groups.

		(mmol/L)		
		Acetic Acid	Propionic Acid	Butyric Acid
NormoGlycemic Group	NormoGlycemic-Control	22.42 ± 0.96 ^b	7.15 ± 0.69 ^b	12.00 ± 3.49 ^a
	NormoGlycemic-La 5–7 Days	13.57 ± 1.30 ^c	4.91 ± 0.15 ^c	6.48 ± 1.03 ^b
	NormoGlycemic-La 5–14 Days	24.71 ± 1.79 ^a	9.25 ± 1.31 ^a	8.78 ± 1.35 ^a

Table 3. Cont.

		(mmol/L)		
		Acetic Acid	Propionic Acid	Butyric Acid
Type 2 Diabetics Group	T2D-Control	39.59 ± 3.31 ^a	5.73 ± 0.51 ^a	4.08 ± 1.30 ^c
	T2D-La 5–7 Days	21.91 ± 1.25 ^b	2.15 ± 0.20 ^b	4.90 ± 0.31 ^b
	T2D-La 5–14 Days	20.94 ± 0.65 ^b	2.26 ± 0.20 ^b	8.56 ± 0.12 ^a

^{a, b, c} Different letters indicate a statistical difference ($p < 0.01$) in the same column.

The fermentation of polysaccharides induced by the intestinal microbiota produces short-chain fatty acids (SCFAs), which are used by the epithelial cells of the colon as nutrition. Propionate, acetate, and butyrate are the major SCFAs produced by bacterial fermentation of dietary fiber in the intestine [54]. SCFAs provide about 6% to 10% of the body's total daily energy [54,55]. Most SCFAs are absorbed in the colon (90–95%), while the remainder (5–10%) is secreted in the feces. About 60% of SCFA absorption occurs via epithelial membrane diffusion, while the remainder is absorbed by active cell transport, specifically by monocarboxylate transporters in colonocytes [56]. Those that are not metabolized by the colonocytes (mainly butyric acid) are transported through the portal circulation and metabolized in the liver before reaching the systemic circulation. However, the distal colon, where most of the intestinal microbiota reside, bypasses the portal circulation, allowing systemic access [57]. Therefore, SCFAs produced by the microbiota may be present in the portal blood, liver, peripheral blood, and feces [58,59]. Low levels of SCFAs in the blood and gastrointestinal system are implicated in diabetes and inflammatory diseases [60]. SCFAs in the intestinal lumen are absorbed by enterocytes and reach the bloodstream. After reaching the bloodstream, SCFAs can affect glucose storage in muscles, liver, and fat. In addition, SCFAs can activate the G-protein-coupled receptor 43 (GPR43) on intestinal epithelial cells to improve intestinal barrier function and prevent inflammatory diseases [61].

It is important to emphasize that, in the diabetic group, after 14 days of treatment with La5, an increase in the production of butyric acid was observed. Among various SCFAs, butyric acid has attracted particular attention [62,63]. Several studies have shown that an impaired glucose metabolism is associated with decreased levels of butyrate-producing bacteria in diabetic patients [63]. Butyric acid is also known to regulate the size and function of the colonial regulatory T-cell pool, improve insulin sensitivity, and reduce the inflammatory response of adipocytes by stimulating glucagon-peptide-1 (GLP-1) production [62]. In animal models, Gao et al. [19] reported that butyrate supplementation (5% w/w in the high-fat diet) prevented the development of insulin resistance and obesity in diet-induced obese mice [19]. In an animal study in diabetic rats, it was found that the cell membrane of the intestinal microbiota is more active in transporting sugars and branched-chain amino acids, but butyric acid synthesis is reduced. Therefore, the oxidative stress reaction directly related to pro-inflammatory reactions is increased [64].

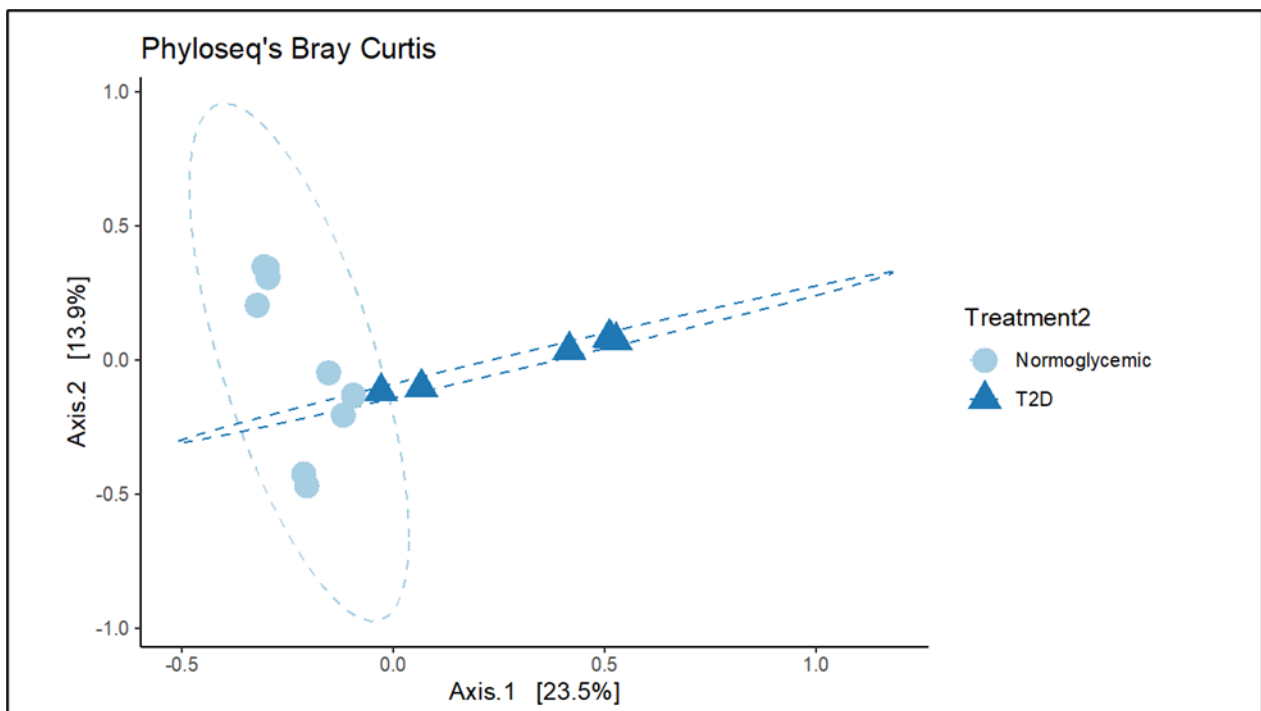
3.3. Richness and Diversity of the Gut Microbiota

There were no statistical differences in the alpha-diversity indexes Chao1 (species richness) and Shannon (diversity) for the groups (NormoGlycemic Group and Type 2 Diabetics Group) or after 14 days of La5 treatment (Table 4). These results agree with a Brazilian study by Leite et al. [65] which compared the microbiota of 20 healthy individuals with 19 individuals with type 2 diabetes. The authors found no significant differences in the richness values (Chao1 and observed OTUs). Importantly, richness generally refers to the number of unique species that are present in a sample [66].

Table 4. Values of alpha-diversity microbiota from the NormoGlycemic Group and Type 2 Diabetics Group in the ascending colons vessels of the SHIME®.

		Chao1	Shannon
NormoGlycemic Group	NormoGlycemic-Control	53.00	2.38
	NormoGlycemic-La 5–7 Days	50.00	2.41
	NormoGlycemic-La 5–14 Days	36.00	2.37
Type 2 Diabetics Group	T2D-Control	50.00	2.28
	T2D-La 5–7 Days	51.00	2.32
	T2D-La 5–14 Days	44.00	2.50

For beta diversity (Figure 2), there were significant differences between the treatment and control period for both groups when calculated by weighted and unweighted UniFrac ($p = 0.001$). In addition, it was possible to observe differences between the microbiota of NormoGlycemic and Type 2 Diabetics Groups ($p = 0.001$). These results show the effect of treatment with *L. acidophilus*—La5 on the composition of the microbiota and the differences between the microbiota of NormoGlycemic and Type 2 Diabetics Groups (Figure 3). Similar results were observed by Leite et al. [66], using the weighted and unweighted UniFrac metric with Bonferroni correction (beta diversity); they observed a statistical difference between gut microbial communities of Type 2 Diabetics and NormoGlycemic patients.

**Figure 2.** PCoA plots of beta diversity with Bray-Curtis dissimilarity measure, in the microbiota of healthy and type 2 diabetic subjects in the SHIME® colons. NormoGlycemic Group: NormoGlycemic-Control: Control period, NormoGlycemic-La 5–7 Days: treatment for 7 days with *L. acidophilus*-La5, NormoGlycemic-La 5–14 Days: treatment for 14 days with *L. acidophilus*-La5. Type 2 Diabetics Group (T2D): T2D-Control: Control Period, T2D-La 5–7 Days: Treatment during 7 days with *L. acidophilus*-La5, T2D-La 5–14 Days: Treatment during 14 days with *L. acidophilus*-La5.

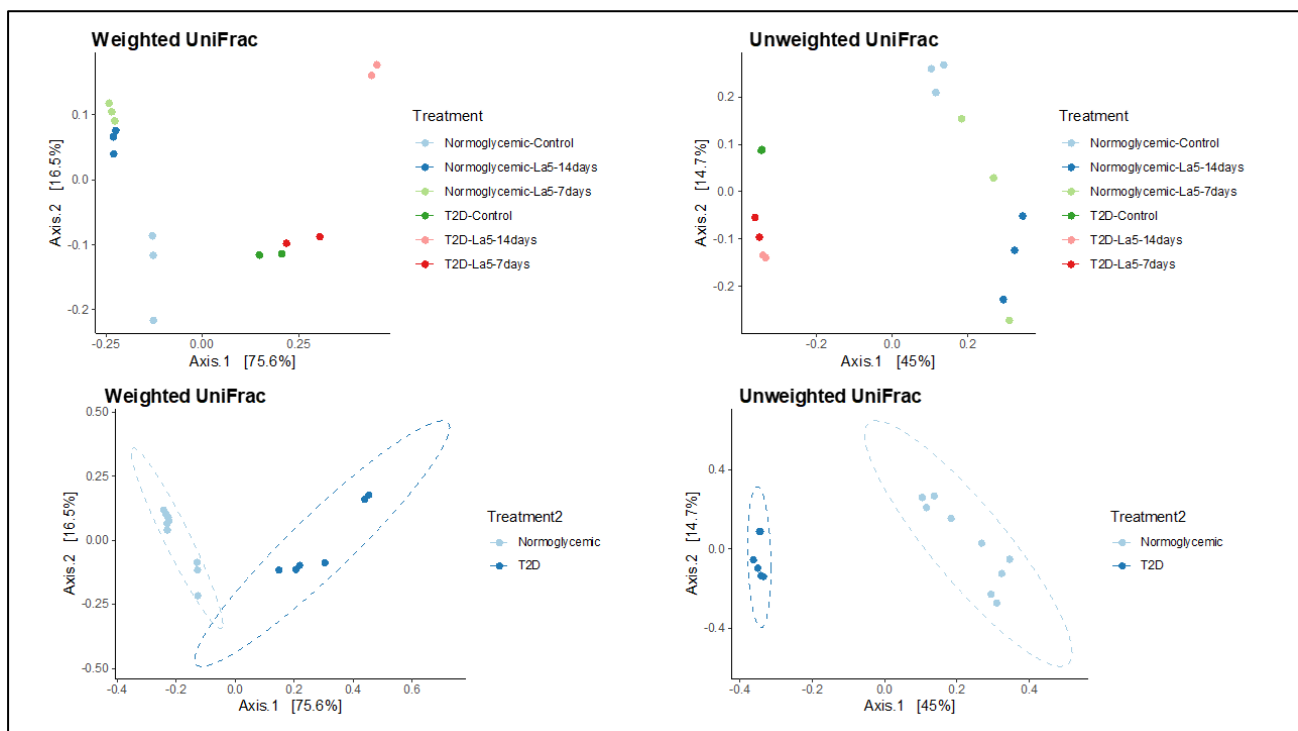


Figure 3. Plots of PCoA with weighted and unweighted UniFrac metrics with Bonferroni correction, microbiota from healthy and type 2 diabetic subjects in the SHIME[®] colons. NormoGlycemic Group: NormoGlycemic-Control: Control period, NormoGlycemic-La 5–7 Days: treatment for 7 days with *L. acidophilus*-La5, NormoGlycemic-La 5–14 Days: treatment for 14 days with *L. acidophilus*-La5. Type 2 Diabetics Group (T2D): T2D-Control: Control Period, T2D-La 5–7 Days: Treatment during 7 days with *L. acidophilus*-La5, T2D-La 5–14 Days: Treatment during 14 days with *L. acidophilus*-La5.

3.4. Influence of *Lactobacillus acidophilus*—La5 on Gut Microbiota Composition

The composition of the gut microbiota of NormoGlycemic and Type 2 Diabetics from the SHIME[®] samples was evaluated using 16S rRNA gene sequencing.

Figure 4 shows the relative abundance of the main phyla found in the gut microbiota during all experimental periods. In both groups (NormoGlycemic and Type 2 Diabetics) the main phyla were *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Proteobacteria*. In the control group, the *Firmicutes*/*Bacteroides* ratio was lower when compared to that of the diabetic group (Figure 5).

Some studies have shown a significant association between changes in the composition profile of the gut microbiota and the development of type 2 diabetes [67]. We understand that disrupted eubiosis of the *Bacteroidetes*/*Firmicutes* phylum is associated with increased intestinal permeability, with infiltration of bacterial by-products through a permeable intestinal barrier and, as a consequence, triggers subsequent inflammatory responses characteristic of diabetes [68]. On the other hand, several bacteria play a protective role, decreasing the risk of developing diabetes by reducing pro-inflammatory markers and maintaining the integrity of the intestinal barrier [69]. For example, *L. fermentum*, *L. plantarum*, and *L. casei*, *Roseburia intestinalis*, *Akkermansia muciniphila*, and *Bacteroides fragilis* have been shown to improve glucose metabolism and insulin sensitivity and suppress pro-inflammatory cytokines [68].

In the control group, the effect on microbiota composition occurred after 7 days of treatment, with an increase in the abundance of *Bacteroidetes* and a decrease in *Firmicutes*. However, in the experiment with the Type 2 Diabetics Group, a modulation of the microbiota was observed that was different from that of the control group, with an increase after 14 days of treatment in *Firmicutes* and decrease in *Bacteroidetes* (Figure 5). Among the main

phyla of the gut microbiota, *Bacteroidetes* have often been assigned a prominent position as indicators of the metabolism and health [70].

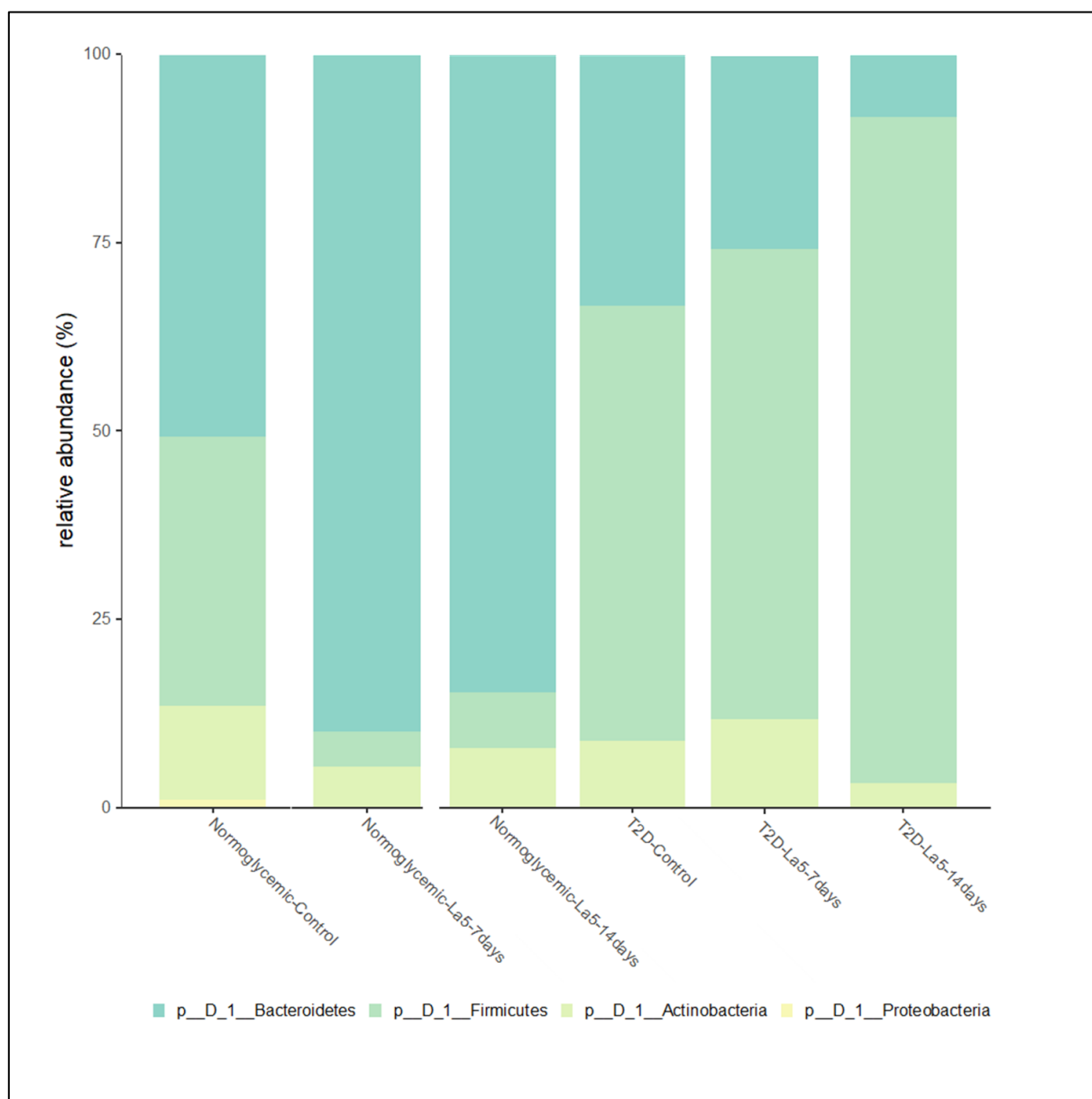


Figure 4. Relative abundance of microbiota phyla from healthy and type 2 diabetic subjects in the SHIME® colons. NormoGlycemic Group: NormoGlycemic-Control: Control period, NormoGlycemic-La 5–7 Days: treatment for 7 days with *L. acidophilus*-La5, NormoGlycemic-La 5–14 Days: treatment for 14 days with *L. acidophilus*-La5. Type 2 Diabetics Group (T2D): T2D-Control: Control Period, T2D-La 5–7 Days: Treatment during 7 days with *L. acidophilus*-La5, T2D-La 5–14 Days: Treatment during 14 days with *L. acidophilus*-La5.

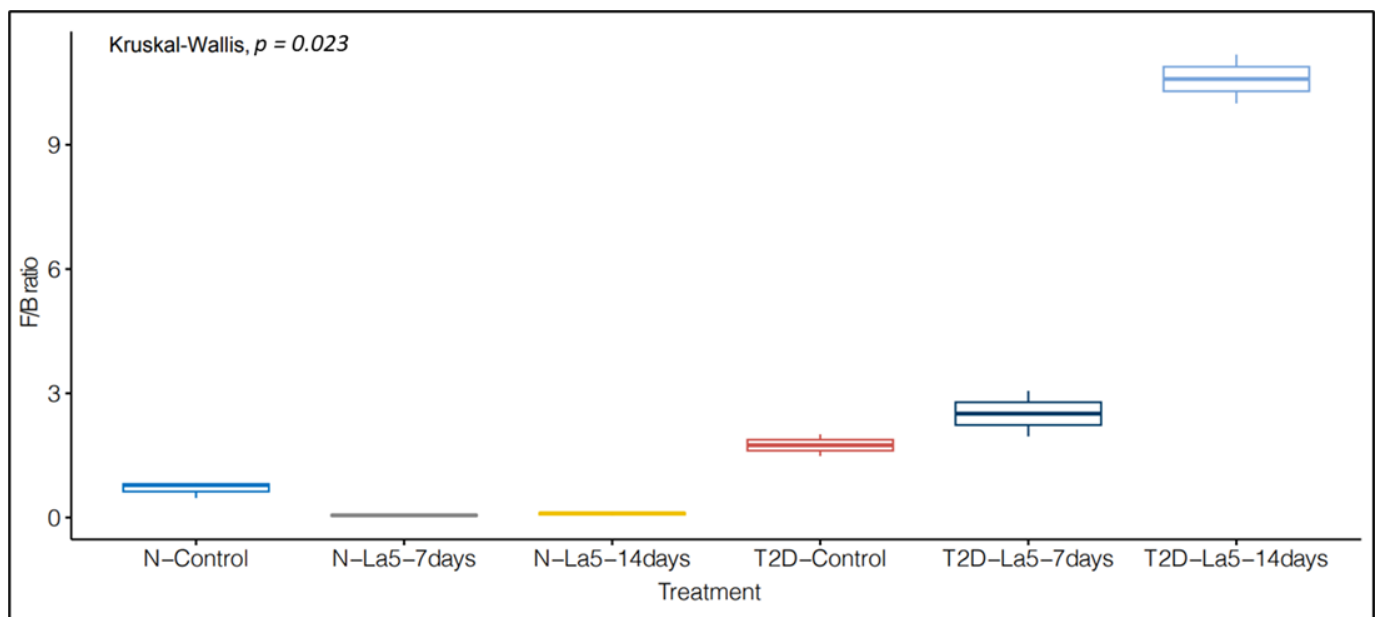


Figure 5. Firmicutes and Bacteroidetes filament relationships of the microbiota of healthy and type 2 diabetic individuals in the colons of SHIME®. Control group (NormoGlycemic): NormoGlycemic-Control: Control period, NormoGlycemic-La 5–7 Days: treatment for 7 days with *L. acidophilus*-La5, NormoGlycemic-La 5–14 Days: treatment for 14 days with *L. acidophilus*-La5. Type 2 Diabetics Group (T2D): T2D-Control: Control Period, T2D-La 5–7 Days: Treatment during 7 days with *L. acidophilus*-La-5, T2D-La 5–14 Days: Treatment during 14 days with *L. acidophilus*-La5.

Regarding the analysis of relative abundance of the main genera (Figure 6), it is possible to observe an alteration in both groups studied; for the control group, after 7 days of treatment with La5, a significant increase ($p > 0.001$) in *Bacteroides* was observed. Regarding the diabetic group, it was clear that the modulation of the microbiota happened after 14 days of treatment with La5, where there was an increase in some important genera for the microbiota of type 2 diabetics, such as *Faecalibacterium* and *Subdoligranulum*.

Bacteroidetes is a complex phylum and the class *Bacteroidia* is composed of five families of bacteria (*Bacteroidaceae*, *Marinilabiliaceae*, *Porphyromonadaceae*, *Prevotellaceae*, and *Rikenellaceae*), each of which is commonly found in the human gut. *Bacteroides* is one of six genera in the highly diverse family *Bacteroidaceae*, composed of several species. *Bacteroides* spp. have a mutualistic relationship with the human host and play an important role in the fermentation of complex sugars, protein metabolism, and deconjugation of bile salts [71].

Another important genus stimulated with La5 treatment in the microbiota of healthy volunteers was *Megasphaera* spp., and it has been reported that this genus has the potential to produce metabolites such as short-chain fatty acids (butyrate, acetate, formate, and caproate), vitamins, and essential amino acids [72]. Interestingly, the genera *Mitsuokella* ssp. and *Megasphaera* ssp., which increased in abundance after La5 treatments, are recognized as butyrate producers and for contributing to the enhancement of anti-inflammatory and immune response [73].

In the microbiota of volunteers with type 2 diabetes, after 14 days of treatment with La5 a significant increase in butyrate-producing bacteria was observed, such as *Subdoligranulum* ($p = 0.00043$) and *Faecalibacterium* ($p = 0.00043$). These two genera presented potential anti-inflammatory effects both in vitro and in vivo [74,75]. These two genera help reduce bacterial translocation, enhance tight-junction protein expression, and stimulate mucin secretion, maintaining intestinal integrity [76].

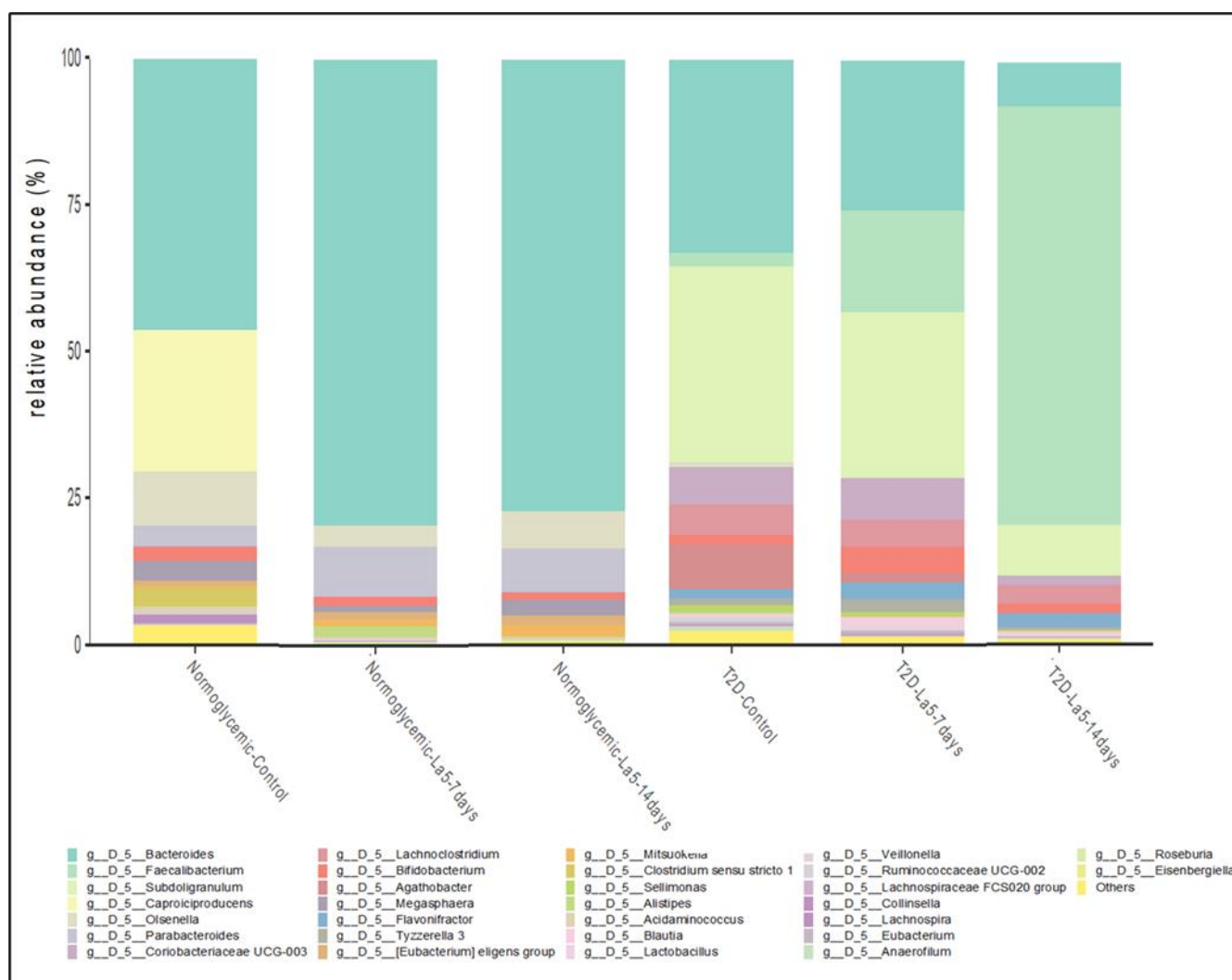


Figure 6. Relative abundance of microbiota genera from healthy and type 2 diabetic subjects in the colons of SHIME®. Control group (NormoGlycemic): NormoGlycemic-Control: Control period, NormoGlycemic-La 5–7 Days: treatment for 7 days with *L. acidophilus*—La5, NormoGlycemic-La 5–14 Days: treatment for 14 days with *L. acidophilus*—La5. Type 2 Diabetics Group (T2D): T2D-Control: Control Period, T2D-La 5–7 Days: Treatment during 7 days with *L. acidophilus*—La5, T2D-La 5–14 Days: Treatment during 14 days with *L. acidophilus*—La5.

Faecalibacterium prausnitzii, besides being an important butyrate producer, protects against inflammation in the gut [77], *F. prausnitzii* are commensal inhabitants of the human large intestine, with demonstrated anti-inflammatory properties in vivo [78]. *Subdoligranulum*, another genus found, belongs to the family *Ruminococcaceae* and is closely related to the genus *Faecalibacterium*. In fact, some studies have shown that treatment of obese and diabetic mice with prebiotics (oligofructose) increased the levels of *A. muciniphila*, but that the levels of *Subdoligranulum* also increased almost fourfold [79]. It was previously observed in obese humans that a subgroup of subjects with slight improvement in their metabolic parameters during caloric restriction had significantly lower abundance of both *Akkermansia* and *Subdoligranulum* than the subgroup with a better metabolic response to the caloric restriction intervention [80]. Interestingly, antidiabetic drugs such as metformin and acarbose increase the relative abundance of fecal *Subdoligranulum* [81,82]. In addition, the genus *Subdoligranulum* correlated negatively with glycated hemoglobin (HbA1c) and positively with HDL cholesterol [83]. In a study on some diseases, *Subdoligranulum* was also strongly associated with immune markers, including CD14 levels, related to chronic

inflammation; one study showed that *Subdoligranulum* was closely related to immune markers and soluble sCD14 [84,85].

In this way, as already reported, a multitude of studies have demonstrated a significant association between changes in the compositional profile of the gut microbiota and the development of diabetes. In particular, disrupted eubiosis of the phylum *Bacteroidetes/Firmicutes* has been associated with increased intestinal permeability, with infiltration of bacterial byproducts across a permeable intestinal barrier triggering subsequent inflammatory responses characteristic of diabetes [86]. On the other hand, several bacteria have been shown to play a protective role, decreasing the risk of developing diabetes by reducing pro-inflammatory markers and maintaining the integrity of the intestinal barrier [83,84]. Therefore, *Lactobacillus acidophilus*—La5 has shown to be a promising strain in the adjuvant treatment of T2D [87].

4. Conclusions

This study using a dynamic colon model showed positive and hopeful results in modulating gut microbiota composition and metabolism using *Lactobacillus acidophilus*—La5 of healthy adults, and especially in dysbiosis (T2D). We reveal decreased production of ammonia ions and increased production of butyric acid, which is an important metabolite for glycemic control in the T2D group. Furthermore, the probiotic promotes a greater diversity of microorganisms present in the simulated intestinal lumen. Our research indicates promise for the use of the probiotic to promote the general improvement of the host as a whole.

Author Contributions: M.K.S. conducted the experiments at the Simulator of Human Intestinal Microbial Ecosystem (SHIME®), worked on conceptualization. F.L.d.O.: worked on conceptualization. M.P.A.M.: worked on conceptualization. V.M. performed the bioinformatics analysis. A.S. performed the SCFA analyses. K.S. was the main supervisor, responsible for the design, writing, and review of the final manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of São Paulo State University (UNESP) (CAAE:30156020.8.0000.5426) for studies involving humans.

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

Data Availability Statement: All data generated by the current project are available upon request.

Conflicts of Interest: The authors declare no conflict of interest, and the funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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