Applied Microbiology and Biotechnology (2018) 102:8827–8840
https://doi.org/10.1007/s00253-018-9234-8

APPLIED GENETICS AND MOLECULAR BIOTECHNOLOGY

Modulation of gut microbiota from obese individuals by in vitro fermentation of citrus pectin in combination with *Bifidobacterium longum* BB-46

Fernanda Bianchi[1,2] · Nadja Larsen[2] · Thatiana de Mello Tieghi[1] · Maria Angela Tallarico Adorno[3] · Witold Kot[4] · Susana Marta Isay Saad[5] · Lene Jespersen[2] · Katia Sivieri[1]

Received: 15 May 2018 / Revised: 29 June 2018 / Accepted: 5 July 2018 / Published online: 18 August 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

This study aimed to evaluate the effects of three treatments, i.e., *Bifidobacterium longum* BB-46 (T1), *B. longum* BB-46 combined with the pectin (T2), and harsh extracted pectin from lemon (T3) on obesity-related microbiota using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®). The effects of the treatments were assessed by the analysis of the intestinal microbial composition (using 16S rRNA gene amplicon sequencing) and the levels of short-chain fatty acids (SCFAs) and ammonium ions (NH_4^+). Treatments T2 and T3 stimulated members of the *Ruminococcaceae* and *Succinivibrionaceae* families, which were positively correlated with an increase in butyric and acetic acids. Proteolytic bacteria were reduced by the two treatments, concurrently with a decrease in NH_4^+. Treatment T1 stimulated the production of butyric acid in the simulated transverse and descending colon, reduction of NH_4^+ as well as the growth of genera *Lactobacillus*, *Megamonas*, and members of *Lachnospiracea*. The results indicate that both *B. longum* BB-46 and pectin can modulate the obesity-related microbiota; however, when the pectin is combined with *B. longum* BB-46, the predominant effect of the pectin can be observed. This study showed that the citric pectin is able to stimulate butyrate-producing bacteria as well as genera related with anti-inflammatory effects. However, prospective clinical studies are necessary to evaluate the anti/pro-obesogenic and inflammatory effects of this pectin for future prevention of obesity.

Keywords *Bifidobacterium longum* BB-46 · Obese microbiota · Pectin · SHIME® model · 16S rRNA sequencing

Introduction

Obesity is a global public health concern and can result in many health complications like insulin resistance, type II diabetes, dyslipidaemia, hepatosteatosis, and hypertension (Flegal et al. 2015). The metabolic syndrome, characterized by the association of risk factors for cardiovascular disease, peripheral vascular disease, and diabetes, is highly prevalent in obese individuals and is directly related to a sedentary lifestyle, along with unhealthy eating behavior (Kushner and Choi 2010).

The recent studies have demonstrated the interplay between the composition of intestinal microbiota and pro-inflammatory response, metabolic disturbances, gut barrier, and energy balance (Ley et al. 2006; Fleissner et al. 2010; Bomhof and Reimer 2015), showing that the human gut microbiota has a crucial role in the onset and establishment of obesity (Rosenbaum et al. 2015). The precise role of the gut

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00253-018-9234-8) contains supplementary material, which is available to authorized users.

✉ Katia Sivieri
sivierik@fcfar.unesp.br

[1] Department of Food Science, UNESP - São Paulo State University, Araraquara, Brazil

[2] Department of Food Science, Faculty of Science, University of Copenhagen, Frederiksberg, Denmark

[3] Department of Hydraulics and Sanitation, School of Engineering of São Carlos, University of São Paulo (USP), São Carlos, SP, Brazil

[4] Department of Environmental Science, Aarhus University, Roskilde, Denmark

[5] Department of Biochemical and Pharmaceutical Technology, Food Research Center, University of São Paulo (USP), São Paulo, SP, Brazil

microbiota in obesity is still being investigated, but it is known that changes in the composition of the gut microbiota as a consequence of the ingestion of high-fat diets may lead to lipogenesis (Bäckhed et al. 2007), increased gut permeability of lipopolysaccharides (LPS), and chronic inflammation (Cani et al. 2008).

The intake of dietary fiber can modulate the microbiota, protecting against several health complications such as large bowel and stomach carcinoma, type 2 diabetes, metabolic syndrome, and cardiovascular disease (Trepel 2004; Anderson et al. 2009). The term "dietary fiber" includes a number of polymeric plant materials (polysaccharides, oligosaccharides, lignin, and compounds associated with the plant cell wall) which have beneficial physiological effects, including laxation as well as attenuation of blood glucose and cholesterol concentrations (DeVries et al. 2001).

Pectins are complex hetero-polysaccharides (Voragen et al. 2009), currently acknowledged as emerging prebiotics which are able to modulate the microbiota, including increases in bacterial species like *Faecalibacterium prausnitzii* or *Roseburia intestinalis* (Gómez et al. 2016). Furthermore, pectins slow the gastric transit, moderate the glycemic index, and help to control energy intake, and in some cases, they may have the potential to reduce the risk of colon cancer and cardiovascular diseases (Olano-Martin et al. 2002). According to Wicker and Kim (2015), pectin is fermented by colonic bacteria generating short-chain fatty acids. Although some studies have linked short-chain fatty acids (SCFAs) to obesity, showing higher total amount of SCFA in the obese microbiota, especially regarding acetic acid (Turnbaugh et al. 2006; Schwiertz et al. 2010; Rahat-Rozenbloom et al. 2014; Perry et al. 2016), there is a strong indication that acetate, propionate, and butyrate have a protective action against weight gain, being considered predominantly anti-obesogenic (Chakraborti 2015; Lu et al. 2016; Morrison and Preston 2016). According Wren and Bloom (2007) and Zhou et al. (2008), the formation of SCFA has been associated with increased expression and production of hormones related to anorexigenic effects.

The SCFA production can also be increased by some probiotic strains as *Bifidobacterium adolescentis*, *Bifidobacterium longum*, and *Bifidobacterium pseudocatenulatum*, which are able to modulate the composition of the microbiota, increasing the production of intestinal metabolites including SCFA (Duncan et al. 2004; Falony et al. 2006; An et al. 2011). Several studies have shown that lactate and /or acetate produced by bifidobacteria is used by some bacterial genera such as *Roseburia*, *Eubacterium*, and *Anaeroestipes*, which convert these metabolites into SCFA (Duncan et al. 2004; Falony et al. 2006). Furthermore, according to An et al. (2011), some specific strains of bifidobacteria are related to lipid-lowing effects and reduced body weight and therefore, may be potential therapeutic candidates for obesity management. Although many authors have been associating different bifidobacteria as well as pectin and other fibers to gut microbial and metabolite composition, few studies exist associating the synbiotic effect of *B. longum* BB-46 and pectin, especially on obesity-related microbiota.

The interactions between the intestinal microbiota community and determined probiotic, prebiotic, and other fibers can be evaluated by both in vivo and in vitro systems. The in vivo models present some limitations such as high cost, invasive research methods, and, in case of animal studies, the impossibility to extrapolate the obtained results to human reality (Parvova et al. 2011). Therefore, many studies have been using in vitro systems, as for example the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®), to study such interactions (Macfarlane and Macfarlane 2007). The SHIME® is a validated in vitro system able to simulate the different parts of the gastrointestinal tract, proven to be very useful in analyzing the human intestinal microbial community (Molly et al. 1994; Kontula et al. 2002).

Therefore, the aim of this study was to investigate the impact of a pectin extracted from lemon and the probiotic strain *B. longum* BB-46, in combination or alone, on fecal microbiota collected from obese adults with the use of the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®).

Materials and methods

Bacterial culture conditions and pectin origin

B. longum BB-46 was provided by Christian Hansen (Copenhagen, DK) as fresh cultures and maintained at − 80 °C in MRS broth with glycerol. The strain was activated in MRS broth supplemented with L-cysteine (0.05%) and cultured at 37 °C for 24 h. The cells were centrifuged (2600 rpm/ 10 min., 4 °C) and washed with saline solution (0.85% (*w*/*v*) NaCl). The harsh extracted LM pectin from lemon was provided by CP Kelco (Lille Skensved, DK).

Microbiota fermentations in the SHIME®

Microbiota fermentations were performed in the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®). The SHIME® was developed by researchers from the University of Ghent (Ghent, Belgium) and validated by Molly et al. (1994). It is a system that enables the mimicking and maintenance of the human gastrointestinal microbial diversity in vitro for several months (Molly et al. 1993). In this system, the pH, residence time, and temperature are controlled by a software (Possemiers et al. 2004). The SHIME® is composed by five double-jacketed vessels. These vessels simulate the stomach, the duodenum, and the ascending, transverse, and descending colon.

The pH of the stomach was automatically adjusted by the addition of NaOH 1 M or HCl 1 M. The duodenum was simulated with 60 mL of artificial pancreatic juice (per liter; 12.5 g of $NaHCO_3$, 6 g of Oxgall, and 0.9 g of pancreatin) at a rate of 4 mL/min for 15 min (Molly et al. 1994; Possemiers et al. 2004). The pH culture of the colon vessels 3 (V3), 4 (V4), and 5 (V5) was automatically adjusted by the addition of NaOH 0.5 M or HCl 0.1 M (Molly et al. 1994; Possemiers et al. 2004).

A magnetic stirrer provided the five vessels to be continuously stirred whereas the temperature was maintained at 37 °C. Continuous anaerobic conditions were provided through daily N_2 flushing for 30 min.

Fecal inoculum

At the beginning of the experiment, the colon vessels (V3, V4, and V5) were inoculated with bacteria from a mixed stool sample of three obese adults (body mass index (BMI) between 30 and 39.9 kg/m^2) and waist circumference > 80 cm). All donors had not consumed probiotic products over the past 3 to 6 months and had no history of antibiotic treatment within a period of 6 months prior to the study.

From the selected donors, 40 g of feces (~ 13.5 g of each donor) was collected and diluted in phosphate buffer (200 mL) containing 0.05 mol/L of Na_2HPO_4, 0.05 mol/L NaH_2PO_4, and 0.1% of Na-thioglicolate (pH = 6.5). Subsequently, after being stirred in a homogenizer (stirrer model 130, Norte Científica, São Paulo, BR) for 10 min, the diluted sample was centrifuged for 15 min at 3000 rpm. From the supernatant, 40 mL was added to vessels 3, 4, and 5, which were already filled with the SHIME® feed [carbohydrate-based medium that allows the adaptation to specific environmental conditions of the ascending, transverse, and descending colon in terms of pH range, retention time, and available carbon sources] at specific volumes, allowing for the adjustment and stabilization of the microbial community (Molly et al. 1994). SHIME® feed is composed of starch (4.0 g/L (Maizena, São Paulo, BR)), mucin (4.0 g/L (Sigma, St. Louis, USA)), yeast extract (3.0 g/L (Kasvi, São José dos Pinhais, BR)), arabinogalactan (1.0 g/L (Sigma, St. Louis, USA)), xylan (1.0 g/L (Sigma, St. Louis, USA)), peptone (1.0 g/L (Kasvi, São José dos Pinhais, BR)), cysteine (0.5 g/L (Sigma, St. Louis, USA)), and glucose (0.4 g/L) (Synth, Diandema, BR).

Experimental protocol in a SHIME® model

The experimental protocol included a 2-week control period after the stool sample inoculation to allow the adaptation of the microbial community to physicochemical and nutritional conditions prevailing in different parts of the colon (Molly et al. 1994) and also to stabilize the microbial community (Possemiers et al. 2004). During this period, 200 mL of the SHIME® feed entered through the system three times a day. After 2 weeks of stabilization (period where no more changes are observed in the microbiota composition and metabolite production.), the protocol was followed by 1 week of treatment with *B. longum* BB-46 (T1), 1 week of treatment with *B. longum* BB-46 and pectin (T2), 1 week of washout period (W), and 1 week of treatment with pectin (T3). *B. longum* BB-46, as well as pectin combined with BB-46 and pectin alone, were applied together with the SHIME® feed (200 mL) twice a day. *B. longum* was added at 10^8 CFU mL^{-1}, and the pectin at 2% (*w*/*v*). The complete protocol is shown in Fig. 1.

Microbiological analysis employing 16S rRNA gene sequencing

Samples from each compartment of the colon were weekly collected for 16S rRNA gene sequencing. Microbiota profiles of each compartment of the SHIME® were determined using tag-encoded 16S rRNA gene fragment amplicon sequencing. Two technical replicates were performed per treatment.

The DNA isolation of each sample was performed using the "PowerLyzer@PowerSoil DNA Isolation Kit" (Qiagen, Valencia, USA). Samples in a volume of 4.0 mL each were centrifuged (rpm), and the pellet freeze-dried. To start the DNA isolation, 700 μL of bead solution was added to the freeze-dried sample, and the next steps were performed according to the kit's manual.

After the DNA isolation, the polymerase chain reaction I (PCR I) was conducted. The V3 region (~ 190 bp) of the 16S rRNA gene was amplified using primers compatible with a Nextera Index Kit (Illumina) (NXt_388_F: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACWCCTACGGGWGGCAGCAG-3′ and NXt_518_R: 5′-GTCTCGTGGGCTCGG AGATGTGTATAAGAGACAGATTACCGCGGCTGCTGG-3′). The PCR was performed using 12 μL of AccuPrime SuperMix II (Life Technologies, Camarillo, USA), 5 μL of genomic DNA (~ 20 ng/μl), and 0.5 μL of each primer (10 μM). Nuclease-free water was added

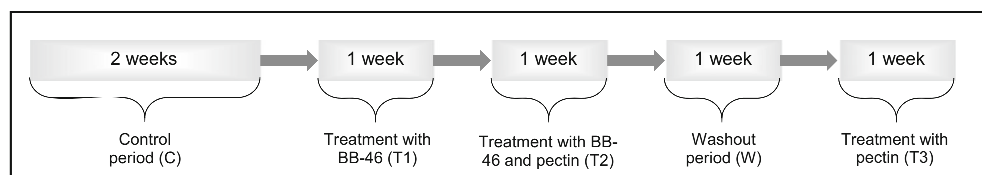

Fig. 1 Experimental SHIME® protocol employed in the treatments with *Bifidobacterium longum* BB-46, pectin, and *Bifidobacterium longum* BB-46 combined with pectin

to complete the volume to 20 μL. The following setup was used: 95 °C for 2 min of initial denaturation, followed by 33 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 15 s, followed by elongation at 68 °C for 30 s, final extension at 68 °C for 4 min, and final cooling to 4 °C (Williams et al. 2017).

To incorporate primers with adapters and indexes, a new PCR was performed (PCR II). PCR II reactions were performed using 2.0 μL of primers P5 and P7 (Nextera Index Kit), 12 μL Phusion High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Tewksbury, USA), 2 μL PCR I product, and nuclease-free water for a total volume of 25 μL. The following setup was used: initial denaturation at 98 °C for 1 min, followed by 13 cycles of 98 °C for 10 s, annealing at 55 °C for 20 s and elongation at 72 °C for 20 s, final extension at 72 °C for 5 min, and cooling to 4 °C. After PCR II, the purification of the amplified fragments, along with adapters and tags, was conducted through AMPure XP beats (Beckman Coulter Genomic, Indianapolis, USA) (Williams et al. 2017).

The sequencing was performed on the Illumina NextSeq instrument as a part of a flowcell using a 2 × 150-cycle MID output kit V2 (Illumina, San Diego, USA). The raw dataset of pair-ended reads and respective quality scores were merged and trimmed with settings, according to Williams et al. (2017). Subsequent analysis steps were conducted using the Quantitative Insight Into Microbial Ecology (QIIME) open-source software package (1.7.0 and 1.8.0) (Caporaso et al. 2011). The UPARSE pipeline was employed in order to purge the dataset of chimeric reads, as well as to construct de novo operational taxonomic units (OTUs). As a reference database, the green genes (13.8) 16S rRNA gene collection was used, as previously described by McDonald et al. (2012). To normalize different depths of sequencing samples, the matrix abundance of taxonomic units of each sample was divided by the total number of pairings after cutting. For rarefied OTU tables (23,000 reads/sample), alpha diversity measures expressed with an observed species (sequence similarity 97% OTUs) value were computed. For this purpose, the alpha rarefaction workflow was employed.

Short-chain fatty acid and ammonium ion (NH_4^+) analyses

Samples were collected weekly from the vessels V3 (ascending colon), V4 (transverse colon), and V5 (descending colon) for SCFA and NH_4^+ analyses throughout the experimental period (control, treatments, and washout). For the determination of SCFA, 2 mL of the samples was centrifuged (14,000 rpm for 5 min), followed by the dilution of 100 μL of the supernatant in 1900 μL of ultrapure water. Next, NaCl (1 g) and crotonic acid (100 μL) were added, as well as isobutanol (70 μL) and 2 M H_2SO_4 (200 μL). The SCFA analysis was conducted using a 2010-model gas chromatograph (Shimadzu, Gifu, JP) equipped with a split/splitless injector, a flame ionization detector, and a CombiPAL automated sampler for headspace analysis. Separation of the SCFAs took place through a HP-INNOWAX column (30 m × 0.25 mm × 0.25 μm) (Agilent Technologies, La Jolla, USA). Hydrogen was used as the carrier gas, the flow rate was set at 1.45 mL/min, and the temperature of the injector and the detector was maintained at 240 °C (Adorno et al. 2014).

NH_4^+ amounts were determined through a selective ion meter (HI 4101 model, Hanna Instruments, Leighton Buzzard, UK) coupled with an ammonia selective ion electrode (Orion 95–12). Samples collected from the colon vessels (10 mL) were transferred to 0.2 mL of an ammonia pH ionic strength adjusting solution (Orion, Thermo Fisher, Millersburg, USA). The analyses were performed in duplicates.

Statistical analysis

The significance of the results was determined using a one-way ANOVA, and individual means were compared through the Tukey test ($p < 0.05$), employing Biostat 5.0 software (IBM, Belém, BR) (Ayres et al. 2007). A simple correspondence analysis was used to test the correlation between the treatments and the microbiota composition using the Minitab Software (State College, USA) (Minitab 2010). Correlation analyses were made to correlate the SCFA production and ammonium ions with specific groups of bacteria using the Spearman correlation test. A value of $p < 0.05$ was considered statistically significant. The Spearman correlation test was conducted using the open-source RStudio software program (RStudio 2017). This program was also used to create a heatmap based on the relative abundances of different genera.

Accession number

The sequences have been deposited at the European Nucleotide Archive (ENA) under the accession number PRJEB23969.

Results

Sequencing characteristics

The sequencing yielded a total of 2,614,738 reads from 30 microbiota samples collected during treatment with *B. longum* BB-46, *B. longum* BB-46 combined with pectin, and pectin alone. These sequencing reads were merged and clustered into operational taxonomic units (OTUs). After normalizing the data, a total of 690,000 sequences were produced, generating 23,000 sequences per sample. On average, 406 OTUs were obtained per sample (ranking from 232 to 616). Rarefaction curves were constructed to evaluate the sequencing depth and the species richness. The curves suggested that sequencing

depth was enough to cover most of the bacteria in the SHIME® samples (Supplemental Fig. S1). As Supplemental Fig. S2 shows, alpha diversity measurement suggested variations in species richness (Chao1) and diversity (Shanon index) between samples. Treatments with pectin (T3) and pectin with *B. longum* BB-46 (T2) showed the lowest richness (index of 271 to 487 during treatment T2 and 309 to 530 during treatment T3) and diversity (index of 3.72 to 4.88 during treatment T2 and 3.93 to 4.75 during treatment T3).

Microbiota composition

Figure 2 shows the main bacterial phyla determined in the microbiota from obese individuals during all the experiment in SHIME® model. A high relative abundance of *Firmicutes* phylum (73%, 61%, and 51% for the ascending, transverse, and descending colon, respectively), followed by *Bacteroidetes* (19%, 27%, and 34% for the ascending, transverse, and descending vessels, respectively), and *Actinobacteria* (8%, 11%, and 13% for the ascending, transverse, and descending vessels, respectively) was observed during the control period. The effects of treatments were similar in the three regions of the colon vessels, with minor differences in abundance proportions. A high increase in *Firmicutes* as well as a decrease in *Bacteroidetes* was observed during the treatment with *B. longum* BB-46 (T1). Treatments T2 (pectin with BB-46) and T3 (pectin alone) stimulated the increase in *Proteobacteria* as well as the reduction in *Bacteroidetes* phylum (Fig. 2). An increase in the abundance of *Firmicutes* was also observed during the treatment with pectin alone (T3).

As observed in Fig. 3a, the increase in *Firmicutes* phylum during the treatment with *B. longum* (T1) was mostly due to the abundance of the *Lachnospiraceae* and *Veillonellaceae* families and a small contribution of *Lactobacillaceae*, while during T3 (pectin alone), this increase was mostly attributed to the high abundance of *Ruminococcaceae*. A correspondence analysis was performed to test the correlation between the different treatments and the microbiota composition in terms of family (Fig. 3b). The two first axes of the correspondence analysis aggregated 61.43% of the total variance, which is sufficient to explain the results. The impact of treatments (T1, T2, and T3) was similar in the three regions of the colon (V3, V4, and V5) and we could clearly see three distinct groups. One group was composed of the control period (C) and the washout period (W), whereas another was composed of the treatment with *B. longum* BB-46 (T1), and the final one contained the treatments T2 (pectin combined with *B. longum* BB-46) and T3 (pectin). These groups were clustered based on the microbiota composition similarity in terms of family. We could observe a relationship between T2 and T3 and the families *Succinivibrionaceae*, *Ruminococcaceae*, and *Erysipelotrichaceae*, as well as between T1 and *Lachnospiraceae*, *Veillonellacea*e, *Lactobacillaceae*, and *Synergistaceae* (Fig. 3b). As Fig. 3b shows, when *B. longum* was combined with pectin, only the pectin effects could be observed, and as a consequence, treatments T2 (pectin with BB-46) and T3 (pectin) were clustered together due to the similarity of the microbiota composition.

Figure 4 shows the relative abundance of *Lachnospiraceae* family during the fermentation with *B. longum* BB-46 (T1), *B. longum* BB-46 and pectin (T2), and pectin (T3) in the SHIME® model. Similar effects were observed in the three regions of the simulated colons. A significant increase in the *Lachnospiraceae* family ($p < 0.01$) was observed during the treatment T1 when compared to the control period, whereas a significant decrease was noticed during the treatments T2 and T3. No significant difference was observed between the last two treatments.

Figure 5 shows the relative abundance of bacterial genera in the obese microbiota during the different fermentations in the SHIME® model. The control and washout periods showed similar bacterial genera composition, as well as treatments T2 (pectin and BB-46) and T3 (pectin). Treatment T1 (with BB-46) showed different genera composition, but closer to the control and washout periods. These results reinforce the idea that both pectin and *B. longum* BB-46 can modulate the obese microbiota in different ways, but when combined in a

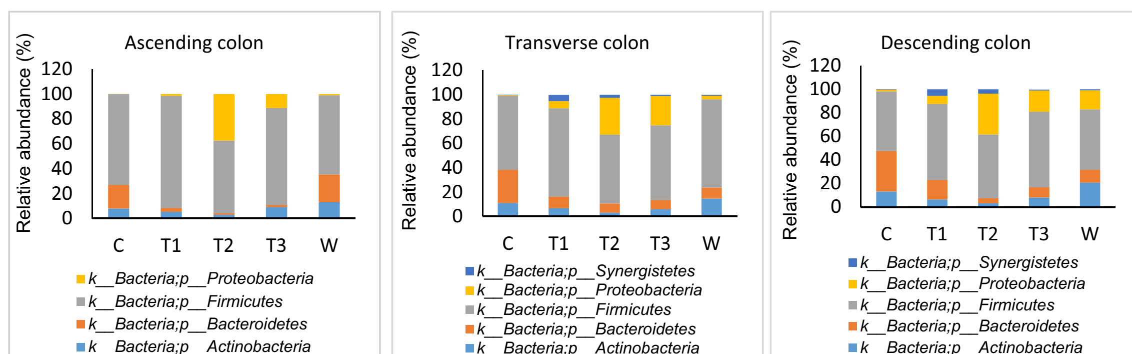

Fig. 2 The main bacterial phyla determined in the microbiota from obese individuals during all the experiments in SHIME® colon vessels. C, control period; T1, treatment with *Bifidobacterium longum* (BB-46); T2, treatment with BB-46 and pectin; T3, treatment with pectin; W, washout period

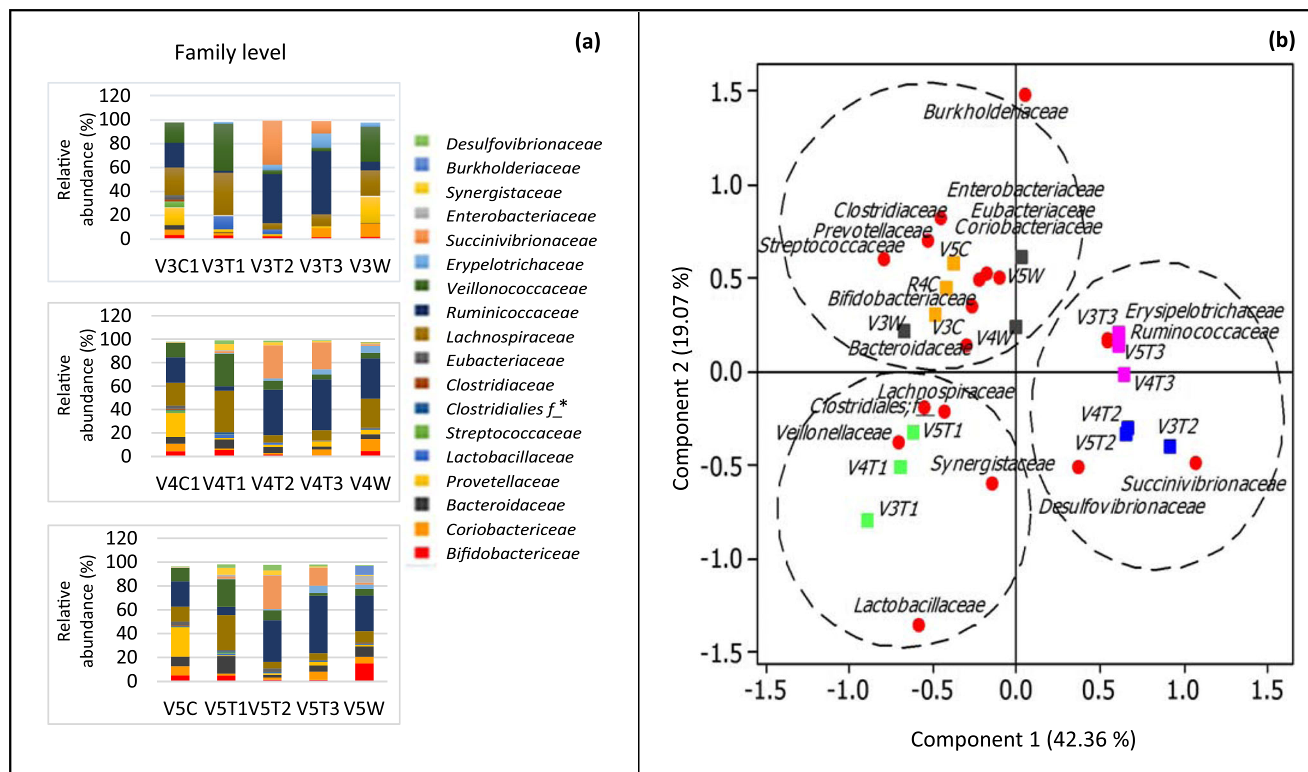

Fig. 3 Microbiota composition at a family level during all the experiments in SHIME® colon vessels. **a** The main bacterial family determined during the experiments in the three simulated colons; **b** simple correspondence analysis showing relationship between the treatments and bacterial families in the three regions of the SHIME® model. C, control period; T1, treatment with *Bifidobacterium longum* (BB-46); T2, treatment with BB-46 and pectin; T3, treatment with pectin; W, washout period; V3, ascending colon; V4, transverse colon; V5, descending colon. The different families are represented by red dots and the treatments by colorful square. Control period is represented by *orange* color and T1, T2, W, and T3 by *green*, *blue*, *black*, and *purple*, respectively. *Unclassified family of *Clostridiales* order

presented setup, the predominant effect of the pectin can be observed. The most dominant genera found during T2 and T3 were the *Succinivibrio* (relative abundance of 28–37%) and an unclassified genera of the *Ruminococcaceae* family (relative abundance of 33–53%). *Blautia* (relative abundance of 14–19%) and *Megamonas* (relative abundance of 19–32%) were the most dominant genera found during treatment T1 (Fig. 5).

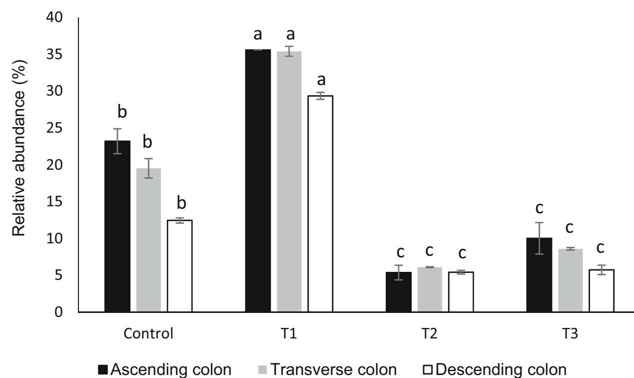

Fig. 4 Relative abundance (%) of the *Lachnospiraceae* family in the microbiota from obese individuals during all the experiments in SHIME® colon vessels. C, control period; T1, treatment with *Bifidobacterium longum* (BB-46); T2, treatment with BB-46 and pectin; T3, treatment with pectin. Different letters represent statistical difference ($p < 0.05$) between the treatments for the same vessel (one-way ANOVA and Tukey post hoc test)

Table 1 shows the relative abundance of bacterial genera with significant changes during the three treatments (BB-46 (T1), BB-46 with pectin (T2), and pectin (T3)) in the SHIME® model. A significant increase ($p < 0.05$) in *Succinivibrio*, *Holdemanella*, *Alteromonadaceae*, unclassified genera of *Ruminococcaceae* (OTUs 1077, 1194, 1027, 1153, 1037, 601, and 576), and *Catenibacterium* genera was found during treatments T2 and T3, when compared to the control period. During treatment T1, a significant increase ($p < 0.05$) in *Blautia*, *Megamonas*, *Succinivibio*, *Holdemanella*, *Lactobacillus* (only ascending and descending colon), *Dorea*, unclassified genera of *Lachnospiracea*e family, *Catenibacterium*, and *Bacteroides* (only descending colon) was found.

Metabolic activity

Figure 6 shows the results obtained for SCFA. *B. longum* BB-46 (T1) had no effect on SCFA production, except for an increase ($p < 0.05$) in butyric acid in the transverse and descending colon. However, a high and significant increase ($p < 0.05$) in acetic and butyric acid was observed during the

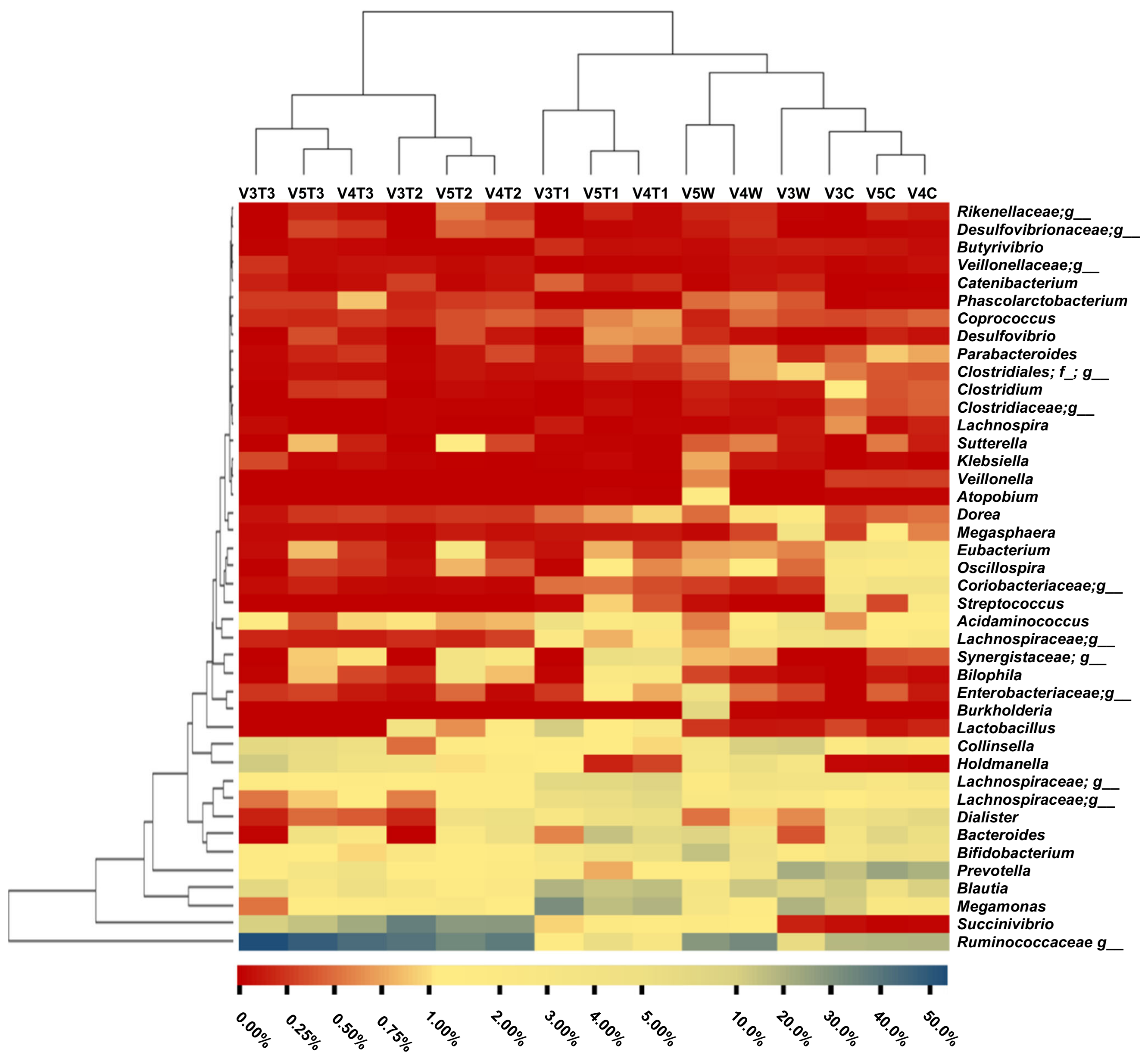

Fig. 5 Relative abundance of bacterial genera (%) in the obese microbiota during all the experiments in the SHIME® model. C, control period; T1, treatment with *Bifidobacterium longum* (BB-46); T2, treatment with BB-46 and pectin; T3, treatment with pectin; W, washout period; V3, ascending colon; V4, transverse colon; V5, descending colon. Unclassified genera are represented by "g_"

treatments with the *B. longum* BB-46 and pectin (T2) and just pectin (T3), with a higher increase in butyric acid, especially during the treatment with pectin (T3) in the three regions of the colon (increase by 7, 4.5, and 12 folds in the ascending, transverse, and descending colon, respectively, comparing to control period). There were no significant changes in propionic acid contents during the different treatments in all colon regions.

As Fig. 7 shows, a significant decrease in NH_4^+ production in all compartments of the colon vessels was observed ($p < 0.05$) during the three different treatments (BB-46, BB-46 with pectin, and pectin) (Fig. 7). However, the largest reduction of NH_4^+ occurred during the treatments with BB-46 combined with pectin (T2) and pectin (T3). There was no statistical difference in ammonium levels between treatments T2 and T3 for all the colon regions evaluated.

Correlation analysis was performed to identify the genera related to production of SCFA or NH_4^+ (Fig. 8). The relative abundance of *Succinivibrio* and seven unclassified genera of *Ruminococcaceae* (OTUs 1077, 1037, 601, 576, 1197, 1153, and 1027) had positive correlations with production of butyric and acetic acid. *Holdemanella* (*Erysipelotrichaceae* family) and an unclassified genera of *Alteromonodaceae* also showed a positive correlation with butyric and acetic acid and a negative

Table 1 Relative abundance (mean ± SD) of bacterial genera with significant changes during all the experiments in SHIME® colon vessels

Genus	Ascending colon					Transverse colon		
	Control	Treatment T1	Treatment T2	Washout	Treatment T3	Control	Treatment T1	Treatment T2
Ruminococcaceae g_ (OTU 1077)	8.11 ± 0.49	0.43 ± 0.00*	18.78 ± 0.94*	2.70 ± 0.04*	25.41 ± 0.96*	7.48 ± 1.03	0.89 ± 0.23*	17.77 ± 2.14*
Ruminococcaceae g_ (OTU 1194)	7.13 ± 0.51	0.37 ± 0.01*	17.93 ± 0.04*	2.41 ± 0.10*	23.48 ± 0.82*	7.08 ± 0.84	0.81 ± 0.17*	16.08 ± 1.55*
Ruminococcaceae g_ (OTU 1027)	0.08 ± 0.00	0.01 ± 0.00*	1.22 ± 0.10*	0.03 ± 0.01	0.59 ± 0.04*	0.10 ± 0.02	0.03 ± 0.01	0.86 ± 0.07*
Ruminococcaceae g_ (OTU 576)	0.08 ± 0.01	0.01 ± 0.00*	0.24 ± 0.10	0.06 ± 0.00	0.28 ± 0.01*	0.16 ± 0.02	0.05 ± 0.05	0.36 ± 0.03*
Ruminococcaceae g_ (OTU 1153)	0.16 ± 0.00	0.03 ± 0.00*	0.36 ± 0.05*	0.07 ± 0.02*	0.38 ± 0.00*	0.20 ± 0.02	0.04 ± 0.02*	0.39 ± 0.00*
Ruminococcaceae g_ (OTU 1037)	0.12 ± 0.02	0.00 ± 0.00*	0.25 ± 0.04	0.06 ± 0.01	0.32 ± 0.00*	0.13 ± 0.04	0.03 ± 0.00	0.30 ± 0.07
Ruminococcaceae g_ (OTU 601)	0.00 ± 0.00	0.00 ± 0.00	0.44 ± 0.03*	0.00 ± 0.00	0.26 ± 0.01*	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.04*
Succinivibrio	0.06 ± 0.01	0.96 ± 0.03*	37.01 ± 2.61*	0.15 ± 0.06	10.45 ± 0.56*	0.03 ± 0.02	1.62 ± 0.02*	28.27 ± 3.33*
Holdemanella	0.03 ± 0.01	1.16 ± 0.01*	4.26 ± 1.80*	3.21 ± 0.99*	11.75 ± 1.46*	0.02 ± 0.00	0.30 ± 0.08*	1.77 ± 0.42*
Lactobacillus	0.32 ± 0.11	11.00 ± 0.01*	3.87 ± 2.23	0.10 ± 0.02	0.00 ± 0.00	0.16 ± 0.00	2.85 ± 1.63	1.55 ± 0.88
Ateromonadaceae	0.00 ± 0.00	0.00 ± 0.00	0.17 ± 0.05*	0.00 ± 0.00	0.14 ± 0.01*	0.00 ± 0.00	0.00 ± 0.00	0.12 ± 0.00*
Catenibacterium	0.00 ± 0.01	0.45 ± 0.00*	0.28 ± 0.02*	0.15 ± 0.02*	0.15 ± 0.00*	0.01 ± 0.00	0.20 ± 0.07*	0.09 ± 0.01*
Blautia	12.24 ± 1.17	19.24 ± 0.01*	3.08 ± 0.50*	8.42 ± 0.11*	7.48 ± 1.72	9.78 ± 1.03	15.98 ± 0.69*	2.22 ± 0.03*
Megamonas	11.05 ± 0.67	31.80 ± 0.00*	1.98 ± 2.39*	19.69 ± 2.14*	0.53 ± 0.12*	2.88 ± 1.11	19.33 ± 3.02*	1.07 ± 0.69
Dorea	0.32 ± 0.00	0.51 ± 0.00*	0.22 ± 0.10	1.76 ± 0.45*	0.09 ± 0.02*	0.51 ± 0.02	0.95 ± 0.04*	0.25 ± 0.02*
Bacteroides	3.66 ± 0.72	0.59 ± 0.00*	0.00 ± 0.00*	0.37 ± 0.03*	0.01 ± 0.01*	5.61 ± 0.65	7.60 ± 1.02	5.04 ± 1.03
*Lachnospiraceae g_* [A]	1.11 ± 0.02	5.28 ± 0.03*	0.57 ± 0.14*	2.68 ± 0.30*	0.54 ± 0.12*	2.06 ± 0.02	7.67 ± 0.97*	1.48 ± 0.07*
Lachnospiraceae g_ other [B]	3.92 ± 0.10	7.73 ± 0.01*	1.09 ± 0.15*	3.88 ± 0.52	1.49 ± 0.27*	3.87 ± 0.37	8.92 ± 0.53*	1.42 ± 0.03*
Clostridium	1.16 ± 0.09	0.06 ± 0.00*	0.00 ± 0.00*	0.10 ± 0.02*	0.00 ± 0.00*	0.43 ± 0.03	0.01 ± 0.00*	0.03 ± 0.00*
Streptococcus	4.69 ± 0.56	0.035 ± 0.03*	0.00 ± 0.00*	0.00 ± 0.00*	0.00 ± 0.00*	2.25 ± 0.55	0.39 ± 0.21*	0.00 ± 0.00*
Bifidobacterium	3.36 ± 0.47	3.20 ± 0.00	2.38 ± 0.15	1.84 ± 0.05*	1.63 ± 0.11*	4.38 ± 0.11	5.47 ± 1.47	1.66 ± 0.32*

Genus	Transverse colon		Descending colon				
	Washout	Treatment T3	Control	Treatment T1	Treatment T2	Washout	Treatment T3
Ruminococcaceae g_ (OTU 1077)	14.29 ± 0.40*	20.45 ± 0.09*	7.55 ± 0.63	1.59 ± 0.03*	14.75 ± 3.26*	11.82 9.66	22.18 ± 1.22*
Ruminococcaceae g_ (OTU 1194)	12.62 ± 0.08*	18.03 ± 0.52*	6.84 ± 0.74	1.40 ± 0.08*	13.59 ± 2.81	10.49 ± 8.61	19.90 ± 1.27*
Ruminococcaceae g_ (OTU 1027)	0.21 ± 0.02	0.76 ± 0.06*	0.10 ± 0.00	0.05 ± 0.01*	0.80 ± 0.02*	0.20 ± 0.141	0.76 ± 0.09*
Ruminococcaceae g_ (OTU 576)	0.22 ± 0.01	0.36 ± 0.02*	0.21 ± 0.01	0.08 ± 0.00*	0.46 ± 0.02*	0.34 ± 0.06	0.46 ± 0.10
Ruminococcaceae g_ (OTU 1153)	0.29 ± 0.01*	0.44 ± 0.06*	0.19 ± 0.06	0.06 ± 0.00	0.32 ± 0.07	0.30 ± 0.17	0.39 ± 0.01*
Ruminococcaceae g_ (OTU 1037)	0.23 ± 0.01	0.28 ± 0.00*	0.12 ± 0.00	0.02 ± 0.01*	0.32 ± 0.01*	0.178 ± 0.14	0.30 ± 0.05*
Ruminococcaceae g_ (OTU 601)	0.01 ± 0.01	0.20 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.23 ± 0.01*	0.04 ± 0.03	0.23 ± 0.04*
Succinivibrio	1.14 ± 0.08*	22.86 ± 3.33*	0.01 ± 0.01	1.88 ± 0.20*	28.08 ± 3.97*	1.74 ± 1.58	14.94 ± 0.14*
Holdemanella	5.65 ± 0.39*	4.34 ± 0.27*	0.03 ± 0.02	0.15 ± 0.01*	1.00 ± 0.31*	3.15 ± 2.98	6.05 ± 0.10*
Lactobacillus	0.09 ± 0.00*	0.00 ± 0.00*	0.09 ± 0.00	1.34 ± 0.12*	0.65 ± 0.28	0.24 ± 0.27	0.00 ± 0.00*
Ateromonadaceae	0.01 ± 0.00	0.14 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.02*	0.03 ± 0.03	0.14 ± 0.02*
Catenibacterium	0.09 ± 0.01*	0.07 ± 0.02*	0.00 ± 0.00	0.13 ± 0.04*	0.02 ± 0.01	0.01 ± 0.00	0.03 ± 0.00*
Blautia	13.07 ± 0.20*	5.22 ± 0.04*	5.00 ± 0.34	13.88 ± 0.85*	1.64 ± 0.03*	3.54 ± 2.89	2.91 ± 0.48*

Table 1 (continued)

Megamonas	1.39 ± 0.09	1.71 ± 0.00	2.66 ± 0.02	16.08 ± 2.46*	2.66 ± 1.44	3.32 ± 3.78	1.14 ± 0.13*
Dorea	1.02 ± 0.15*	0.27 ± 0.02*	0.45 ± 0.02	0.72 ± 0.00*	0.25 ± 0.01*	0.48 ± 0.15	0.25 ± 0.03*
Bacteroides	4.15 ± 0.55	2.41 ± 0.24*	8.16 ± 0.15	14.67 ± 0.64*	2.30 ± 0.61*	8.75 ± 0.92	5.10 ± 0.80*
*Lachnospiraceae g_* [A]	2.96 ± 0.01*	1.06 ± 0.05*	2.13 ± 0.01	5.94 ± 0.48*	1.52 ± 0.27*	2.53 ± 0.80	0.91 ± 0.06*
Lachnospiraceae g_ other [B]	4.05 ± 0.03	1.62 ± 0.11*	2.82 ± 0.01	7.33 ± 0.04*	1.49 ± 0.07	1.98 ± 0.02*	1.30 ± 0.16*
Clostridium	0.09 ± 0.00*	0.26 ± 0.04	0.38 ± 0.01	0.03 ± 0.01*	0.05 ± 0.01*	0.15 ± 0.15	0.24 ± 0.03*
Streptococcus	0.00 ± 0.00*	0.00 ± 0.00*	0.33 ± 0.05	0.94 ± 0.00*	0.00 ± 0.00*	0.06 ± 0.08	0.00 ± 0.00*
Bifidobacterium	4.47 ± 0.30	0.97 ± 0.03*	4.84 ± 0.10	4.68 ± 1.22	1.35 ± 0.37*	14.87 ± 15.78	1.15 ± 0.22*

Significant increases or decreases compared to the control are indicated by asterisk ($p < 0.05$). *T1*, treatment with *Bifidobacterium longum* BB46; *T2*, treatment with BB46 and pectin; *T3*, treatment with pectin. [A] Unclassified genera of *Lachnospiraceae* family; [B] different unclassified genera of *Lachnospiraceae* family

correlation with ammonium ions. The relative abundance of *Streptococcus*, *Bacteroide*s, and *Clostridium* positively correlated with the levels of ammonium ions. *Succinivibrio* and three unclassified genera of *Ruminococcaceae* (OTUs 1077, 601, and 1194) showed negative correlation with NH_4^+ (Fig. 8).

Discussion

In this study, we evaluated the effects of three treatments, i.e., *B. longum* BB-46 (T1), *B. longum* BB-46 combined with the pectin (T2), and harsh extracted pectin from lemon (T3), on obesity-related microbiota using a Simulator of the Human Intestinal Microbial Ecosystem. A high increase in *Ruminococcaceae* (mainly OTUs 1077, 1194, and 1027—unclassified genera of *Ruminococcaceae)* and *Succinivibrionaceae* members (mainly *Succinivibrio* genus) was observed during the treatments T2 (pectin with BB-46) and T3 (pectin). Both families are able to degrade pectin as well as other carbohydrates such as starch (Duncan et al. 2007; Santos and Thompson 2014; Tian et al. 2017). The pectin probably stimulated the increase of *Ruminococcaceae* and *Succinivibrionaceae* members during the treatments T2 and T3, inhibiting the growth of several bacterial species due to a competitive advantage, which might explain the low bacterial diversity (in all colon vessels) during these two treatments.

The increase in *Succinivibrionaceae* and *Ruminococcaceae* members is, however, considered as beneficial, since both families are associated with several health benefits (Louis et al. 2010; Li et al. 2012; Nakayama et al. 2017). Members of the *Succinivibrionaceae* family (*Proteobacteria* phylum) have a protective role against gut inflammation and are able to efficiently transport molecules implicated in immune recovery (Li et al. 2012). Moreover, investigating the impact of dietary habits on the gut microbiota, Nakayama et al. (2017) showed that the genus *Succinivibrio* had a negative correlation with total fat intake. *Ruminococcaceae*, the other dominant family in this study (during T2 and T3), include members with potential specialization in ecological niches, such as the ability to generate energy from fermentable substrates available in the colon using different routes (Arumugam et al. 2011). According to Louis et al. (2010), members of this family have been associated with the maintenance of the gut health and the production of butyric acid. In addition, by investigating the correlation between changes in the body weight over time and the gut microbiome composition, Menni et al. (2017) showed that the family *Ruminococcaceae* was nominally protective against weight gain. Such findings are interesting for the present study, since we used fecal samples from obese people. Tian et al. (2017) as well as Gómez et al. (2016) also reported an increase in *Ruminococcaceae* members during fermentation of citrus pectin, but none of them observed changes in *Succinivibrionaceae* members. These studies were, however,

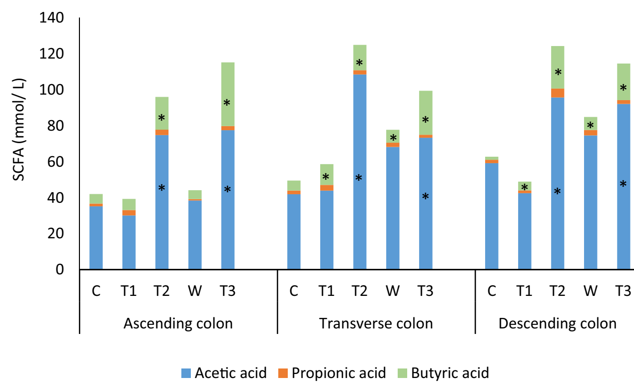

Fig. 6 Production of acetic, propionic, and butyric acids by microbiota from obese individuals during all the experiments in SHIME® colon vessels. Significant increases compared to the control are indicated by asterisk ($p < 0.05$) (one-way ANOVA and Tukey post hoc test). C, control period; T1, treatment with *Bifidobacterium longum* (BB-46); T2, treatment with BB-46 and pectin; T3, treatment with pectin; W, washout period

performed with fecal samples from piglets and lean individuals, respectively.

In this study, we observed an increase ($p < 0.05$) in acetic and butyric acids during the treatments with *B. longum* BB-46 and pectin (T2) and pectin alone (T3). According to Santos and Thompson (2014), members of the *Succinivibrionaceae* family can ferment carbohydrates to succinate and acetate, which may explain the high increase in acetic acid contents during treatments T2 and T3. Correlation analysis between the abundances of the gut microbiome and SCFAs revealed that *Succinivibrio* correlated positively with acetic acid.

The family *Ruminococcaceae* includes the major butyrate-producing species (Louis et al. 2010), which may explain the high increase in the butyric acid production, especially during treatment T3 (pectin) in vessel 3. According to Gómez et al. (2016), some pectins and oligosaccharides derived from pectin have been identified as emerging prebiotics due to their intestinal microbiota modulation ability, including the increase in some bacteria from the *Ruminococcaceae* family such as *F. prausnitzii*. In this study, there was no increase in *F. prausnitzii* during the treatments but rather an increase in the OTUs 1194, 1153, 1077, 1037, 1027, 601, and 576 (unclassified genera of *Ruminococcaceae*) was observed during treatments T2 and T3. Correlation analysis between the abundance of the gut microbiome and SCFAs revealed that the different unclassified genera of the *Ruminococcaceae* family correlated positively with butyric acid levels, confirming the relationship between *Ruminococcaceae* members and butyric acid production.

In this study, we also observed a positive correlation between the levels of butyric acid and two bacterial genera: an unclassified genera of *Alteromonadaceae* family and *Eubacterium biforme*, reclassified as *Holdemanella biformis* (De Maesschalck et al. 2014). *H. biformis* is considered butyrate producers (Schwiertz et al. 2010), which can explain the result. On the other hand, there is no scientific evidence that members of *Alteromonadaceae* family are butyric producer; however, a high correlation between butyric acid levels and members of this family was found in this study. Members of *Alteromonadaceae* family are often associated to nutrient-rich environments with the ability to degrade several complex polysaccharides such as agar, chitin, cellulose, β-glucan, laminarin, pectin, pullulan, starch, and xylan (López-Pérez and Rodriguez-Valera 2014). This way, the results indicate that members of *Alteromonadaceae* probably used the pectin as a substrate to generate SCFA.

Once the acetic and butyric acids seem to be predominantly anti-obesogenic (Chakraborti 2015; Morrison and Preston 2016), their increase in the colon region, especially from obese people, is desirable. Butyrate has been found to increase mitochondrial activity, prevent metabolic endotoxemia, improve insulin sensitivity, possess anti-inflammatory potential, increase the intestinal barrier function, and protect against diet-induced obesity without causing hypophagia (Chakraborti 2015), while acetate appears to stimulate leptin secretion in adipocytes (Zaibi et al. 2010). Although there were no significant changes in propionic acid contents during the different treatments, propionate has also been found to be involved in obesity, inhibiting the cholesterol synthesis and regulating the body weight through a stimulatory effect on anorexigenic gut hormones (Chakraborti 2015).

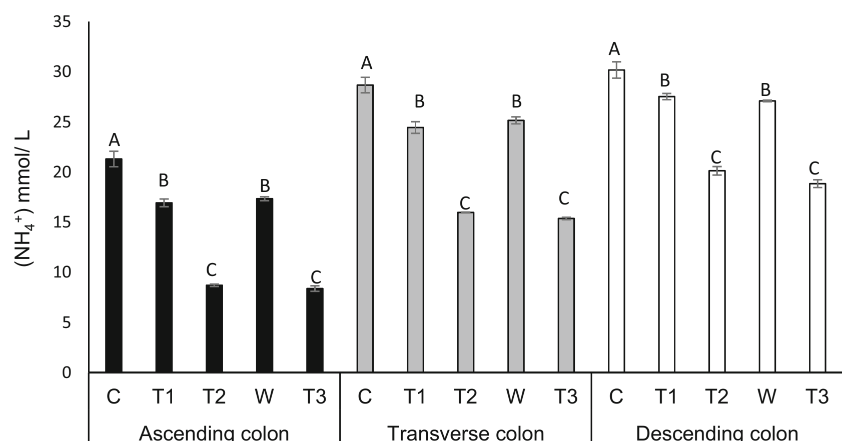

Fig. 7 NH_4^+ production (mmol/L) by microbiota from obese individuals during all the experiments in SHIME® colon vessels. C, control period; T1, treatment with *Bifidobacterium longum* (BB-46); T2, treatment with BB-46 and pectin; T3, treatment with pectin; W, washout period. Different letters represent statistical difference ($p < 0.05$) between the treatments for the same vessel (one-way ANOVA and Tukey post hoc test)

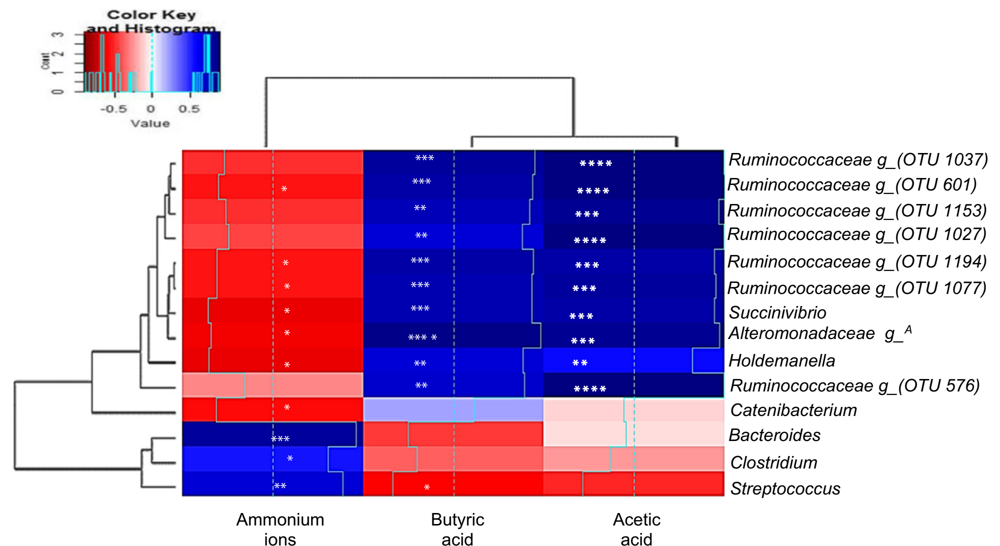

Fig. 8 Correlation between SCFA production (butyric and acetic acids), ammonium ions, and bacterial genera. Significant correlations are indicated by one asterisk ($p < 0.05$), two asterisks ($p < 0.01$), three asterisks ($p < 0.001$), and four asterisks ($p < 0.0001$) (Spearman correlation). [A]Unclassified genera of *Alteromonadaceae* family

Despite the significant increase in the *Lachnospiraceae* members (related to butyric and propionic acids production (Louis et al. 2010)), as well as *Lactobacillus* (related to acetic acid production (Tachedjian et al. 2017)) during treatment T1, only an increase in butyric acid (transverse and descending colon) was observed, whereas the levels of acetic and propionic acids were maintained. We believe that certain SCFAs, such as acetic acid produced during treatment T1, have probably been used by specific groups of bacteria, which converted them into other metabolites. The gut harbors a complex microbial community, where many interactions exist, including bacterial cross-feeding interactions. According to Ríos-Covián et al. (2016), bacterial cross-feeding has a large impact on the final balance of SCFA production since some groups of microorganisms can utilize the end products from the metabolism of another bacterial group, like for example, the conversion of acetic acid produced by specific bacteria into butyrate for other groups (Louis et al. 2010).

In this study, a significant decrease in ammonium ions was observed during all the treatments (T1, T2, and T3), especially T2 and T3. The concentration of ammonium ions in the intestine mainly results from amino acid deamination and urea hydrolysis by intestinal bacteria (Davila et al. 2013). According to Smith and MacFarlane (1998), the addition of fermentable carbohydrates to microbial populations is directly linked to the reduction of NH_4^+ in the intestine. This relationship, according to Ito et al. (1993), can be explained by the inhibition of the peptides and amino acid fermentation in favor of carbohydrate fermentation by the intestinal microbiota. In this study, however, the reduction of NH_4^+ seems to be more related to the decrease in proteolytic bacteria than to the switch of substrate, since the levels of ammonium ions were positively correlated with the proteolitic bacteria *Clostridium*, *Streptococcus*, and *Bacteroides.* The proteolytic activity in the large intestine is mainly attributed to the *Bacteroides*, *Clostridium*, *Propionibacterium*, *Streptococcus*, and *Lactobacillus* genera, which use amino acids as sources of nitrogen, carbon, and energy, generating NH_4^+ as one of the intermediate or final metabolites (Macfarlane and Cummings 1991). A significant reduction in *Clostridium* spp. was observed during all the treatments ($p < 0.05$) as well as *Streptococcus* and *Bacteroides* during treatments T2 and T3 (Table 1), reducing thus the levels of ammonium ions.

Together with considerable decrease in proteolytic bacteria, treatment T1 (with *B. longum* BB-46) resulted in an increase of *Bacteroides* spp. (vessel 5) and *Lactobacillus* spp. (Table 1). This fact probably explains the lower reduction in ammonium ion production during the treatment T1 compared to treatments T2 and T3.

The reduction in NH_4^+ in the colon is considered to be beneficial as these ions may alter the morphology and intermediate metabolism of the intestinal cells, increasing DNA synthesis and promoting the development of tumors (Ichikawa and Sakata 1998; Davila et al. 2013). In addition, Hughes et al. (2008) demonstrated that NH_4^+ can increase cell permeability in the colonocytes, causing several host diseases.

In this study, it was also observed that pectin (T3) and pectin in combination with BB-46 (T2) inhibited the growth of *Lachnospiraceae.* Some studies have shown an association between obesity and the *Lachnospiraceae* family. Kameyama and Itoh (2014) identified a specific *Lachnospiraceae* bacterium (strain AJ110941) involved in metabolic disorders. They concluded that intestinal colonization by a *Lachnospiraceae* contributes to the development of diabetes in obese mice. According to Ravussin et al. (2012), rats fed with a high-fat diet present high populations of *Lachnospiraceae* members, and a reduction is observed after weight loss. Changes in bacterial populations depend on many variables such as competition between bacteria for substrates, synthesis of antimicrobial agents, and bacterial metabolism (Mao et al. 2012). We suppose that the decrease on *Lachnospiraceae* members can

probably be related to the *Ruminococcaceae* increase. Both families have members able to produce butyric acid (Onrust et al. 2015) and can probably need similar substrates to survive, thus creating a competitive environment.

In summary, the dynamic view of the microbiome and microbial metabolites, exhibited by *B. longum* BB-46, pectin, and the combination of *B. longum* BB-46 and pectin, provided interesting insights into the interplay of probiotic and pectin with the microbiota. The results indicate that *B. longum* BB-46 and pectin have a different impact on obesity-related microbiota, but when combined, the predominant effect of the pectin can be observed. Treatments with pectin and pectin combined with *B. longum* BB-46 showed a high increase in bacteria with potential anti-inflammatory effects (*Succinivibrionaceae* members), an increase in SCFA, and a decrease in the *Lachnospiraceae* family. Based on literature, these findings indicate that the studied pectin can probably have a protective role on obesity. However, further clinical studies are necessary to evaluate the anti/pro-obesogenic and inflammatory effects of this pectin.

Acknowledgments The study is part of the "Bioactive components from by-products of food processing used in a synbiotic approach for improving human health and well-being (BioSyn)" project, within the frame of the international thematic project "Strategic Research Collaboration in Food Science in the State of São Paulo, Brazil and Denmark – 2013" FAPESP/DCSR. The authors wish to thank Fundação de Amparo à Pesquisa do Estado de São Paulo and DCSR for the financial support and fellowships. The authors also wish to thank Thomas Lesser (Chr. Hansen, Denmark) and Karin Meyer Hansen (CP Kelco, Denmark) for providing, respectively, the *B. longum* BB-46 strain and the pectin from lemon and for the valuable discussions of the study results.

Funding This article was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Projects 2013/50506-8; 2015/13965-0; 2015/08228-6; 2016/20336-1), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and the Danish Council for Strategic Research (DCSR) (project BioSyn, no. 3050-00005B).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Studies using fecal donations from human volunteers do not require medical ethical committee approval in Brazil since they are considered as noninvasive.

References

Adorno MAT, Hirasawa JS, Varesche MBA (2014) Development and validation of two methods to quantify volatile acids (C2-C6) by GC/FID: headspace (automatic and manual) and liquid-liquid extraction (LLE). Am J Anal Chem 05:406–414. https://doi.org/10.4236/ajac.2014.57049

An HM, Park SY, Lee DK, Kim JR, Cha MK, Lee S, Lim H, Kim K, Ha N (2011) Antiobesity and lipid-lowering effects of *Bifidobacterium* spp. in high fat diet-induced obese rats. Lipids Health Dis 10:116. https://doi.org/10.1186/1476-511X-10-116

Anderson JW, Baird P, Davis RH, Ferreri S, Knudtson M, Koraym A, Waters V, Williams CL (2009) Health benefits of dietary fiber. Nutr Rev 67:188–205

Arumugam M, Raes J, Pelletier E, Le Paslier D, Batto J-M, Bertalan M, Borruel N, Casellas F (2011) Enterotypes of the human gut microbiome. Nature 473:174–180. https://doi.org/10.1038/nature09944.Enterotypes

Ayres M, Ayres JM, Ayres DL, Santos AAS (2007) Bioestat 5.0: Aplicações estatísticas nas áreas das ciências biomédicas. Instituto de Desenvolvimento Sustentável Mamirauá, Belém.

Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI (2007) Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci 104:979–984. https://doi.org/10.1073/pnas.0605374104

Bomhof MR, Reimer RA (2015) Pro- and prebiotics: the role of gut microbiota in obesity. In: Venema K, do Carmo AP (eds) Probiotics and prebiotics: current research and future trends. Caister Academic Press, Poole, pp 363–380

Cani PD, Bibiloni R, Knauf C, Neyrinck AM, Delzenne NM (2008) Changes in gut microbiota control metabolic diet-induced obesity and diabetes in mice. Diabetes 57:1470–1481. https://doi.org/10.2337/db07-1403

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci 108:4516–4522. https://doi.org/10.1073/pnas.1000080107

Chakraborti CK (2015) New-found link between microbiota and obesity. World J Gastrointest Pathophysiol 6:110–119. https://doi.org/10.4291/wjgp.v6.i4.110

Davila AM, Blachier F, Gotteland M, Andriamihaja M, Benetti PH, Sanz Y, Tomé D (2013) Intestinal luminal nitrogen metabolism: role of the gut microbiota and consequences for the host. Pharmacol Res 68:95–107

De Maesschalck C, Van Immerseel F, Eeckhaut V, De Baere SD, Cnockaert M, Croubels S, Haesebrouck F, Ducatelle R, Vandamme P (2014) *Faecalicoccus acidiformans* gen. nov., sp. nov., isolated from the chicken caecum, and reclassification of *Streptococcus pleomorphus* (Barnes et al. 1977), *Eubacterium biforme* (Eggerth 1935) and *Eubacterium cylindroides* (Cato et al. 1974) as *Faecalicoccus pleomorphus* comb. nov., *Holdemanella biformis* gen. nov., comb. nov. and *Faecalitalea cylindroides* gen. nov., comb. nov., respectively, within the family *Erysipelotrichaceae*. Int J Syst Evol Microbiol 64:3877–3884. https://doi.org/10.1099/ijs.0.064626-0

DeVries JW, Camire ME, Cho S, Craig S, Gordon D, Jones JM, Li B, Lineback D, Prosky L, Tungland BC (2001) The definition of dietary fiber. Cereal Foods World 46:112–126. https://doi.org/10.3402/fnr.v54i0.5750

Duncan SH, Louis P, Flint HJ (2004) Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. Appl Environ Microbiol 70:5810–5817. https://doi.org/10.1128/AEM.70.10.5810-5817.2004

Duncan SH, Louis P, Flint HJ (2007) Cultivable bacterial diversity from the human colon. Lett Appl Microbiol 44:343–350

Falony G, Vlachou A, Verbrugghe K, De Vuyst L (2006) Cross-feeding between *Bifidobacterium longum* BB536 and acetate-converting, butyrate-producing colon bacteria during growth on oligofructose. Appl Environ Microbiol 72:7835–7841. https://doi.org/10.1128/AEM.01296-06

Flegal KM, Panagiotou OA, Graubard BI (2015) Estimating population attributable fractions to quantify the health burden of obesity. Ann Epidemiol 25:201–207. https://doi.org/10.1016/j.annepidem.2014.11.010

Fleissner CK, Huebel N, Abd El-Bary MM, Loh G, Klaus S, Blaut M (2010) Absence of intestinal microbiota does not protect mice from diet-induced obesity. Br J Nutr 104:919–929. https://doi.org/10.1017/S0007114510001303

Gómez B, Gullón B, Yáñez R, Schols H, Alonso JL (2016) Prebiotic potential of pectins and pectic oligosaccharides derived from lemon peel wastes and sugar beet pulp: a comparative evaluation. J Funct Foods 20:108–121. https://doi.org/10.1016/j.jff.2015.10.029

Hughes R, Kurth MJ, McGilligan V, McGlynn H, Rowland I (2008) Effect of colonic bacterial metabolites on caco-2 cell paracellular permeability in vitro. Nutr Cancer 60:259–266. https://doi.org/10.1080/01635580701649644

Ichikawa H, Sakata T (1998) Stimulation of epithelial cell proliferation of isolated distal colon of rats by continuous colonic infusion of ammonia or short-chain fatty acids is nonadditive. J Nutr 128:843–847

Ito M, Kimura M, Deguchi Y, Yajima T, Kan T (1993) Effects of transgalactosylated intestinal microflora and their on the human metabolism. J Nutr Sci Vitaminol (Tokyo) 39:279–288

Kameyama K, Itoh K (2014) Intestinal colonization by a *Lachnospiraceae* bacterium contributes to the development of diabetes in obese mice. Microbes Environ 29:427–430. https://doi.org/10.1264/jsme2.ME14054

Kontula P, Nollet L, Saarela M, Vilpponen-Salmela T, Verstraete W, Mattila-Sandholm T, Von Wright A (2002) The effect of lactulose on the survival of *Lactobacillus rhamnosus* in the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) and in vivo. Microb Ecol Health Dis 14:90–96. https://doi.org/10.1080/08910600260081739

Kushner RF, Choi SW (2010) Prevalence of unhealthy lifestyle patterns among overweight and obese adults. Obesity 18:1160–1167. https://doi.org/10.1038/oby.2009.376

Ley RE, Turnbaugh PJ, Klein S, Gordon JI (2006) Microbial ecology: human gut microbes associated with obesity. Nature 444:1022–1023. https://doi.org/10.1038/4441022a

Li RW, Wu S, Li W, Navarro K, Couch RD, Hill D, Joseph F, Urban J (2012) Alterations in the porcine colon microbiota induced by the gastrointestinal nematode *Trichuris suis*. Infect Immun 80:2150–2157. https://doi.org/10.1128/IAI.00141-12

López-pérez M, Rodriguez-Valera F (2014) The family *Alteromonadaceae*. In: Rosenberg E, EF DL, Lory S, Stackebrandt E, Thompson F (eds) The prokaryotes. Springer-Verlag Berlin Heidelberg, Berlin, pp 69–92

Louis P, Young P, Holtrop G, Flint HJ (2010) Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA: acetate CoA-transferase gene. Environ Microbiol 12:304–314. https://doi.org/10.1111/j.1462-2920.2009.02066.x

Lu Y, Fan C, Li P, Lu Y, Chang X, Qi K (2016) Short chain fatty acids prevent high-fat-diet-induced obesity in mice by regulating G protein-coupled receptors and gut microbiota. Sci Rep 6:1–13. https://doi.org/10.1038/srep37589

Macfarlane GT, Cummings JP (1991) The colonic flora, fermentation and large bowel digestive function. In: Phillips SF, Pemberton JH, Shorter GR (eds) The large intestine: physiology, pathophysiology and disease. Raven Press, New York, p 923

Macfarlane GT, Macfarlane S (2007) Models for intestinal fermentation: association between food components, delivery systems, bioavailability and functional interactions in the gut. Curr Opin Biotechnol 18:156–162. https://doi.org/10.1016/j.copbio.2007.01.011

Mao S, Zhang R, Wang D, Zhu W (2012) The diversity of the fecal bacterial community and its relationship with the concentration of volatile fatty acids in the feces during subacute rumen acidosis in dairy cows. BMC Vet Res 8:237. https://doi.org/10.1186/1746-6148-8-237

McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P (2012) An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J 6:610–618. https://doi.org/10.1038/ismej.2011.139

Menni C, Jackson MA, Pallister T, Steves CJ, Spector TD, Valdes AM (2017) Gut microbiome diversity and high-fibre intake are related to lower long-term weight gain. Int J Obes 41:1099–1105. https://doi.org/10.1038/ijo.2017.66

Minitab (2010) Minitab 17 Statistical Software [Computer software]. Minitab, Inc., State College. http://www.minitab.com. Accessed 26 Oct 2017

Molly K, Woestyne MV, Verstraete W (1993) Development of a 5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem. Appl Microbiol Biotechnol 39:254–258. https://doi.org/10.1007/BF00228615

Molly K, Woestyne MV, De SI, Verstraete W (1994) Validation of the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) reactor using microorganism-associated activities. Microb Ecol Health Dis 7:191–200. https://doi.org/10.3109/08910609409141354

Morrison DJ, Preston T (2016) Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. Gut Microbes 7:189–200. https://doi.org/10.1080/19490976.2015.1134082

Nakayama J, Yamamoto A, Palermo-Conde LA, Higashi K, Sonomoto K, Tan J, Lee YK (2017) Impact of westernized diet on gut microbiota in children on Leyte island. Front Microbiol 8:1–18. https://doi.org/10.3389/fmicb.2017.00197

Olano-Martin E, Gibson GR, Rastall RA (2002) Comparison of the in vitro bifidogenic properties of pectins and pectic-oligosaccharides. J Appl Microbiol 93:505–511. https://doi.org/10.1046/j.1365-2672.2002.01719.x

Onrust L, Ducatelle R, Van Driessche K, De Maesschalck C, Vermeulen K, Haesebrouck F, Eeckhaut V, Van Immerseel F (2015) Steering endogenous butyrate production in the intestinal tract of broilers as a tool to improve gut health. Front Vet Sci 2:1–8. https://doi.org/10.3389/fvets.2015.00075

Parvova I, Danchev N, Hristov E (2011) Animal models of human diseases and their significance for clinical studies of new drugs. J Clin Med 4:19–29

Perry RJ, Peng L, Barry NA, Cline GW, Zhang D, Cardone RL, Petersen KF, Kibbey RG, Goodman AL (2016) Acetate mediates a microbiome-brain-β cell axis promoting metabolic syndrome. Nature 534:213–217. https://doi.org/10.1038/nature18309

Possemiers S, Verthé K, Uyttendaele S, Verstraete W (2004) PCR-DGGE-based quantification of stability of the microbial community in a simulator of the human intestinal microbial ecosystem. FEMS Microbiol Ecol 49:495–507. https://doi.org/10.1016/j.femsec.2004.05.002

Rahat-Rozenbloom S, Fernandes J, Gloor GB, Wolever TMS (2014) Evidence for greater production of colonic short-chain fatty acids in overweight than lean humans. Int J Obes 38:1525–1531. https://doi.org/10.1038/ijo.2014.46

Ravussin Y, Koren O, Spor A, LeDuc C, Gutman R, Stombaugh J, Knight R, Ley RE, Leibel RL (2012) Responses of gut microbiota to diet composition and weight loss in lean and obese mice. Obesity 20:738–747. https://doi.org/10.1038/oby.2011.111

Ríos-Covián D, Ruas-Madiedo P, Margolles A, Gueimonde M, De los Reyes-Gavilán CG, Salazar N (2016) Intestinal short chain fatty acids and their link with diet and human health. Front Microbiol 7:1–9. https://doi.org/10.3389/fmicb.2016.00185

Rosenbaum M, Knight R, Leibel RL (2015) The gut microbiota in human energy homeostasis and obesity. Trends Endocrinol Metab 26:493–501

RStudio (2017) RStudio: integrated development environment for R (Version 1.1.383) [Computer software]. Boston, MA. http://www.rstudio.org/. Accessed 16 Sept 2017

Santos EO, Thompson FE (2014) The family *Succinivibrionaceae*. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F

(eds) The prokaryotes: *Gammaproteobacteria*, 4th edn. Springer-Verlag, Berlin Heidelberg, pp 639–648

Schwiertz A, Taras D, Schäfer K, Beijer S, Bos NA, Donus C, Hardt PD (2010) Microbiota and SCFA in lean and overweight healthy subjects. Obesity 18:190–195. https://doi.org/10.1038/oby.2009.167

Smith EA, MacFarlane GT (1998) Enumeration of amino acid fermenting bacteria in the human large intestine: effects of pH and starch on peptide metabolism and dissimilation of amino acids. FEMS Microbiol Ecol 25:355–368. https://doi.org/10.1016/S0168-6496(98)00004-X

Tachedjian G, Aldunate M, Bradshaw CS, Cone RA (2017) The role of lactic acid production by probiotic *Lactobacillus* species in vaginal health. Res Microbiol 168:782–792. https://doi.org/10.1016/j.resmic.2017.04.001

Tian L, Bruggeman G, van den Berg M, Borewicz K, Scheurink AJW, Bruininx E, de Vos P, Smidt H, Schols HA, Gruppen H (2017) Effects of pectin on fermentation characteristics, carbohydrate utilization, and microbial community composition in the gastrointestinal tract of weaning pigs. Mol Nutr Food Res 61:10–10. https://doi.org/10.1002/mnfr.201600186

Trepel F (2004) Dietary fibre: more than a matter of dietetics. I. Compounds, properties, physiological effects. Wien Klin Wochenschr 116:465–476

Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444:1027–1031. https://doi.org/10.1038/nature05414

Voragen AGJ, Coenen GJ, Verhoef RP, Schols HA (2009) Pectin, a versatile polysaccharide present in plant cell walls. Struct Chem 20:263–275. https://doi.org/10.1007/s11224-009-9442-z

Wicker L, Kim YK (2015) Pectin and health. In: Caballero B, Finglas P, Toldra F (eds) Encyclopedia of food and health. Academic, Cambridge, pp 289–293

Williams AR, Hansen TVA, Krych L, Ahmad HFB, Nielsen DS, Skovgaard K, Thamsborg SM (2017) Dietary cinnamaldehyde enhances acquisition of specific antibodies following helminth infection in pigs. Vet Immunol Immunopathol 189:43–52. https://doi.org/10.1016/j.vetimm.2017.06.004

Wren AM, Bloom SR (2007) Gut hormones and appetite control. Gastroenterology 132:2116–2130. https://doi.org/10.1053/j.gastro.2007.03.048

Zaibi MS, Stocker CJ, O'Dowd J, Davies A, Bellahcene M, Cawthorne MA, Brown AJH, Smith DM, Arch JRS (2010) Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. FEBS Lett 584:2381–2386. https://doi.org/10.1016/j.febslet.2010.04.027

Zhou J, Martin RJ, Tulley RT, Raggio AM, McCutcheon KL, Shen L, Danna SC, Tripathy S, Hegsted M, Keenan MJ (2008) Dietary resistant starch upregulates total GLP-1 and PYY in a sustained day-long manner through fermentation in rodents. Am J Physiol Endocrinol Metab 295:1160–1166. https://doi.org/10.1152/ajpendo.90637.2008