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Potential Efficacy of *Bacillus coagulans* BACO-17 to Modulate Gut Microbiota in Rats Fed High-Fat Diet

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Abstract: This study aimed to evaluate the potential efficacy of *Bacillus coagulans* BACO-17 in ameliorating body fat accumulation as well as gut microbiota dysbiosis in animals, which were given a high-fat diet to mimic the adverse effect of an unhealthy dietary pattern. Compared with normal control, high-fat consumption resulted in significant ($p < 0.05$) elevations in weight gain (168%), feed efficiency (176%), visceral fat accumulation (228%), and a lesser total fecal short-chain fatty acids (SCFAs) (−27.5%). A significant shift of fecal *Fimicutes*:*Bacteroidetes* ratio from 1.13 to 3.14 was also observed. After 12 weeks of experiment, a supplementation of *B. coagulans* BACO-17 at high dose (9 log CFU/day) along with a high-fat diet could exert an apparent fat reduction ability by decreasing weight gain (by 23.7%) and visceral fat mass (by 24.0%). It was found that *B. coagulans* BACO-17 was able to increase fecal SCFA concentrations and revert *Fimicutes*:*Bacteroidetes* ratio back to the level comparable with the normal control. It could play a probiotic effect by increasing and decreasing the abundance of *Muribaculaceae* and *Allobaculum*, respectively. Therefore, a supplementation of adequate amount of *B. coagulans* BACO-17 might confer a concreted amelioration of deteriorated bacteria profiles and body fat accumulation due to high-fat consumption.

Keywords: *Bacillus coagulans* BACO-17; high-fat diet; fat accumulation; microbiota

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1. Introduction

Based on the statistic of World Health Organization, over 600 million adults are obese [1]. Overweight and obesity are key factors that contribute to the prevalence of long-term illnesses such as cancer, cardiovascular diseases, musculoskeletal disorders, hypertension, and type 2-diabetes around the world [1]. Many interventional studies have suggested that that appropriate dietary and long-term lifestyle interventions together with moderate-intensity exercise are crucial cornerstone to weight management success, whereas it is in fact challenging to maintain such a healthy lifestyle [2,3].

A high dietary fat consumption has been demonstrated to increase the concentration of lipopolysaccharide and cause endotoxemia, which is closely related to obesity [4]. Evidence has indicated that high dietary fat consumption was associated with intestinal microbiota dysbiosis [5]. Dysbiosis of gut microbiota can lead to irritable bowel syndrome, coeliac disease, cardiovascular disease, and metabolic syndrome [6]. Supplementation of probiotic potentially provides a strategy to modulate the gut microbiota composition and inhibit pathogenic bacteria [7]. Recent studies [8] showed that supplementation of some probiotic species alone or in combinations (e.g., *Lactobacillus plantarum*, *L. acidophilus*, and others) might improve gastrointestinal barrier function in rats given a high-fat diet.

Several *Bacillus* strains have been commonly used in humans as dietary probiotic supplements [9]. *B. coagulans* is a spore-forming probiotic, and is widely used in commercial food products. It was capable of consuming reactive oxygen species in the intestinal lumen and improving the intestinal environment through an elevation of fecal short-chain fatty

acid (SCFA) concentration [10], which is an important regulatory factor to regulate lipid metabolism [11]. *B. coagulans* could modulate intestinal microbiota by enhancing the growth of lactic acid bacteria, exerting an antagonistic effect on pathogenic bacteria in gut, and to a certain extent leading to a displacement of fecal enterococci and *Escherichia coli* in the gut [12,13]. Many studies have also substantiated different beneficial effects of *B. coagulans* including immune system stimulation, reducing serum cholesterol, alleviating diarrhea-predominant irritable bowel syndrome, and treating gastrointestinal disease [14,15]. It is interesting that different probiotics strains within the same genus and species might have distinct effects on the host [16]. To date, the potential influence of *B. coagulans* in animals with high-fat consumption remain to be elucidated.

The present study was to evaluate the potential efficacy of *B. coagulans* BACO-17 in ameliorating body fat accumulation in rats given a high-fat diet. After feeding animals with two different doses of *B. coagulans* BACO-17 for 12 weeks, its influences on body weight gain, feed efficiency, visceral fat, liver lipids, fecal SCFAs, other and fecal parameters were accessed. Fecal microbiome analysis was also conducted and the changes in fecal microbiota composition were compared in this study.

2. Materials and Methods

2.1. Animal and Experimental Design

A pure strain of *B. coagulans* BACO-17 (5×10^9 spores per gram dry powder) was provided by Syngen Biotech Co., Ltd. (Taiwan) (Supplementary Figures S1 and S2). Twenty 8-week-old male Sprague-Dawley rats were obtained from the BioLASCO Company (Taiwan). The animal study protocol was approved by the Animal Care and Use Committee of National Chung Hsing University in accordance with the 3Rs Principles (IACUC approval number: 109-105).

The rats were raised individually in stainless steel screen-bottomed cages. The animal house was maintained at 22 ± 1 °C with a 12 h light/dark cycle. After acclimation for two weeks, the animals weighing 363.0–378.7 g on average were randomly assigned to 4 groups, including one normal control (NC), one high-fat control (HF), and two high-fat sample groups. NC and HF groups were fed with chow diet and high-fat diet, respectively. As for the two high-fat sample groups, two milliliters of *B. coagulans* BACO-17 solution (3.5 and 4.5 log CFU/mL) was given to rats which were designated as low dose (BCL) and high dose (BCL) groups, respectively. More specifically, rats in the BCL and BCH groups were given a dose of 7 and 9 log CFU/day, respectively, which cover the commonly seen range of probiotics in the market. The composition of high-fat diet consisted of chow, lard, soybean oil, and sweetened condensed milk (Supplementary Table S1).

Body weights and food intakes were recorded daily. In the last 3 days of experimental period, fecal samples were collected and kept at −20 °C until used. The rats were fasted for 12 h before being sacrificed at the end of 12-week experiment. Visceral fat pads including mesenteric, epididymal, and perirenal fats were excised, blot-dried, and weighed.

2.2. Feed Efficiency

At the end of 12-week experiment, feed efficiency for each dietary group was calculated with the equation [17] as shown below:

$$\text{Feed efficiency (\%)} = \text{body weight change (g)/food consumption (g)} \times 100\%$$

2.3. Determination of Total Visceral Fat

Total visceral fat refers to the sum of the perirenal, mesenteric, and epididymal fat pads. Each visceral fat pad collected was weighted. The percentage of total visceral fat was presented by the following formula [17]:

$$\text{Total visceral fat (\%)} = \text{total visceral fat (g)/final body weight (g)} \times 100\%$$

2.4. Total Liver Lipids Analysis

Liver sample collected from each rat was freeze-dried and ground into powder, and 0.2 g of the powder was homogenized with methanol (1.5 mL) for 30 s using a homogenizer (T18 Basic Ultra-Turrax®, IKA, Staufen im Breisgau, Germany). An aliquot of 3 mL hexane was added and vortexed. Into this solvent mixture, 1.5 mL of saline (0.9% NaCl) was added and vortexed. After being centrifuged at 1530× *g* for 15 min, the supernatant was collected and transferred into another test tube, fecal sample-containing bottom layer was further vortex-extracted with 4.5 mL of hexane and saline mixture (2:1, *v*/*v*), followed by centrifugation. After combining the first and second supernatants, it was vortex-mixed with 1.5 mL of saline again. After centrifugation, the supernatant was desolventized to dryness using nitrogen gas, and then the total liver lipid content was quantified gravimetrically.

2.5. Determination of Fecal Moisture and Total Fecal Lipids

In the last 3 days of feeding period, fecal samples from each rat were collected, freeze-dried, and ground into powder. The total fecal lipids in 0.2 g of powdered fecal sample were extracted and quantified gravimetrically using the same method as described in the Section 2.4. For the determination of fecal moisture, the fresh fecal sample, which was placed on an aluminum foil tray, was dried to a constant weight in an oven at 105 °C.

2.6. Analysis of Gut Microbiota

Gut microbiota in freshly collected fecal samples from each rat was analyzed by 16S rRNA gene sequencing and bioinformatics analysis based on the methods described by Hsieh et al. [18]. Fecal DNA was first extracted with the CatchGene ™ Stool DNA kit (CatchGene Co., Ltd., Taipei, Taiwan) by following the manufacturer's protocol. Amplicons libraries were pooed in equimolar amounts and purified amplicons with 300 bp paired-end were sequenced on an Illumina MiSeq platform. Subsequently, the paired-end reads were concatenated into longer tags through FLASH v.1.2.7 [19]. Using UCHIME, the chimera removal of quality-filtered tags was further performed to collect effective tags. By using UPARSE, the effective tags were clustered into operational taxonomic units (OTUs) at 97% identity. Taxonomy was analyzed by using the ribosomal database project classifier algorithm (version 2.2). The alpha and beta diversity indices were analyzed with QIIME and were compared between two groups (Wilcoxon test) or among various groups (Kruskal test). Statistical differences of bacterial clades between two groups ($p < 0.05$) were analyzed by using Welch's t-test in Statistical Analysis of Metagenomic Profiles (STAMP). Principal coordinate analysis (PCoA) was performed to illustrate the beta diversity of bacterial communities. Using linear discriminant analysis effect size (LEfSe), the statistically significant bacterial taxa between groups were identified. In the LEfSe analysis, the Wilcoxon rank-sum test and non-parametric Kruskal–Wallis test were used to detect differentially abundant taxa among different dietary groups. The threshold on the logarithmic LDA score for discriminative features was set to 4.0.

2.7. Determination of SCFAs

Based on the procedure described by Wu et al. [20], homogenization with cold saline (0.9%, *w*/*v*) at a ratio of 1:10 (*w*/*v*) was performed on freshly collected fecal samples. After 10 min of centrifugation at 1006× *g*, an aliquot of supernatant (2 mL) was mixed with 50% (*w*/*v*) sulfuric acid (20 µL) and isocaproic acid (10 µL). The SCFAs in the mixture were extracted with diethyl ether. The fecal SCFAs were analyzed using a column (Agilent J and W HP-INNO Wax GC Column, 30 m, 0.25 mm. 0.25 µm). Helium was used as the carrier gas, and the flow rate was kept constant at 7 mL/min. The initial oven temperature was set at 80 °C for 1 min and then raised at a rate of 20 °C/min to 140 °C, held at 140 °C for 1 min and raised again at a rate of 20 °C/min to 220 °C; maintained at 220 °C for another 2 min. The injector and detector temperatures were set at 140 °C and 250 °C, respectively.

2.8. Statistical Analysis

Statistical analysis was performed using SPSS (Version 20.0; SPSS, Armonk, NY, USA). Apart from gut microbiota analysis, all data obtained from rats were analyzed by one-way analysis of variance (ANOVA) by Duncan's multiple range test. The value of $p < 0.05$ was considered significant.

3. Results and Discussion

3.1. Body Weight Gain, Food Intake, and Feed Efficiency

Table 1 presents a summary of final weights, body weight gain, total food and fat intakes, total calorie intake, and feed efficiency. After 12 weeks of experiment, final weights of the three high-fat groups (ranging from 617.7 to 698.3 g) were significantly ($p < 0.05$) higher than NC group (554.1 g). As the weight gain of HF group (321.1 g) was markedly ($p < 0.05$) higher than NC group (increasing by 68%), it implied that the high-fat diet used in this study could induce obesity effectively. Compared with HF group, the consumption of *B. coagulans* BACO-17 at low dose (7 log CFU/day) rendered an apparent ($p < 0.05$) decrease in weight gain (by 13.5%) while a further increase of dosage to 9 log CFU/day could further ($p < 0.05$) push the reduction of weight gain by 23.7%.

Table 1. Summary of body weight gain, total food and fat intakes, total calorie intake, and feed efficiency among different groups.

	NC	HF	BCL	BCH
Body weight gain (g)	191.1 ± 14.0^{d}	321.1 ± 29.0^{a}	277.8 ± 23.1^{b}	245.1 ± 25.0^{c}
Total food intake (g)	2122.1 ± 56.9^{a}	2034.8 ± 143.4^{a}	1986.8 ± 185.7^{ab}	1817.1 ± 119.5^{b}
Total fat intake (g)	106.1 ± 2.8^{c}	457.8 ± 32.3^{a}	447.0 ± 41.8^{ab}	408.8 ± 26.9^{b}
Total calorie intake (kcal)	7215.3 ± 193.5^{c}	8749.8 ± 616.5^{a}	8543.2 ± 798.4^{ab}	7813.4 ± 514.0^{bc}
Feed efficiency (%)	9.0 ± 0.6^{c}	15.8 ± 0.9^{a}	14.0 ± 1.0^{b}	13.5 ± 0.9^{b}

[a–d.] Values (mean ± SD, $n = 5$) with different superscripts in the same row are significantly different, $p < 0.05$. NC: normal control; HF: high-fat control; BCL: high-fat diet + low dose of *B. coagulan* BACO-17 (7 log CFU/day); BCH: high-fat diet + high dose of *B. coagulan* BACO-17 (9 log CFU/day).

After the 12-week feeding period, the total food intake of the BCH group (1817.1 g) was significantly ($p < 0.05$) lower than the other diet groups (1986.8–2122.1 g). Regarding the total fat and total calorie intakes, the three high-fat groups had markedly ($p < 0.05$) higher dietary fat consumption (408.8–457.8 g) and calorie intake (7813.4–8749.8 kcal) in contrast to NC group (106.1 g and 7215.3 kcal, respectively). It was believed that an intake of high-fat foods induced short-term positive energy balances, thus having an influence on satiation and post-ingestive appetite inhibition [21]. Among the three high-fat groups, the significantly ($p < 0.05$) lower levels of total fat and energy intakes in BCH group resulted from its apparently lesser amount of total diet consumption. Yadav et al. [22] demonstrated that probiotics can promote the release of glucagon-like peptide 1 (GLP-1) in the intestine, hence suppressing appetite and reducing food intake.

As for the feed efficiency (%), Table 1 presents that significant ($p < 0.05$) increases in feed efficiencies (150–176%) were noted in the three high-fat groups relative to NC group (9.0). Fat, evidently, was a major factor that caused elevation in feed efficiencies. As the comparable feed efficiencies between BCH and BCL groups (14.0 and 13.5, respectively) were apparently ($p < 0.05$) lower than HF group (15.8), it was believed that the supplementation of *B. coagulans* BACO-17 could effectively attenuate feed efficiency.

3.2. Total Visceral Fat and Liver Total Lipids

In Table 2, rats in all high-fat diet groups had apparently ($p < 0.05$) higher total visceral fat accumulation (9.2–12.1 g/100 g body weight) than the NC group (5.3 g/100 g body weight). A significant increase by 73.6% to 128% was noted. The results reflected that the composition of the high-fat diet used in this study was effective in inducing an accumulation of visceral fat including mesenteric, epididymal, and perirenal fats. Despite that, the administration of *B. coagulans* BACO-17 at a relatively higher dose (9 log CFU/day) together

with a high-fat diet could exert an apparent fat reduction ability. A 24.0% decrease in total visceral fat was noted in BCH group compared with normal control. More specifically, a significant ($p < 0.05$) decline in the levels of perirenal and epididymal fats in the BCH group (25.7% and 25.5%, respectively) against the HF group (5.5 and 3.5 g/100 g, respectively) was noted. The changes in total visceral fat parallel the changes in total food, fat, and energy intakes (Table 1). Although the use of probiotics to treat overweight and obesity remains debated, many studies reported that some of the specific strains included in the genus *Bifidobacterium* and *Lactobacillus* possessed strain-specific probiotic effect on helping reduce body weight in overweight and obese populations [23]. Another study by Cao et al. [24] also found that the supplementation of probiotic (*B. licheniformis*) was able to suppress the accumulation of epididymal fat. It was believed that the decreased fat and energy intakes resulting from the supplementation of *B. coagulans* BACO-17 were part of the major factors leading to the attenuated accumulation of body fat.

Table 2. Relative size of visceral fat pads (g/100 g body weight) and liver total lipids (mg/g) among different groups.

Visceral Fats	NC	HF	BCL	BCH
Total visceral fat	5.3 ± 0.7^{c}	12.1 ± 1.5^{a}	11.2 ± 1.6^{a}	9.2 ± 0.9^{b}
Perirenal fat	2.3 ± 0.1^{c}	5.5 ± 0.7^{a}	4.8 ± 0.6^{ab}	4.1 ± 0.6^{b}
Mesenteric fat	1.4 ± 0.3^{b}	3.2 ± 0.7^{a}	3.0 ± 0.8^{a}	2.4 ± 0.4^{a}
Epididymal fat	1.7 ± 0.3^{c}	3.5 ± 0.4^{a}	3.4 ± 0.5^{a}	2.6 ± 0.4^{b}
Liver total lipids (mg/g)	156.3 ± 8.7^{c}	294.8 ± 50.4^{a}	257.5 ± 30.9^{ab}	234.0 ± 30.1^{b}

[a–c.] Values (mean ± SD, $n = 5$) with different superscripts in the same row are significantly different, $p < 0.05$. NC: normal control; HF: high-fat control; BCL: high-fat diet + low dose of *B. coagulan* BACO-17 (7 log CFU/day); BCH: high-fat diet + high dose of *B. coagulan* BACO-17 (9 log CFU/day).

Table 2 shows that the administration of high-fat diets induced significantly ($p < 0.05$) higher lipids accumulation in liver tissues in all three high-fat groups (88.6–49.7%) relative to the NC group (156.3 mg/g). Among those high-fat groups, apparently ($p < 0.05$) lower level of liver total lipids in BCH group (234.0 mg/g) was noted. This trend of lipids reduction parallels the decline in total visceral fat.

3.3. Changes in Fecal Parameters

Figure 1a shows that the administration of high-fat diets caused a 2.4- to 2.5-fold reduction ($p < 0.05$) of fresh fecal weight from 12.1 g/day (NC group) to 4.8–5.1 g/day. The drastic drop in fecal mass was probably resulted from the high-fat intake associated gastrointestinal motility disorders. As shown in Figure 1b, the feeding of high-fat diet was also found to cause a significant drop in fecal moisture (by 8%) in the HF group as compared with the NC group (53.5 g/100 g feces). It was noteworthy that the supplementation of *B. coagulans* BACO-17 could lead to a substantial increase of fecal moisture back to the level comparable with the normal control. Other authors [10,20] have also observed a remarkable increase in fecal moisture in rats fed strains of *B. coagulans*, and the increment was ascribed to a profuse production of organic acids in feces.

Compared with the fecal fat content in NC group (181.1 mg/day), apparently lower levels of total fecal fat excretion per day (30.9–38.4%) were observed among the three high-fat groups (Figure 1c). It was ascribed to the drastic drop in fecal output (2.4- to 2.5-fold reduction) in these groups. However, as for the fecal fat content calculated by per gram basis, it was interesting to note that the fecal fat excretions among the high-fat groups (45.9–55.1 mg/g) were notably ($p < 0.05$) increased by 42.5–71.1% relative to the normal control (32.2 mg/g) (Figure 1d). A higher fecal fat output was to a certain degree associated with a lesser extent of dietary fat absorption [25]. It was thus believed that the supplementation of *B. coagulans* BACO-17 at high dose (9 log CFU/day) could enhance fecal fat excretion in rats with high-fat consumption.

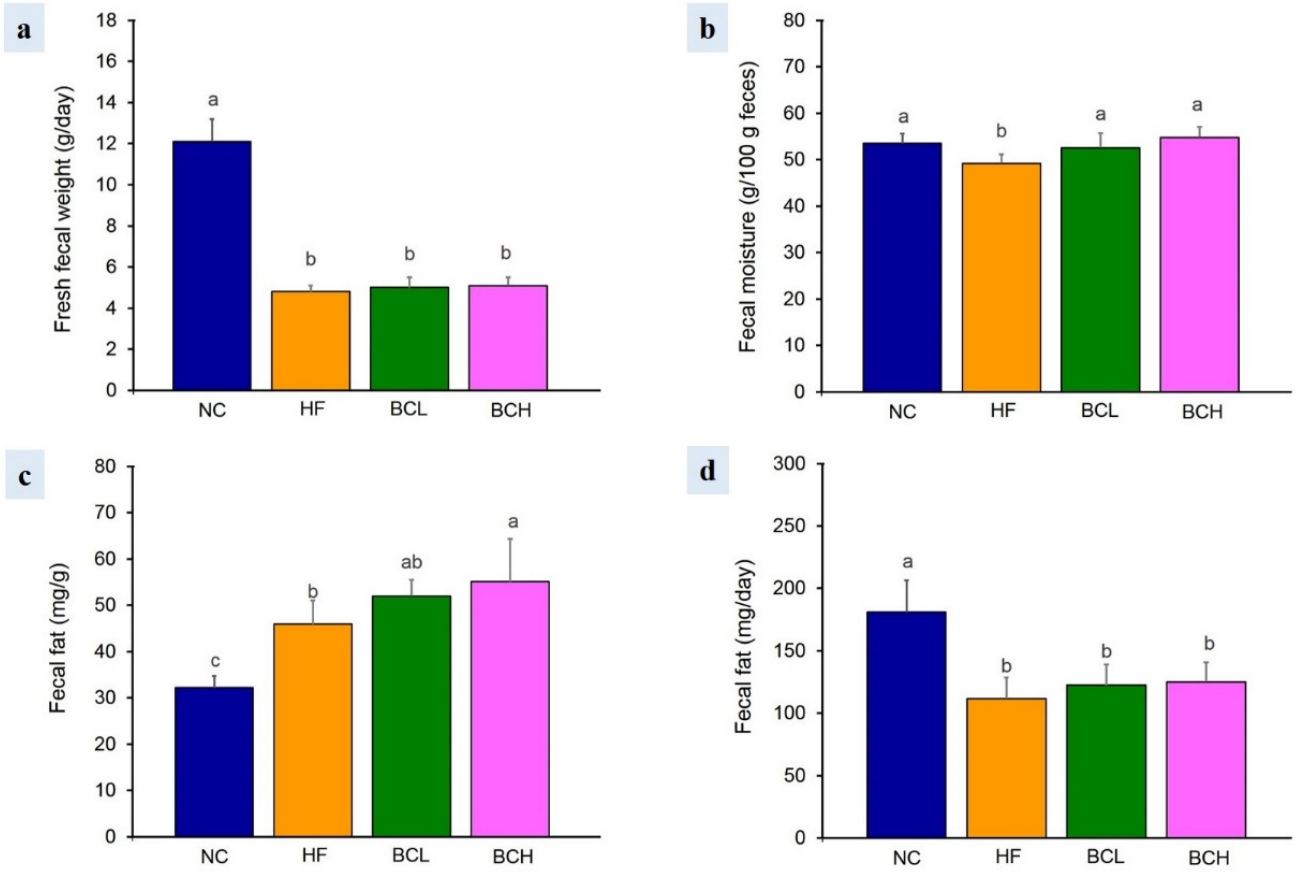

Figure 1. Variations in (**a**) fresh fecal weight, (**b**) fecal moisture, (**c**) fecal fat (mg/g), and (**d**) fecal fat (mg/day) within rats fed chow diet (NC group), high-fat diet (HF group), high-fat diet + low dose of *B. coagulan* BACO-17 (7 log CFU/day) (BCL group), and high-fat diet + high dose of *B. coagulan* BACO-17 (9 log CFU/day) (BCH group) (n = 5 per group). a–c: Bars with different letters are significantly different, $p < 0.05$.

The fecal SCFAs profiles as shown in Table 3 reveal that the consumption of HF diet result in a significant ($p < 0.05$) reduction in the level of total SCFAs by 27.5% (193.1 μmol/g) as compared with NC diet (266.5 μmol/g). The results agreed with the observations made by Jakobsdottir et al. [26] that high-fat dietary pattern would lead to a reduced production of fecal SCFAs. Table 3 demonstrates the capability of *B. coagulans* BACO-17 in restoring the low efficiency of SCFAs production induced by high-fat consumption. The administration of *B. coagulans* BACO-17 in both BCL and BCH groups was capable of enhancing the production of fecal SCFAs concentration back to the level comparable with the normal control. These metabolic by-products from carbohydrate fermentation provide energy to gut epithelium cells and strengthen intestinal integrity [27,28]. To be specific, drastic ($p < 0.05$) elevations of both acetic and butyric acid levels were observed in the BCH group (1.5-fold and 1.4-fold, respectively) versus the HF group. The findings from Aoki et al. [29] demonstrated that fecal acetic acid level negatively correlated with the abundance of visceral fat and adipocyte size. Yadav et al. [22] also suggested that the increase of fecal butyrate concentration could stimulate the release of hormone GLP-1 and subsequently suppress appetite and reduce food intake. Furthermore, these increased levels of fecal SCFAs which could trigger peristaltic reflex and shorten intestinal transit time [30] might partly explain the higher fecal moisture content in the *B. coagulans* BACO-17-fed groups via impeded water reabsorption in gut.

Table 3. Changes in the concentrations of fecal short chain fatty acids among different groups.

SCFAs	NC	HF	BCL	BCH
Total SCFAs (μmol/g)	266.5 ± 28.9 [a]	193.1 ± 37.5 [b]	266.3 ± 29.9 [a]	274.0 ± 38.6 [a]
Acetic acid (μmol/g)	186.5 ± 26.2 [a]	119.2 ± 41.4 [b]	177.2 ± 33.0 [a]	182.0 ± 32.2 [a]
Propanoic acid (μmol/g)	59.0 ± 21.0	40.6 ± 16.9	43.4 ± 18.6	44.0 ± 7.7
Butyric acid (μmol/g)	21.1 ± 3.9 [c]	33.4± 12.8 [b]	45.6 ± 6.7 [a]	48.0 ± 7.9 [a]

[a–c.] Values (mean ± SD, n = 5) with different superscripts in the same row are significantly different, $p < 0.05$. NC: normal control; HF: high-fat control; BCL: high-fat diet + low dose of *B. coagulan* BACO-17 (7 log CFU/day); BCH: high-fat diet + high dose of *B. coagulan* BACO-17 (9 log CFU/day).

In Table 3, a negative relationship between elevated fecal SCFA concentrations and decreased visceral fat weights was observed. Literature by Yadav et al. [22] suggested that the administration of probiotic could modulate the gut microbiota-SCFA-hormone axis and subsequently help treat obesity in mice. Earlier works from Kimura et al. [31,32] also reported that the SCFAs produced in the gut lumen might elevate the metabolism of unincorporated lipids through the SCFA-mediated sympathetic activation, repress insulin signaling in adipocytes, and suppress fat accumulation in adipose tissue. It was therefore inferred that the apparent SCFA production of fecal SCFAs by *B. coagulans* BACO-17 might be one of the contributing factors to ameliorate the accumulation of visceral fat and possibly body weight gain in rats.

3.4. Profile of Gut Microbiota

To investigate the influence of *Bacillus coagulans* BACO-17 on gut microbiota dysbiosis in rats fed high-fat diet, fecal samples collected from all groups were analyzed by 16S rRNA sequencing. In Figure 2a,b, *Firmicutes* and *Bacteroidetes* were the most predominant phyla among all groups, followed by *Actinobacteria*, *Verrucomicrobia* and *Proteobacteria* which contributed comparably smaller proportions to the intestinal community profile. Our study showed that high-fat diet caused an apparent ($p < 0.05$) variation in *Firmicutes*:*Bacteroidetes* ratio ranging from 1.13 to 3.14 (Figure 3). The higher ratio of *Firmicutes* to *Bacteroidetes* is commonly recognized to be associated with obesity, but recently remains controversial due to some opposite results reported by others [33]. Undoubtedly, variations in genders, age, gene, environment, dietary, lifestyle, and specific populations might play important roles in the association between *Firmicutes*:*Bacteroidetes* ratio and obesity [33].

Linear discriminant analysis score (LDA score) and cladogram of bacterial clades have identified that *Ruminococcaceae* (belonging to *Firmicutes*) was considered to be the dominated family in HF group, whereas *Muribaculaceae* (belonging to *Bacteroidetes*) was the key microbiome in NC group (Figure 4a,b). More precisely, the abundance of *Ruminococcaceae* was found to have a more marked elevation at the expansion of *Muribaculaceae* in response to high-fat feeding (Figure 4c). The bacteria of *Firmicutes* phylum were in general more relevant to the development of obesity than those in *Bacteroidetes* phylum due to their better capacity for fermenting and degrading non-absorbed carbohydrates in gastrointestinal tract. Thus, it was therefore believed that the energy deposition from ingested foods occurred more easily, resulting in a higher chance of gaining weight [33]. Remarkably, a species, *Lactobacillus reuteri*, was highlighted in the HF group (Figure 4a), implying that high-fat consumption might be positively correlated to the abundance of this bacterium. The first evidence concerning the association between *Lactobacillus* and obesity in humans was reported by Million et al. [34], indicating that *L. reuteri* from *Lactobacillus* genus was mainly associated with the development of obesity.

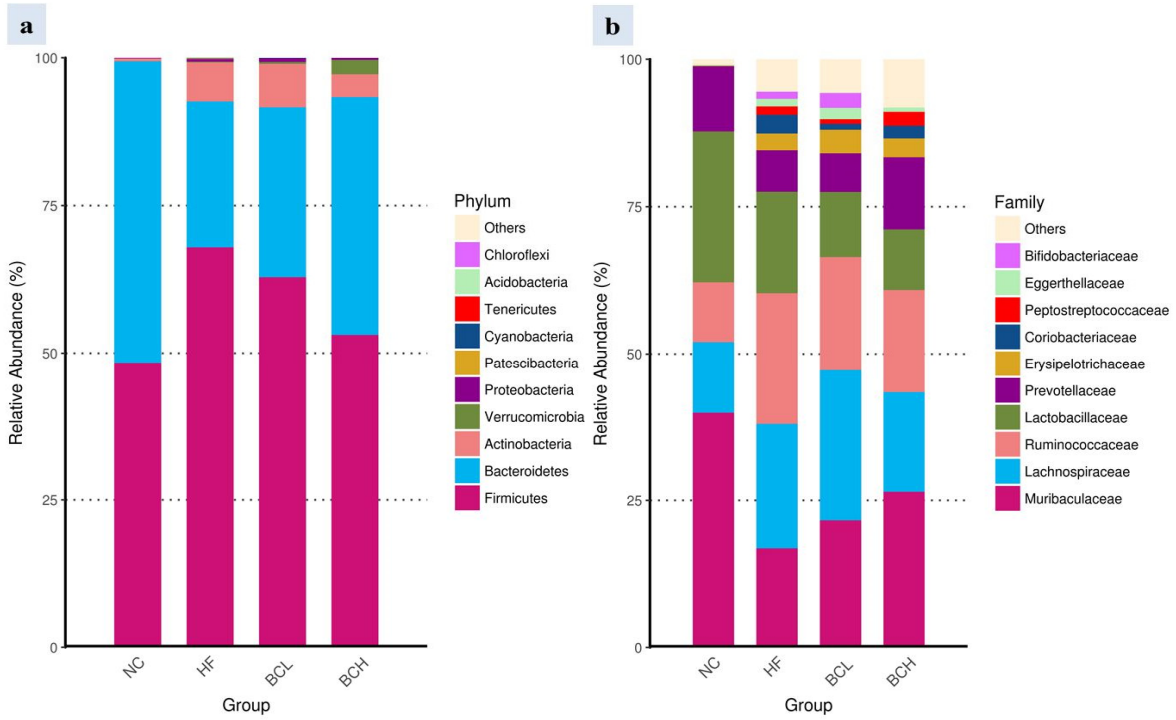

Figure 2. Analysis of bacterial composition in gut microbiota within rats fed chow diet (NC group), high-fat diet (HF group), high-fat diet + low dose of *B. coagulan* BACO-17 (7 log CFU/day) (BCL group), and high-fat diet + high dose of *B. coagulan* BACO-17 (9 log CFU/day) (BCH group) (n = 5 per group). The top 10 relative abundance of the bacterial taxon shown at the level of (**a**) phylum and (**b**) family.

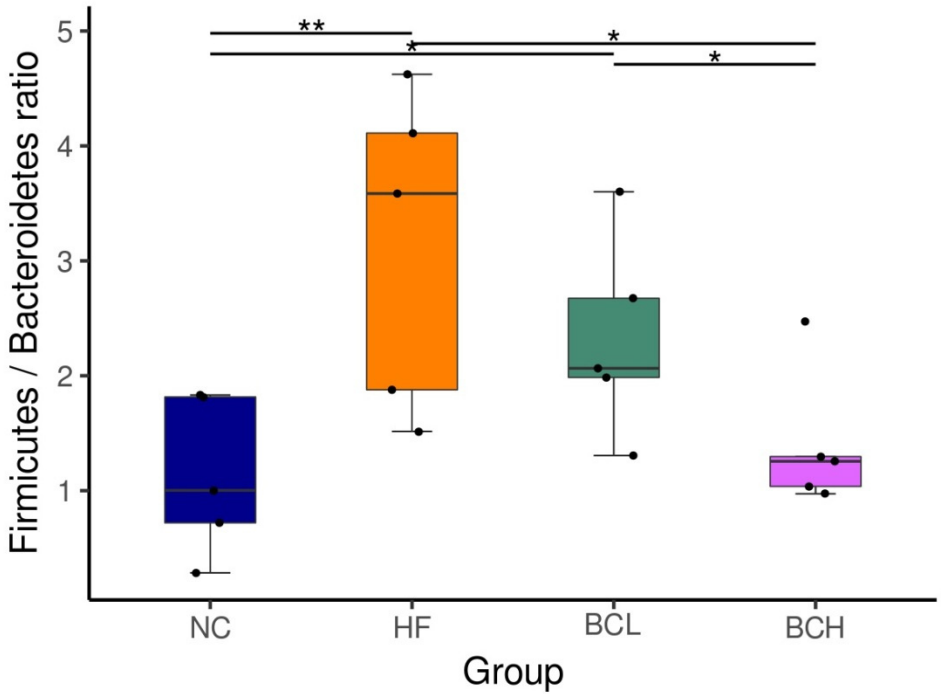

Figure 3. The ratio of *Firmicutes* to *Bacteroidetes* within rats fed chow diet (NC group), high-fat diet (HF group), high-fat diet + low dose of *B. coagulan* BACO-17 (7 log CFU/day) (BCL group), and high-fat diet + high dose of *B. coagulan* BACO-17 (9 log CFU/day) (BCH group) (n = 5 per group). Levels of statistical significance are denoted as follows: *, $p < 0.05$, and **, $p < 0.01$.

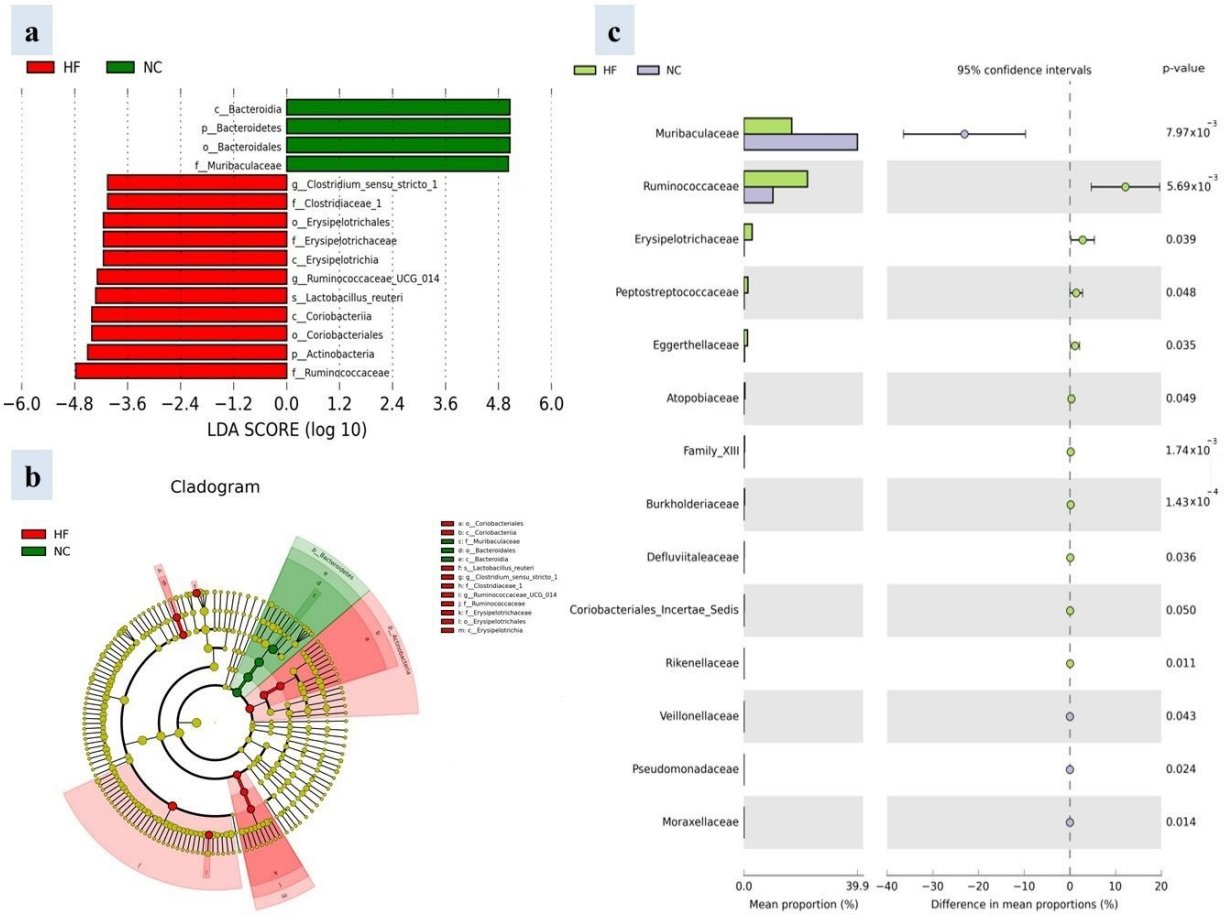

Figure 4. (**a**) Linear discriminant analysis effect size analysis (LEfSe) with linear discriminant analysis (LDA) score > 4 between normal control (NC) and high-fat control (HF) groups. (**b**) Cladogram of gut microbiota between NC and HF groups. (**c**) STAMP analysis of bacterial taxon shown at family level between NC and HF groups. The value of $p < 0.05$ was considered significant ($n = 5$ per group).

Figure 5 illustrates that some specific genera were significantly ($p < 0.05$) enriched after high-fat intake. These genera included *Ruminococcaceae UCG-014*, *Oscillibacter* and *Ruminiclostridium 9* (belonging to *Ruminococcaceae* family), *Prevotellaceae UCG-001* (belonging to *Prevotellaceae* family), *Allobaculum* (belonging to *Erysipelotrichaceae* family), *Romboutsia* (belonging to *Peptostreptococcaceae* family), and *Lachnospiraceae UCG-006*, *Roseburia* (belonging to *Lachnospiraceae* family), and others. *Ruminococcaceae* and *Lachnospiraceae* from *Firmicutes* phylum, which indigenously inhabited in cecum and colon, could ferment and cleavage the indigestive polysaccharides into SCFAs, especially butyrate. It might perhaps partly explain the relatively higher levels of fecal butyric acid in high-fat diet groups, as compared with NC group (Table 3). The relatively higher fecal butyric acid levels in BCL and BCH groups might be attributed to the ability of *B. coagulans* to produce butyric acid [10,20]. A previous study revealed that mice with obesity and diabetic leptin-resistant had a higher abundance of *Ruminococcaceae* than in lean mice [35]. Another study [36] also showed that high-fat feeding rendered an increase in the proportion of *Ruminococcaceae* and *Lachnospiraceae* compared with chow diet group.

A relative larger proportion of *Firmicutes* family *Erysipelotrichaceae*, which were implicated in obesity and metabolic disorder [36], was detected in HF group, while these bacteria were considered to be negligible in NC group (Figure 4c). Zheng et al. [37] reported that high fat intake drove a taxonomic shift in gut community, especially *Allobaculum* in *Erysipelotrichaceae* family. The abundance of this bacterium was positively correlated with the production of angiopoietin-like protein 4 (ANGPTL4). This protein was found able

to avoid excessive fatty acid uptake and lipid accumulation via the inhibition of lipoprotein lipase. This might thus explain the phenomenon that a relative higher proportion of *Allobaculum* genus was observed in rats fed with HF diet (Figure 5). Similarly, Fleissner and co-workers also found an increased abundance of *Erysipelotrichaceae* in the fecal samples from rats fed a high-fat diet or western-style diet [38].

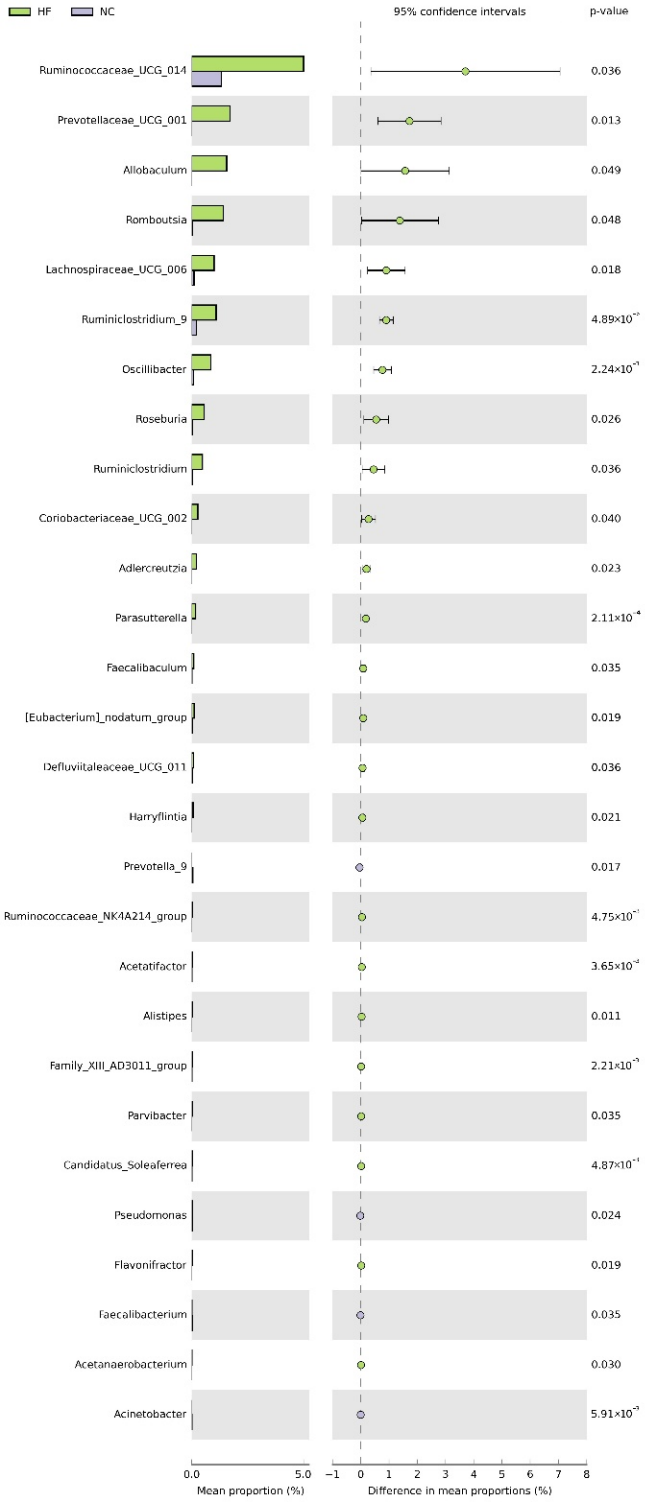

Figure 5. STAMP analysis of bacterial taxon shown at the genus level between normal control (NC) and high-fat control (HF) groups. The value of $p < 0.05$ was considered significant ($n = 5$ per group).

The richness and diversity of a bacterial community are usually measured using alpha diversity analysis. In this study, alpha diversity metrics including ACE and chao1 index were adapted for estimating the total number of species in a community, while the indices of shannon and simpson were used for measuring microbial diversity in a sample. Several studies concerning the diet-induced dysbiosis of gut microbiota have reported a phenomenon that high-fat consumption would lead to a decrease of bacterial diversity in mice models [39,40]. However, despite high-fat consumption, four alpha-diversity indices in the HF group, on the contrary, revealed a higher richness and diversity of microbial community relative to NC group (Table 4). The alpha-diversity indices of ACE, chao1, shannon, and simpson increased from 257.73, 254.47, 3.97, and 0.84 to 354.43, 350.19, 5.80, and 0.96, respectively. These results were consistent with the findings reported by Lecomte and colleagues, indicating that the alpha diversity of gut microbiota would be increased by high-fat intake [36]. Despite having a high-fat diet, our results demonstrated that the supplementation of *B. coagulans* BACO-17 was able to reverse the richness of microbial community back to the condition comparable to the NC group, but the microbial diversity in BCH group seemed to remain unaltered at higher microbial diversity.

Table 4. The alpha diversity among different groups.

	NC	HF	BCL	BCH
ACE index	257.73 ± 25.75^{a}	345.43 ± 21.87^{b}	353.30 ± 16.49^{b}	272.65 ± 45.43^{a}
Chao1 index	254.47 ± 25.49^{a}	350.19 ± 24.12^{b}	354.30 ± 19.33^{b}	274.27 ± 45.61^{a}
Shannon index	3.97 ± 0.97^{a}	5.80 ± 0.28^{bc}	6.06 ± 0.35^{c}	5.09 ± 0.35^{b}
Simpson index	0.84 ± 0.11^{a}	0.96 ± 0.01^{b}	0.97 ± 0.02^{b}	0.94 ± 0.01^{b}

[a–c.] Values (mean ± SD, $n = 5$) with different superscripts in the same row are significantly different, $p < 0.05$. NC: normal control; HF: high-fat control; BCL: high-fat diet + low dose of *B. coagulan* BACO-17 (7 log CFU/day); BCH: high-fat diet + high dose of *B. coagulan* BACO-17 (9 log CFU/day).

Principal coordinate analysis (PCoA) based on unweighted Unifrac distance was conducted to analyze community diversity among all animal groups. As illustrated in Figure 6, the clustered samples of each group are roughly separated into two parts (PC1 = 40.8%, PC2 = 13.6%). An obvious discrepancy in the microbial community was observed between each taxon of NC and HF groups, suggesting that the composition of gut microbiota was altered by high-fat consumption. Although a larger distance between the NC and HF groups was generated, the probiotic intervention could revert the bacterial community of the BCH group back to a similar pattern as that of the NC group.

It was noteworthy that the supplementation of high dose of *B. coagulans* BACO-17 (9 log CFU/day) effectively ($p < 0.05$) reduced the *Firmicutes*:*Bacteroidetes* ratio as compared with the HF group, ranging from 3.14 to 1.41 (Figure 3). More specifically, an intervention with *B. coagulans* BACO-17 at high dose enhanced the level of *Bacteroidetes* family *Muribaculaceae* (Figure 7), which were found more abundant in anti-obesity mice than in obese mice as reported by Cao and colleagues [41]. We also found that the proportion of *Akkermansia* was lower in rats from all groups, except in the BCH group (Figure 7). It was supposed that dietary probiotic supplementation of *B. coagulans* BACO-17 at a high dose might promote the growth of *Akkermansia* in rats during the ingestion of high-fat diet. The bacteria of *Akkermansia* family, particularly in *Akkermansia muciniphila*, seemed to be inversely linked to the plasma level of fasting glucose, visceral fat deposition, and the diameter of subcutaneous adipocyte in human suffering obesity [42]. On the other hand, the supplementation of adequate amount of *B. coagulans* BACO-17 might be in favor of reducing the abundance of *Allobaculum* (Figure 8a), thus facilitating the adipolysis and alleviating fat accumulation in adipose tissue. Furthermore, a downward trend in the proportion of *L. reuteri* was observed in BCL and BCH groups (Figure 8b). It could be speculated that the ingestion of *B. coagulans* BACO-17 could bring down the population of *L. reuteri*, which was believed to be associated with obesity [34]. Taken together, it was believed that the intervention of *B. coagulans* BACO-17 at high dose had a potential to recover the composition of gut microbiota disrupted by high-fat feeding.

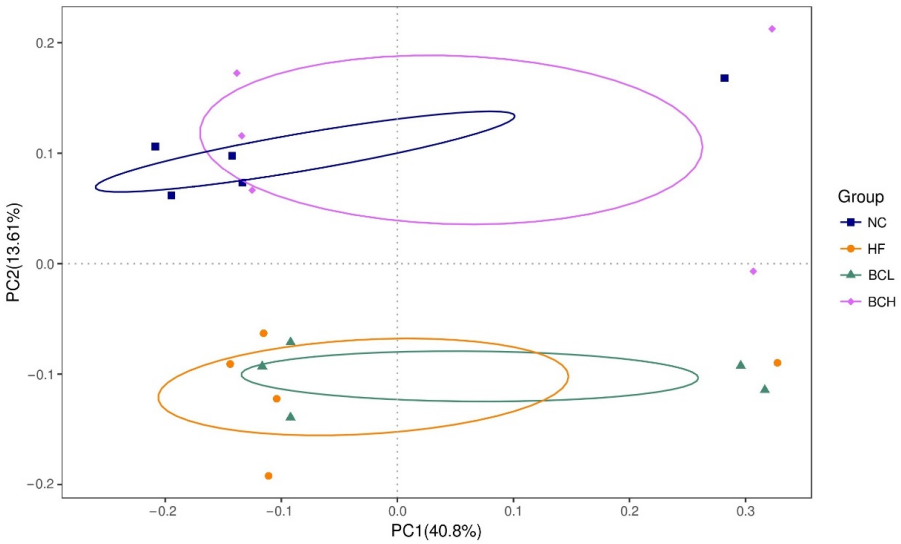

Figure 6. Beta diversity of gut microbiota within rats fed chow diet (NC group), high-fat diet (HF group), high-fat diet + low dose of *B. coagulan* BACO-17 (7 log CFU/day) (BCL group), and high-fat diet + high dose of *B. coagulan* BACO-17 (9 log CFU/day) (BCH group) (n = 5 per group). Principal coordinate analysis (PCoA) plotted for the measurement of similarity of microbial community among all groups.

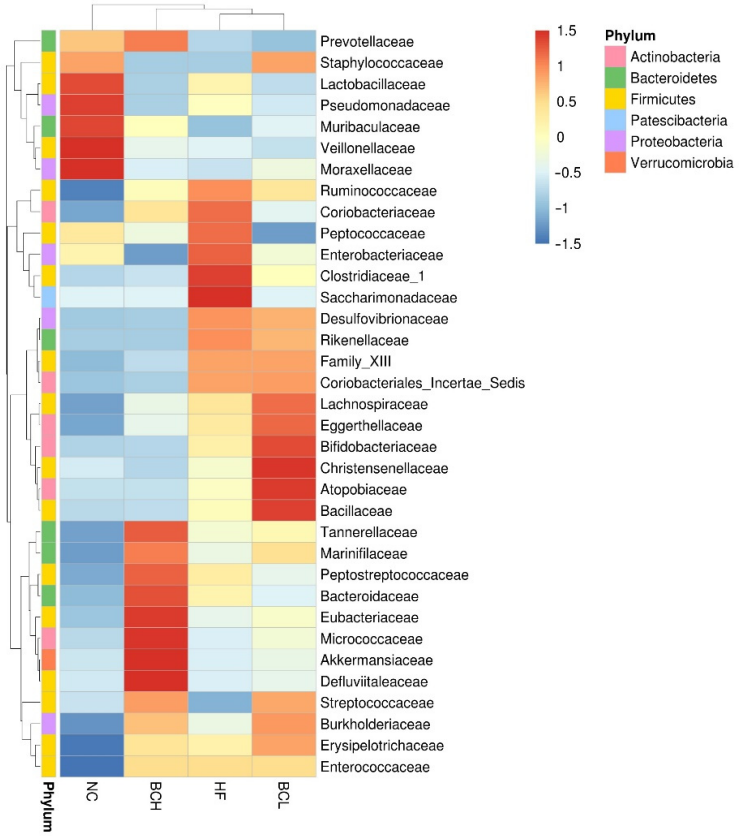

Figure 7. Heatmap analysis for the top 35 relative abundances of bacterial family in fecal samples of rats fed chow diet (NC group), high-fat diet (HF group), high-fat diet + low dose of *B. coagulan* BACO-17 (7 log CFU/day) (BCL group), and high-fat diet + high dose of *B. coagulan* BACO-17 (9 log CFU/day) (BCH group) (n = 5 per group).

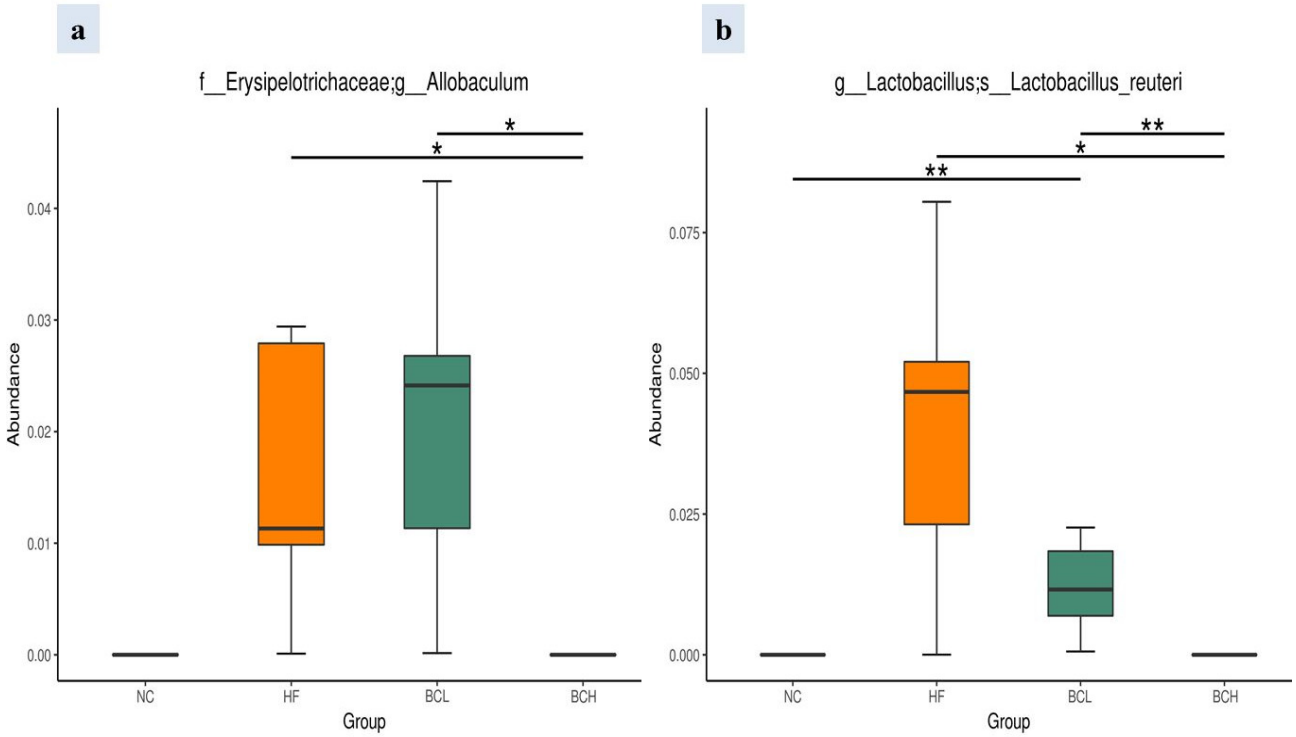

Figure 8. Identifications of the abundance of (**a**) *Allobaculum* genus and (**b**) *Lactobacillus reuteri* in fecal samples of rats fed chow diet (NC group), high-fat diet (HF group), high-fat diet + low dose of *B. coagulan* BACO-17 (7 log CFU/day) (BCL group), and high-fat diet + high dose of *B. coagulan* BACO-17 (9 log CFU/day) (BCH group) by metagenomeSeq analysis. *, $p < 0.05$ and **, $p < 0.01$ ($n = 5$ per group).

4. Conclusions

In conclusion, high-fat diets induced higher levels of weight gain and fat accumulation in rats. Compared with high-fat control, a supplementation of *B.* coa*gulans* BACO-17 at high dose (9 log CFU/day) was capable of attenuating weight gain and accumulation of visceral fat and liver lipids via reduced food intake. It could also counteract some undesirable impacts in fecal parameters by rendering an increase in fecal moisture and fecal fat output as well as restoring fecal total SCFA level. High-fat feeding shaped the structure of gut microbiota into a deteriorated bacteria profile via increasing *Firmicutes* and decreasing *Bacteroidetes*, particularly in *Ruminococcaceae* and *Muribaculaceae*, respectively. The supplementation of *B. coagulans* BACO-17 at high dose was also found capable of regulating the obesity-associated microbiomes to an appropriate level, especially *Muribaculaceae* and *Allobaculum*. The present study suggested that the supplementation of *B. coagulans* BACO-17 as a probiotic could be a potential way to retard body fat accumulation and also to promote intestinal health in individuals having a high-fat dietary pattern, but clinical trials are needed to verify the effect.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr10122692/s1, Figure S1: (A) Agarose gel electrophoresis of the amplified target of 16S rRNA gene from *Bacillus coagulans* BACO-17, and (B) the primers used for 16S rRNA gene analysis; Figure S2: Bacterial identification of *Bacillus coagulans* BACO-17. (A) Partial sequence of 16S rRNA gene, and (B) top listed BLAST results; Figure S3. Visceral fat mass (A) perirenal fat, (B) mesenteric fat, and (C) epididymal fat among different groups; Table S1: Composition of experimental diets [43].

Author Contributions: Conceptualization, C.-F.C., C.-Y.S. and W.-J.C.; methodology, C.-F.C., C.-Y.S., Z.C., H.-F.C. and C.-Y.L.; formal analysis, C.-Y.S., Z.C., H.-F.C., D.-K.L. and C.-Y.L.; data curation, Z.C., C.-Y.S., Y.-C.W., H.-F.C., C.-Y.L., D.-K.L. and A.-L.T.; writing-original draft preparation, Z.C., C.-Y.S., Y.-C.W., C.-F.C., C.-Y.L. and A.-L.T.; writing-review and editing, C.-F.C., C.-Y.S., Z.C., Y.-C.W. and H.-F.C.; supervision, C.-F.C.; project administration, C.-F.C. and W.-J.C.; funding acquisition, C.-F.C. and W.-J.C. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

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Conflicts of Interest: All contributing authors declare no conflict of interest.

References

1. Obesity and Overweight. Available online: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (accessed on 8 December 2022).
2. Apovian, C.M.; Aronne, L.J.; Bessesen, D.H.; McDonnell, M.E.; Murad, M.H.; Pagotto, U.; Ryan, D.H.; Still, C.D. Pharmacological management of obesity: An endocrine society clinical practice guideline. *J. Clin. Endocrinol. Metab.* **2015**, *100*, 342–362. [CrossRef] [PubMed]
3. Sun, N.N.; Wu, T.Y.; Chau, C.F. Natural Dietary and Herbal Products in Anti-Obesity Treatment. *Molecules* **2016**, *21*, 1351. [CrossRef] [PubMed]
4. Zhao, R.; Long, X.; Yang, J.; Du, L.; Zhang, X.; Li, J.; Hou, C. Pomegranate peel polyphenols reduce chronic low-grade inflammatory responses by modulating gut microbiota and decreasing colonic tissue damage in rats fed a high-fat diet. *Food Funct.* **2019**, *10*, 8273–8285. [CrossRef]
5. Liu, H.Y.; Walden, T.B.; Ahl, D.; Nyman, M.; Bertilsson, S.; Phillipson, M.; Holm, L. High-fat diet enriched with bilberry modifies colonic mucus dynamics and restores marked alterations of gut microbiome in rats. *Mol. Nutr. Food Res.* **2019**, *63*, 1–13. [CrossRef] [PubMed]
6. Carding, S.; Verbeke, K.; Vipond, D.T.; Corfe, B.M.; Owen, L.J. Dysbiosis of the gut microbiota in disease. *Microb. Ecol. Health Dis.* **2015**, *26*, 1–9. [CrossRef]
7. Gerritsen, J.; Smidt, H.; Rijkers, G.T.; de Vos, W.M. Intestinal microbiota in human health and disease: The impact of probiotics. *Genes Nutr.* **2011**, *6*, 209–240. [CrossRef]
8. Azad, M.A.K.; Sarker, M.; Li, T.; Yin, J. Probiotic species in the modulation of gut microbiota: An overview. *BioMed Res. Int.* **2018**, *2018*, 1–8. [CrossRef]
9. Konuray, G.; Erginkaya, Z. Potential use of *Bacillus coagulans* in the food industry. *Foods* **2018**, *7*, 92. [CrossRef]
10. Saw, C.Y.; Chang, T.J.; Chen, P.Y.; Dai, F.J.; Lau, Y.Q.; Chen, T.Y.; Chau, C.F. Presence of *Bacillus coagulans* spores and vegetative cells in rat intestine and feces and their physiological effects. *Biosci. Biotechno. Biochem.* **2019**, *83*, 2327–2333. [CrossRef]
11. He, J.; Zhang, P.; Shen, L.; Niu, L.; Tan, Y.; Chen, L.; Zhao, Y.; Bai, L.; Hao, X.; Li, X.; et al. Short-chain fatty acids and their association with signaling pathways in inflammation, glucose and lipid metabolism. *Int. J. Mol. Sci.* **2020**, *21*, 6356. [CrossRef]
12. Haldar, L.; Gandhi, D.N. Effect of oral administration of *Bacillus coagulans* B37 and *Bacillus pumilus B9* strains on fecal coliforms, *Lactobacillus* and *Bacillus* spp. in rat animal model. *Vet. World* **2016**, *9*, 766–772. [CrossRef] [PubMed]
13. Abhari, K.; Saadati, S.; Yari, Z.; Hosseini, H.; Hedayati, M.; Abhari, S.; Alavian, S.M.; Hekmatdoost, A. The effects of *Bacillus coagulans* supplementation in patients with non-alcoholic fatty liver disease: A randomized, placebo-controlled, clinical trial. *Clin. Nutr. ESPEN* **2020**, *39*, 53–60. [CrossRef] [PubMed]
14. Sudha, M.R.; Jayanthi, N.; Aasin, M.; Dhanashri, R.D.; Anirudh, T. Efficacy of *Bacillus coagulans* Unique IS2 in treatment of irritable bowel syndrome in children: A double blind, randomised placebo controlled study. *Benef. Microbes* **2018**, *9*, 563–572. [CrossRef] [PubMed]
15. Cao, J.; Yu, Z.; Liu, W.; Zhao, J.; Zhang, H.; Zhai, Q.; Chen, W. Probiotic characteristics of *Bacillus coagulans* and associated implications for human health and diseases. *J. Funct. Foods* **2020**, *64*, 1–11. [CrossRef]

16. Campana, R.; Van Hemert, S.; Baffone, W. Strain-specific probiotic properties of lactic acid bacteria and their interference with human intestinal pathogens invasion. *Gut Pathog.* **2017**, *9*, 1–12. [CrossRef] [PubMed]
17. Kao, W.M.; Chang, C.R.; Chang, T.J.; Li, S.Y.; Chen, W.J.; Chau, C.F. Inclusion of fructooligosaccharide and resistant maltodextrin in high fat diets promotes simultaneous improvements on body fat reduction and fecal parameters. *Molecules* **2018**, *23*, 2169. [CrossRef]
18. Hsieh, C.E.; You, F.N.; Dai, F.J.; Tung, Y.C.; Chen, W.J.; Chu, H.F.; Wu, S.H.; Chau, C.F. *Bacillus coagulans* BC198 and *Lactobacillus paracasei* S38 in combination reduce body fat accumulation and modulate gut microbiota. *CyTA–J. Food* **2020**, *18*, 764–775. [CrossRef]
19. Magoč, T.; Salzberg, S.L. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **2011**, *27*, 2957–2963. [CrossRef]
20. Wu, M.Z.; Sun, T.C.; Huang, Y.W.; Wu, Y.C.; Chen, W.J.; Chu, H.F.; Liu, C.Y.; Chau, C.F. *Bacillus coagulans* BACO-17 alone or in combination with galacto-oligosaccharide ameliorates *Salmonella*-induced diarrhea and intestinal inflammation. *Processes* **2022**, *10*, 2123. [CrossRef]
21. Macdiarmid, J.I.; Blundell, J.E. Dietary under-reporting: What people say about recording their food intake. *Eur. J. Clin. Nutr.* **1997**, *51*, 199–200. [CrossRef]
22. Yadav, H.; Lee, J.H.; Lloyd, J.; Walter, P.; Rane, S.G. Beneficial metabolic effects of a probiotic via butyrate-induced GLP-1 hormone secretion. *J. Biol. Chem.* **2013**, *288*, 25088–25097. [CrossRef] [PubMed]
23. Álvarez-Arraño, V.; Martín-Peláez, S. Effects of Probiotics and Synbiotics on Weight Loss in Subjects with Overweight or Obesity: A Systematic Review. *Nutrients* **2021**, *13*, 3627. [CrossRef] [PubMed]
24. Cao, G.T.; Dai, B.; Yan, Y.; Xu, Y.L.; Wang, Y.X.; Yang, C.M. *Bacillus licheniformis*, a potential probiotic, inhibits obesity by modulating colonic microflora in C57BL/6J mice model. *J. Appl. Microbiol.* **2019**, *127*, 1–9. [CrossRef] [PubMed]
25. Jun, S.C.; Jung, E.Y.; Kang, D.H.; Kim, J.M.; Chang, U.J.; Suh, H.J. Vitamin C increases the fecal fat excretion by chitosan in guinea-pigs, thereby reducing body weight gain. *Phytother. Res.* **2010**, *24*, 1234–1241. [CrossRef] [PubMed]
26. Jakobsdottir, G.; Xu, J.; Molin, G.; Ahrne, S.; Nyman, M. High-fat diet reduces the formation of butyrate, but increases succinate, inflammation, liver fat and cholesterol in rats, while dietary fibre counteracts these effects. *PLoS ONE* **2013**, *8*, e80476. [CrossRef]
27. Chávez-Carbajal, A.; Nirmalkar, K.; Pérez-Lizaur, A.; Hernández-Quiroz, F.; Ramírez-del-Alto, S.; García-Mena, J.; Hernández-Guerrero, C. Gut microbiota and predicted metabolic pathways in a sample of Mexican women affected by obesity and obesity plus metabolic syndrome. *Int. J. Mol. Sci.* **2019**, *20*, 438. [CrossRef]
28. Vacca, M.; Celano, G.; Calabrese, F.M.; Portincasa, P.; Gobbetti, M.; De Angelis, M. The controversial role of human gut Lachnospiraceae. *Microorganisms* **2020**, *8*, 573. [CrossRef]
29. Aoki, R.; Kamikado, K.; Suda, W.; Takii, H.; Mikami, Y.; Suganuma, N.; Hattori, M.; Koga, Y. A proliferative probiotic *Bifidobacterium* strain in the gut ameliorates progression of metabolic disorders via microbiota modulation and acetate elevation. *Sci. Rep.* **2017**, *7*, 1–10. [CrossRef]
30. Grider, J.R.; Piland, B.E. The peristaltic reflex induced by short-chain fatty acids is mediated by sequential release of 5-HT and neuronal CGRP but not BDNF. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2007**, *292*, 429–437. [CrossRef]
31. Kimura, I.; Inoue, D.; Maeda, T.; Hara, T.; Ichimura, A.; Miyauchi, S.; Kobayashi, M.; Hirasawa, A.; Tsujimoto, G. Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 8030–8035. [CrossRef]
32. Kimura, I.; Ozawa, K.; Inoue, D.; Imamura, T.; Kimura, K.; Maeda, T.; Terasawa, K.; Kashihara, D.; Hirano, K.; Tani, T.; et al. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat. Commun.* **2013**, *4*, 1–12. [CrossRef] [PubMed]
33. Stojanov, S.; Berlec, A.; Štrukelj, B. The influence of probiotics on the *Firmicutes*/*Bacteroidetes* ratio in the treatment of obesity and inflammatory bowel disease. *Microorganisms* **2020**, *8*, 1715. [CrossRef] [PubMed]
34. Million, M.; Maraninchi, M.; Henry, M.; Armougom, F.; Richet, H.; Carrieri, P.; Valero, R.; Raccah, D.; Vialettes, B.; Raoult, D. Obesity-associated gut microbiota is enriched in *Lactobacillus reuteri* and depleted in *Bifidobacterium animalis* and *Methanobrevibacter smithii*. *Int. J. Obes.* **2012**, *36*, 817–825. [CrossRef] [PubMed]
35. Geurts, L.; Lazarevic, V.; Derrien, M.; Everard, A.; Van Roye, M.; Knauf, C.; Cani, P.D. Altered gut microbiota and endocannabinoid system tone in obese and diabetic leptin-resistant mice: Impact on apelin regulation in adipose tissue. *Front. Microbiol.* **2011**, *2*, 149. [CrossRef]
36. Lecomte, V.; Kaakoush, N.O.; Maloney, C.A.; Raipuria, M.; Huinao, K.D.; Mitchell, H.M.; Morris, M.J. Changes in gut microbiota in rats fed a high fat diet correlate with obesity-associated metabolic parameters. *PLoS ONE* **2015**, *10*, e0126931. [CrossRef]
37. Zheng, Z.; Lyu, W.; Ren, Y.; Li, X.; Zhao, S.; Yang, H.; Xiao, Y. *Allobaculum* involves in the modulation of intestinal ANGPTLT4 expression in mice treated by high-fat diet. *Front. Nutr.* **2021**, *8*, 242. [CrossRef]
38. Fleissner, C.K.; Huebel, N.; Abd El-Bary, M.M.; Loh, G.; Klaus, S.; Blaut, M. Absence of intestinal microbiota does not protect mice from diet-induced obesity. *Br. J. Nutr.* **2010**, *104*, 919–929. [CrossRef]
39. Tang, W.; Pan, L.; Cheng, J.; Wang, X.; Zheng, L.; Wang, S.; Wang, H. High-fat-diet-induced gut microbiome changes in mice. *Stress Brain* **2022**, *2*, 17. [CrossRef]
40. Dalby, M.J.; Ross, A.W.; Walker, A.W.; Morgan, P.J. Dietary uncoupling of gut microbiota and energy harvesting from obesity and glucose tolerance in mice. *Cell Rep.* **2017**, *21*, 1521–1533. [CrossRef]

41. Cao, W.; Chin, Y.; Chen, X.; Mi, Y.; Xue, C.; Wang, Y.; Tang, Q. The role of gut microbiota in the resistance to obesity in mice fed a high fat diet. *Int. J. Food Sci. Nutr.* **2020**, *71*, 453–463. [CrossRef]
42. Dao, M.C.; Everard, A.; Aron-Wisnewsky, J.; Sokolovska, N.; Prifti, E.; Verger, E.O.; Kayser, B.; Levenez, F.; Chilloux, J.; Hoyles, L.; et al. *Akkermansia muciniphila* and improved metabolic health during a dietary intervention in obesity: Relationship with gut microbiome richness and ecology. *Gut* **2016**, *65*, 426–436. [CrossRef] [PubMed]
43. Weisburg, W.G.; Barns, S.M.; Pelletier, D.A.; Lane, D.J. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **1991**, *173*, 697–703. [CrossRef] [PubMed]