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# The Effects of *Rhodobacter sphaeroides* on the Composition of Gut Microbiota and Short-chain Fatty Acids in Mice

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**Abstract** This study aims to determine the effects of *Rhodobacter sphaeroides* 8513 on the physiological and biochemical indicators, short-chain fatty acids, and gut microbiota of healthy BALB/c mice. Mice were divided into the control group and *R. sphaeroides* group ($5\times10^8$ CFU/mL). After 28 days of continuous gavage, fecal samples were collected for high-throughput sequencing, then mice were sacrificed and the serum was taken for biochemical analysis. Liver, kidney, and spleen were obtained for organ coefficient calculation, and cecum contents were collected for short-chain fatty acid analysis by gas chromatography. The results showed that there was no significant difference in body weight gain, alanine aminotransferase (ALT) and urea nitrogen (BUN) levels between the control group and *R. sphaeroides* group, while *R. sphaeroides* significantly decreased the aspartate aminotransferase (AST) and creatinine (CRE) level. No side effect of *R. sphaeroides* treatment on the basic physiological health of mice was observed. *R. sphaeroides* significantly increased the content of acetic acids in the gut of mice, while there were no significant changes in the concentration of butyric and propionic acid. High-throughput sequencing analysis indicated that there was a significant difference in α-diversity between these 2 groups, while there was no significant difference in β-diversity between them. *R. sphaeroides* not only increased the abundance of anaerobic bacteria of *Rhodospirillaceae*, *Desulfovibrionaceae*, and *Helicobacter* in the gut of mice, but also increased the abundance of the predominant microflora in the gut such as *Bacteroidaceae* (*Bacteroides*), *Prevotellaceae*, *Clostridiales_vadinBB60_group* (*Closporaceae*). These results suggested that *R. sphaeroides* 8513 could modulate the microbial ecology in the gut of mice.

***Keywords:*** *Rhodobacter sphaeroides, gut microbiota, short-chain fatty acids, high-throughput sequencing, mice*

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## 1. Introduction

The physiological role of gastrointestinal ecology has become an important subject in the field of nutrition research in recent years [1,2,3]. The gastrointestinal tract is home to a diverse microbiota of about $10^{14}$ bacteria in a symbiotic manner, which plays an essential role in host physiology and metabolic health [4,5,6]. In addition to maintaining the integrity of the intestinal immune barrier and performing essential catabolic and biotransformation functions, the gut microbiota also produce small bioactive molecules that mediate interactions with the host and contribute fundamentally to overall health [6,7,8]. An individual's microbiota composition could be influenced by their maternal genetics and environmental factors such as diet, lifestyle including physical exercise and exposure to antibiotics [4,9,10]. The dysbiosis of the gut microbiome was associated with several pathologies including chronic metabolic disease [11,12], inflammatory bowel disease [13,14], autoimmune disease [15,16], and cognitive diseases [17,18,19]. Since gut microbiota is closely related to host health and disease, regulating gut microbiota has become an important approach to improve host health.

The administration of probiotics has been reported to be one of the most widely used approaches to

modulate gut microbiota. Probiotics are defined as "live micro-organisms, which when consumed in adequate amounts, confer a health effect on the host" [20]. *Lactobacillus* and *Bifidobacterium* are the most commonly used genera as probiotics. The underlying mechanism may be that probiotics can promote the abundance of beneficial microbiota, resist pathogenic microbiota colonization, produce vitamins, antioxidants and short-chain fatty acids (SCFAs), exert barrier effect by competing for binding sites or inhibition of adhesion, and protect gut epithelial cells and immune cells [11,21].

*Rhodobacter sphaeroides* is a gram-negative, purple nonsulfur photosynthetic bacteria belongs to the α-3 subgroup of *Proteobacteria* [22]. As a safe and non-toxic photosynthetic bacteria, *R. sphaeroides* has been widely studied in its unique biological transformation functions [23,24,25]. Besides, *R. sphaeroides* serves as a new platform for producing bioactive substance including coenzyme Q10, carotenoids, sesquiterpene, fatty acids, and 5- aminolevulinic acid [26,27,28,29,30]. Owing to its excellent adaptability to the aerobic or anaerobic environment and fermentation, or photosynthesis conditions, *R. sphaeroides* has attracted considerable attention in food, breeding, environmental, and pharmaceutical industries [22,24,31,32]. A commercial carotenoid product from the extract of probiotic *R. sphaeroides* mutant strain WLAPD911 has been used to enhance tilapia growth performance via immune regulation [33]. *R. sphaeroides* (CGMCC No. 8513) exhibited significant protective activity against $H_2O_2$-induced oxidative stress in Caco-2 cells [34]. Besides, superfine powder of *R. sphaeroides* was proved to enhance the immunological function of mice and to improve the antioxidant effect of intestinal epithelial cells. These studies were implicit that *R. sphaeroides* may play a beneficial role in gut health. However, few studies have evaluated the effect of *R. sphaeroides* on the gut microbiota. In this study, the BALB/c mice were gastric gavage with *R. sphaeroides* 8513 for 28 successive day, and fecal samples were harvested to test the modulating effect of *R. sphaeroides* on the composition of gut microbiota in mice by high-throughput sequencing. Cecum contents were collected to test short-chain fatty acids by gas chromatography. Besides, the related physiological and biochemical indicators have also been studied.

# 2. Materials and Methods

## 2.1. Bacterial Strains and Cultural Conditions

A mutant strain *R. sphaeroides* 8513 was preserved by the China General Microbiological Culture Collection Center. *R. sphaeroides* 8513 was maintained as frozen (-80 °C) stocks in peptone-yeast extract-glucose medium (10 g/L tryptone, 10 g/L yeast extract, 20 g/L glucose and 7 g/L NaCl, pH 7.2) supplemented with 20% (v/v) glycerol and on nutrient agar (Difco) slopes at 4 °C. It was transferred at least twice consecutively using a 5% (v/v) inoculums in nutrient broth at 32 °C, 180 r/min for 24 h before fermentation. Then it was transferred using a 10% (v/v) inoculums in the fermentative medium (4 g/L sodium malate, 20 g/L glucose, 7 g/L $(NH_4)_2SO_4$, 10 g/L yeast extract, 0.9 g/L $K_2HPO_4$, 0.6 g/L $KH_2PO_4$, 10 mL/L growth factor solution -Vitamin $B_1$ 1.0 g/L, VPP 1.0 g/L, Vitamin H 0.016 g/L, pH 7.0) at 32 °C, 180 r/min for 40 h. The bacteria were harvested by centrifugation at 8000 r/min for 10 min (Eppendorf, German) and washed by sterile phosphate buffered saline (PBS, pH 7.4) twice, finally resuspended with PBS.

## 2.2. Animal Experimental Design

Animals used in this study were cared for in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health, and all experimental procedures were approved by Animal Ethics Committee of China Agricultural University (Beijing, China) with the license number SYXK (Beijing) 2010–0036 under standard laboratory condition. Specific-pathogen-free, male 8-week-old BALB/c mice free from specific pathogens were purchased from Vital River Laboratories Inc. (Beijing, China). After acclimatization for two weeks in controlled conditions of temperature (22 ± 2 °C), relative humidity (40%-60%), and a 12 h light-dark cycle with access to sterile standard normal chow (Beijing Huafukang Bioscience CO. INC., Beijing, China) and water *ad libitum*, animals were randomly divided into 2 groups of 12 mice each. The control group was orally administrated sterile PBS (pH 7.4), while the *R. sphaeroides* group was orally given the *R. sphaeroides* ($5\times10^8$ CFU/mL) suspension at 15 mL/kg body weight once daily over a 28 days' period.

## 2.3. Biochemical and Clinical Assays

The weight of all mice was recorded once a week, and daily behaviors observation was conducted daily throughout the experimental period. Before mice were sacrificed, fresh feces were obtained, and quickly frozen in liquid nitrogen and stored at -80 °C for further analysis. Moreover, Blood was collected for blood chemistry analysis just before euthanization. The liver, kidney, and spleen were excised and weighted.

## 2.4. Fecal DNA Extraction and Sequencing

Genomic DNA was extracted from fecal samples by Kit (Tiangen, China) according to the manufacturer's instructions. Isolated fecal DNA was used as a template for the amplification of the 16S rRNA gene V3-V4 region with universal primer 338F (5'-ACTCCTACGGGAGGCAGC AG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT -3'), which were conjunct with unique 24 pairs base barcode to tag each PCR production. The PCR amplification was performed in duplicate with a reaction volume of 50 μL, which contained 5 μL of 10×KOD-buffer, 5 μL of dNTP mixture (2 mM of each), 2 μL of forward and reverse primer (1 mM of each), and 2 μL of $MgSO_4$(25 mM) 1 μL KOD -Plus-(1.0 U/μL), and 1 μL of template DNA, add water to the total reaction volume of 50 μL. The cycling parameters were as follows: initial denaturation at 95 °C followed by 34 cycles of 94 °C for 30 s, 58 °C for 30 s, 68 °C for 60 s with a final extension at 68 °C for 5 min. The PCR amplification

products were detected by 1% agarose gel (1×TAE) electrophoresis (Bio-Rad, USA) to make sure correctly amplified fragment. The sequencing was performed by a MiSeq platform (Illumina, San Diego, CA, USA) at Majorbio Co., (Shanghai, China). A RDP classifier (version 2.2) was used to conduct taxonomic annotation. Nonmetric multidimensional scaling (NMDS) plots and ANOSIM were applied in analyzing the variation between different groups utilizing the PAST version 3.0 software program. Community structure variance analysis was conducted with LEFSe software using the default parameters (http://huttenhower.sph.harvard.edu/galaxy/).

## 2.5. Analysis of SCFAs in Cecum Contents

Short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate, were determined by gas chromatography (HP7890) with FID detector. A mixed standards of SCFAs containing acetate, propionate and butyrate (Sigma Chemical Co., St. Louis, MO, USA) were used, and 2-Methylpentanoic acid was used as internal standard. 1 μL of sample to be analyzed was injected using a CP-Wax52CB-CP884 capillary column (30 m, 32 mm×0.25 μm) with starting temperature 140 °C for 2 min, then increased to 160 °C by 4 °C/min, lasting for 2 min, then increased to 230 °C by 20 °C/min. The temperature of the detector is 230 °C. The split ratio of carrier gas (high purity nitrogen) and hydrogen are both 40 mL/min, and combustion air 400 mL/min.

# 3. Results

## 3.1. Effect of *R. sphaeroides* on the Growth of Mice

During the experiment period, the health of mice was closely monitored. There were no illnesses, infections or deaths during the *R. sphaeroides* administration. Both the control group and the treatment group were in good condition. The mice were active and in good spirits, and their hair was smooth and shiny. There were no effects on the appetite and water intake of mice. Body weight was recorded once a week throughout the 4-week gavage. The results showed (Figure 1) that there was no significant difference in body weight gain between the two groups, indicating that *R. sphaeroides* treatment did not influence the growth of the mice.

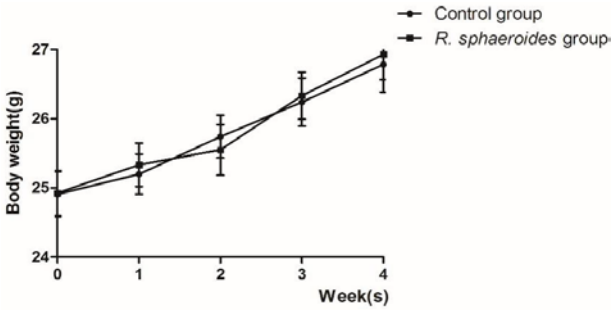


**Figure 1.** Effect of *R. sphaeroides* on the body weight of the mice

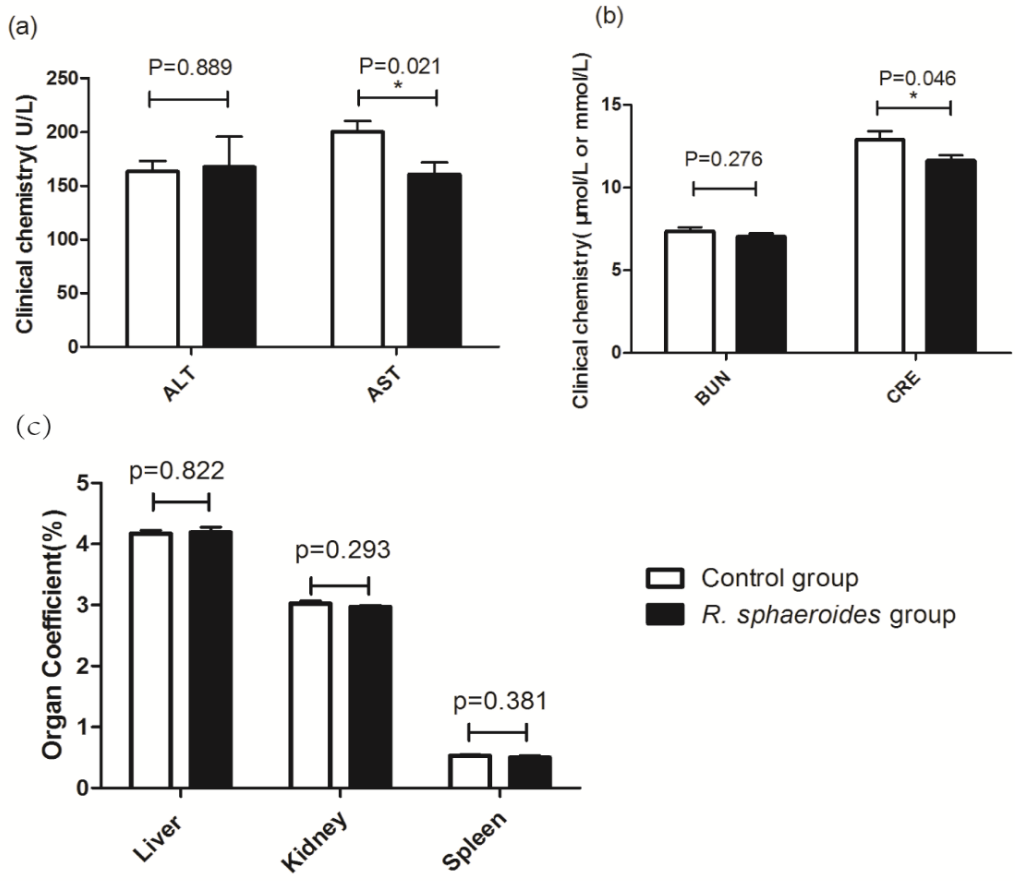


**Figure 2.** Effect of *R. sphaeroides* on mice organs

### 3.2. Effect of *R. sphaeroides* on the Function of Mice Organs

There was no significant difference in alanine aminotransferase (ALT) and urea nitrogen (BUN) levels between the control group and *R. sphaeroides* group. While *R. sphaeroides* significantly decreased the aspartate aminotransferase (AST) and creatinine (CRE) level. There were no significant effects of *R. sphaeroides* on the organ coefficient of liver, kidney, and spleen (Figure 2).

### 3.3. The Effect of *R. sphaeroides* on SCFAs of Mice

SCFAs concentrations in the control and *R. sphaeroides* group were shown in Figure 3. After the administration of *R. sphaeroides*, the productions of acetic acid increased significantly compared with the control group ($P$ = 0.038 < 0.05). There was also a subtle increase in butyric and propionic acids, but there was no significant difference between the control and *R. sphaeroides* group ($P$ = 0.907 > 0.05 and $P$ = 0.522, respectively).

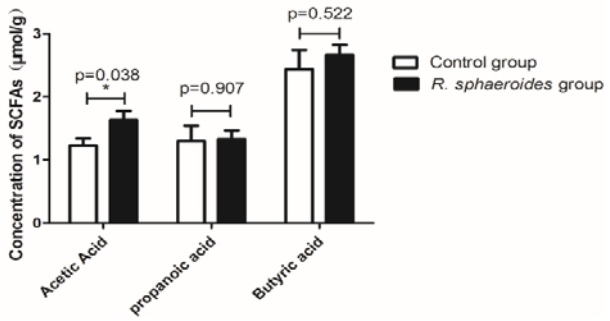


**Figure 3.** Effect of *R. sphaeroides* on concentration of SCFAs of mice

### 3.4. The Effect of *R. sphaeroides* on the Composition of Gut Microbiota in Mice

The bacterial community diversity and composition of gut microbiota in the control and *R. sphaeroides* group were identified by high-throughput sequencing technology based on 16S rRNA gene sequencing of the V3-V4 hypervariable region. An average of 23929 clean reads was obtained per sample. After operational taxonomic unit (OTU)-based cluster and taxonomy annotation, these clean reads were classified into 90 taxa that were annotated to the genus or family level.

Non-metric multidimensional scaling (NMDS) plots of those data were shown in Figure 4a. In this dimensional space, the sample of each group was reflected in the form of point. The differences between different groups were reflected by the distance of different groups and points. *R. sphaeroides* treatment resulted in divergences in the community structure of the gut microbiota compared with the control group. The Simpson α-diversity and Bray-Curtis β-diversity indices were used to reflect the evenness and richness between different microbiota communities intuitively (Figure 4b and Figure 4c). There was no significant difference in β-diversity between the *R. sphaeroides* group and the control group ($P$ = 0.460 > 0.05). However, there was a significant difference in α-diversity between these 2 groups ($P$ = 0.000 < 0.001), indicating that the distribution of OTUs abundance was more uneven in *R. sphaeroides* group with more prominent Simpson a-diversity index value. The analysis of similarities (ANOSIM) confirmed the similarity and divergence. These results indicated that *R. sphaeroides* treatment affected the gut microbiota of mice.

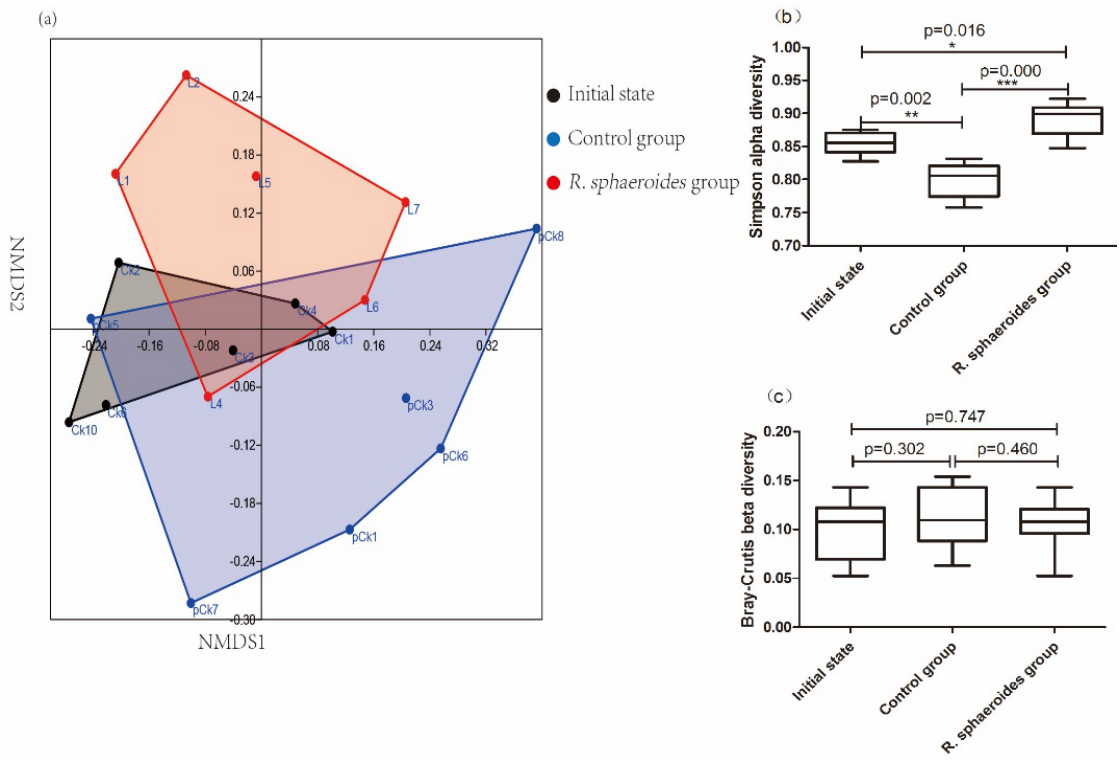


**Figure 4.** Effects of *R. sphaeroides* on the community structure of gut microbiota in mice

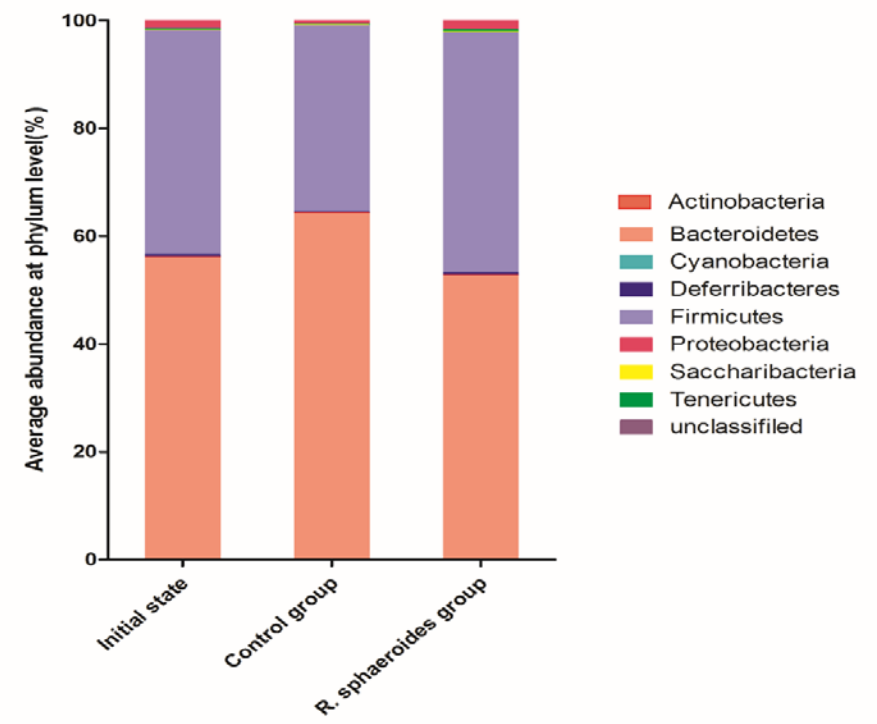


**Figure 5.** Barplots of relative abundances of bacterial phyla in *R.sphaeroides* group and Control group

**Table 1. Effect of *R. sphaeroides* on gut microbiota of mice at phylum level**

| Phylum | Abundance (%) | | |
|---|---|---|---|
| | Inistial state | Control group | *R. sphaeroides* group |
| *Actinobacteria* | 0.13±0.017 | 0.16±0.028 | 0.20±0.020 |
| *Bacteroidetes* | 56.10±1.43 | 64.22±2.04 | 52.60±1.35 |
| *Cyanobacteria* | 0.02±0.00 | 0.02±0.00 | 0.02±0.00 |
| *Deferribacteres* | 0.34±0.079 | 0.22±0.042 | 0.47±0.108 |
| *Firmicutes* | 41.49±1.42 | 34.50±2.07 | 44.45±1.34 |
| *Proteobacteria* | 1.38±0.299 | 0.54±0.084 | 1.69±0.18* |
| *Tenericutes* | 0.25±0.034 | 0.26±0.046 | 0.39±0.032 |
| *Saccharibacteria* | 0.16±0.022 | 0.07±0.014 | 0.16±0.021 |
| Unclassified | 0.13±0.035 | 0.01±0.00 | 0.01±0.00 |

*P < 0.05 between compared groups, **P < 0.01 between compared groups. Value = mean ± SD (N = 6).

## 3.5. Relative Taxa Abundances in the Microbiota of Mice with Different Treatment

Of a total of 8 identified bacterial phyla (Figure 5), *Bacteroidetes* and *Firmicutes* dominated the gut microbiota of mice (cumulative abundance > 96%). *Bacteroidetes*, which accounted for 64.22% in the control group and 52.60% in *R. sphaeroides* group, respectively, were the most abundant phylum in the 2 groups. *Firmicutes* were the second, occupying 34.50% and 44.55% of the whole sequence on average, respectively. They play an essential role in maintaining the structural and functional stability of intestinal microbiota according to plenty of research [35,36]. *Proteobacteria* composed the third dominant phylum in the gut microbiota, followed by *Actinobacteria*, *Cyanobacteria*, *Deferribacteres*, *Tenericutes*, and *Saccharibacteria* with less abundance. Compared with the control group, *R. sphaeroides* treatment significantly increased the abundance of *Proteobacteria* (1.69%, $P < 0.05$). Besides, there was also a slight increase in the abundance of *Tenericutes*, *Deferribacteres*, and *Saccharibacteria* in the *R. sphaeroides* group. The relative abundances of those bacterial phyla were shown in Table 1. There was a significant difference in the low-abundance bacterial phylum (*Proteobacteria*) between the control group and *R. sphaeroides* treatment groups.

## 3.6. Effect of *R. sphaeroides* on the Specific Taxa of Gut Microbiota in Mice

To further detect the multilevel impact of *R. sphaeroides* on the structure of gut microbiota, LEfSe online tool was used to identify the specific microbiota communities (from phylum to genus) that were influenced by *R. sphaeroides* treatment (Figure 6). The *R. sphaeroides* treatment significantly increased *α-Proteobacteria* at the class level compared with the control group. Besides, there was also a significant increase in *Desulfovibrionales*, *Rhodospirillales*, and *Campylobacterales* at the order level. As for family level, *R. sphaeroides* treatment significantly increased the abundance of *Bacteroidaceae*, *Christensenellaceae*, *Clostridiales vadinBB60 group*, *Prevotellaceae*, *Rhodospirillaceae*, *Desulfovibrionaceae*, *Helicobacteraceae*, and *Ruminococcaceae*. At the genus level, *Bacteroides*, *Helicobacter*, *Christensenellaceae_g_norank*, *clostridiales_vadinbb60_group_g_norank*, *Family_XIII_AD3011_group*, *Acetatifactor*, *Coprococcus_3*, *Lachnoclostridum*, *Lachnospiraceae_6*, *Ruminococcaceae_g_norank*, and *Ruminococcaceae_g_unclassified* all increased significantly. While *R. sphaeroides* treatment decreased the *[Eubacterium]_oxidoreducens_group* and *Prevotellaceae_g_unclassified*.

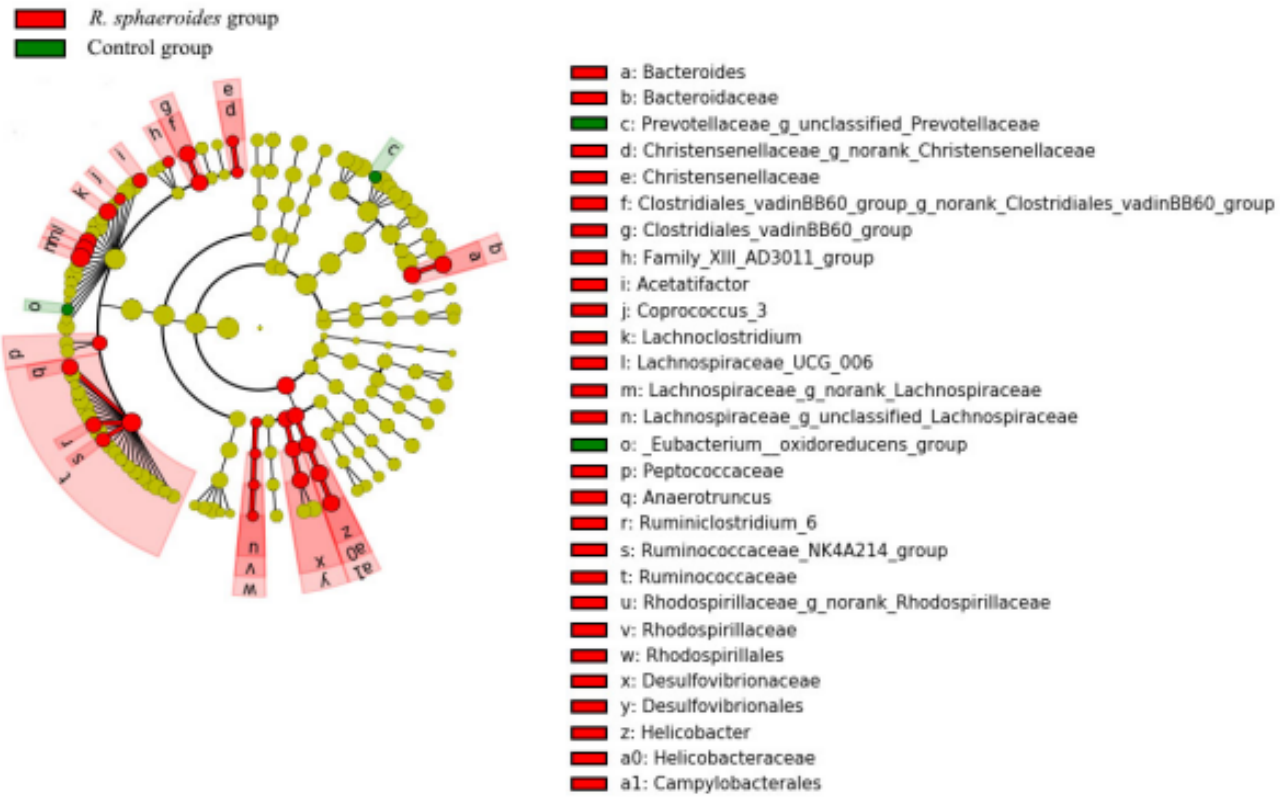


**Figure 6.** The structural changes of gut microflora affected by *R. sphaeroides* according to the LEfSe analysis

## 4. Discussion

In this study, *R. sphaeroides* 8513 was used to investigate its effects on gut microbiota in BALB/c mice. There was no side effect of *R. sphaeroides* treatment on the basic physiological health of mice. The gas chromatography was used to investigate the SCFAs in the cecum contents, and 16S rRNA high throughput sequencing was used to investigate the gut microbiota of fecal samples in different groups. SCFAs are the important metabolites of gut microbiota, which is given priority to acetic acid, propionic acid, and butyric acid, accounting for about 85% of the total amount of intestinal SCFAs [37]. SCFAs is mainly produced by anaerobic bacteria in the colon fermentation of dietary fiber, resistant starch, oligosaccharides, edible gums, and other sugars that are not easy to digest [38,39]. SCFAs can not only be used for maintaining the metabolic demand of their producers, but also they can cross through intestinal epithelial barrier and participates in the life activities of the host [40]. For example, butyric acid plays an important role in maintaining intestinal homeostasis and preventing colon cancer due to its conversion into beta-hydroxybutyric acid, which provides energy to the epithelial cells of the colon and cecum. In recent researches, acetic acid has also been proven to have beneficial effects on host. Hernandez *et al* [41] reviewed that acetate beneficially affects host energy and substrate metabolism via secretion of the gut hormones like glucagon-like peptide-1 and peptide YY, which, thereby, affects appetite, via a reduction in whole-body lipolysis, systemic pro-inflammatory cytokine levels, and via an increase in energy expenditure and fat oxidation. Wu *et al* [42] elucidated that acetate has promoted intestinal IgA response to microbiota mediated by GPR43, which has a crucial role in maintenance of intestinal homeostasis and in protecting the intestines from inflammation. Besides, acetate has been proven to promote resolution of neutrophilic inflammation in a model of gout in mice [43]. *R. sphaeroides* treatment significantly increased the content of acetic acid in the cecum contents of mice, and there was also a slight increase in propionic acid and butyric acid content, though no significant changes were observed. We can infer that there may be a positive effect of *R. sphaeroides* administration on intestinal homeostasis and physical health by promoting the production of SCFAs. *R. sphaeroides* treatment significantly increased the content of acetic acid in the cecum contents of mice, and there was also a slight increase in propionic acid and butyric acid content, though no significant changes were observed. We can infer that there may be a positive effect of *R. sphaeroides* administration on intestinal homeostasis and physical health by promoting the production of SCFAs. The SCFAs were produced by a series of bacteria, including *Blautia*, *Allobaculum*, *Prevotella* and *Butyricimonas* [44]. We hypothesis that *R. sphaeroides* treatment may influence redox state or shape the microbiota of the intestine, thus providing favorable conditions for anaerobic fermentation of colon and cecum micro-organisms.

16S rRNA gene sequencing and bioinformatics were used for gut microbiota analysis. In all fecal samples, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* account for more than 98% of the total intestinal microflora, followed by a small amount of *Actinomycetes*, *Cyanobacteria*, *Deferribacteres* and *Tenericutes* (Figure 5). *Bacteroidetes*

and *Firmicutes* dominated (> 96%) the gut microbiota, which played an important role in maintaining the stability of the structure and function of community (Table 1). It has been indicated that *Bacteroidetes* and *Firmicutes* account for as much as 99% of species in the gut microbiota of healthy adults [4]. Gu *et al [45]* used the high-throughput pyrosequencing method to systematically analyze C57BL/6 mice along with different segments of the gastrointestinal tract for the first time. The results illustrated that there were significant differences in the microbial community structure among different sections of the intestinal tract: *Lactobacillaceae* dominated the stomach and small intestine, while *Bacteroidaceae*, *Prevotellaceae*, *Rikenellaceae*, *Lachnospiraceae* and *Ruminococcaceae* dominated the large intestine and feces, respectively [45]. The concept of "Core microbial groups" in different sections was proposed. Those core microbiota groups are closely related to the health of mammals, and the interaction between host and micro-organisms is related to physiological reactions such as metabolic, immune homeostasis of mammals [11,46]. *R. sphaeroides* had no significant effect on the OTUs diversity since there were no significant differences in β-diversity. However, the α-diversity index of gut microbiota in mice treated with *R. sphaeroides* significantly increased, indicating the uniformity of gut microbiota decreased. *R. sphaeroides* treatment significantly increased the abundance of *Proteobacteria* (1.69%, $P < 0.05$). Numerous studies have explored the relationship between gut microbiota and disease, those specific microbiota communities have become potential markers for disease diagnosis and therapeutic targets [47,48]. For example, *Escherichia coli, Clostridium, Enterococcus, Enterotoxigenic bacteroides* and *Helicobacter pylori* are reported to be involved in colorectal cancer carcinogenesis [49]. TMAO (Trimethylamine N-oxide) which is an important gut microbe-dependent metabolite involves in the mechanisms of atherosclerotic cardiovascular diseases from the perspective of inflammation, inflammation-related immunity, cholesterol metabolism, and atherothrombosis [50]. *Akkermansia muciniphila* is reported to improve host health by affecting glucose metabolism, lipid metabolism, and intestinal immunity [50]. *Lachnospiraceae* can produce butyric acid, and *Proteobacteria* can reduce the bacterial endotoxin LPS-producing bacteria [51]. Gao *et al* explored the structure of gut microbiota of colonic cancer rats and healthy rats, and the results showed that the diversity of the *Lachnospiraceae Ruminococcaceae*, *Lactobacillus intestinalis*, *Paraprevotella*, *Lactobacillus murinus*, *Lactobacillus*, *Prevotella*, *Lactobacillus crispatus*, and *Lachnospiracea incertae sedis* decreased significantly in colonic cancer rats, while the potential pathogen *Coprobacillus* increased significantly. Also, a great number of studies have shown that the bacteria in the gut microbiota such as *Ruminococcus*, *ruvotella*, *Oscillospira*, *Bifidobacterium*, *Lactobacillus*, *Streptococcus*, *Faecalibacterium*, *Blautia*, and *Lachnospira* are negatively correlated with the occurrence of metabolic diseases such as obesity and diabetes and that dietary fiber and low-fat diet can increase the proportion of these bacteria [52].

We further investigated the effect of *R. sphaeroides* administration on the specific taxa of gut microbiota by LEFSe analysis. The results showed that *R. sphaeroides* treatment significantly increased the intestinal *Rhodospirillaceae*, *Desulfovibrionaceae*, and *Helicobacter* in *Proteobacteria*, which belong to anaerobic bacteria. Researches showed that *Desulfovibrionaceae* and *Helicobacter* were potential digestive pathogens [53,54]. Besides, *Mycoplasma* and *Anaeroplasma* are typical anaerobic bacteria, when oxygen contents fall in the environment, the proportion of these anaerobic bacteria increases [55,56]. *R. sphaeroides* can produce carotenoids, superoxide dismutase (SOD), coenzyme Q10, ornithine, 5-aminolevulinic acid (ALA), D-psicose and other bioactive substances [57,58,59,60,61]. Our studies indicated that *R. sphaeroides* superfine powder has immunomodulatory activity and can enhance the immune function of mice. Besides, *In vitro* cell experiments were conducted to study the antioxidant activity of *R. sphaeroides*, and the results showed that *R. sphaeroides* had antioxidant activity, which could coexist with Caco-2 cells and improve the antioxidant activity of Caco-2 cells under oxidative stress [34]. We hypothesize that *R. sphaeroides* may exert an antioxidant activity in the gut and increase the anaerobic bacteria *Mycoplasma* and *Anaeroplasma* abundance. However, in this study, we didn't detect the oxides such ROS (reactive oxygen species) and CAT (catalase) to confirm this hypothesis. There was also an increase in the intestinal probiotics of *Bacteroidaceae*, *Bacteroides*, and *Clostridiales_vadinBB60_group*, which reflects the regulatory effect of *R. sphaeroides* on intestinal health.

## 5. Conclusions

In conclusion, our results have shown that oral administration of *R. sphaeroides* 8513 not only increased the abundance of anaerobic bacteria *Rhodospirillaceae*, *Desulfovibrionaceae*, and *Helicobacter* in the gut of mice, but also increased the abundance of the predominant microflora in the gut such as *Bacteroidaceae (Bacteroides)*, *Prevotellaceae*, *Clostridiales_vadinBB60_group (Closporaceae)*. *R. sphaeroides* treatment significantly increased the content of acetic acids and α-diversity of the gut of mice. No side effect of *R. sphaeroides* treatment on the basic physiological health of mice was observed. These results suggested that *R. sphaeroides* 8513 could modulate the microbial ecology in the gut of mice. However, futher researches are needed for investigating the metabolomics of cecal contents, and we can also study the REDOX level of intestine by testing SOD(superoxide dismutase), CAT(antioxidant enzymes catalase), GPx (glutathione peroxidase), hydroxyl free radical, and superoxide anion etc. Besides, we can also check for intestinal permeability assay (e.g., FITC dextran) and inflammatory markers (TNF-alpha, IL6) after *R. sphaeroides* treatment.

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