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Effects of red meat diet on gut microbiota in mice

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Abstract

We aimed to evaluate the effect of red meat diet on gut microbiota in mice. Balb/c mice at weaning were randomized into control group and red meat groups with different proportions (25%, 50%, and 75%). Mice were fed with a standard pellet diet as control group, while those were fed with different proportions of red meat diet as red meat groups. After 8 weeks, they were sacrificed and their intestinal contents were obtained for 16S rRNA sequencing and bioinformatics analysis. Our results showed that there were significant-structural differences among the four groups. The top-two most abundant phylum were Firmicutes and Bacteroidetes. In the red meat groups, the abundance of Bacteroidetes was increased, but the abundance of Firmicutes was decreased. At the family level, Bacteroidaceae and Family XIII were significantly higher in the high-dose group than those in the control group. There were also significant differences in abundance of many genera. In conclusion, different proportions of red meat diet may lead to changes in gut microbial flora in mice. These changes may be pathological and may be related to the frequent occurrence of many diseases.

Keywords: red meat diet; 16S rRNA sequencing; gut microbiome; mice; bioinformatics analysis

Practical Application: We evaluated the effect of red meat diet on gut microbiota in mice. We found that different proportions of red meat diet may lead to changes in gut microbial flora in mice. These changes may be pathological and may be related to the frequent occurrence of many diseases.

1 Introduction

The interaction between the gut microbiota and the host is crucial for maintaining homeostasis and body health (Jiang & Schnabl, 2020). Microbiota facilitates carbohydrate digestion (Makki et al., 2018), bile acid metabolism (Wang et al., 2020), maintenance of the integrity of gut barrier against pathogen infection (Mendes et al., 2019), and vitamin synthesis (Yatsunenko et al., 2012). However, the gut microbiota is associated with the pathogenesis of many diseases, including Colon Cancer (Birt & Phillips, 2014), Cardiovascular (Scarmozzino et al., 2020) and liver diseases. The composition of the gut microbiota may be affected by many factors in the host, including physiology, pathology, living environment, immune system and lifestyle (Butel et al., 2018). Among them, diet plays a significant role (Steer et al., 2000). Foods are mainly digested and absorbed in the stomach and small intestine, but a substantial quantity of foods may enter the large intestine and alter the diversity of gut bacteria (van Hylckama Vlieg et al., 2011; Rist et al., 2013).

It has revealed that fiber- and vegetable-rich diets and physical activity may contribute to reduced incidences of many diseases. However, the consumptions of red and processed meat or alcoholic beverages are related to elevated incidences of many diseases (Birt & Phillips, 2014), such as colon cancer (Abu-Ghazaleh et al., 2021), inflammatory bowel disease (Li et al., 2020) and cardiovascular disease (Zhong et al., 2020). In humans, high consumption of red meat and reduced content of nondigestible carbohydrates in daily diet may increase the risks of inflammatory bowel disease and colorectal cancer (Hou eta al., 2011; Alexander & Cushing 2011). It has been hypothesized that the reason may be due to the changes in colonic microflora (Ijssennagger et al., 2015; O'Keefe et al., 2015). Evidence shows that dietary intervention of the human gut microbiota is feasible and has been proven as efficacious in voluntary trials (Steer et al., 2000).

The relationship between microbiota and food is bidirectional. While the food in-take can alter the gut microbiota, the modified microbiota in turn produce a variety of metabolites, which could be beneficial or detrimental (Dey 2019). In mouse models of colitis, microbial colonization is required for the development of active inflammation (Hudcovic et al., 2001). Their composition and diversity will change with different diet (Wu et al., 2011). Therefore, it is possible to reduce the risk of developing colitis through controlled diet, which requires a profound understanding of the relationship between diet and the gut microbiota.

The Illumina instruments provide the highest yield and quality data currently (Zhang et al., 2018). Therefore, in this study, we aimed to evaluate the effect of red meat diet on gut microbiota in mice.

2 Materials and methods

2.1 Samples

The protocols were approved by the institutional ethical committee of Xinjiang Medical University. Different proportions of red meat diets were obtained from the Animal Experimental

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Center of Xinjiang Medical University. The proportions of red meat were 0%, 25%, 50%, and 75%, which were served as diets for control group, low-dose group, medium-dose group and high-dose group, respectively. All the specific configuration of red meat diet was shown in Table1.

A total of 24 healthy balb/c mice (aged 3 weeks; male and female each half; Certificate number, SCXK(XIN)2018-0002) were obtained from the Animal Experimental Center of Xinjiang Medical University (Xinjiang, China). The animals were housed in standard feeding environment. Mice were randomized into control group (mice number 1-1,1-2,1-3,1-4,1-5, and 1-6), low-dose group (mice number 2-1,2-2,2-3,2-4,2-5, and 2-6), medium-dose group (mice number 3-1,3-2,3-3,3-4,3-5, and 3-6) and high-dose group (mice number 4-1,4-2,4-3,4-4,4-5, and 4-6). The mice were provided with water and fed with ad libitum. After 8 weeks, all mice were sacrificed by cervical dislocation. The flowchart was shown in Figure 1.

Table 1. The red meat diet in different groups.

Composition	Control group	Low-dose group	Medium-dose group	High-dose group
tyrosine	20	20	0	0
red meat	0	25	50	75
corn starch	41.72	16.7	11.7	2.19
sucrose	10.95	10.95	10.95	10.95
sunflower seed oil	17.66	17.68	17.68	2.19
lard	2.55	2.55	2.55	2.55
Alpha-cellulose	2	2	2	2
L-cysteine	0.3	0.3	0.3	0.3
choline	0.17	0.17	0.17	0.17
mineral	3.5	3.5	3.5	3.5
vitamin	1	1	1	1
DL-methionine	0.15	0.15	0.15	0.15
Total	100	100	100	100

2.2 Sequencing

Fecal samples were collected aseptically from the ileum, cecum and distal colon. Intestinal contents were obtained for further microbial community analysis. DNA samples were extracted using the E.Z.N.A.® stool DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) following the manufacturer' s protocols. DNA samples were detected by 1% agarose gel electrophoresis. The V3-V4 region of the bacteria 16S ribosomal RNA gene were amplified by PCR. The reaction parameters of PCR are as follows: 95 °C for 5 min, followed by 27cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s and a final extension at 72 °C for 10 min. Specific primers are as follows. 341F 5'-barcode- CCTAYGGGRBGCASCAG-3' and 806R 5'- GGACTACNNGGGTATCTAAT-3'. PCR reactions were performed by TransStart Fastpfu DNA Polymerase (TransGen, China) using GeneAmp PCR System 9700 (ABI,U.S.). Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions. Amplicons were further quantified using QuantiFluor™ -ST (Promega, U.S.). The pooled DNA product was used to construct Illumina Pair-End library following Illumina's genomic DNA library preparation procedure by using NEB NextUltra DNA Library Prep Kit for Illumina (NEB, USA). The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific, CA, USA) and Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA). Then the amplicon library was paired-end sequenced (2 × 250) on an Illumina MiSeq platform (MingkeBio (Hangzhou) Co., Ltd., Hangzhou, China) according to the standard protocols.

2.3 Bioinformatics analysis

Paired-end reads generated on Illumina platform were carried out with QuantiFluor™ -ST. Joined reads that have ≥97% proximity were assigned to OTUs. Prepare typical sequences of OTU and analyze the divergences of dominants. Alpha diversity was considered as Observed-species, Chao1, Shannon and Simpson. Beta diversity analysis was performed using UniFrac (Lozupone et al., 2011) to compare the results of the principal component analysis (PCA) with the community ecology package. R-forge (Vegan 2.0 package was used to generate a PCA figure.

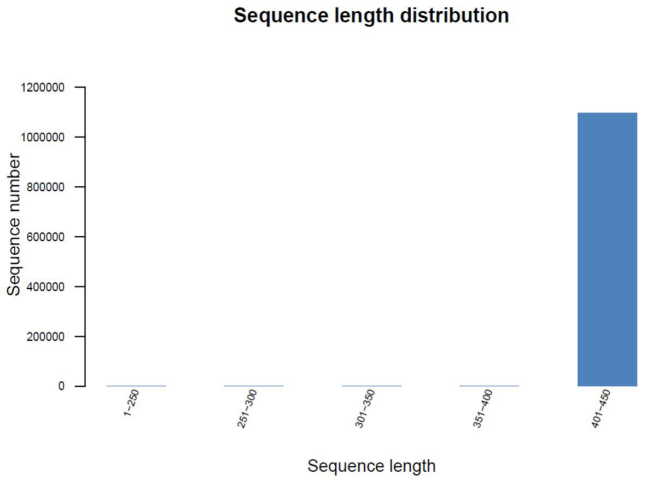

Figure 1. All the trimmed sequences length. X-axis is the sequence length and Y-axis is the sequence number.

Venn diagrams were implemented by Venn Diagram software, while Mantel test, Redundancy analysis (RDA) and Heatmap figures were performed by R package vegan.

2.4 Statistical analysis

The quality control and sequence filtering of amplicons were performed according to the barcode matching and sequence overlapping with QIIME. OTUs were clustered with 97% similarity cutoff using Usearch (version 10) and chimeric sequences were identified and removed using UCHIME. The phylogenetic affiliation of each 16S rRNA gene sequence was analyzed by RDP Classifier against the silva (SSU123)16S rRNA database using confidence threshold of 70% (Amato et al., 2013).

Community compositional was analyzed using linear discriminant analysis (LDA) and effect size (LEfSe) (Segata et al., 2011). Differences between groups were analyzed by one-way analysis of variance (ANOVA) test followed by Student's t-test.

Data are presented as means ± standard deviation (SD). $P < 0.05$ was considered significant. A P-value between 0.05 and 0.1 was considered a tendency. Statistical analysis was performed using SPSS 20 (International Business Machines, corp., Armonk, NY, USA).

3 Results and discussion

3.1 Statistics of trimmed sequences

Lengths of all the trimmed sequences were shown in Figure 1. The proportion of the high-quality sequence was more than 95%. The sequence length was almost between 401 and 450 bp, with the average value of 414.15 bp..

3.2 Rarefaction curves

The species richness was evaluated as previous described (Schloss et al., 2011). As shown in Figure 2, the rarefaction curves of all the samples were common risen rapidly and then flattened. Only the 1-2 and 1-5 samples showed saturation. Similarly, they had the lower diversity and richness than others.

3.3 Distribution and accuracy of statistical analyses

As shown in Table 2, after filtering, entire 1,098,609 quality sequences from enrichment samples were obtained, with an average of 44520 ± 9209 reads per sample. There were 13960 OTUs with 97% similarity. The community diversity characteristics of the 4 reactors were also showed in Table 2. Chao index indicated the OTU richness The Chao, Shannon and Simpson indices suggested that the species diversity in the control group was decreased than red meat diet groups, and those in both medium-dose group and high-dose group were higher than other groups (Table 3). Samples in the control group can decrease the growth of specific microorganisms due to the lower OTU numbers (465 ± 28). Although most of the intestinal microbiota is the dominant flora, there are still harmful ones. Along with the increased proportion of red meat in diet, the number of dominant flora was decreased while the harmful flora was increased, which can lead to an elevated α-diversity of the intestinal flora.

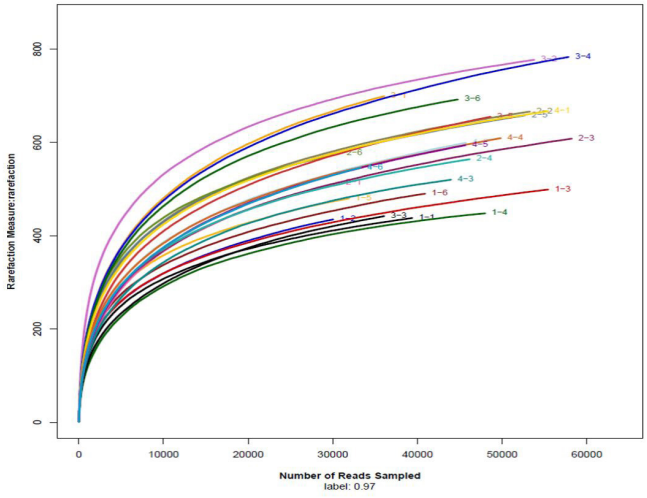

Figure 2. The rarefaction curves of all the samples. X-axis is the number of readers sampled and Y-axis is rarefaction measure. Different colors represent mice in different groups.

3.4 Shannon-Wiener and rank-abundance curve analysis

Shannon-Wiener index is taken into account species richness and proportion of each species (Magwira et al., 2012). As shown in Figure 3a, the Shannon-Wiener curve of all samples monotonically climbed until reaching a plateau. Figure 3b showed the rank-abundance curves. The width reflected the abundance.

3.5 Venn diagram and principal coordinate analysis

There were 948, 1072, 1123 and 1030 OTUs in the control group, low-dose group, medium-dose group and high-dose group, respectively (Figure 4). Among the four groups, there were 603 common OTUs. 77 unique OTUs were found in the control group, which was the largest in the four groups. This could be a hint of the beneficial effect of diet.

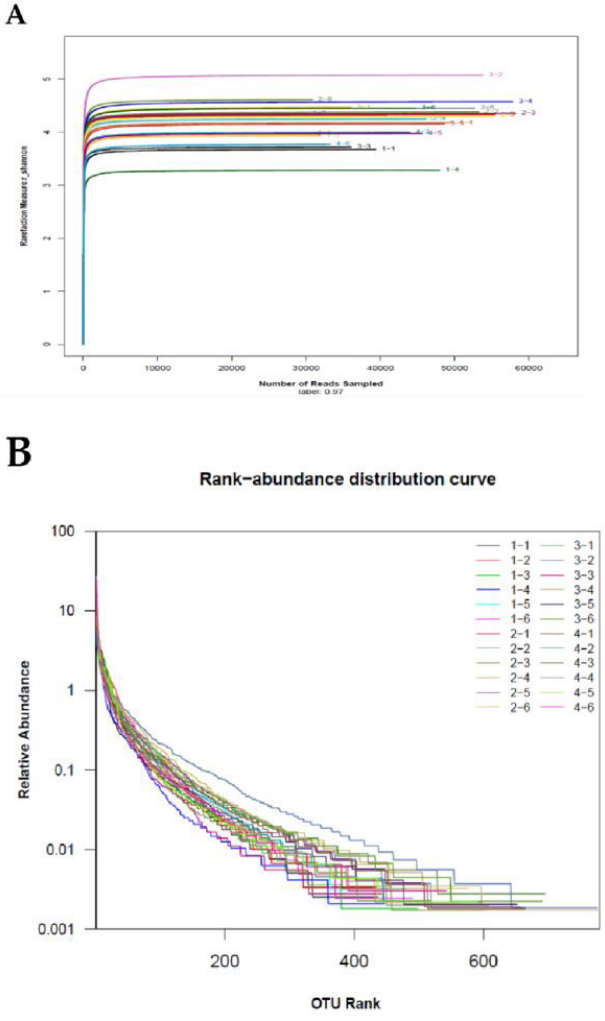

Figure 3. Shannon-Wiener and rank-abundance curve analysis. (a) the Shannon-Wiener curve of all samples; X-axis is the number of readers sampled and Y-axis is rarefaction measure. (b) the rank-abundance curves of all samples. X-axis is the OUT Rank and Y-axis is relative abundance. The width of the curve reflects the species richness. Different colors represent mice in different groups.

Table 2. The community diversity characteristics of OTUs.

Sample ID	Reads	0.97				
		OTU	Chao	Coverage	Shannon	Simpson
1-1	39378	438	566	0.997384	3.67	0.0674
			-515,652		(3.66,3.69)	(0.0661,0.0687)
1-2	30038	435	566	0.996172	4.35	0.0254
			-516,647		(4.34,4.37)	(0.0249,0.026)
1-3	55429	499	634	0.997835	4.34	0.0255
			-583,715		(4.32,4.35)	(0.0251,0.0259)
1-4	47981	448	509	0.998145	3.28	0.1119
			-484,552		(3.26,3.3)	(0.11,0.1138)
1-5	31901	480	641	0.996301	3.93	0.0828
			-579,741		(3.91,3.95)	(0.0803,0.0852)
1-6	40893	490	613	0.997066	4.32	0.0274
			-567,687		(4.3,4.33)	(0.0269,0.0278)
2-1	30734	514	640	0.99564	3.98	0.0468
			-595,709		(3.96,4)	(0.0457,0.0479)
2-2	53273	666	807	0.997222	4.38	0.0321
			-759,881		(4.37,4.4)	(0.0315,0.0327)
2-3	58219	608	768	0.997406	4.36	0.0246
			-713,851		(4.34,4.37)	(0.0243,0.025)
2-4	46163	564	706	0.997119	4.25	0.0302
			-655,785		(4.24,4.26)	(0.0297,0.0307)
2-5	52696	658	838	0.997153	4.45	0.0353
			-776,934		(4.44,4.47)	(0.0345,0.0361)
2-6	30820	577	698	0.996009	4.61	0.0242
			-653,769		(4.59,4.63)	(0.0236,0.0248)
3-1	36069	699	849	0.995259	4.47	0.0328
			-800,920		(4.45,4.48)	(0.032,0.0336)
3-2	53789	777	879	0.99749	5.08	0.013
			-843,934		(5.06,5.09)	(0.0127,0.0132)
3-3	36063	442	534	0.996867	3.72	0.062
			-499,590		(3.7,3.74)	(0.0607,0.0633)
3-4	57835	783	972	0.996767	4.58	0.027
			-9,131,058		(4.56,4.59)	(0.0265,0.0275)
3-5	48625	655	867	0.996319	4.15	0.0383
			-799,969		(4.14,4.17)	(0.0376,0.039)
3-6	44757	692	805	0.996805	4.45	0.0254
			-766,864		(4.44,4.47)	(0.025,0.0258)
4-1	55401	667	854	0.99722	4.3	0.0349
			-790,952		(4.29,4.32)	(0.0343,0.0356)
4-2	45717	599	762	0.99661	4.23	0.0307
			-707,846		(4.22,4.25)	(0.0302,0.0311)
4-3	43952	520	621	0.997361	4	0.0442
			-583,682		(3.98,4.01)	(0.0434,0.045)
4-4	49831	609	811	0.996789	4.18	0.0374
			-742,915		(4.16,4.19)	(0.0368,0.0381)
4-5	45657	594	764	0.996539	3.98	0.051
			-707,850		(3.96,3.99)	(0.0499,0.0521)
4-6	33257	546	732	0.995309	3.77	0.0819
			-669,828		(3.75,3.79)	(0.0797,0.084)

OTU, operational taxonomic unit; ID, identification.

3.6 Community structure analysis

Table 4 showed that there were 21 phyla, 42 classes, 92 orders, 140 families, 268 genera and 335 species in the communities of all samples. The dominant phyla of all groups were Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria, while the dominant classes of all groups were Clostridia, Bacteroidia, Deltaproteobacteria and Bacilli. At the phylum level, the most predominant phylum was Firmicutes that contributed 59.33%,

Table 3. Alpha diversity index of fecal samples of mice in each group.

Groups	Chao index	Coverage index	Shannon index	Simpson index
Control	588.17 ± 50.515	0.997150 ± 0.000799	3.9817 ± 0.44061	0.056733 ± 0.036481
Low-dose	742.83 ± 74.497*	0.996758 ± 0.000739	4.3383 ± 0.21198	0.0322 ± 0.008349
Medium-dose	817.67 ± 149.414**	0.996585 ± 0.000749	4.4083 ± 0.45314	0.033083 ± 0.016509
High-dose	757.33 ± 79.397**	0.996638 ± 0.000729	4.0767 ± 0.19664	0.046683 ± 0.018684

*P < 0.05; **P < 0.01 compared with the control group.

Table 4. The community structures of the observed samples at phylum levels.

Phylum	Control group	Low-dose group	Medium-dose group	High-dose group
Firmicutes	59.33 ± 15.95	58.04 ± 15.4	52.58 ± 13.69	45.58 ± 8.72
Bacteroidetes	25.41 ± 18.01	26.87 ± 20.75	32.35 ± 12.98	39.85 ± 13.53
Proteobacteria	12.3 ± 15.1	9.82 ± 7.92	10.67 ± 5.66	7.69 ± 3.89
Actinobacteria	0.85 ± 0.68	2.98 ± 1.77*	1.95 ± 2.11	1.35 ± 2.09
Patescibacteria	0.58 ± 0.69	1.35 ± 0.53	1.77 ± 1.49*	1.56 ± 0.79
Verrucomicrobia	0.78 ± 1.28	0 ± 0	0.13 ± 0.17	0.63 ± 1.42
Epsilonbacteraeota	0.38 ± 0.28	0.16 ± 0.18	0.2 ± 0.2	2.52 ± 4.42
Deferribacteres	0.22 ± 0.22	0.01 ± 0.01*	0.05 ± 0.07	0.16 ± 0.24
Tenericutes	0.11 ± 0.11	0.33 ± 0.35	0.18 ± 0.17	0.6 ± 0.62
Cyanobacteria	0.02 ± 0.03	0.39 ± 0.79	0.04 ± 0.08	0.03 ± 0.05
Acidobacteria	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.01 ± 0.01
Chloroflexi	0 ± 0	0 ± 0	0.01 ± 0	0 ± 0
Gemmatimonadetes	0 ± 0.01	0 ± 0	0.01 ± 0.01	0 ± 0
Nitrospirae	0 ± 0	0.01 ± 0.01	0.02 ± 0.01*	0 ± 0.01
Unclassified	0 ± 0	0.01 ± 0.02	0.01 ± 0.01	0.01 ± 0.02

*P < 0.05 compared with the control group.

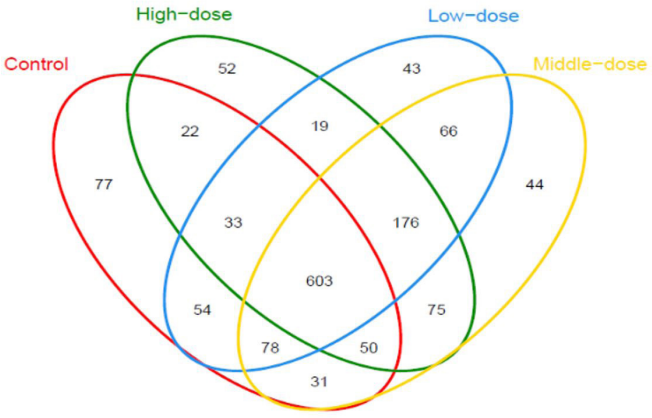

Figure 4. The Venn diagram. Red represents control group; green represents high-dose group; blue represents low-dose group and yellow represents middle-dose group.

58.04%, 52.58% and 45.58% of the fecal microbiota in the control, low-dose, medium-dose and high-dose groups, respectively. The second predominant phylum was Bacteroidetes, contributing 25.4%, 26.87%, 32.35% and 39.85%, respectively. There were increased proportion of Bacteroidetes (by 6.95% and 14.44%, respectively) in both medium-dose and high-dose groups. However, the proportion of Firmicutes was substantially reduced in these two groups (by 6.75% and 13.74%, respectively). The results were shown in Figure 5.

As shown in Table 5, it demonstrated that the dominant family of all groups were Lachnospiraceae, Ruminococcaceae, Muribaculaceae, Desulfovibrionaceae. Lachnospiraceae, Ruminococcaceae, Muribaculaceae, Burkholderiaceae, Erysipelotrichaceae, Lactobacillaceae, Marinifilaceae, Caulobacteraceae and Peptococcaceae were lower in the high-dose group than those in the control group. Clostridiaceae 1, Enterococcaceae and Peptostreptococcaceae were significantly lower in the medium-dose and high-dose group than those in the control group (P < 0.01). Two family were notably higher in high-dose group than control group, including Bacteroidaceae (P < 0.01) and Family XIII (P < 0.01). Moreover, Desulfovibrionaceae, Rikenellaceae, Prevotellaceae and Helicobacteraceae were higher in the high-dose group than those in the control group. At genera levels, Parasutterella, Marvinbryantia, [Eubacterium] nodatum group, Bacteroides, Lachnospiraceae UCG-001, Adlercreutzia, Prevotellaceae UCG-001, Ruminococcaceae UCG-013, Negativibacillus, Faecalitalea, Lachnospiraceae UCG-008 and [Eubacterium] brachy group were higher in the high-dose group than those in the control group (P < 0.05) (Figures 6-8).

The gastrointestinal tracts in humans and other animals harbor a diverse array of microorganisms, which play fundamental-roles in health and disease. Imbalance in the gut microbiota, namely dysbiosis, can lead to various diseases, ranging from metabolic and cardiovascular disorders to cancer and gastrointestinal tract disorders. Approaches to improve gut dysbiosis, including dietary intervention, intake of probiotics and fecal microbiota transplantation, are emerging strategies to

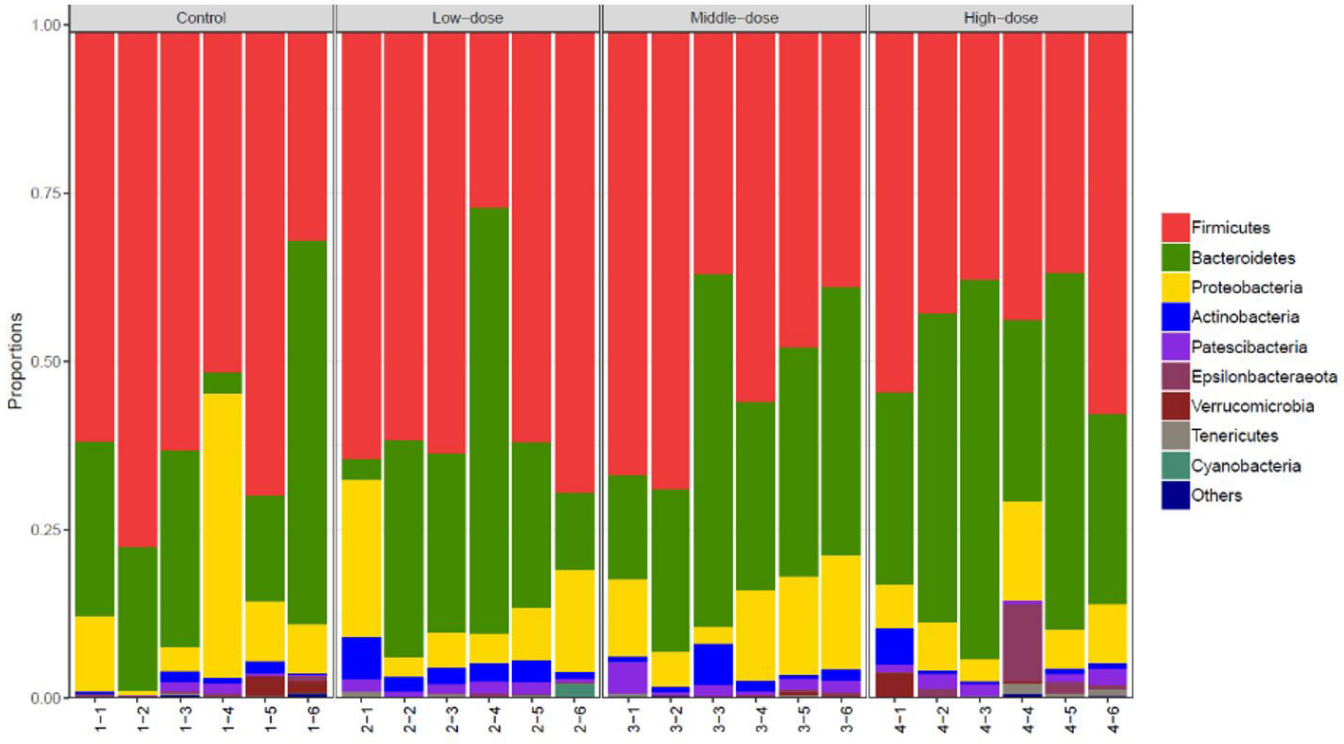

Figure 5. The community structures at phylum levels. X-axis is the mice in different groups and Y-axis is proportions. Different colors represent different bacteria.

Table 5. The community structures of the observed samples at family levels.

Family	Control group	Low-dose group	Middle-dose group	High-dose group
Lachnospiraceae	34.1 ± 10.93	36.9 ± 14.16	32.84 ± 13.53	31.39 ± 9.22
Ruminococcaceae	15.57 ± 13.25	12.97 ± 3.54	13.32 ± 6.61	9.83 ± 4.42
Muribaculaceae	14 ± 8.9	19.78 ± 15.41	23.03 ± 13.78	11.62 ± 6.71
Desulfovibrionaceae	6.56 ± 4.1	5.9 ± 4.57	8.64 ± 4.83	6.84 ± 3.61
Rikenellaceae	5.02 ± 4.54	3.18 ± 2.82	2.7 ± 1.39	8.69 ± 3.24
Burkholderiaceae	4.73 ± 11.56	0.17 ± 0.17	1.69 ± 2.3	0.64 ± 0.55
Erysipelotrichaceae	2.54 ± 2.22	2.33 ± 1.8	0.36 ± 0.41	0.13 ± 0.1
Clostridiaceae 1	2.41 ± 5.6	0.01 ± 0.01*	0 ± 0**	0 ± 0**
Lactobacillaceae	2.18 ± 2.48	3.86 ± 3.68	4.74 ± 6.25	1.01 ± 1.11
Prevotellaceae	2.11 ± 2.85	0.2 ± 0.23	0.35 ± 0.53	2.57 ± 3.7
Marinifilaceae	2.11 ± 2.13	1.19 ± 1.36	0.83 ± 1.04	0.29 ± 0.1
Tannerellaceae	1.4 ± 2.22	0.39 ± 0.63	0.6 ± 0.54	1.85 ± 1.17
Bacteroidaceae	0.73 ± 0.88	2.11 ± 1.87	4.83 ± 6.15	14.82 ± 6.77**
Family XIII	0.23 ± 0.22	0.57 ± 0.35	0.67 ± 0.33	2.11 ± 1.12**
Caulobacteraceae	0.21 ± 0.34	1.97 ± 1.85*	0.11 ± 0.16	0.07 ± 0.06
Eggerthellaceae	0.73 ± 0.69	2.84 ± 1.64*	1.88 ± 2.06	1.33 ± 2.07
Enterococcaceae	0.12 ± 0.16	0 ± 0	0 ± 0**	0 ± 0.01
Helicobacteraceae	0.38 ± 0.28	0.16 ± 0.18	0.2 ± 0.2	2.51 ± 4.42
Mollicutes RF39_norank	0.05 ± 0.07	0.14 ± 0.12	0.1 ± 0.15	0.11 ± 0.12
Peptococcaceae	0.35 ± 0.24	0.21 ± 0.23	0.34 ± 0.34	0.13 ± 0.05
Peptostreptococcaceae	0.91 ± 1.42	0.75 ± 1.01	0.05 ± 0.07*	0 ± 0**

*P < 0.05; **P < 0.01 compared with the control group.

treat diseases (Cheung et al., 2020). Diet can significantly reshape gut microbial composition (Brown et al., 2012). For instance, compared to European children, increased Bacteroidetes and decreased Firmicutes and Enterobacteriaceae in rural African children were mainly attributed to differences in dietary patterns between the two populations. This difference was probably

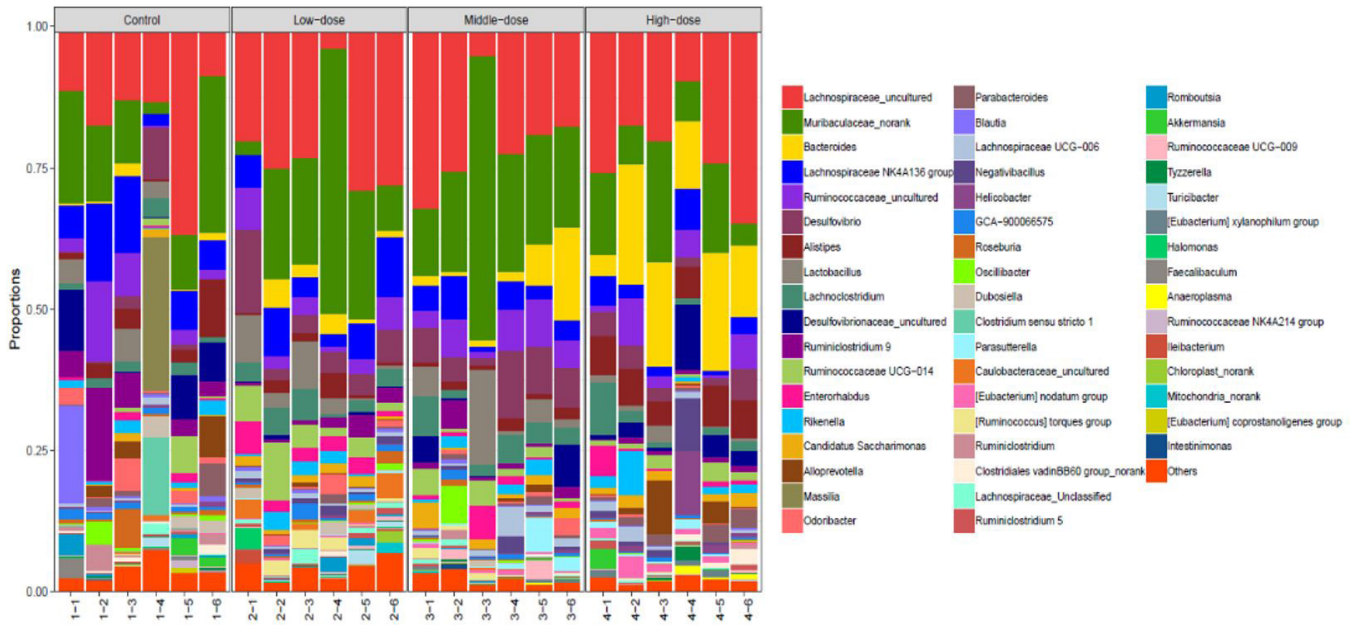

Figure 6. The community structures at genera levels. X-axis is the mice in different groups and Y-axis is proportions. Different colors represent different bacteria.

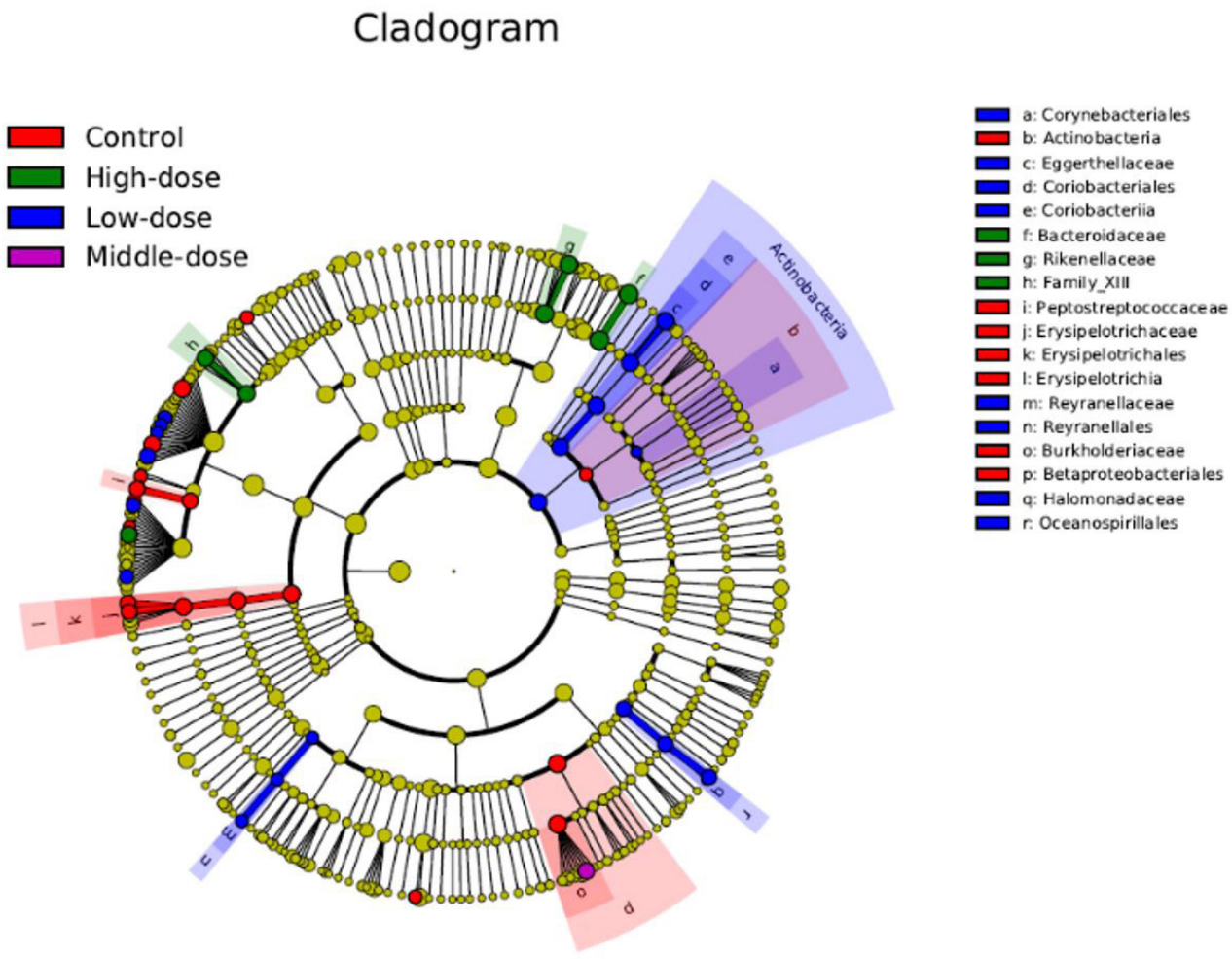

Figure 7. Significantly different species were compared in the four groups.

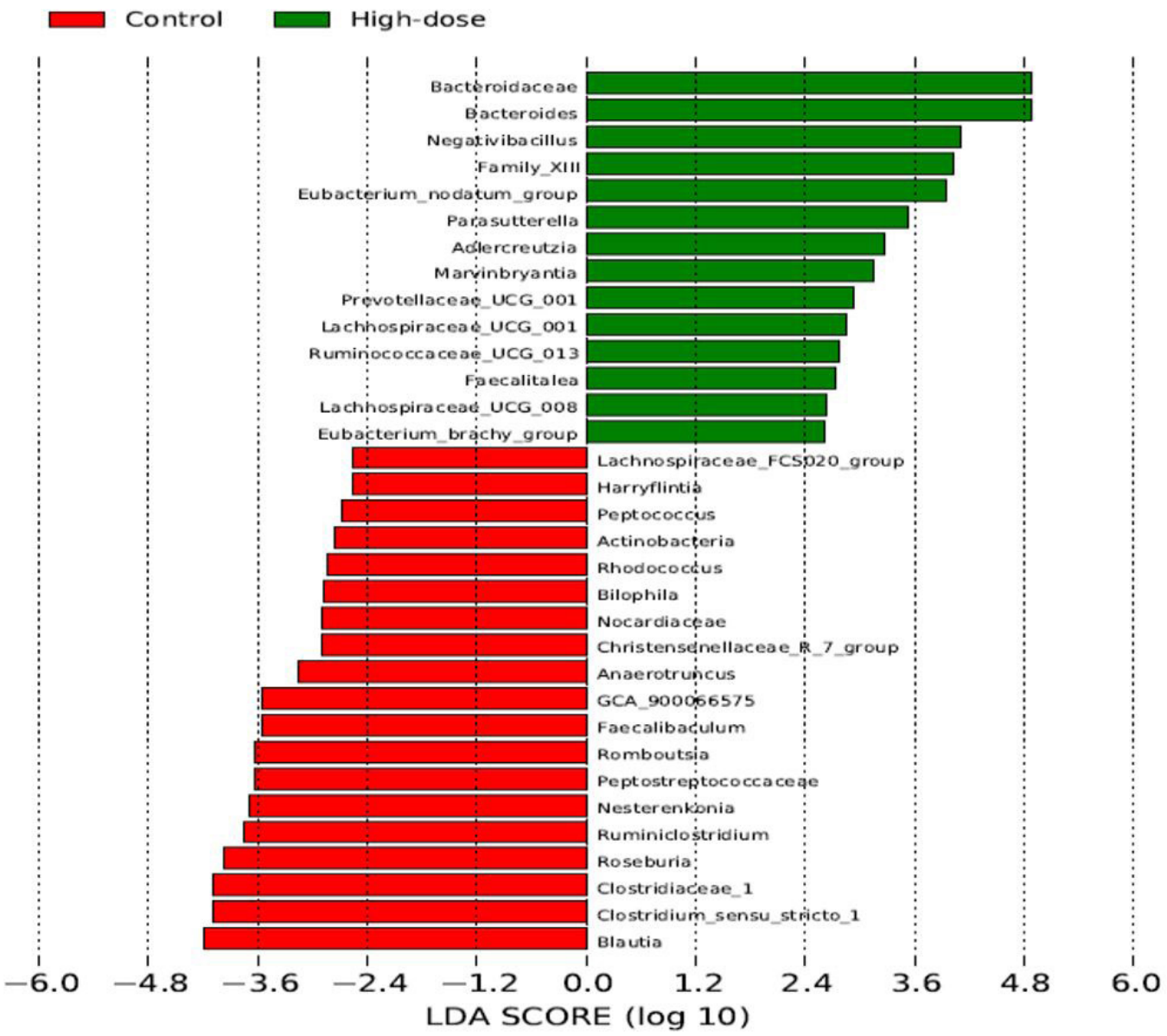

Figure 8. Significantly different species were compared in the high-dose group than in the control group.

explained by the higher red meat content of the food in Europe (De Filippo et al., 2010). In this study, at the phylum level, there were four major phyla (Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria) in all samples. We found that Firmicutes and Bacteroides were the most abundant flora in mice' intestines. Bacteroidetes and Firmicutes are leading groups of bacteria involved in metabolizing undigested food (Parkar et al., 2013). Moreover, the abundance of Bacteroidetes in the high-dose group was higher than that in the control group (39.85% vs. 25.40%), while the abundance of Firmicutes in the high-dose group was lower than that in the control group (49.58% vs. 59.33%). This is consistent with those researches on humans. Both short- and long-term studies have shown a higher proportion of Bacteroides in fecal microbiota after the consumption of a "Western diet" rich in animal protein and fat, and low in fibre (Wu et al., 2011; David et al., 2014).

Compared to omnivores, vegetarians have significantly lower microbial counts of Bacteroides and Bifidobacterium specie, while the total cell numbers remain unchanged (Zimmer et al., 2012). In this study, the contents of Bacteroidaceae and Family XIII were significantly higher in the high-dose group than the control group. The abundance of Lactobacillus was low in all groups, contributing 2.18%, 3.86%, 4.74% and 1.01%, respectively. It's noteworthy that the lowest occurred in the high-dose group. Lactobacillus has been considered as a key player in host metabolic balance (Arora et al., 2012). It is a common probiotic that can inhibit pathogens by fermenting food to produce lactic acid, which lowers the pH value in the intestine environment.

Moreover, Lachnospiraceae is a highly abundant bacterial family within the gut microbiota in human, whose members may protect against human colon cancer by producing butyric acid, a substance that is important for both microbial and host epithelial cell growth (Meehan & Beiko, 2014; Yang et al., 2020; Huang et al., 2020; Hwang et al., 2019; Grom et al., 2020). In this study, the compositions of Lachnospiraceae in the control, low-dose, medium-dose and high-dose groups were 34.1%, 36.9%, 32.84% and 31.39%, respectively. Lachnospiraceae FCS020 group, Lachnospiraceae UCG-001 and Lachnospiraceae UCG-008 were significantly higher in the low-dose group than other groups. These results indicated that the red meat diet may reform the gut microbiota, and refinement of diet regarding the proportion of red meat could help prevent colon cancer.

On the genus level, there were several species with widely varying abundance in community heatmap, including Romboutsia, Roseburia, Lachnospiraceae FCS020 group, Clostridium sensu stricto 1, Anaerotruncus, Bilophila, Blautia, GCA-900066575, Christensenellaceae R-7 group, Nesterenkonia, Harryflintia, Peptococcus, Rhodococcus, Faecalibaculum, and Ruminiclostridium. They were all dominant bacterium in the control group. Mouse models revealed that Faecalibacterium prausnitzii had anti-inflammatory properties, and in patients with Crohn Disease, reductions in ileal F. prausnitzii abundance were associated with disease recurrence (Sokol et al., 2008). In patients with ulcerative disease (UC), from stool samples, reductions in F. prausnitzii abundance are associated with an increased number of disease flares, a shorter time in remission, and a higher disease extent (pancolitis versus proctitis) in adult UC patients (Varela et al., 2013). In our experiment, the abundance of the Faecalibaculum was the highest in the control group, and was gradually decreased with the increase of red meat diet. Therefore, red meat diet may alter intestinal flora and, as a result, increase the risk of inflammatory bowel disease by altering intestinal flora.

Our experiment also demonstrated that the red meat diet was associated with significant changes in the composition of intestinal flora at genus level in mice, including increased relative abundance of Bacteroidaceae and Rikenellaceae, and decreased ratio of Peptostreptococcaceae and Blautia and Erysipelotrichaceae. We found a higher abundance of the genus Roseburia in the control group, which was consistent with previously reported studies (Chen et al., 2014; Morgan et al., 2012; Rehman et al., 2016). The genus Roseburia includes known butyrate producing organisms, and butyrate can interact with the intestinal epithelium to produce an anti-inflammatory environment (Louis et al., 2014) and modulate intestinal barrier function (Kelly et al., 2015). In patients with UC, a decrease in short-chain fatty acid production is correlated with reductions in Roseburia abundance (Machiels et al., 2014; Kumari et al., 2013). Therefore, the loss of Roseburia may lead to the uncontrolled colonic inflammation of UC. In general, the red meat diet changes the intestinal flora, which causes a decrease in Roseburia. This pathway may explain why people are prone to ulcerative colitis prefer red meat.

The mice in the control group had a higher abundance of Clostridiaceae-1, Clostridium spp, which contributed to complex carbohydrate breakdown in the gut and produces short chain acids. It is beneficial to intestinal epithelial cells (Kaoutari et al., 2013). The Lachnospiraceae and Ruminococcaceae are two of the most abundant families from the order Clostridiales found in the mammalian gut environment, and they have been demonstrated to be associated with the maintenance of gut health (Zhang et al., 2018). In this study, we found that Clostridiaceae-1 and Ruminococcaceae were significantly higher in the control and low-dose group compared with other groups. It further confirmed our hypothesis that less red meat intake might have a healthier gut flora.

4 Conclusions

Remarkably, the profiling of the gut microbiota communities with various diets in this investigation showed that administration of red meat diet for 8 weeks caused a reshaping of the intestinal bacteria composition towards an unhealthier microbial community. The red meat diet changed fecal microbiota content significantly by enriching harmful bacteria and/or depleting the beneficial ones. Therefore, it is necessary to continue the evaluation of the diet as a modulator of the gut microbiota, which could provide potential treatment strategies for diseases such as colitis and cancers.

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