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ORIGINAL RESEARCH

Microbial Disruptions in Inflammatory Bowel Disease: A Comparative Analysis

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Objective: The fecal microbiota was studied in patients with inflammatory bowel disease (IBD), and the characteristics of gut microbiota were compared among patients with different subtypes and stages of IBD, aiming to identify the gut microbiota associated with IBD.

Methods: Fecal samples were collected from 41 IBD patients (18 patients with ulcerative colitis [UC] and 23 patients with Crohn's disease [CD]) in the Department of Gastroenterology of East China Hospital, Fudan University between January 2021 and January 2022. In addition, fecal samples were collected from 20 healthy volunteers. The fecal microbiota was subjected to 16S rRNA gene sequencing, followed by bioinformatics analysis.

Results: There was significant difference in the fecal microbiota between IBD patients and controls. The abundance and diversity of fecal microbiota in the IBD patients were significantly lower than in controls. The relative abundance of *Subdoligranulum, Ruminococcus, Anaerostipes* and *Lachnospira* was reduced markedly in the IBD patients. As compared to controls, the relative abundance of *Streptococcus* increased dramatically in the UC patients. The relative abundance of *Lachnoclostridium, Fusobacterium, Cloacibacillus* and *Erysipelatoclostridium* significantly increased in the CD patients. As compared to CD patients, the relative abundance of *Alistipes* was reduced markedly in the UC patients; the relative abundance of *Faecalibacterium, Roseburia* and *Haemophilus* was reduced dramatically in the CD patients. In addition, significant difference was also noted in the fecal microflora between patients with active IBD and those with IBD in remission period. In active IBD patients, the relative abundance of *Roseburia, Coprococcus* and *Ruminiclostridium* was reduced significantly.

Conclusion: There is intestinal microbiota imbalance in IBD patients, and the abundance of *Roseburia, Coprococcus* and *Ruminiclostridium* is reduced significantly in the active period of IBD, which may be related to the active IBD.

Keywords: inflammatory bowel disease, ulcerative colitis, Crohn's disease, gut microbiota, 16S rRNA gene sequencing

Introduction

Inflammatory bowel disease (IBD) is a chronic, relapsing intestinal inflammatory disease and can be divided into ulcerative colitis (UC) and Crohn's disease (CD). Environment, genetic susceptibility, abnormal immune response of the intestinal mucosa, and gut microbiota are involved in the occurrence and development of IBD. In recent years, increasing evidence has shown that intestinal microbiota disorder is closely related to the pathogenesis of IBD.[1]

The global incidence of IBD is increasing over year, and the annual incidence of IBD varies from 0.1/100000 to 58/100000 in different regions.[2] The incidence of CD is 6.3/100000–23.8/100000 each year in North America, but it is about 54/100000[3,4] each year in the Asia Pacific region, and the incidence of IBD is closely related to the regions and socio-economic development. With the rapid economic development in Asia Pacific region (especially China) and the change of lifestyle, such as the industrialization, urbanization, air pollution, popularization of western diet (high calorie diet), the

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incidence of IBD is growing rapidly, but the incidence of IBD has become relatively stable in the industrialized regions such as Europe.

A meta-analysis[5] investigated 48 studies, in which there were 2221 IBD patients (1206 CD patients and 1015 UC patients) and 2063 healthy controls. Among them, 34 studies investigated the microbiota of CD patients, and 29 studies examined the microbiota of UC. All 3 studies of Christensenellaceae and Coriobacteriaceae and 6 of 11 studies of Faecalibacterium prausnitzii reported a decreased amount of those organisms compared with controls, whereas 2 studies each of Actinomyces, Veillonella, and Escherichia coli revealed an increased amount in patients with Crohn's disease. For patients with ulcerative colitis, Eubacterium rectale and Akkermansia were decreased in all 3 studies, whereas E coli was increased in 4 of 9 studies. The microbiota diversity was either decreased or not different in patients with IBD vs controls. To data, increasing studies have indicated that regulation of gut microbiota will become an important strategy for the treatment of IBD, but the association between specific microorganisms and IBD has not identified yet.

In this study, 16s rRNA high-throughput sequencing technology was employed to sequence the fecal microbiota in patients diagnosed with IBD. The characteristics of fecal microbiota were compared between IBD patients and healthy controls, between patients with active IBD and those in remission period, and between UC patients and CD patients. This study aimed to investigate the changes in the fecal microbiota of IBD patients and identify specific fecal microbiota related to IBD, which may elucidate the role of gut microbiota in the pathogenesis of IBD and provide evidence on the treatment of IBD by regulating intestinal microbiota.

Subjects and Groups

Subject Recruitment

From January 2021 to January 2022, 41 IBD patients (including 18 UC patients and 23 CD patients) who met the inclusion and exclusion criteria were recruited from the Department of Gastroenterology, East China Hospital, Fudan University. In addition, 20 healthy volunteers who met the inclusion and exclusion criteria and received physical examination in our hospital were also recruited as controls in the same period. IBD patients were divided into UC group and CD group. IBD patients were divided into active IBD (IBD-A) and remissive IBD (IBD-R) group. Healthy subjects served as controls (HC) group. The gender, age, height and body weight were recorded. This study was approved by the Ethics Committee of East China Hospital, Fudan University (No. ISRCTN: 2023K002).

Inclusion and Exclusion Criteria

The inclusion criteria for IBD were as follows: IBD was diagnosed according to the Consensus on the Diagnosis and Treatment of Inflammatory Bowel Disease (2018 • Beijing) developed by the Digestive Disease Branch of the Chinese Medical Association;[6] patients had no mental illness and could co-operate with this study; informed consent was obtained before study in accordance with the Declaration of Helsinki. Patients had no mental illness and could co-operate with this study.

The exclusion criteria for IBD were as follows: IBD was not diagnosed clinically, or the types of IBD (UC or CD) were unknown; subjects received treatment with probiotics, prebiotics, synbiotics, or other intestinal microbiota regulators in the prior month, or received fecal microbiota transplantation; subjects received antibiotic treatment; patients had mental illness, other severe injury to the major organs (such as heart, brain, kidney and others) or malignant tumors.

The inclusion criteria for healthy controls were as follows: subjects were 18–75 years old; subjects had no evident organic diseases. The exclusion criteria for healthy controls were as follows: subjects received antibiotic treatment within prior month. Subjects received treatment with probiotics, prebiotics, synbiotics, or other intestinal microbiota regulators in the prior month, or received fecal microbiota transplantation.

Assessment of Disease Activity

The activity of UC was assessed with Mayo ulcerative colitis endoscopic index[7] and the activity of CD with simplified Crohn's Disease Activity index.[8]

Sample Collection

Subjects were trained for the standard collection of fecal samples (5 g/subject). Fecal samples were collected into AxyPrepDNA gel recovery kit and stored at −80°C within 2 h.

Detection of Fecal Microbiota

Miseq library was established with 61 fecal samples (including genomic DNA extraction, PCR amplification, recovery with AxyPrepDNA gel recovery kit, and real-time fluorescence quantification with FTC-3000TM real-time PCR), and then illumina high-throughput sequencing and bioinformatics analysis were carried out. The two-step PCR amplification was employed and the V4-V5 region of 16S rRNA of bacteria was used as the target for PCR amplification.

F: 5'-TTCCCTACACGACGCTCTTCCGATCT-barcode F1 −3'
F: 5'-AATGATACGGCGACCACCGAGATCTACAC-
TCTTTCCCTACACGACGCTC −3'
R: 5'-GAGTTCCTTGGCACCCGAGAATTCCA- barcodeR1 −3'
R: 5'-CAAGCAGAAGACGGCATACGAGAT- barcodeR2 -
GTGACTGGAGTTCCTTGGCACCCGAGA-3'

Bioinformatics Analysis of Gut Microbiota

The non-parametric test was used to compare the relative abundance of microbiota in different groups at the levels of phylum, genus, and species, and the microbiota with significant differences in the relative abundance were identified in each group. Two groups were compared using the Wilcox test, and three or more groups were compared using the Kruskal test. For three or more groups, microbiota with difference in relative abundance among multiple groups was further analyzed between two groups using post hoc test. Microbiota with significant difference ($P < 0.05$) was selected and displayed in a bar chart. If there were more than 20 microbiotas with significant difference, the top 20 were selected for the delineation of bar chart. The non-parametric Kruskal–Wallis rank sum test was used to analyze the difference in LEfSe between groups.

Statistical Analysis

This study used R language 3.4.1 and SPSS version 25.0 (SPSS, Chicago, IL, USA) for statistical analysis. The quantitative data with normal distribution were compared with *t*-test or one way analysis of variance (ANOVA), and are expressed as mean ± standard deviation (SD). Data without normal distribution were compared with non-parametric tests (such as Mann–Whitney *U*-test or Kruskal–Wallis test) and are presented as M (P25, P75). Qualitative data were compared with Chi square test or Fisher's exact test, and are expressed as frequency or percentage. Delineation was done with GraphPad Prism 8.4.3.686 and R language 3.4.1 ((SPSS, Chicago, IL, USA)). A value of $P < 0.05$ was considered statistically significant.

Results

Demographic Characteristics of Subjects in Different Groups

A total of 41 IBD patients (including 18 UC patients and 23 CD patients) and 20 healthy volunteers were recruited into present study. As shown in Table 1, there were no significant differences in the gender, age and body mass index (BMI) among UC patients, CD patients and healthy controls ($P > 0.05$).

Table 1 Demographic Characteristics of Subjects in Different Groups

Variables	UC group	CD group	Control group	P
Gender (M/F)	14:4	14:9	8:12	0.06
Age (years)	51.94±14.72	44.96±18.28	50±6.97	0.385
BMI (kg/m²)	22.33±3.47	22.06±3.47	24.02±3.16	0.141

Abbreviations: BMI, body mass index; CD, Crohn's disease; F, female; M, male; UC, ulcerative colitis.

Gut Microbiota Among UC Patients, CD Patients and Controls

In the present study, the Chao index, ACE index and Shannon index in the UC patients and CD patients were significantly lower than in the healthy controls ($P < 0.05$). As compared to the CD patients, the Chao index and ACE index were reduced significantly ($P < 0.05$), but there were no significant differences in the Shannon index and Simpson index. This indicated that UC patients and CD patients had lower abundance and lower diversity of gut microbiota as compared to healthy controls. In addition, the abundance in the UC patients was lower than in the CD patients, but there was no marked difference in the diversity between UC patients and CD patients.

Gut Microbiota Among IBD-A Patients, IBD-R Patients and Controls

In the IBD-A group and IBD-R group, the Chao index, ACE index and Shannon index were significantly lower than in the healthy controls ($P < 0.05$). In addition, the Chao index and Shannon index in the IBD-A group were markedly lower than in the IBD-R group ($P < 0.05$). The mean Simpson index in the IBD-A group was significantly higher than in the IBD-R group ($P < 0.05$). These findings indicated that patients in the IBD-A group and IBD-R group had significantly lower abundance and diversity as compared to healthy controls. Moreover, as compared to IBD-R patients, the abundance and diversity of gut microbiota were reduced markedly in the IBD-A patients.

Beta Diversity of Gut Microbiota

PCoA diagram were made based on the principal coordinate analysis of weighted unifrac. Based on the dispersion of samples in different groups, preliminary comparisons were made on the intra-group and inter-group differences in the composition of gut microbiota among groups. Then, similarity analysis (ANOSIM) was used to further examine the Beta diversity of gut microbiota among different groups.

Gut Microbiota Among UC Patients, CD Patients and Controls

As shown in Figure 1A, samples in the HC group had clustered distribution, but those in the UC group and CD group displayed dispersed distribution. This indicated the composition of gut microbiota in the healthy controls had small intra-

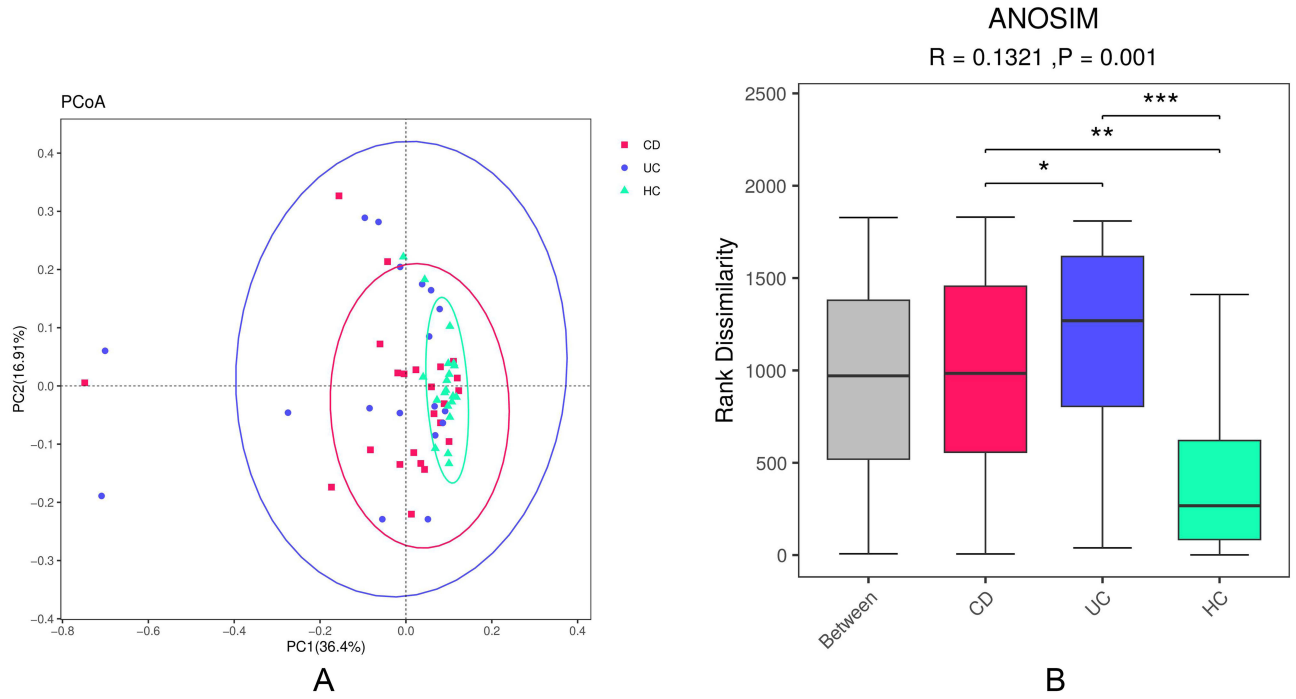

Figure 1 Beta diversity of gut microbiota among UC patients, CD patients and controls. (**A**) PCoA diagram; (**B**) ANOSIM analysis of weighted unifrac; *P<0.05, **P<0.01; ***P<0.001.

group difference, but this difference was relatively large in the UC patients and CD patients. As compared to the CD group, this difference was even larger in the UC group. ANOSIM analysis showed significant difference among UC patients, CD patients and controls ($R > 0$, $P < 0.05$) (Figure 1B). This indicated significant difference in the Beta diversity of gut microbiota among UC patients, CD patients and controls, and the composition of gut microbiota was significantly different among these subjects.

Gut Microbiota Among IBD-A Patients, IBD-R Patients and Controls

As shown in Figure 2A, clustered distribution of gut microbiota was noted in the controls and IBD-R patients, but dispersed distribution was observed in the IBD-A group. This indicated small intra-group difference in the composition of gut microbiota in the healthy controls and IBD-R patients, but it was larger in the IBD-A patients. ANOSIM analysis showed marked difference among IBD-A patients, IBD-R patients and controls ($R > 0$, $P < 0.05$) (Figure 2B). These findings indicated significant difference in the Beta diversity among IBD-A patients, IBD-R patients and controls, and there was marked difference in the composition of gut microbiota among these subjects.

Composition of Gut Microbiota Among UC Patients, CD Patients and Controls

The composition and relative abundance of bacteria in the gut microbiota were compared among between UC, CD, and HC groups at the levels of phylum and species.

Composition of Gut Microbiota at Phylum Level

The top 10 bacteria with the highest abundance were displayed at the phylum level in Figures 3 and 4. The top 3 bacteria were *Bacteroidetes, Firmicutes* and *Proteobacteria*, and the sum of their abundances was higher than 94% in three groups. In addition, the relative abundance of *Proteobacteria* in the UC patients and CD patients was significantly higher than in the healthy controls ($P < 0.05$).

Moreover, at the phylum level, marked difference in the relative abundance was also noted in the *Fusobacteria, Actinobacteria* and *Synergistetes* among UC patients, CD patients and healthy controls. In the CD patients and UC

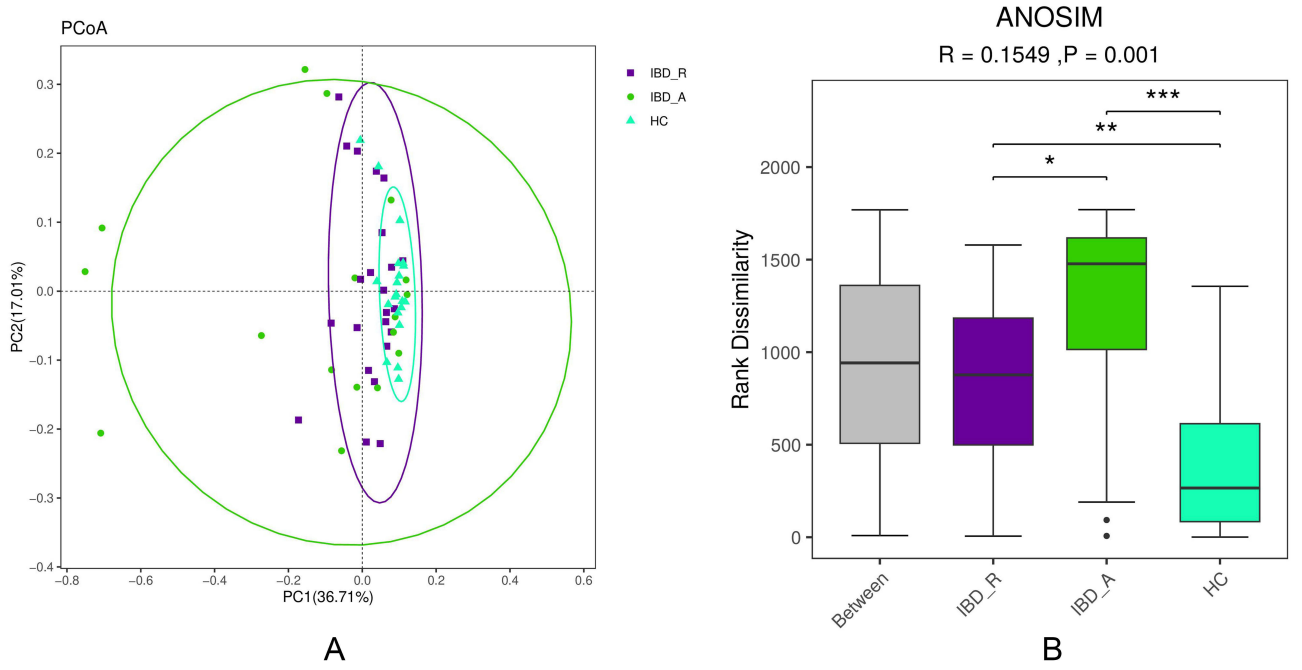

Figure 2 Beta diversity of gut microbiota among IBD-A patients, IBD-R patients and controls. (**A**) PCoA diagram; (**B**) ANOSIM analysis of weighted unifrac; *P<0.05, **P<0.01; ***P<0.001.

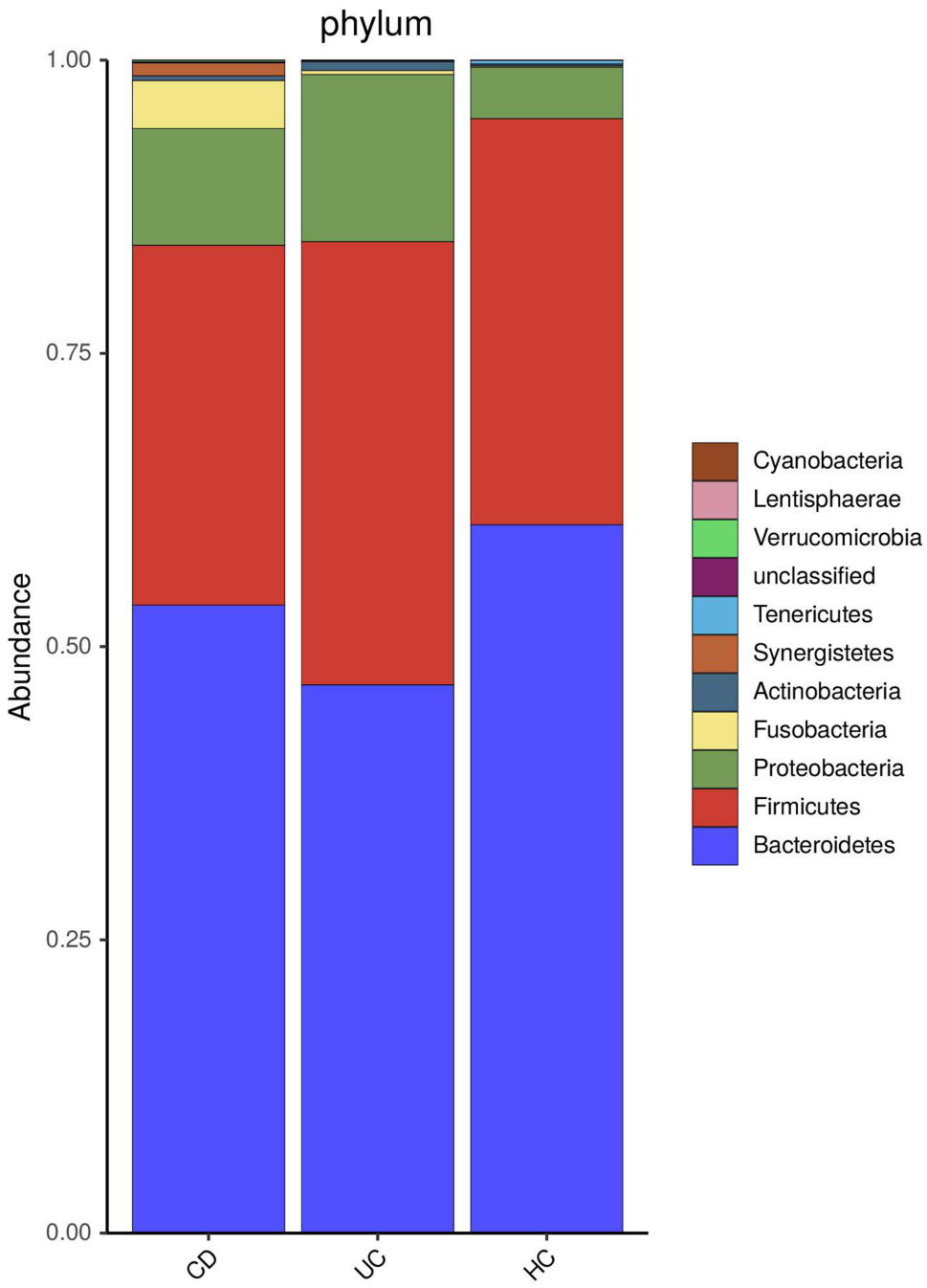

Figure 3 Composition of gut microbiota at phylum level among UC patients, CD patients and healthy controls.

patients, the relative abundance of *Actinobacteria* increased dramatically ($P < 0.05$), and this increase was more evident in the UC patients ($P < 0.05$).

Composition of Gut Microbiota at Species Level

The bacteria with relative abundance higher than 0.5% were analyzed in the UC group, CD group and HC group. Results showed *Bacteroides_vulgatus* (13.22% vs 16.43% vs 15.00%, $P = 0.577$) and *Bacteroides_thetaiotaomicron* (8.08% vs 7.77% vs 5.69%, $P = 0.846$) had the highest abundance in the UC group, CD group and HC group, and the relative abundance was higher than 5% in three groups. In addition, the relative abundance of *Escherichia_coli* was

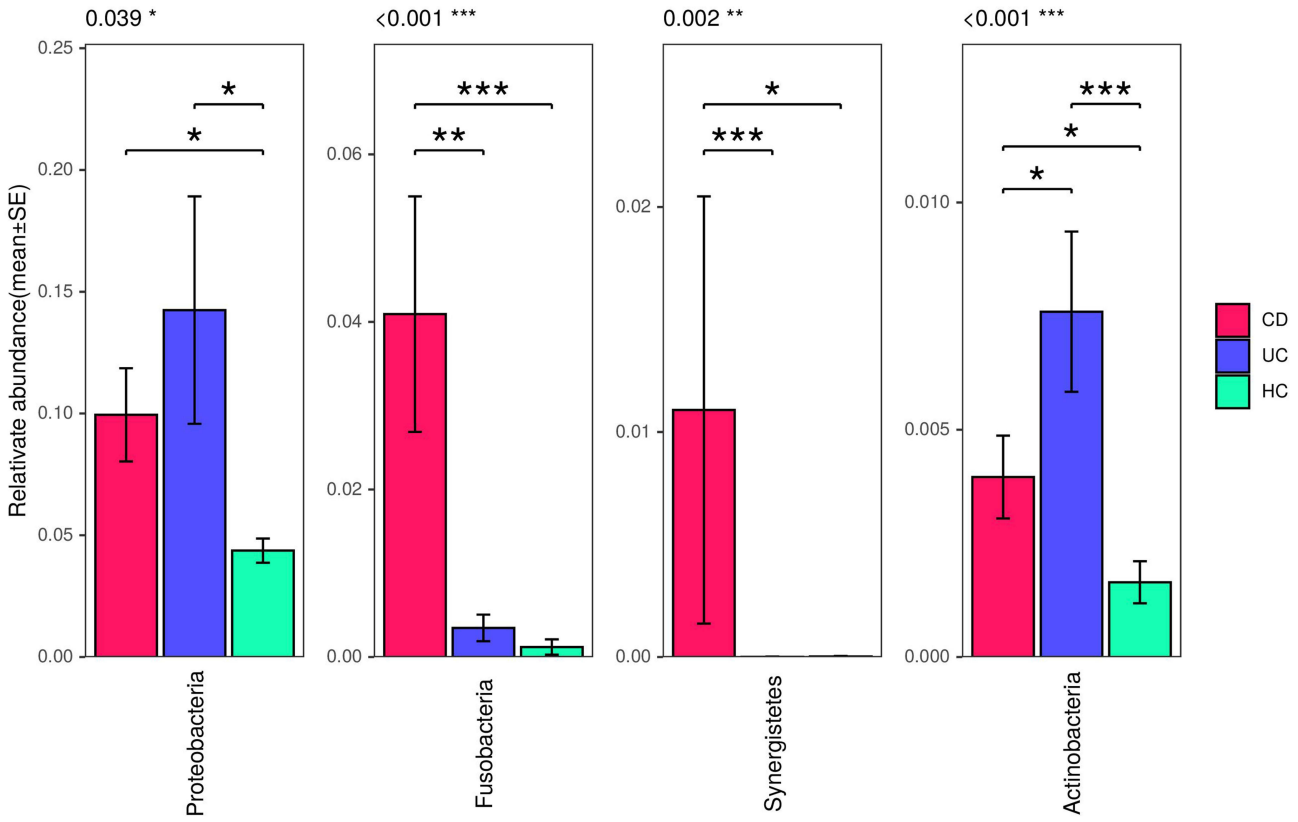

Figure 4 Bacteria with significant difference at phylum level among UC patients, CD patients and healthy controls. Note: * P<0.05; ** P<0.01; *** P<0.001.

6.31% and 4.27% in the UC patients and CD patients, respectively, which were significantly higher than in the controls (0.50%).

The bacteria with significant difference among three groups were further analyzed at the species level, and the top 20 bacteria with the highest abundance were selected for further analysis (Figure 5). Results showed, as compared to the HC group, the abundance of *Bacteroides_massiliensis, Parabacteroides_merdae* and *Bacteroides_cellulosilyticus* was reduced significantly in the UC group, the abundance of *Roseburia_hominis* was reduced markedly in the CD group, and the abundance of *Roseburia_inulinivorans* and *Anaerostipes_hadrus* was reduced significantly in both UC patients and CD patients. In addition, the abundance of *Lactobacillus_salivarius, Cloacibacillus_evryensis, Erysipelatoclostridium_ramosum* and *Fusobacterium_sp._RMA_1065* increased markedly in the CD group, but that of *Escherichia_coli* increased dramatically in bother UC group and CD group.

Moreover, the abundance of butyrate producing *Faecalibacterium_prausnitzii* was reduced significantly in bother UC group and CD group (0% vs 0.01%). In the HC group, the abundance of *Faecalibacterium_prausnitzii* was 0.02%, which was significantly higher than in the UC group and CD group ($P < 0.001$).

Composition of Gut Microbiota Among IBD-A Group, IBD-R Group and HC Group

The composition and relative abundance of bacteria in the gut microbiota were compared among IBD-A, IBD-R, and HC groups at the levels of phylum and species.

Comparison of Gut Microbiota at Phylum Level

The top 10 bacteria with the highest abundance at the phylum level were further analyzed (Figure 6). Results showed the top 3 bacteria among IBD-A group, IBD-R group and HC group were *Bacteroidetes, Firmicutes* and *Proteobacteria*; the sum of their abundances was higher than 94% in three groups. In addition, the abundance of *Proteobacteria* in the IBD-A

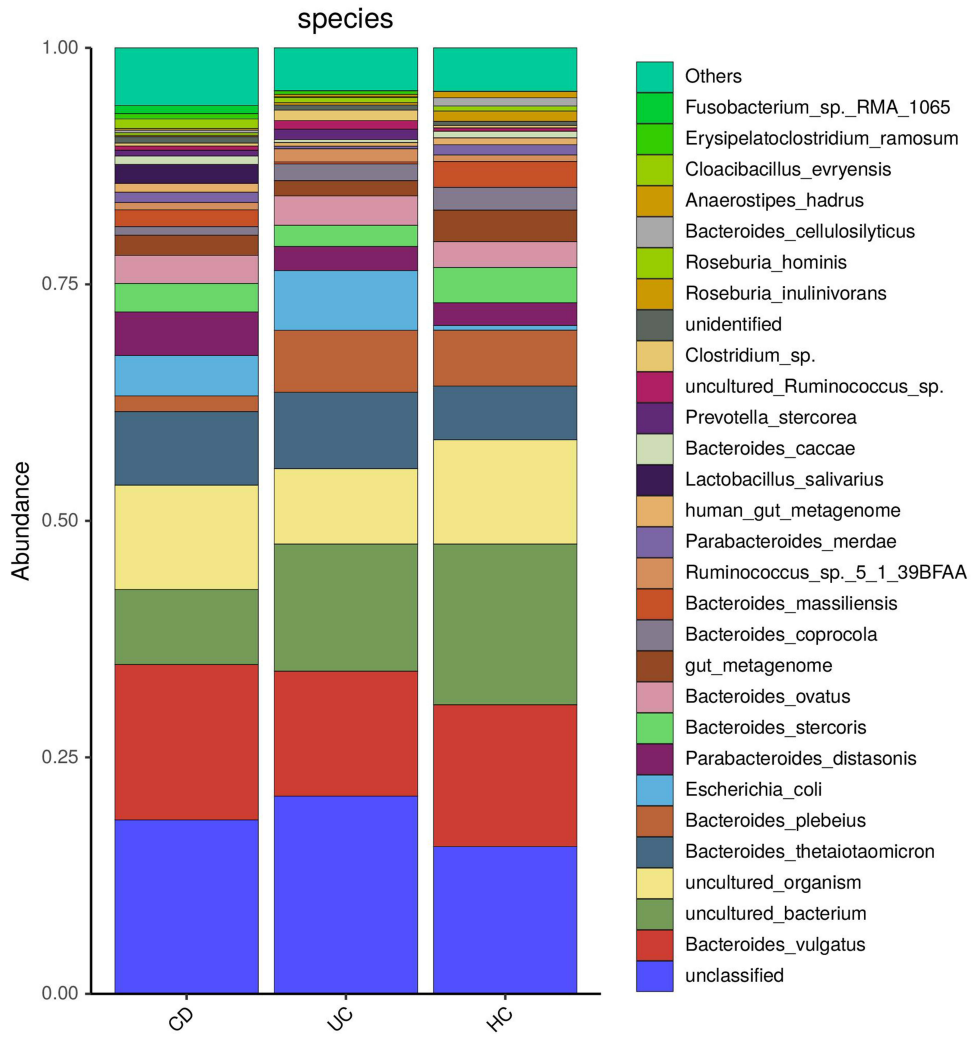

Figure 5 Bacteria with significant difference at species level among UC patients, CD patients and controls.

group and IBD-R group was significantly higher than in the HC group. Moreover, at the phylum level, the abundance of *Fusobacteria* and *Actinobacteria* in the IBD-A group and IBD-R group was significantly higher than in the HC group.

Comparison of Gut Microbiota at Species Level

At the species level, bacteria with relative abundance higher than 0.5% among IBD-A group, IBD-R group and HC group were further analyzed (Figure 7). Results showed *Bacteroides_vulgatus* (13.39% vs 16.61% vs 15.00%, P = 0.543) and *Bacteroides_thetaiotaomicron* (11.72% vs 5.12% vs 5.69%, P = 0.850) had the highest abundance in the UC group, CD group and HC group, and its abundance was higher than 5% in three groups.

At the species level, the bacteria with significant difference were selected and the top 20 bacteria were further analyzed (Figure 7). Results showed the abundance of *Roseburia_hominis* was reduced significantly, and the abundance of *Clostridium_sp*. increased markedly in the IBD-A group as compared to the HC group and IBD-R group. As compared to the HC group, the relative abundance of *Parabacteroides_merda* in the IBD-A group was reduced significantly, but the relative abundance of *Erysipelatoclostridium_ramosum* and *Fusobacterium_sp._RMA_1065* increased in the IBD-R group. The relative abundance of *Roseburia_inulinivorans, Bacteroides_cellulosilyticus* and *Anaerostipes_hadrus* reduced significantly in both IBD-A group and IBD-R group, but the abundance of *Escherichia_coli* and *Clostridium_neonatale* increased dramatically in both IBD-A group and IBD-R group.

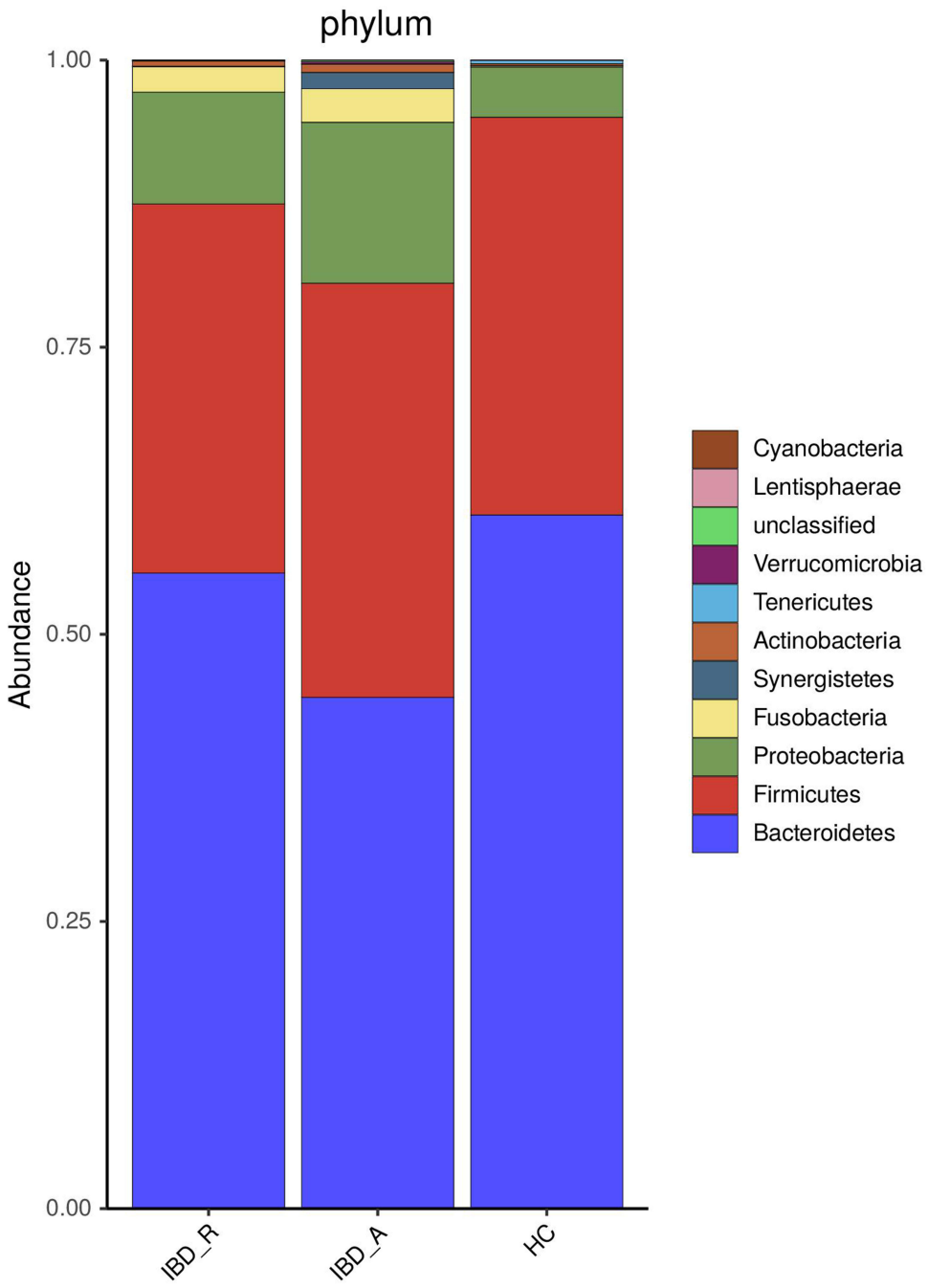

Figure 6 Composition of gut microbiota at phylum level among IBD-A group, IBD-R group and HC group.

As compared to the HC group and IBD-R group, the abundance of *Coprococcus_comes* reduced significantly and that of *Coprococcus_sp._HPP0074* increased markedly in the IBD group. As compared to the HC group, the abundance of *Dorea_longicatena* reduced significantly, and the abundance of *Odoribacter_splanchnicus* and *Blautia_obeum* reduced markedly in both IBD-A group and IBD-R group.

Discussion

IBD is a chronic, inflammatory intestinal disease, but its pathogenesis is still poorly understood. In recent years, with the development of technologies such as 16S rRNA gene sequencing and metagenomic analysis, the role of gut microbiota in the pathogenesis of IBD has become a hot topic. To date, no conclusive evidence has suggested that the triggering and

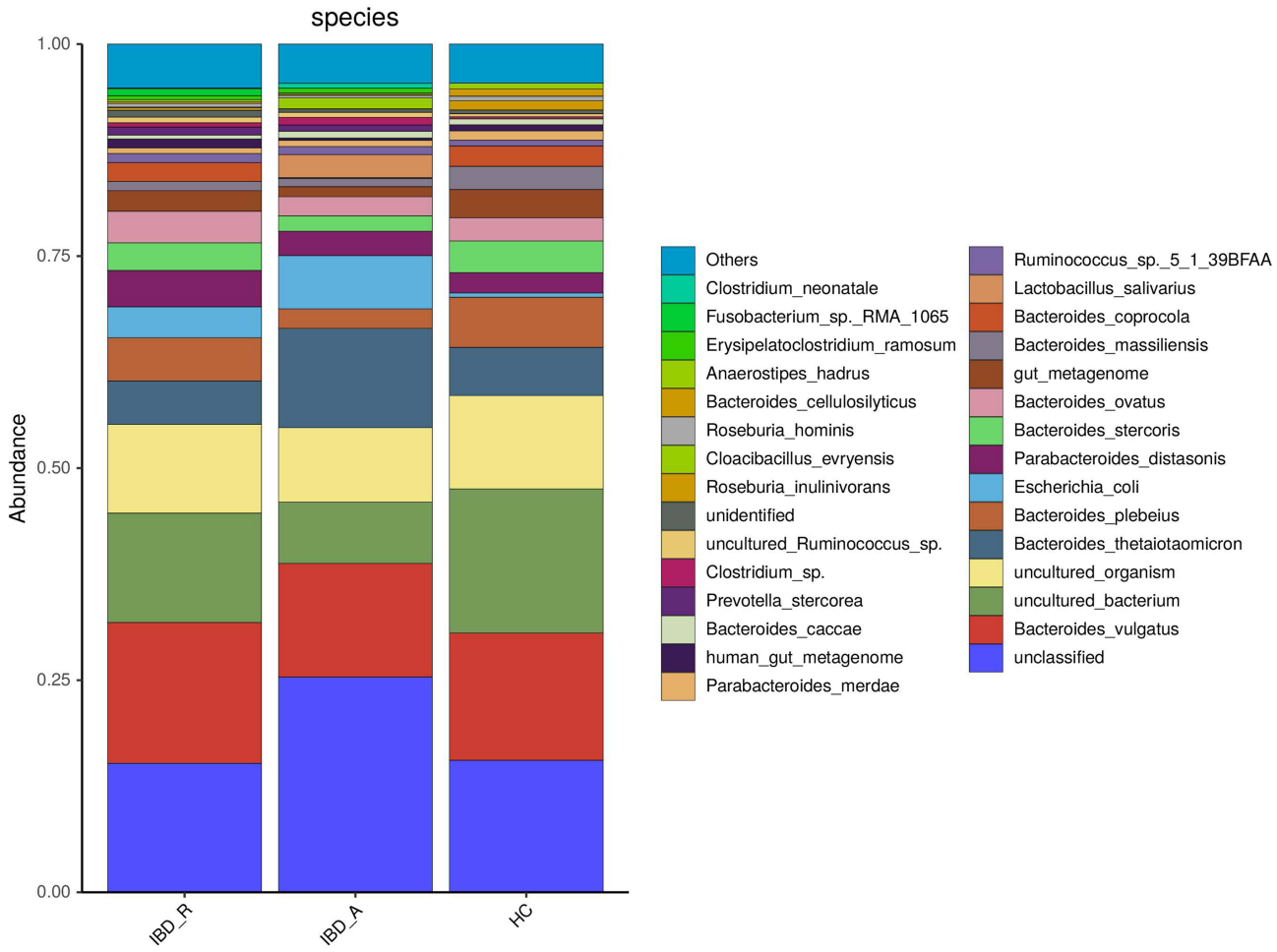

Figure 7 Composition of gut microbiota at species level among IBD-A group, IBD-R group and HC group.

aggravation of intestinal barrier damage in IBD patients are related to a specific pathogenic microorganism, and most studies have indicated that the overall imbalance of intestinal microbiota is related to the pathogenesis of IBD. In recent years, some studies have shown that the increases in oxidative stress and nitrification stress in the gut of IBD patients affect the abundances of specialized anaerobic bacteria and facultative anaerobic bacteria.[9,10] In the present study, the characteristics of gut microbiota were investigated in the UC patients, CD patients, IBD-A patients, and IBD-R patients by using 16S rRNA gene sequencing.

The available findings about the Alpha and Beta diversity of gut microbiota among UC patients, CD patients, and healthy controls are inconsistent. Our study showed that the abundance (Chao index and ACE index) and diversity (Shannon index) of gut microbiota in the UC and CD groups were significantly lower than in the healthy control group, which was similar to the results reported by Wang et al.[11] In addition, our study also revealed that the abundance of gut microbiota in the UC patients was lower than in the CD patients, but there was no significant difference in the diversity of gut microbiota between UC patients and CD patients. However, Kiernan et al[12] reported that the Shannon index in the UC group was higher than in the CD group ($P < 0.01$). Ma et al[13] failed to find a significant difference in the Alpha diversity of gut microbiota among UC patients, CD patients, and healthy individuals. This discrepancy may be related to some factors, such as differences in dietary habits, age, disease status, disease activity, and living environment of enrolled patients. Our results showed a marked difference in the composition of gut microbiota among UC patients, CD patients, and healthy controls (ANOSIM: UC group vs HC group, $R = 0.210$, $P = 0.001$; CD group vs HC group, $R = 0.125$, $P = 0.005$; UC group vs CD group, $R = 0.080$, $P = 0.033$). This indicates a significant difference in the Beta diversity of gut microbiota among UC patients, CD patients, and healthy controls. Kiernan et al[10] reported similar results, but Ma et al[8]

found the composition of gut microbiota was similar between UC patients and CD patients (ANOSIM: P = 0.133). This discrepancy may be related to the differences in the source of samples and methodology.

Our results showed the Alpha diversity of gut microbiota tended to reduce with the progression of IBD: healthy control group > IBD-R group > IBD-A group and a significant difference was observed among these groups (P < 0.05). This was consistent with previous findings.[14] There is evidence showing that the microbial composition is similar between IBD-A patients and IBD-R patients,[15] and the composition of microbiota was hard to distinguish based on the principal component analysis. Our results showed a marked difference in the Beta diversity of gut microbiota among IBD-A patients, IBD-R patients, and healthy controls (ANOSIM: IBD-A group vs IBD-R group, R=0.128, P = 0.012; IBD-A group vs HC group, R = 0.260, P = 0.001; IBD-R group vs HC group, R = 0.117, P = 0.005). Some investigators further compared the composition of gut microbiota among IBD patients with different disease activities, and results showed a marked difference in the Beta diversity among patients with mild, moderate, and severe UC (ANOSIM: mild vs moderate, R = 0.093, P = 0.016; moderate vs severe, R = 0.136, P = 0.015; mild vs severe, R = 0.332, P = 0.001). This indicates that the disease activity also affects the composition of gut microbiota of UC patients, which is consistent with our findings.

Our results showed that the sum of relative abundance of three dominant bacteria (*Bacteroidetes, Firmicutes*, and *Proteobacteria*) in the UC group, CD group, and HC group was higher than 94%. Studies have revealed that *Firmicutes*, especially *Clostridia*, is rich in bacteria that can produce short-chain fatty acids (SCFAs), which can increase regulatory T cells and enhance the immune tolerance in the intestinal mucosa. The relative abundance of *Firmicutes* generally is reduced in the IBD patients, but some other studies fail to identify the significant change in the relative abundance of *Firmicutes* in the IBD patients.[16] In the present study, the relative abundance of *Firmicutes* in the UC group and CD group was not reduced significantly as compared to the HC group, but the abundance of SCFA-producing bacteria was reduced (such as *Faecalibacterium* and *Roseburia*). The abundance of *Proteobacteria* was significantly higher in the UC patients and CD patients than in the healthy controls (P < 0.05), which were consistent with results reported by Ma et al[14] and Kiernan et al.[12] There are several types of opportunistic bacteria in *Proteobacteria*, and some have been confirmed to deteriorate intestinal inflammation in IBD animal models, including the facultative anaerobic bacteria in the *Escherichia* and *Klebsiella*.[17] At the phylum level, the abundance of *Fusobacteria* and *Synergistetes* increased significantly in the CD group (P < 0.05), which was consistent with previous findings.[18–20] Studies have indicated that *Fusobacterium* in the *Fusobacteria* is adherent to the intestinal mucosa and may invade the intestinal epithelial cells, resulting in the deterioration of inflammation.[21,22] In the present study, the abundance of *Actinobacteria* increased significantly in the UC group and CD group (P < 0.05), and this increase was more evident in the UC group (P < 0.05). This was consistent with results reported by Salimi et al,[21] Alam et al[22] and Forbes et al.[23] However, Soltys et al[24] reported that the relative abundance of fecal *Actinobacteria* in the IBD patients showed seasonal change and was related to the serum level of 25-hydroxyvitamin D. In winter and spring, the serum level of 25-hydroxyvitamin D in the IBD patients was lower, and the relative abundance of *Actinobacteria* decreased; in summer and autumn, the serum level of 25-hydroxyvitamin D and the relative abundance of *Actinobacteria* increased in the IBD patients.

In the present study, the top 3 bacteria in the IBD-A group, IBD-R group, and HC group were *Bacteroidetes, Firmicutes*, and *Proteobacteria* based on their abundance, and the sum of their abundances was higher than 94% in three groups. In addition, the abundance of *Proteobacteria* in the IBD-A group was higher than in the IBD-R group and HC group although there was no significant difference. Ma et al reported the abundance of *Proteobacteria* in the active CD patients was also slightly higher than in the remissive CD patients.[14] However, Forbes et al[24] found that *Proteobacteria* was more likely to be detectable in the activated UC patients as compared to the active CD patients. In recent two studies,[12,13] results showed *Proteobacteria* gained growth advantages from host-derived nitrogen, which exacerbated colitis in the mouse model. The sources of host-derived nitrogen include nitric oxide produced by immune cells and byproducts of metabolism (such as urea). In addition, Zhu et al[25] found tungstate treatment could inhibit the replication of *Proteus*, which improved colitis in mice. These findings indicate the increase in the abundance of *Proteobacteria* is related to the activity of colitis. In the present study, the relative abundance of *Proteobacteria* was comparable between IBD-A group and IBD-R group, which may be ascribed to the difference in the proportion of CD and UC patients in the IBD-A group and IBD-R group. In the present study, the relative abundance of *Fusobacteria* and *Actinobacteria* was

significantly higher than in the healthy controls, but no significant difference was noted between IBD-A group and IBD-R group, which was inconsistent with results reported by Forbes et al.[23]

Our study showed that the relative abundance of *Roseburia, Coprococcus*, and *Ruminiclostridium* significantly decreased in the IBD-A group as compared to the healthy control group and IBD-R group, which was consistent with previously reported.[25] *Roseburia* can upregulate antimicrobial peptides, improve intestinal innate immunity, and induce the differentiation of regulatory T cells, improving intestinal barrier function. In addition, butyric acid-producing *Roseburia* provides substrates for the β-oxidation in the intestinal mucosal cells and is involved in maintaining the anaerobic environment in the intestine.[26] *Roseburia* is one of the bacteria closely related to the disease activity of IBD. *Coprococcus* is also a type of important bacteria that can produce butyric acid. Studies have shown that bacteria in the *Coprococcus* may help suppress the immune responses and therefore inhibit the severity of allergic reactions, and thus it can be used as a microbial biomarker to evaluate human gastrointestinal health.[27] *Ruminiclostridium* is a specialized anaerobic bacterium that participates in the decomposition of cellulose. Although their role in host physiology has not been widely studied, a study indicates that *Ruminiclostridium* may act in the gut-brain axis.[28]

Taken together, our study indicates that the relative abundances of *Roseburia, Coprococcus*, and *Ruminiclostridium* in fecal bacteria of IBD patients are closely related to the disease activity of IBD, providing a theoretical basis for the treatment of IBD with probiotics in the future. However, the diet, living environment, and lifestyle of enrolled patients were not analyzed in this study, which may bias our results.

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Disclosure

The authors report no conflicts of interest in this work.

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