Fecal microbiota profile in patients with inflammatory bowel disease in Taiwan

Tien-En Chang
Taipei Veterans General Hospital
Jiing-Chyuan Luo (jcluo@vghtpe.gov.tw)
Taipei Veterans General Hospital https://orcid.org/0000-0003-4306-5464
Ueng-Cheng Yang
National Yang-Ming University
Yi-Hsiang Huang
Taipei Veterans General Hospital
Ming-Chih Hou
Taipei Veterans General Hospital
Fa-Yauh Lee
Taipei Veterans General Hospital

Research article

Keywords: Dysbiosis, gut microbiota, inflammatory bowel disease, next-generation sequencing, ulcerative colitis, Crohn's disease

Posted Date: August 26th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-51769/v1

Version of Record: A version of this preprint was published at Journal of the Chinese Medical Association on April 12th, 2021. See the published version at https://doi.org/10.1097/JCMA.0000000000000532.

Abstract

Background Inflammatory bowel disease (IBD) is a chronic inflammatory disease that associated with complicated interaction between immune, gut microbiota and environmental factors in a genetically vulnerable host. Dysbiosis is often seen in patients with IBD. Our aim is to investigate the fecal microbiota in patients with IBD and compared to healthy controls in Taiwan.

Methods In this cross-sectional study, we investigated fecal microbiota in 20 patients with IBD and 48 healthy controls. Fecal samples from both IBD patients and controls were analyzed by next-generation sequencing method and relevant software.

Results The IBD group showed lower bacterial richness and diversity compared to the control group. The principal coordinate analysis also revealed significant structural difference between the IBD group and the control group. These findings were consistent whether the analysis was based on operational taxonomic unit or amplicon sequence variant. However, no significant difference was found when comparing the composition of fecal microbiota between ulcerative colitis (UC) and Crohn's disease (CD). Further analysis showed that Lactobacillus, Enterococcus, Bifidobacterium and Veillonella were dominant in the IBD group, while Faecalibacterium and Subdoligranulum were dominant in the control group at genus level. When comparing UC, CD and control group, Lactobacillus, Bifidobacterium and Enterococcus were identified as dominant genera in the UC group. Fusobacterium and Escherichia_Shigella were dominant in the CD group. Faecalibacterium and Subdoligranulum were dominant in the control group.

Conclusions Compared to the healthy control, the IBD group showed dysbiosis with a significant decreased in both richness and diversity of gut microbiota.

Background

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a chronic progressive inflammatory disorder that affect gastrointestinal tract. [1, 2] The prevalence of IBD increases globally. [3] Although the incidence of IBD is low in Taiwan, the incidence and prevalence of IBD kept raising in the past decades. [4] The urge of understanding the pathogenesis of IBD and to facilitate the treatment strategy of IBD is growing. The most accepted hypothesis of IBD pathogenesis is an abnormal immune response against the gut microbiota that triggered by external environment in a genetically vulnerable host. [2, 5-7]

Dysbiosis of the gut microbiota alters host–microbiota interaction and the host immune system. Growing evidence has shown that dysbiosis is associated with several diseases, such as IBD, metabolic syndrome, and cardiovascular disease. [7, 8] The composition of microbiota in IBD is altered compared with that in healthy subjects. [9, 10]

The alteration of gut microbiota, including change of composition and reduce of diversity, in IBD patients was found in many studies. Among them, decrease of *Firmicutes* and increase of *Proteobacteria* were

reported mostly. [7, 11-14] By fermenting resistant starch or indigestible carbohydrates, intestinal bacteria produce short-chain fatty acids (SCFAs). SCFAs are major anions in the colon, mainly as acetate, propionate, and butyrate. Butyrate is energy source for colonic epithelial cells. The levels of SCFAs are significantly decreased in IBD and may be crucial in intestinal and immune homeostasis. [15, 16]

The aim of our study is to investigate the fecal microbiota in patients with IBD and compared to healthy controls in Taiwan and to estimate the relationship between the microbiota and IBD.

Methods

Patients

A total of 20 patients diagnosed with IBD were enrolled for this study. The diagnosis of IBD is based on medical history, clinical evaluation, typical endoscopic, and histological findings, and the exclusion of an infectious etiology. Detailed clinical and laboratory data, including smoking, alcohol drinking, the underlying co-morbidities, such as diabetes, dyslipidemia, and medication including mesalazine, steroid, azathioprine, biological agents were recorded at out-patient clinical departments. Anthropometric measurements (including body height and weight, waist circumference, body mass index [BMI], and blood pressure [BP]) were taken by an experienced nursing staff. Blood tests including complete blood count, differentiated count, C-reactive protein, stool routine and stool calprotectin were measured. Mayo score for UC and Crohn's disease activity index (CDAI) for CD were calculated.

Patients who had severe cardiovascular, pulmonary, hepatic, or renal disease, who had gastrointestinal tract surgery, and who have taken proton pump inhibitors (PPI), histamine-2 receptor antagonists, nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, or probiotics within 2 weeks of sample collection were excluded.

Forty-eight patients without prior IBD history and no surgery history for GI tract were recruited from out-patient department and during physical checkup as the control group with same exclusion criteria as IBD group.

The present study complied with the standards of the Declaration of Helsinki and current ethical guidelines and was approved by our hospital’s Institutional Review Board (approval No. 2018-07-013B).

Stool bacterial genomic DNA extraction and PCR amplification

Fresh stool samples were collected. Bacterial genomic microbial DNAs was extracted for direct use in 16S rRNA gene sequencing. [17, 18] The amounts and quality of isolated genomic DNA was determined with NanoDrop ND-1000 (Thermo Scientific, Wilmington, DE, USA). Genomic DNA was stored at –80 °C prior to 16S rRNA sequencing. One microliter of sample DNA (10pg~500 ng) was used as template in a PCR reaction for bacteria 16S rRNA variable region V3–V4. The primer set for the reaction was chosen with 341F_V3_illumina (5’- CCTACGGGNGGCWGCAG-3’) and 805R_V4_illumina (5’-GACTACHVGGGTATCTAATCC -3’). [17] PCR consisted of an initial denaturation at 94°C for 2 min, 30

cycles of 92°C for 20 sec, 55°C for 30 sec and 68°C for 1 min for amplification, 68°C for 1 min to finish replication on all templates, and stored at 4°C. Dual-indexes (barcodes) was used for each sample before sequencing and next-generation sequencing was performed by the Illumina MiSeq Desktop Sequencer following the standard protocol. [18]

Data processing and statistical analysis

The raw sequencing reads were assembled using FLASH v.1.2.7. [19] The quality of reads was assessed by QIIME 1.9.1 pipeline and low-quality reads (Q <20) were truncated. [20] Operational taxonomic unit (OTU) were clustered at 97% sequence identity using the UPARSE function in the USEARCH v.7 pipeline. [21, 22] The RDP classifier (v.2.2) algorithm was employed to annotate the taxonomy of each 16S rRNA gene sequence based on the information of Silva Database v.132, [23-25] with a confidence threshold of 80%.

The α-diversity indices evaluating gut microbial community richness (the observed OTUs and Chao1 indices) and community diversity (the Shannon and Simpson indices) were calculated using QIIME pipeline. Principal coordinate analysis (PCoA) based on Bray-Curtis distance was conducted using the R package. (v.2.15.3). [26] Analysis of similarities (ANOSIM) and permutational multivariate analysis of variance (PERMANOVA) were used to determine whether the community structures significantly vary among and within groups.

Welch's t-test was performed using the STAMP software (v2.1.3). [27] Statistically significant difference in the relative abundances of taxa were recognized and calculated by the use of the linear discriminant analysis (LDA) effect size (LEfSe) analysis. [28] In the present study, taxa with LDA score (log 10) > 4 was considered significant.

As comparison, we also processed the reads with QIIME 2 v.2019.10 pipeline. [29] Deblur was used for denoising. [30] The amplicon sequencing variants (ASV) was identified and the taxonomic features were classified by the information of Silva Database v.132. α-diversity and β-diversity were also evaluated between the IBD group and the control group, and also between the UC and CD groups.

All data were expressed as means ± standard deviation. If some parameters were not normally distributed, nonparametric analysis was used. Results was compared between groups depending on the type of data analyzed using either the Fisher's Exact, Student's t, or nonparametric Mann-Whitney U tests. Statistical analyses were performed using Sample Power release 2.0 and SPSS for Windows version 14.0 (both by SPSS Inc, Chicago, IL, USA). All p values were two-tailed, and a p value <0.05 was considered statistically significant.

Results

Baseline clinical characteristics of study participants

The characteristics of 14 patients with UC, 6 patients with CD and 48 healthy controls were demonstrated in Table 1. The mean age was 49.8 ± 14.1 years old in IBD group and 49.9 ± 11.6 in controls. Among the 14 patients with UC, 9 patients were in remission (Mayo score 0-2), 4 patients had active disease with mild inflammation (Mayo score 3-5), and 1 patient had active disease with moderate inflammation (Mayo score 6-10). For the 6 patients with CD, 5 patients were in remission (CDAI < 150) and 1 patient had moderate inflammation (CDAI=300) when entered the study. In the 20 study subjects, 10 patients had taken azathioprine, five had received biological agents (including one CD patient for concurrent rheumatic arthritis) at the data and samples collection. Only one patient with UC did not receive any medication for disease control at the time of stool collection.

Table 1. Baseline clinical characteristics of study subjects

	IBD (n=20)	Control (n=48)
Women/ men, n (%)	3/17	19/29
Age, years	49.8 ± 14.1	49.9 ± 11.6
BMI, kg/m^2	21.5 ± 9.4	23.2 ± 2.8
CRP, mg/dL	0.54 ± 0.51	0.05± 0.07
WBC, /ul	6266.7 ± 1822.4	5313.2 ± 1267.9
Hb, g/dl	13.6 ± 2.2	13.7 ± 1.30
Mayo score for UC n (%)	total:14 (100)	
0-2	9 (64.3)	
3-5	4 (28.6)	
6-10	1 (7.1)	
>10	0 (0)	
CDAI for CD n=6	78.5 ± 111.9 (11-300)	Mean± SD (range)
Medication		
Mesalazine, n (%)	18 (90)	
Steroid, n (%)	2 (10)	
Immunotherapy, n (%)	10 (50)	
Biologic agents, n (%)	5 (25)	
Enema, n (%)	9 (45)	

Data are expressed as the mean ± standard deviation (SD); IBD: inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; BMI, body–mass index; CRP, C-Reactive protein; WBC, white blood cell; Hb,

hemoglobin; CDAI, Crohn's disease activity index.

The microbiota composition in patients with IBD compared with healthy controls

After 16S rRNA gene sequencing and quality filtering, 5.4 million reads from a total of 6.5 million pair-end reads were obtained. A total of 835 OTUs were identified from 68 stool samples.

The α-diversity was estimated using different indices. The richness of microbiota was estimated by observed OTUs and Chao1 indices and the evenness was evaluated by Shannon and Simpson index. Compared to the control group, the IBD group had significantly lower richness (observed OTUs, $p = 0.02$; Chao1, $p = 0.03$). The IBD group was also less evenness than the control (Shannon, $p < 0.01$; Simpson, $p < 0.01$) (Fig.1).

The overall structure of the fecal microbiome among the two groups were evaluated. PCoA showed a separation between the control group and the IBD group, which revealed significant differences in bacterial genera abundance (Fig. 2). Significant intercommunity differences among the two groups were demonstrated by ANOSIM (R = 0.3344, $p = 0.001$) and PERMANOVA analyses ($p < 0.001$).

Phyla *Bacteroidetes*, *Firmicutes* and *Proteobacteria* consisted more than 90% of stool microbiota in both control and IBD groups. Although the IBD group had higher percentage of *Firmicutes* (41.5% vs. 37.9%) and lower proportion of *Bacteroidetes* (40.9% vs. 51.3%) compared to the control group, the *Firmicutes to Bacteroidetes* ratio was not statistically different between the two groups.

There were nine dominant classes were found in both groups, including *Bacteroidia*, *Clostridia*, *Negativicutes*, *Gammaproteobacteria*, *Bacilli*, *Fusobacteriia, Verrucomicrobiae*, *Actinobacteria*, *Coriobacteriia*, and *Deltaproteobacteria*. Bacterial class *Bacilli* belonging to *Firmicutes* (5.5% vs. 0.2%, $p = 0.03$) and *Actinobacteria* belonging to *Actinobacteria* (2.4% vs. 0.5%, $p < 0.01$) were significantly higher in the IBD group compared to the control group. In the contrast, bacterial class *Bacteroidia* belonging to *Bacteroidetes* (40.9% vs. 51.3%, $p = 0.04$) and *Deltaproteobacteria* belonging to *Proteobacteria* (0.2% vs. 0.7%, $p < 0.01$) were significantly lower in the IBD group compared to the control group.

In genus level, *Faecalibacterium* (2.6% vs. 7.6%, $p < 0.01$), *Subdoligranulum* (0.1% vs. 1.9%, $p < 0.01$), *Parabacteroides* (1.6% vs. 3.2%, $p = 0.03$), *Ruminococcaceae UCG-002* (0.4% vs. 1.3%, $p < 0.01$) and *Paraprevotella* (0.1% vs. 0.9%, $p = 0.03$) were significant lower in the IBD group compared to the controls. *Bifidobacterium* (2.3% vs. 0.5%, $p < 0.01$), *Ruminococcus gnavus group* (1.1% vs. 0.2%, $p < 0.01$), *Streptococcus* (1.0% vs. 0.2%, $p = 0.02$) and *Blautia* (0.9% vs. 0.3%, $p = 0.01$) were significant higher in the IBD group compared to the control group. Analysis of composition of microbiomes (ANCOM) in genus level revealed that *Lactobacillus, Sellimonas* and *Bifidobacterium* were dominant in the IBD group and *Subdoligranulum* was dominant the control group. *Bifidobacterium longum subsp. Longum* was significantly increased in the IBD group compared to controls.

The discriminant analysis by using LEfSe method was applied to recognize the key taxa accountable for the difference between the two groups. The recognized taxa were emphasized on the cladogram along with their LDA scores. *Lactobacillus* and *Veillonella* were identified as dominant genera in the stool microbiome of the IBD group, whereas genera *Faecalibacterium* was dominant in the control group (Fig.3).

The microbiota composition analysis with ASV

A total of 2,034 ASV were identified from 68 stool samples with total frequency of 976,619. The mean frequency per feature was 480.1. Similar to the analysis based on OTU clusters, the IBD group had lower richness and lower evenness compared to control group (observed OTUs, $p< 0.01$; Shannon, $p< 0.01$). The β-diversity between the two groups also had significant difference in Unweighted UniFrac distance ($p = 0.03$) and Bray-Curtis distance ($p< 0.01$). Furthermore, PCoA presented a significant separation between the control group and the IBD group, which revealed significant differences in bacterial genera abundance (Fig.4 A). ANCOM revealed that *Lactobacillus and Subdoligranulum* were the most dominant genera in the IBD group and the control group, respectively.

Additionally, the α-diversity of the CD, UC and control groups were analyzed. The CD group remained significantly lower α-diversity compared to the control group (observed OTUs, $p< 0.01$; Shannon, $p< 0.01$). However, the α-diversity revealed no significant difference between the UC and control group (observed OTUs, $p = 0.08$; Shannon, $p = 0.06$), and between the CD and UC group (observed OTUs, $p = 0.06$; Shannon, $p = 0.32$).

The β-diversity between the CD group and the control group also had significant difference in Unweighted UniFrac distance ($p< 0.01$) and Bray-Curtis distance ($p< 0.01$). Similar results were found between the UC group and the control group (Unweighted UniFrac distance, $p< 0.01$; Bray-Curtis distance, $p< 0.01$). Yet, the β-diversity showed no significant difference between the CD group and the UC group. PCoA revealed significant separation between the control group and the CD group, and also between the control group and the UC group (Fig. 4B).

By using the LEfSe method, *Lactobacillus, Bifidobacterium* and *Enterococcus* were identified as dominant genera in the UC group. *Fusobacterium* and Escherichia_Shigella were dominant in the CD group. *Faecalibacterium* and *Subdoligranulum* were dominant in the control group.

Discussion

In our study, we found that the IBD group had a significant reduction in both richness and diversity of gut microbiota compared to the control group. The composition of gut microbiota was significantly different between the IBD patients and healthy controls. Our findings are similar with previous studies. [13, 14]

In healthy individuals, the gut microbiota and the host present a symbiotic relationship. The epithelial barrier could accommodate the commensal microbiota while the epithelial cells defy the invading

microorganisms by secreting antimicrobial peptides.

The development of IBD is thought to be associated with environmental exposures in genetically susceptible individuals that may induce abnormal immune response toward gut microbiota and cause intestinal inflammation. Through genome-wide association studies, hundreds of IBD-associated loci were found successively. [13, 31, 32] Those gene loci involve in the mucosal barrier function, innate immunity, cytokine signaling, autophagy and the interleukin (IL)-23-Th17 pathway. [13, 32, 33] The disruption of susceptibility genes also associated with impaired immune response to bacterial ligands and metabolites, and uprising inflammation. [13, 34, 35] The presence of environmental stress, gut microbiota or genetic predisposition alone is insufficient to cause IBD, but the interaction between those factors does.

Imbalance of microbial diversity within gut are known to occur in patients with IBD. The gut microbial composition of IBD has been studied, and diverse results were found. At phylum level, decrease of *Actinobacteria* and *Firmicutes* in CD patients was frequently reported, but the change was not consistent in UC patients. [14] Increased abundance in *Proteobacteria* in IBD patients was noted. [13, 14] At family level, decrease of *Ruminnococcaceae* had been found in IBD patients. [13, 36] In a study of treatment-naïve pediatric CD patients, the CD patients had increase of *Enterobacteriaceae, Pasteurellacae, Veillonellaceae*, and *Fusobacteriaceae*, and decrease of *Erysipelotrichales, Bacteroidales*, and *Clostridiales* compared to controls. [37] In the present study, decrease of *Ruminnococcaceae* and increase of *Enterobacteriaceae* and *Veillonellaceae* were found in the IBD patients, which were practically consistent with previous studies. [13, 36, 37] We found significant decrease of α-diversity in the IBD group compared to the controls.

Decrease of butyrate-producing species such as *Faecalibacterium prausnitzii* and *Roseburia hominis* in both UC and CD patients were also found. [15, 38, 39] As above mentioned, butyrate belongs to SCFA and is fermented from fiber-rich diets by *Firmucutes*, [40] including family *Lachnospiraceae*, *Ruminococcaceae* and *Erysipelotrichaceae*. [41] Butyrate activate target cells via G-protein-coupled receptors, and participate in the maintenance of intestinal barrier, influence the production of proinflammatory cytokines and modulate immune response. [34, 42, 41, 40] Reduction of butyrate and butyrate-producing bacteria probably deteriorate the disrupted mucosal barrier and unconfined inflammation in IBD. In the present study, we identified that the IBD group had decrease abundance in *Faecalibacterium* and *Subdoligranulum* compared to the control group, both genera have capabilities to produce butyrate. [41] However, the concentration of butyrate or other bacterial metabolite were not exanimated in the present study.

In the present study, we found that *Lactobacillus, Bifidobacterium* and *Enterococcus* were dominant in the IBD group. Furthermore, we found *Bifidobacterium longum subsp. Longum* abundant in the IBD group compared to controls. Both *Lactobacillus* and *Bifidobacterium* were assumed to be probiotics. However, the change of *Lactobacillus* and *Bifidobacterium* in IBD patients were conflicted. [9, 36, 37, 43-46] There were studies from USA and Europe that suggested the abundance of *Lactobacillus* and *Bifidobacterium*

had decreased in IBD. [37, 36, 43] In contrast, Wang et al. suggested both *Lactobacillus* and *Bifidobacterium* were increased in active IBD patients. [44] In a Japanese study, *Bifidobacterium* was found to be decreased in active CD patients and increased in healthy patients and IBD patients with remission or maintenance phase. [45] Furthermore, Sha et al. suggested the difference of *Lactobacillus* between the IBD patients and healthy controls were insignificant. [46] Another study revealed that *Bifidobacterium longum* may either trigger anti-inflammatory or pro-inflammatory process by stimulating the production of IL-10 or tumor necrosis factor-alpha. [47] Likewise, *Lactobacillus* had conflicted reports about its role on inflammation. [48, 49] *Lactobacillus* was thought to have anti-inflammatory potential by secreting Lactocepin. [48] Yet, Tsilingiri et al. found that *Lactobacillus* could be harmful in active IBD due to its inflammatory activities. [49] Whether *Lactobacillus* and *Bifidobacterium* had a pathogenic potential in IBD, an association with disease activity or different ethnicity should be further evaluated.

In the present study, it is our strength that we used different methods to analyze the data of 16s rRNA sequencing. [20, 29] No significant conflict was found while evaluate the microbial composition of the IBD and control group. The dominant genera identified in each group were consistent. There were several limitations of this study. First, the included IBD patients were heterogenous, including 14 UC patients and 6 CD patients. The disease activity and the treatment they received were also different. The composition of gut microbiota may be varied in patient who had remitted or active IBD. However, because of the small number of included IBD patients, subgroup analysis for disease activity and treatment were not performed. Second, the composition of gut microbiota is easily affected by environmental factors such as antibiotics, diet and obesity. [6, 50] The diet patients consumed before collecting stool samples were not recorded. Also, the medication patients had taken were basically according to their medical records though the drugs bought in the counter or taken from other clinic were reviewed and medication with PPI, histamine-2 receptor antagonists, NSAIDs, antibiotics, or probiotics within 2 weeks of sample collection were prohibited. Third, the control group of this study had higher BMI and higher rate of female when compared to the IBD group. Forth, the metatranscriptomic data, metabolomics, and metabolite measurements such as SCFA were not performed in this study.

In conclusion, the IBD group had a significant reduction in richness and diversity of gut microbiota compared to the control group. The composition of gut microbiota was significantly different between the IBD patients and healthy controls. *Lactobacillus* and *Bifidobacterium* were found to increase in the IBD group. Whether these genera had an impact on inflammation and influence of IBD should be further assessed.

Declarations

Declaration of conflict of interest: The authors stated that there were no conflicts of interest in this study.

Declaration of funding interests: This study was funded by the grants from Taipei Veterans General Hospital (V108C-024, VN 108-04, V108D41-001-MY3-2) and Ministry of Science and Technology of Taiwan (MOST 108-2314-B-010-050 & MOST 109-2314-B-075-034) to JCL.

References

1. Abraham C, Cho JH. Inflammatory bowel disease. N Engl J Med. 2009;361(21):2066-78. doi:10.1056/NEJMra0804647.
2. Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. Annu Rev Immunol. 2010;28:573-621. doi:10.1146/annurev-immunol-030409-101225.
3. Kaplan GG, Ng SC. Understanding and Preventing the Global Increase of Inflammatory Bowel Disease. Gastroenterology. 2017;152(2):313-21 e2. doi:10.1053/j.gastro.2016.10.020.
4. Yen HH, Weng MT, Tung CC, Wang YT, Chang YT, Chang CH et al. Epidemiological trend in inflammatory bowel disease in Taiwan from 2001 to 2015: a nationwide populationbased study. Intest Res. 2019;17(1):54-62. doi:10.5217/ir.2018.00096.
5. Sartor RB. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. Nat Clin Pract Gastroenterol Hepatol. 2006;3(7):390-407. doi:10.1038/ncpgasthep0528.
6. Ananthakrishnan AN. Epidemiology and risk factors for IBD. Nat Rev Gastroenterol Hepatol. 2015;12(4):205-17. doi:10.1038/nrgastro.2015.34.
7. Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut microbiota in the pathogenesis of inflammatory bowel disease. Clin J Gastroenterol. 2018;11(1):1-10. doi:10.1007/s12328-017-0813-5.
8. Goldsmith JR, Sartor RB. The role of diet on intestinal microbiota metabolism: downstream impacts on host immune function and health, and therapeutic implications. J Gastroenterol. 2014;49(5):785-98. doi:10.1007/s00535-014-0953-z.
9. Nishino K, Nishida A, Inoue R, Kawada Y, Ohno M, Sakai S et al. Analysis of endoscopic brush samples identified mucosa-associated dysbiosis in inflammatory bowel disease. J Gastroenterol. 2018;53(1):95-106. doi:10.1007/s00535-017-1384-4.
10. Sartor RB, Wu GD. Roles for Intestinal Bacteria, Viruses, and Fungi in Pathogenesis of Inflammatory Bowel Diseases and Therapeutic Approaches. Gastroenterology. 2017;152(2):327-39 e4. doi:10.1053/j.gastro.2016.10.012.
11. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci U S A. 2007;104(34):13780-5. doi:10.1073/pnas.0706625104.
12. Manichanh C, Rigottier-Gois L, Bonnaud E, Gloux K, Pelletier E, Frangeul L et al. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut. 2006;55(2):205-11. doi:10.1136/gut.2005.073817.
13. Sheehan D, Moran C, Shanahan F. The microbiota in inflammatory bowel disease. J Gastroenterol. 2015;50(5):495-507. doi:10.1007/s00535-015-1064-1.
14. Pittayanon R, Lau JT, Leontiadis GI, Tse F, Yuan Y, Surette M et al. Differences in Gut Microbiota in Patients With vs Without Inflammatory Bowel Diseases: A Systematic Review. Gastroenterology. 2020;158(4):930-46 e1. doi:10.1053/j.gastro.2019.11.294.

15. Fujimoto T, Imaeda H, Takahashi K, Kasumi E, Bamba S, Fujiyama Y et al. Decreased abundance of Faecalibacterium prausnitzii in the gut microbiota of Crohn's disease. J Gastroenterol Hepatol. 2013;28(4):613-9. doi:10.1111/jgh.12073.
16. Andoh A. Physiological Role of Gut Microbiota for Maintaining Human Health. Digestion. 2016;93(3):176-81. doi:10.1159/000444066.
17. Nossa CW, Oberdorf WE, Yang L, Aas JA, Paster BJ, Desantis TZ et al. Design of 16S rRNA gene primers for 454 pyrosequencing of the human foregut microbiome. World J Gastroenterol. 2010;16(33):4135-44. doi:10.3748/wjg.v16.i33.4135.
18. Lin XH, Huang KH, Chuang WH, Luo JC, Lin CC, Ting PH et al. The long term effect of metabolic profile and microbiota status in early gastric cancer patients after subtotal gastrectomy. PLoS One. 2018;13(11):e0206930. doi:10.1371/journal.pone.0206930.
19. Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011;27(21):2957-63. doi:10.1093/bioinformatics/btr507.
20. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7(5):335-6. doi:10.1038/nmeth.f.303.
21. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods. 2013;10(10):996-8. doi:10.1038/nmeth.2604.
22. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26(19):2460-1. doi:10.1093/bioinformatics/btq461.
23. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73(16):5261-7. doi:10.1128/AEM.00062-07.
24. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41(Database issue):D590-6. doi:10.1093/nar/gks1219.
25. Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C et al. The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. Nucleic Acids Res. 2014;42(Database issue):D643-8. doi:10.1093/nar/gkt1209.
26. Jiang XT, Peng X, Deng GH, Sheng HF, Wang Y, Zhou HW et al. Illumina sequencing of 16S rRNA tag revealed spatial variations of bacterial communities in a mangrove wetland. Microb Ecol. 2013;66(1):96-104. doi:10.1007/s00248-013-0238-8.
27. Parks DH, Tyson GW, Hugenholtz P, Beiko RG. STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics. 2014;30(21):3123-4. doi:10.1093/bioinformatics/btu494.
28. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12(6):R60. doi:10.1186/gb-2011-12-6-r60.
29. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37(8):852-7.

doi:10.1038/s41587-019-0209-9.

30. Amir A, McDonald D, Navas-Molina JA, Kopylova E, Morton JT, Zech Xu Z et al. Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns. mSystems. 2017;2(2):e00191-16. doi:10.1128/mSystems.00191-16.
31. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 2012;491(7422):119-24. doi:10.1038/nature11582.
32. Knights D, Lassen KG, Xavier RJ. Advances in inflammatory bowel disease pathogenesis: linking host genetics and the microbiome. Gut. 2013;62(10):1505-10. doi:10.1136/gutjnl-2012-303954.
33. Blander JM, Longman RS, Iliev ID, Sonnenberg GF, Artis D. Regulation of inflammation by microbiota interactions with the host. Nat Immunol. 2017;18(8):851-60. doi:10.1038/ni.3780.
34. Schirmer M, Garner A, Vlamakis H, Xavier RJ. Microbial genes and pathways in inflammatory bowel disease. Nat Rev Microbiol. 2019;17(8):497-511. doi:10.1038/s41579-019-0213-6.
35. Shanahan F. The host-microbe interface within the gut. Best Pract Res Clin Gastroenterol. 2002;16(6):915-31. doi:10.1053/bega.2002.0342.
36. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biol. 2012;13(9):R79. doi:10.1186/gb-2012-13-9-r79.
37. Gevers D, Kugathasan S, Denson LA, Vazquez-Baeza Y, Van Treuren W, Ren B et al. The treatment-naive microbiome in new-onset Crohn's disease. Cell Host Microbe. 2014;15(3):382-92. doi:10.1016/j.chom.2014.02.005.
38. Machiels K, Joossens M, Sabino J, De Preter V, Arijs I, Eeckhaut V et al. A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. Gut. 2014;63(8):1275-83. doi:10.1136/gutjnl-2013-304833.
39. Hedin CR, McCarthy NE, Louis P, Farquharson FM, McCartney S, Taylor K et al. Altered intestinal microbiota and blood T cell phenotype are shared by patients with Crohn's disease and their unaffected siblings. Gut. 2014;63(10):1578-86. doi:10.1136/gutjnl-2013-306226.
40. Sun M, Wu W, Liu Z, Cong Y. Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases. J Gastroenterol. 2017;52(1):1-8. doi:10.1007/s00535-016-1242-9.
41. Martin-Gallausiaux C, Marinelli L, Blottiere HM, Larraufie P, Lapaque N. SCFA: mechanisms and functional importance in the gut. Proc Nutr Soc. 2020:1-13. doi:10.1017/S0029665120006916.
42. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013;504(7480):446-50. doi:10.1038/nature12721.
43. Vigsnaes LK, Brynskov J, Steenholdt C, Wilcks A, Licht TR. Gram-negative bacteria account for main differences between faecal microbiota from patients with ulcerative colitis and healthy controls. Benef Microbes. 2012;3(4):287-97. doi:10.3920/BM2012.0018.

44. Wang W, Chen L, Zhou R, Wang X, Song L, Huang S et al. Increased proportions of Bifidobacterium and the Lactobacillus group and loss of butyrate-producing bacteria in inflammatory bowel disease. J Clin Microbiol. 2014;52(2):398-406. doi:10.1128/JCM.01500-13.
45. Andoh A, Kuzuoka H, Tsujikawa T, Nakamura S, Hirai F, Suzuki Y et al. Multicenter analysis of fecal microbiota profiles in Japanese patients with Crohn's disease. J Gastroenterol. 2012;47(12):1298-307. doi:10.1007/s00535-012-0605-0.
46. Sha S, Xu B, Wang X, Zhang Y, Wang H, Kong X et al. The biodiversity and composition of the dominant fecal microbiota in patients with inflammatory bowel disease. Diagn Microbiol Infect Dis. 2013;75(3):245-51. doi:10.1016/j.diagmicrobio.2012.11.022.
47. Medina M, Izquierdo E, Ennahar S, Sanz Y. Differential immunomodulatory properties of Bifidobacterium logum strains: relevance to probiotic selection and clinical applications. Clin Exp Immunol. 2007;150(3):531-8. doi:10.1111/j.1365-2249.2007.03522.x.
48. von Schillde MA, Hormannsperger G, Weiher M, Alpert CA, Hahne H, Bauerl C et al. Lactocepin secreted by Lactobacillus exerts anti-inflammatory effects by selectively degrading proinflammatory chemokines. Cell Host Microbe. 2012;11(4):387-96. doi:10.1016/j.chom.2012.02.006.
49. Tsilingiri K, Barbosa T, Penna G, Caprioli F, Sonzogni A, Viale G et al. Probiotic and postbiotic activity in health and disease: comparison on a novel polarised ex-vivo organ culture model. Gut. 2012;61(7):1007-15. doi:10.1136/gutjnl-2011-300971.
50. Lewis JD, Chen EZ, Baldassano RN, Otley AR, Griffiths AM, Lee D et al. Inflammation, Antibiotics, and Diet as Environmental Stressors of the Gut Microbiome in Pediatric Crohn's Disease. Cell Host Microbe. 2015;18(4):489-500. doi:10.1016/j.chom.2015.09.008.

Figures

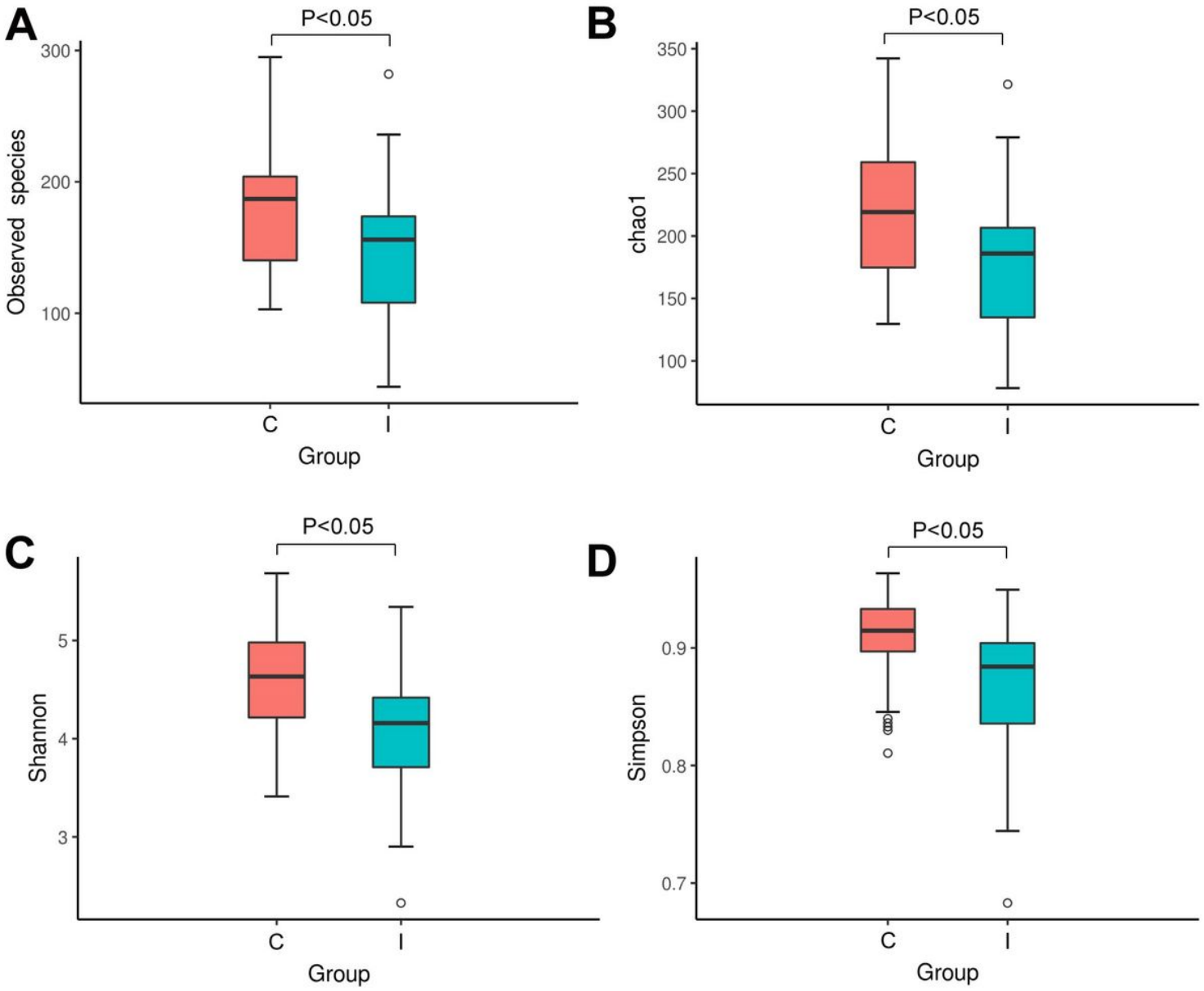

Figure 1

The diversity of fecal microbiota between patients with inflammatory bowel disease (IBD) and controls Observed OTUs revealed significant difference between the IBD group and control group ($p = 0.02$, Fig. 1A). Chao1 estimated significant difference between the IBD group and control group ($p = 0.03$, Fig. 1B). The control group showed higher bacterial diversity, as estimated by the Shannon diversity index and Simpson index, when compared with the IBD group ($p < 0.01$, Fig. 1C& 1D). The boxes (containing 50% of all values) show the median (horizontal line across the middle of the box) and the interquartile range, whereas the blackspots represent the 10th and the 90th percentiles. C: control; I: IBD

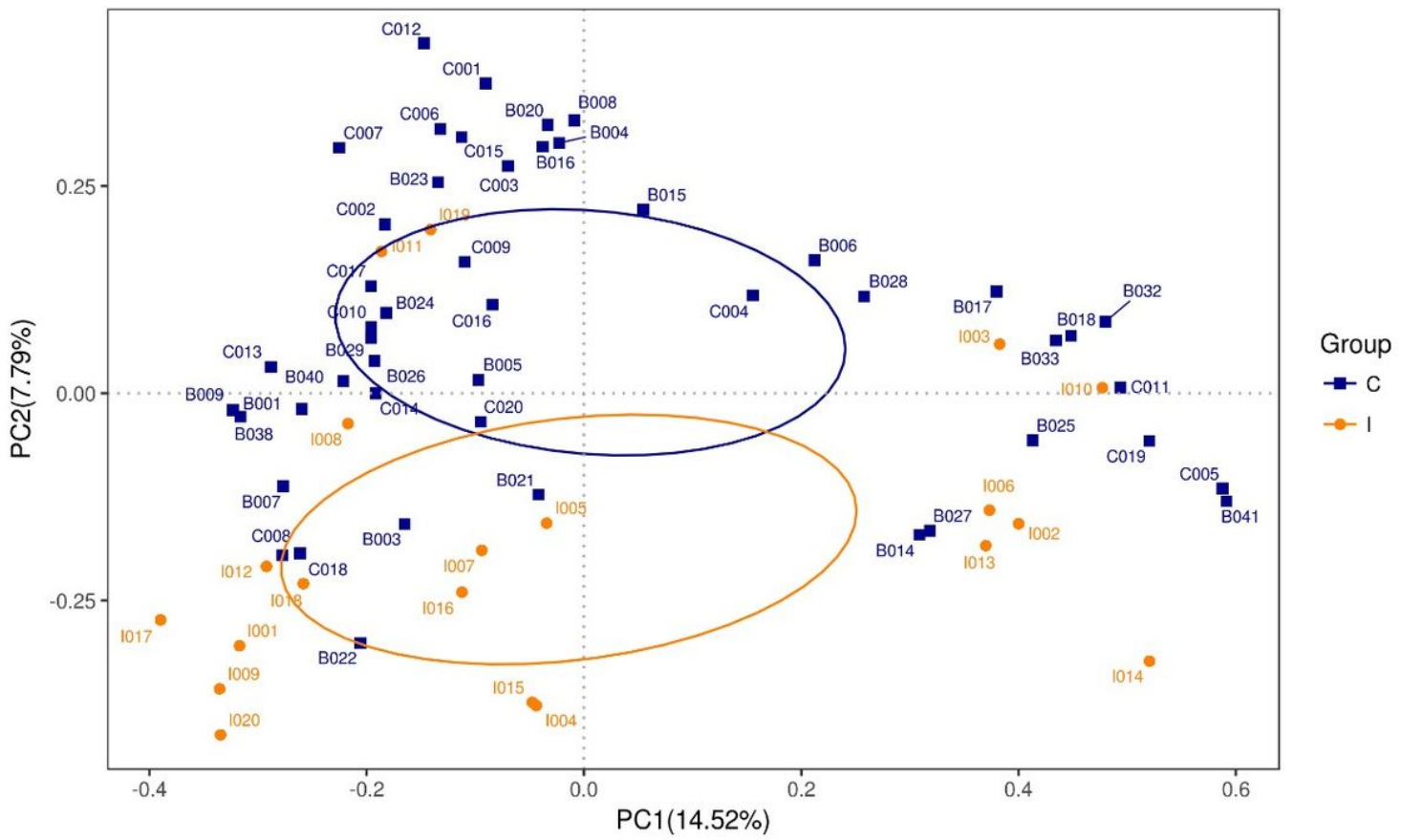

Figure 2

Principal coordinate analysis (PCoA) of bacterial genera abundance Gut microbial genera composition of the control and the inflammatory bowel disease (IBD) groups was analyzed with PCoA. ANOSIM revealed significant differences in the structures of both groups (R = 0.3344, p= 0.001). PERMANOVA analyses also showed significant differences between the two groups (p< 0.001). The axes represent the three dimensions explaining the greatest proportion of variance in the communities. Each symbol represents a sample.

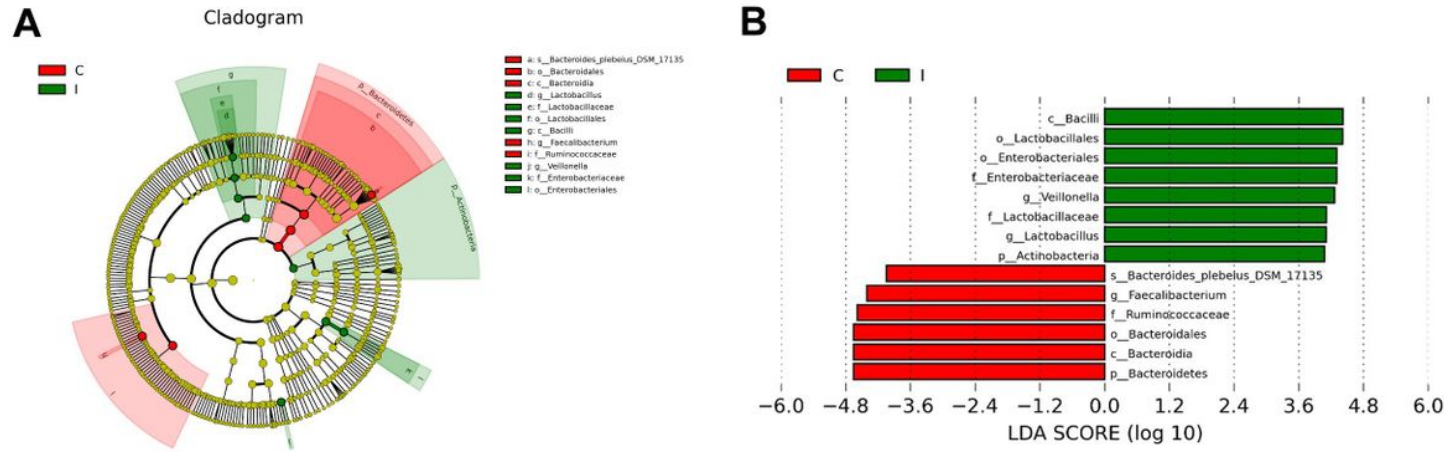

Figure 3

Known taxa abundance reported by LEfSe in the bacterial community. Cladogram showing taxa with the higher differences in relative abundances between the control and the inflammatory bowel disease (IBD) groups. The circle sizes in the cladogram plot were proportional to the bacterial abundances. From the inside to the outside, the circles represent the phylum, class, order, family, and genus. Only taxa with an LDA score of > 4 and p< 0.05 in the Wilcoxon signed-rank test were shown (Fig. 3A). A logarithmic LDA-score cutoff of 4.0 was used to identify significant taxonomic differences between the fecal microbiomes of controls and patients with IBD (Fig. 3B).

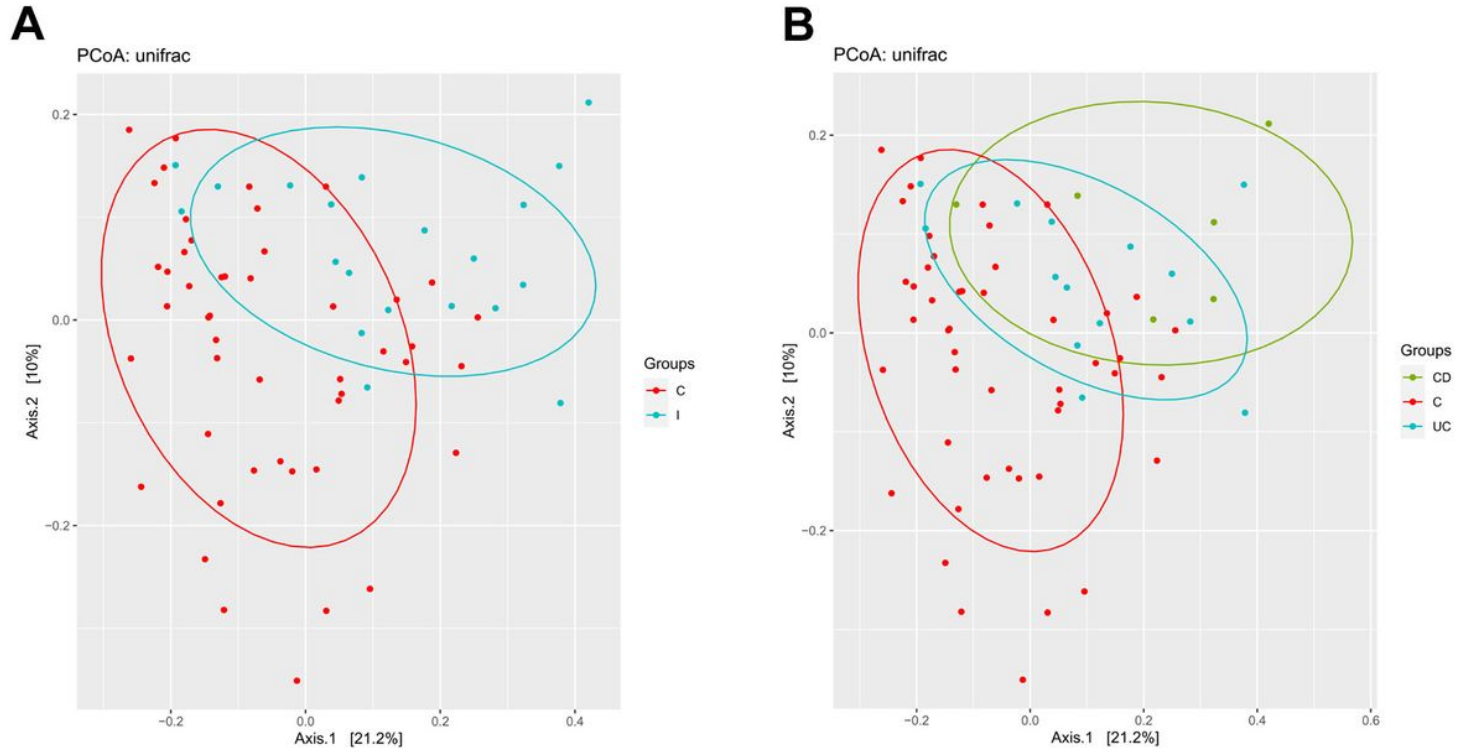

Figure 4

Principal coordinate analysis (PCoA) of bacterial genera abundance Based on the base of amplicon sequencing variant (ASV) by using QIIME2, comparison between the inflammatory bowel disease (IBD) group and the control group showed significant differences (Fig. 4A). PCoA revealed significant separation between the control group and the CD group, and also between the control group and the UC group (Fig. 4B).