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Research Article

Enrichment of Lactic Acid-Producing Bacteria in the Fecal Microbiota of Patients with Ulcerative Colitis in North India

Garima Juyal,[1,2] **Ajit Sood**,[3] **Vandana Midha**,[4] **Arshdeep Singh**,[3] **Dharmatma Singh**,[3] **Ramit Mahajan**,[3] **Vijay Verma**,[5,6] **Rakesh Bhatnagar**,[1,7] **and Mohan C. Joshi**[8]

[1]*School of Biotechnology, Jawaharlal Nehru University, New Delhi, India*
[2]*Department of Biotechnology, Bennett University, Greater Noida, Uttar Pradesh, India*
[3]*Department of Gastroenterology, Dayanand Medical College and Hospital, Ludhiana, India*
[4]*Department of Medicine, Dayanand Medical College and Hospital, Ludhiana, India*
[5]*Division of Medical Bioinformatics, Indian Council of Medical Research, New Delhi, India*
[6]*North Florida Research and Education Centre, Institute of Food and Agricultural Sciences, University of Florida, Quincy 32351, USA*
[7]*Amity University, Rajasthan, Jaipur, India*
[8]*Multidisciplinary Centre for Advance Research and Studies (MCARS), Jamia Millia Islamia, New Delhi, India*

Correspondence should be addressed to Garima Juyal; garimajuyal@gmail.com

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Previous studies have established the relationship between the gut microbiota and ulcerative colitis (UC); however, there is a scarcity of data on the fecal microbiome profile of patients with UC in the Indian population. This study aimed to examine the fecal microbiome profile of north Indian patients with UC ($n = 105$), including with active disease ($n = 64$) and in remission ($n = 41$), compared to healthy controls ($n = 36$), using 16S rRNA gene sequencing. Both relative abundance analysis and linear discriminant analysis effect size (LEfSe) revealed a significant enrichment of lactic acid-producing facultative anaerobic pathobionts, namely, *Bifidobacterium*, *Lactobacillus*, and *Streptococcus*, in patients with UC (both with active disease and those in remission). Additionally, a significant decrease was observed in anaerobic genera responsible for the synthesis of short-chain fatty acids (SCFAs), especially butyrate, such as *Blautia*, *Roseburia*, *Lachnospiraceae*, and *Ruminococcaceae*. Differential metabolic pathway analysis using PICRUSt2 confirmed a loss of SCFAs production and increased lactose and nitrate metabolism in UC patients. Biochemical analysis of fecal samples confirmed the increased colonization of nitrate-reducing microbes in UC patients, suggesting inflammation-driven dysbiosis. LEfSe and PICRUSt2 analyses revealed an enrichment of *Prevotella* and *Blautia* microbes and the upregulation of two specific metabolic pathways, sulfolactate degradation and reductive acetyl coenzyme A pathway-I, which can distinguish UC patients in remission from those with active disease. Our findings caution against the use of lactic acid-producing bacteria (LABs) and recommend the exploration of butyrate-producing microbes as probiotics to restore SCFAs levels in UC patients.

1. Introduction

Historically considered to be a disease of the Western world, ulcerative colitis (UC) has emerged as a global disease. The origin of UC is multifactorial, with chronic inflammation believed to stem from intricate interactions involving genetic factors, environmental influences, and the intestinal microbiota. The conventional treatment strategies focus on inducing and maintaining remission, as well as preventing disease-related complications by targeting the dysregulated immune system [1, 2]. The intestinal microbiota plays an important role in the pathogenesis of UC [3]. In fact, the restoration of a healthy microbiome achieved through fecal microbial transplant has been evaluated as an effective

complementary therapy for UC [4]. Given the differences in genetic predisposition to IBD across ethnically diverse populations, as well as the role of gene-environment interactions in the development and phenotypic expression of UC, it is imperative to characterize the gut microbiome from genetically, culturally, and socially diverse populations [3, 5–7]. Despite the inconsistencies in fecal microbial profiles reported in patients with UC from various parts of the world, the demonstration of low bacterial diversity, proliferation of Proteobacteria and Actinobacteria, and depletion of Firmicutes and Bacteroidetes remain common features across studies, providing compelling evidence for gut microbial dysbiosis in patients with UC [3, 5, 8, 9]. While longitudinal studies have revealed relative intraindividual stability in the fecal microbial profile over time, there is a scarcity of data concerning the composition of the gut microbiota in relation to disease activity [10, 11].

In the current study, we analysed the fecal microbial composition in UC patients and healthy controls of North Indian (NI) origin. Since UC is an emerging disease in developing countries, in contrast to the Western world where it has reached a plateau (prevalence equilibrium), we propose that characterizing the gut microbiome of a UC cohort from a developing country like India provides a unique opportunity to identify early microbial markers. We examined the gut microbiome profiles of 105 patients with UC, which encompassed 64 individuals with active UC and 41 with UC in remission. These profiles were then compared with those of 36 healthy controls, employing 16S rRNA gene sequencing. Our findings revealed reduced microbial diversity in UC patients, specifically a decrease in microbes that produce short-chain fatty acids (SCFAs), compared to healthy controls. Interestingly, contrary to findings in Western populations, we observed an enrichment of lactic acid bacteria (LABs), specifically *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*, in UC patients. Linear discriminant analysis effect size (LEfSe) analysis confirmed enrichment of these microbes in UC patients with active disease and also showed an enrichment of *Prevotella_7* and *Blautia* in UC patients in remission. Further, biochemical analysis revealed an increased abundance of nitrate-reducing microbes in the UC patients compared to healthy controls. Differential metabolic profiling confirmed a decrease in the potential for short-chain fatty acid production and an increase in lactose and nitrate metabolic potential in UC patients. Moreover, we identified 16 unique metabolic pathways distinguishingUC patients with active disease from those in remission, of which two, namely, sulfolactate degradation and reductive acetyl coenzyme A pathway I (homoacetogenic bacteria), were increased in patients in remission. Our findings not only compiled a comprehensive catalogue of microbial genera found in UC patients and healthy individuals from northern India but also raised concerns about the suitability of lactic acid bacteria as probiotics in this region. As a potential alternative therapeutic strategy, we suggest exploring the potential benefits of utilizing butyrate-producing microbes.

2. Methods

2.1. Study Design. This study was performed as a collaborative work between two centers, Dayanand Medical College and Hospital, Ludhiana, and Jawaharlal Nehru University, New Delhi. The fecal samples for microbiota analysis were collected from patients with UC attending the outpatient clinic at Dayanand Medical College and Hospital, a tertiary care teaching hospital in North India, between January 2016 and December 2018. The Institutional ethics review board of both the participating centers (Jawaharlal Nehru University, New Delhi, and Dayanand Medical College and Hospital, Ludhiana) approved the study (IEC numbers 2017/SERB Young Scientist/125 and DMCH/R&D/2015/238, respectively). Written informed consent was obtained from each participant.

2.2. Study Population. Consenting adults (aged 18 years or more) with an established diagnosis of UC (according to the European Crohn's and Colitis Organisation (ECCO) and the European Society of Gastrointestinal and Abdominal Radiology (ESGAR) guidelines) were enrolled [12]. The clinical disease characteristics, such as disease duration, disease extent, and concomitant treatment, were recorded for all the participants. Disease activity was described using the Mayo Clinic score. The Mayo Clinic score includes stool frequency, blood in stools, endoscopic assessment of disease activity, and physician's global assessment of the disease, each scored from 0 to 3. Clinical remission was defined as total Mayo Clinic score ≤ 2, while a Mayo Clinic score > 2 indicated active disease.

Patients with age < 18 years; Crohn's colitis; indeterminate colitis; *Clostridium difficile* infection; comorbid illnesses such as severe heart, lung, or neurological disease; current or past malignancy; pregnancy; and history of antibiotic, probiotic, or nonsteroidal anti-inflammatory drug use in the 12 weeks preceding enrolment were excluded. Adult (aged 18 years or more), unrelated volunteers from the community, with no comorbidities or disorders known to be associated with gut microbiota dysbiosis were enrolled as healthy controls.

2.3. Fecal Sample Collection, DNA Extraction, and Metagenome Sequencing. The patients and controls were told about the stool collection protocols in a face-to-face meeting, with ample opportunities to clear the doubts, if any. The fecal samples were collected at least 24 hours before colonoscopy/sigmoidoscopy to avoid colon-preparation-induced alterations in the gut microbial profile. The fecal samples were collected by the patients and controls at their respective homes and stored immediately after collection at 2-8°C in the ice box provided to each participant. Upon arrival at the hospital, within 4-6 hours of collection, the fecal sample was aliquoted (to avoid future freeze-thaw cycles), labelled with a unique identifier, and stored at −80°C for subsequent microbiota analysis. Fecal DNA was extracted from using DNeasy PowerLyzer PowerSoil Kit (Qiagen/MO BIO cat#12855-50) as per the manufacturer's protocol. For gut microbial profiling, the V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified and sequenced on the Illumina MiSeq platform.

2.4. Taxonomy Assignment. Paired-end reads were merged using Fast Length Adjustment of Short (FLASH) reads with

a minimum and maximum overlap of 30 bp and 250 bp, respectively [13]. Low base quality (average $Q \geq 34$) reads were removed using FASTX toolkit 0.0.13 (http://hannonlab.cshl.edu/fastx_toolkit/). Dereplication was performed in vectorized search (*VSEARCH*). A *de novo* approach in *VSEARCH* was used to remove chimeric sequences [14]. The bacterial composition of the sample was achieved using closed reference operational taxonomic unit (OTU) picking method in UCLUST [15]. A sample that had an OTU count of 1 in a single sample (identified as singletons) was removed. Taxonomy assignment was done using the ribosomal database project (RDP) classifier against Silva 132 database at 97% similarity [16].

2.5. Microbial Diversity and LEfSe Analysis. The raw OTUs as well as metadata files were uploaded on the web interface-enabled metagenome data analysis tool, MicrobiomeAnalyst [17]. As first quality control test, low-quality data reads as well as with low variance were removed using default setting. OTU counts were rarefied to minimum library size across group, and scaling (total sum) of the data was done without any transformation. Relative OTU abundance across samples was plotted, and the Bonferroni-corrected Mann–Whitney p value < 0.05 was considered significant. The Bonferroni correction was applied while determining the p value, because the sample size of patients with active disease was more than those in remission or healthy controls. Rarefaction curve, alpha diversity indices (Simpson, Shannon, Chao1, and observed species), and beta diversity (Bray-Curtis dissimilarity) was generated using default setting of Microbiome Analyst. The taxonomic diversity of fecal samples was plotted relative to median abundance at phylum and genus levels using default setting (merging of taxa < 10). Subsequently, linear discriminant analysis effect size (LEfSe) analysis was performed using Microbiome Analyst at the genus level for UC patients and healthy controls. In the LEfSe analysis, microbial genera with >2.0 LDA score with a p value of <0.05 (FDR adjusted) were considered significant [18]. A histogram of the LDA scores is computed and plotted for differential relative abundance of microbial genera between UC patients with active disease and for those in remission in comparison to healthy controls.

2.6. Functional Analysis of Microbial Community. The raw OTUs as well as metadata file were subjected to PICRUSt2 analysis, which predicted abundance of metabolic pathways observed in UC patients and healthy controls [14]. The predicted pathway counts were uploaded on an online analysis web tool, iDEP.96 [15]. Briefly, the raw number of metabolic pathways was transformed into log2 (EdgeR) (default setting). To test whether differential abundance of metabolic pathway could discriminate these three subgroups, hierarchical clustering analysis and principal component analysis across groups based upon the median pathway abundance were performed, and heatmap and PCA plot were generated in iDEP using the default setting. Subsequently, using DESeq2 method and differentially abundant pathways with >2-fold change (log), a$p$value of <0.05 (FDR adjusted) was considered significant and analysed further. The fold change (log) and Venn diagram of differentially abundant pathways across groups were generated. For selected few metabolic pathways, differential abundance across groups was plotted using Past3b, and Mann–Whitney p value with Bonferroni correction was calculated.

2.7. Nitrate Broth Assay. The fecal samples were grown in nitrate broth (HiMedia, cat#M439S) after homogenization in 1× phosphate buffer saline (pH = 7.0). Debris were removed by spinning at 12,000 rpm, and 100 microliter of supernatant was inoculated into 1 ml of nitrate broth. After 24-48-hour incubation at 37°C, 100 microliters of freshly prepared sulfanilic acid and alpha naphthylamine were added to the culture. The broth colour was observed at 620 nm. A change in the colour of the broth indicated production of nitrite and correlated with presence of nitrate-reducing bacteria in the sample.

3. Results

3.1. Baseline Characteristics and Sequencing Reads. A total of 105 patients with UC and 36 healthy controls were included in the study. The mean age of patients with UC and healthy controls was 37.7 and 34 years, respectively. Majority of the patients with UC had left sided colitis, with a median disease duration of 1 year (0.5 - 4.0). A total of 64 (60%) patients had active disease, while the remaining patients had disease in remission (Table 1).

Sequencing of V3-V4 region yielded a total of 1043380.25 ± 253558.85 and 724038.25 ± 509205.2 paired-end reads for healthy controls, and patients with UC, respectively. After removing low-quality reads, chimera sequences, and singletons, a total of 944561.03 ± 231923.41 and 657270 ± 458609.12 sequencing reads were obtained in healthy controls, and patients with UC, respectively. The sequence reads were normally distributed across samples (Shapiro-Wilk test, $p < 0.001$). (Supplementary Table-1).

3.2. Diversity of the Gut Microbiome. Both UC patients with active disease and those in remission showed significantly lower alpha diversities compared to healthy controls. This was confirmed by various indices, such as the Simpson ($p = 5.9e-10$ and $p = 1.3e-06$), Shannon index ($p = 7.5e-12$ and $p = 1.7e-07$), observed species/richness ($p = 6.5e-10$ and $p = 1.3e-06$), and ChaoI ($p = 7.1e-06$ and $p = 6.9e-07$), for patients with active disease and those in remission compared to healthy controls, respectively. However, there were no significant differences in the diversity indices between patients with active disease and disease in remission (Figures 1(a)–1(d)). Also, rarefaction curve also showed lower species richness in UC patients with active disease and in remission (Figure 1(e)). A tight clustering on the beta diversity analysis (Bray-Curtis), indicating higher gut microbial compositional similarity, was observed in healthy controls as opposed to a scattered distribution among patients with UC, implying significant difference in the composition between groups ($p = 0.001$ PERMANOVA). Also, a significant overlap between

Table 1: Baseline characteristics of the enrolled population.

	Ulcerative colitis ($n = 105$)	Healthy controls ($n = 36$)
Age (years) (mean ± SD)	37.7 ± 14.04	34.0 ± 12.65
Males (n) (%)	55 (52.38)	21 (58.33)
Disease duration (years) (median, IQR)	1 (0.5-4)	
Disease extent (n) (%)		
Proctitis	28 (26.66)	
Left-sided colitis	57 (54.28)	
Pancolitis	20 (19.04)	
Disease activity (n) (%)		
Active disease	64 (60.95)	
Remission	41 (39.04)	
Concomitant therapy (n) (%)*		
5-ASA	105 (100)	
Thiopurines	49 (46.66)	
Corticosteroids	66 (62.85)	
Biologics	9 (8.57)	

IQR: interquartile range; 5-ASA: 5-aminosalicylates. *A patient can be on combination of two or more therapies.

patients with active disease and disease in remission implies no compositional changes in microbial community (Figure 2).

3.3. Composition of the Gut Microbiome. In healthy controls, the major six taxa present in the fecal microbiome were Firmicutes (60.42%), Bacteroidetes (22.7%), Actinobacteria (11.34%), Proteobacteria (3.32%), Tenericutes (1.76%), and others (0.59%). Among patients with active UC, the major five taxa present in the fecal microbiome were Firmicutes (58.54%), Actinobacteria (19.42%), Bacteroidetes (15.08%), Proteobacteria (6.16%), and others (0.81%). In patients with disease in remission, the major five taxa present in the fecal microbiome were Firmicutes (54.62%), Bacteroidetes (19.65%), Actinobacteria (19.51%), Proteobacteria (3.46%), and others (2.76%). The relative abundance of Bacteroidetes was higher in patients with disease in remission compared to those with active disease. Additionally, the Tenericutes phylum was absent in UC patients, both with active disease and in remission, when compared to healthy controls (Figure 3).

A high diversity and richness in taxonomic diversity at the genus level was observed in healthy controls compared to patients with ulcerative colitis (UC). This difference was also evident in the reduced number of microbial genera were present in UC patients. Specifically, in healthy controls, 31 microbial genera represented with a median abundance greater than 0.39%. However, in UC patients with active disease, the number of microbial genera was 13, representing genera with a median abundance greater than 0.85%. In patients with remission, the number of microbial genera was 12, representing genera with a median abundance greater than 1.2% (Table 2). When comparing the groups, we identified a total of twelve common microbial genera among patients and healthy controls, albeit with varying abundance. Out of these twelve, two genera were categorized as "other" and "others." The remaining ten genera were predominantly associated with the Firmicutes phylum ($n = 8$), while one genus each belonged to Actinobacteria and Bacteroidetes. The ten microbial genera identified were *Lactobacillus*, *Streptococcus*, *Faecalibacterium*, *Collinsella*, *Blautia*, *Dialister*, *Dorea*, and *Roseburia* (Firmicutes), *Bifidobacterium* (Actinobacteria), and *Prevotella* (Bacteroidetes). However, there was no statistically significant difference in the relative abundance of *Collinsella* and *Faecalibacterium* between healthy controls and UC patients in either active disease or remission (Figure 4). The remaining microbial genera, which represented a median abundance of over 0.85% of total operational taxonomic units (OTUs) in healthy individuals, were either absent or present in low abundance in UC patients. It is possible that these genera were filtered out during data normalization and filtering processes, due to very low or stochastic abundance across UC patients. Majority of these genera, such as *Ruminococcaceae*, *Bacteroides*, *Roseburia*, *Coprococcus*, *Ruminococcus*, *Alloprevotella*, *Prevotella_2*, Lachnospiraceae_NK4A136_group, and *Rikenellaceae_RC9_gut_group*, are well-known producers of short-chain fatty acids (SCFAs). The genus *Romboutsia* exhibited higher median abundance in UC patients with active disease (3.8%) compared to healthy individuals (0.77%), while it was absent in patients with remission (Table 2).

The most notable observation was a significant increase in the abundance of *Lactobacillus* and *Bifidobacterium* in both UC patients, including those with active disease and those in remission compared to healthy controls. Additionally, the abundance of *Streptococcus* was significantly higher in UC patients with active disease compared to healthy controls ($p = 0.001$). *Lactobacillus* and *Bifidobacterium* are being employed as alternative treatments for IBD patients globally. Therefore, the increased abundance of these genera among UC patients, both in active disease and remission, was quite surprising. Although not statistically significant, there was also a higher abundance of *Faecalibacterium* in UC patients, both with active disease and those in remission, compared to healthy controls (Figure 5). By employing LEfSe analysis, we further assessed the diverse abundance of microbial genera, revealing a microbial signature that can differentiate between UC patients (both with active disease and in remission) and healthy controls based on their composition and abundance. LEfSe analysis revealed the top 25 microbial genera based on a minimum LDA log value of 2 and a p value less than 0.05 (FDR adjusted). Among these, 17 genera were associated with healthy controls, six were associated with UC patients with active disease, and three were associated with patients in remission. Notably, the top three genera, namely, *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*, exhibited enrichment in UC patients with active disease, further confirming our earlier observations. Additionally, *Enterococcus*, *Klebsiella*, and *Escherichia_Shigella* were also significantly associated with patients with active disease. The remaining 17 genera, including *Roseburia*, *Ruminococcaceae*, *Dialister*, *Prevotella-9*, *Enterococcus*, *Rikenellaceae_RC9_gut*, *Ruminococcus*, *Alloprevotella*, *Lachnospiraceae*, *Coprococcus*, and *Dorea,* showed

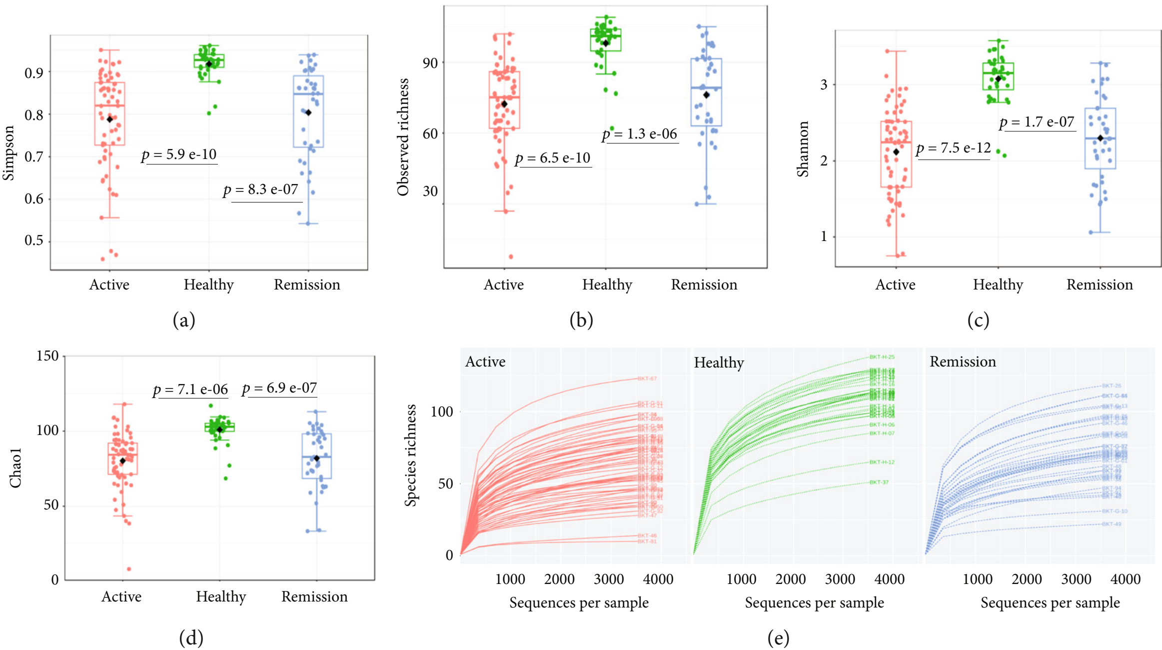

Figure 1: Alpha diversity metrics of fecal bacterial communities in UC patients with active disease, in remission and healthy controls. Whisker box plots illustrate (a) Simpson, (b) observed richness, (c) Shannon, (d) Chao1, and (e) rarefaction curves representing observed species richness at various sequencing depths. The calculated p value is Mann–Whitney with Bonferroni correction.

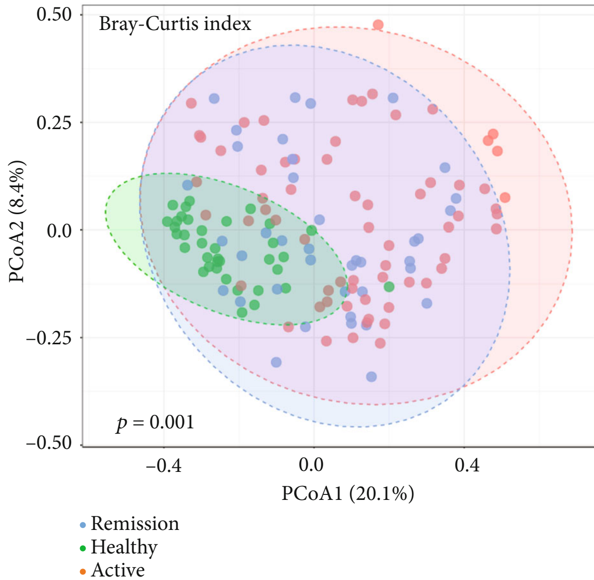

Figure 2: Beta diversity analysis based on the overall structure of the fecal microbiome of principal coordinate analysis (PCoA) of UC patients and healthy controls based on the Bray-Curtis dissimilarity ($p = 0.001$ PERMANOVA).

enrichment in healthy controls. These findings lend support to the observed significant differences in the relative abundance of these genera (Figure 5). Interestingly, LEfSe analysis also identified two genera, namely, *Prevotella-7* and *Blautia*, that were significantly enriched in patients in remission (Figure 6).

3.4. Predictive Functional Profiling of Gut Microbial Communities of UC Patients and Healthy Controls. To monitor variations in metabolic potential resulting from variations in the abundance of microbial genera among UC patients, including those with active disease and those in remission, compared to healthy controls, PICRUSt2 analysis was performed. A total of 417 pathways were predicted across healthy controls and UC patients, both with active disease and in remission. These pathways were further subjected to differential abundance in metabolic pathways using iDEP (integrated differential expression and pathway analysis). The patwhays abundance data (input raw data) was transformed and subjected to principal component analysis (PCA) and DEG analyses. PCA analysis of the transformed data revealed a clustering pattern consistent with that observed in the Bray-Curtis dissimilarity PCoA. Notably, the healthy control group exhibited a distinct cluster attributed to differential metabolic abundance, while the subgroups within the ulcerative colitis (UC) cohort displayed a more diffuse and overlapping clustering trend (Figure 7(a)). The DEG1 analysis revealed that a total of 42 metabolic pathways were upregulated, while 11 were downregulated when comparing patients in remission to healthy controls. Additionally, 38 metabolic pathways were upregulated, and 4 were downregulated when comparing UC patients with active disease to those in remission. Furthermore, the comparison between UC patients with active disease and healthy controls revealed that 77 metabolic pathways were upregulated and 17 were downregulated (Figure 7(b)). Venn diagram was generated to represent the overlap and differencesin metabolic pathways between UC patients (both with active disease and in remission) and healthy controls. It showed that 35 pathways were unique to the comparative

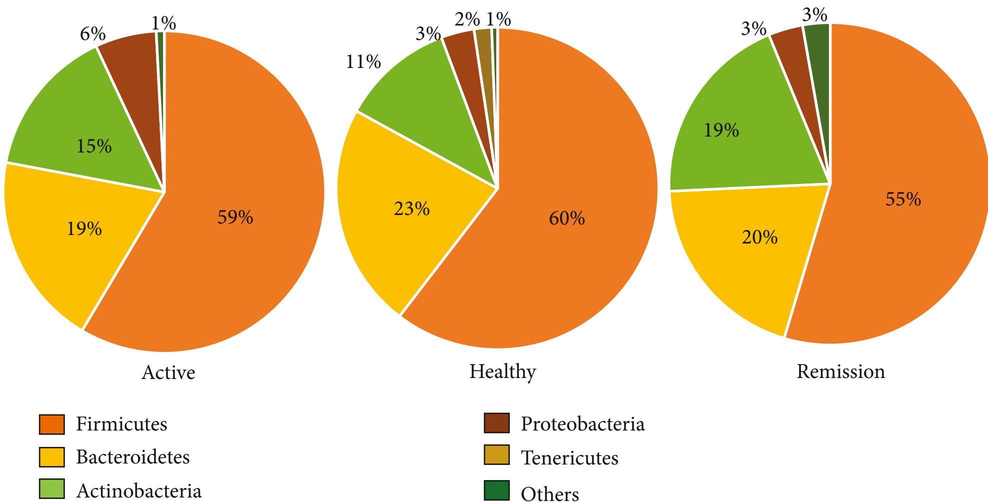

Figure 3: Taxonomic diversity of fecal microbiota composition at the phylum level among UC patients compared to healthy controls.

analysis between active UC and healthy controls, 16 pathways were unique to the comparison between active UC and remission, and 2 pathways were unique to the comparison between remission and healthy controls (Figure 7(c) and Table 3). Among the 35 pathways unique to the comparative group of active UC and healthy controls, 12 were underrepresented, and 23 were overrepresented. In the comparative group of active UC and remission, 2 pathways were underrepresented, and 14 were overrepresented. In the comparative group of remission and healthy controls, one pathway was underrepresented, and one pathway was overrepresented (Figures 7(d)–7(f)). Further analysis of these pathways revealed a loss of short-chain fatty acid (SCFA) producing pathways in UC patients compared to healthy controls, specifically in PWY-650 and CENTFERM-PWY. It is interesting to note that most metabolic pathways were reduced in patients in remission compared to those with active disease, except for two pathways, CODH-PWY and PWY 6641. These pathways are involved in sulfolactate degradation and the reductive acetyl coenzyme A pathway I (homoacetogenic bacteria). Additionally, compared to healthy controls, patients in remission showed an increased metabolic potential for the CMC120CT pathway. Among the comparative groups, four pathways were found to be common. These included PWY-6470 (peptidoglycan biosynthesis V), PWY-6396 (super pathway of 2,3-butanediol biosynthesis), P125-PWY (superpathway of (R,R)-butanediol biosynthesis), and GALLATE-DEGRADATION-II-PWY (gallate degradation II). Interestingly, both UC patients with active disease and those in remission exhibited a significant increase in metabolic potential for LACTOSECAT-PWY, which is involved in lactose degradation (Figures 7(g)–7(i)).

4. Discussion

The emergence of ulcerative colitis (UC) has been linked to the disturbance of gut microbial dysbiosis. This disruption affects the equilibrium between the gut microbiota and the host immune system, leading to the disruption of both metabolic and immune homeostasis [5, 19–24]. Published studies on ulcerative colitis (UC) in India have mainly focused on analyzing the gut microbiota using methods such as quantifying specific bacteria in tissue biopsies or fecal samples. Additionally, some studies have performed 16S rRNA analysis on a limited set of tissue biopsy samples [25–28]. In this study, we analysed the fecal microbiota profile of North Indian patients with UC in relation to disease activity and compared it to the gut microbial composition of healthy controls.

The comparative fecal microbiome analysis of North Indian patients with UC and heathy controls who had similar dietary and lifestyle habits (predominantly vegetarians, wheat bread and dairy consumers, and nonsmokers) revealed enrichment of two anaerobic lactic acid bacilli, namely, *Bifidobacterium* and *Lactobacillus*, in patients with UC (Figures 4 and 5 and Table 2). This is in contrast to the majority of earlier studies from west [5, 29–31], although a similar trend has been reported previously in patients with UC from China, Russia, and Japan [32–34]. Also, a recent study from India has also reported an enrichment of *Bifidobacterium* in the fecal samples of patients with amoebic colitis patient [2, 26]. The observed differences in gut microbiota composition between UC patients compared to healthy controls in different populations provide further evidence for the potent role of host genetics in shaping the composition of the gut microbiota. This hypothesis is supported by recent studies that have used a range of approaches, including transethnic analyses, heritability studies, mouse microbial quantitative trait locus (QTL) mapping, and association studies, to investigate the genetic factors that influence the gut microbiota composition [5, 7, 10, 33, 35, 36].

Both *Bifidobacterium* and *Lactobacillus* are commensal bacteria and are frequently used as probiotics in the treatment of inflammatory bowel disease (IBD) [37, 38] Moreover, our findings, along with the reported enrichment of *Bifidobacterium* and *Lactobacillus* in UC patients from different populations such as China, Japan, and Russia, rule out the influence of dietary habits. Although 5-aminosalicylates affect the host gut pH and promote the growth of *Bifidobacterium* and

Table 2: Microbial genus abundance and characteristics.

Genera	Median abundance			Characteristics
	Active	Healthy	Remission	
Other	9.59	12.09	11.86	
Prevotella_9	6.54	10.47	7.06	Gram negative, anaerobe, propionate
Others	23.28	8.82	34.75	
Dialister	2.86	6.78	2.44	Gram negative, anaerobe, propionate
Roseburia	0	6.45	1.53	Anaerobe, butyrate producer
Faecalibacterium	6.37	5.95	6.21	Gram positive, anaerobe, mesophilic, butyrate
Bifidobacterium	12.51	5.91	12.03	Gram positive, anaerobe, opportunistic pathogen, lactic acid-producing bacteria (LAB)
Ruminococcaceae_UCG_002	0	5.66	0	Anaerobe, butyrate producer
Collinsella	4.7	3.85	3.74	
Lactobacillus	11.74	3.53	10.17	Gram positive, aerotolerant anaerobes, opportunistic pathogen, LAB
Uncultured_bacterium	0	3.03	0	
Bacteroides	6.36	2.74	0	Gram negative, obligate anaerobe
Rikenellaceae_RC9_gut_group	0	2.58	0	
Streptococcus	10.19	2.3	6.61	Gram positive, most are facultative anaerobes, opportunistic pathogen, LAB
Uncultured	0	2.27	0	
Ruminococcus_2	0	2.17	0	Anaerobe, butyrate producer
Blautia	1.41	1.87	2.41	Gram positive, anaerobe, butyrate producer
Alloprevotella	0	1.66	0	Gram negative, anaerobe, propionate
Dorea	0.87	1.63	1.2	Gram positive, anaerobe, propionate
Ruminococcaceae_UCG_014	0	1.35	0	Anaerobe, butyrate producer
Escherichia_Shigella	0	1.18	0	
Prevotella_2	0	1.14	0	Gram negative, anaerobe, propionate
Lachnospiraceae_NK4A136_group	0	0.99	0	Gram positive, anaerobe, butyrate producer
Ruminococcaceae_UCG_005	0	0.82	0	Anaerobe, butyrate producer
Prevotella_7	0	0.81	0	Gram negative, anaerobe, propionate
Ruminococcus_1	0	0.8	0	Anaerobe, butyrate producer
Coprococcus_2	0	0.79	0	Anaerobe, butyrate producer
Romboutsia	3.58	0.77	0	Gram positive, obligate anaerobe
Christensenellaceae_R_7_group	0	0.71	0	
Enterorhabdus	0	0.5	0	
Ruminococcaceae_NK4A214_group	0	0.39	0	Anaerobe, butyrate producer

Lactobacillus, and wheat consumption has also been linked to their enrichment [39, 40], it is worth noting that the widespread use of 5-aminosalicylates and common dietary ingredients, such as wheat, rice, and milk-based products, suggests a limited influence of both on the observed microbial changes. Moreover, animal model studies have indicated that an increase abundance can turn these commensal microbes to opportunistic pathogens and trigger an immune response [41, 42]. In addition to *Bifidobacterium* and *Lactobacillus*, our study found a significant enrichment of *Streptococcus*, a lactic acid-producing bacteria, in patients with ulcerative colitis (UC) (Figure 5). This increase in *Streptococcus* abundance has also been observed in UC patients from different populations [43, 44]. The absence of proton pump inhibitor (PPI) use in our patients confirms that the observed increase in *Streptococcus* abundance is not related to drug use, as reported in previous studies [45, 46]. Notably, all of these genera are also part of the oral microbiome, which supports the emerging evidence suggesting a potential link between the severity of gut inflammation and an increased abundance of oral bacteria [47, 48]. This raises the question of whether the increased abundance of these commensal bacteria contributes to the onset of the disease or if it is a consequence of disease progression. Our subsequent data provide support for the hypothesis that the increase in abundance is a consequence of disease progression.

It has been reported that patients with persistent inflammation accumulate nonfermentable nitrate, providing a growth advantage to nitrate-reducing bacteria that utilize nitrate as an energy source. This mechanism potentially exacerbates inflammation [49, 50]. Our biochemical analysis of stool samples revealed an increased abundance of nitrate-

Figure 4: Taxonomic diversity of fecal microbiome composition in healthy controls and patients with active disease and disease in remission at the genus level.

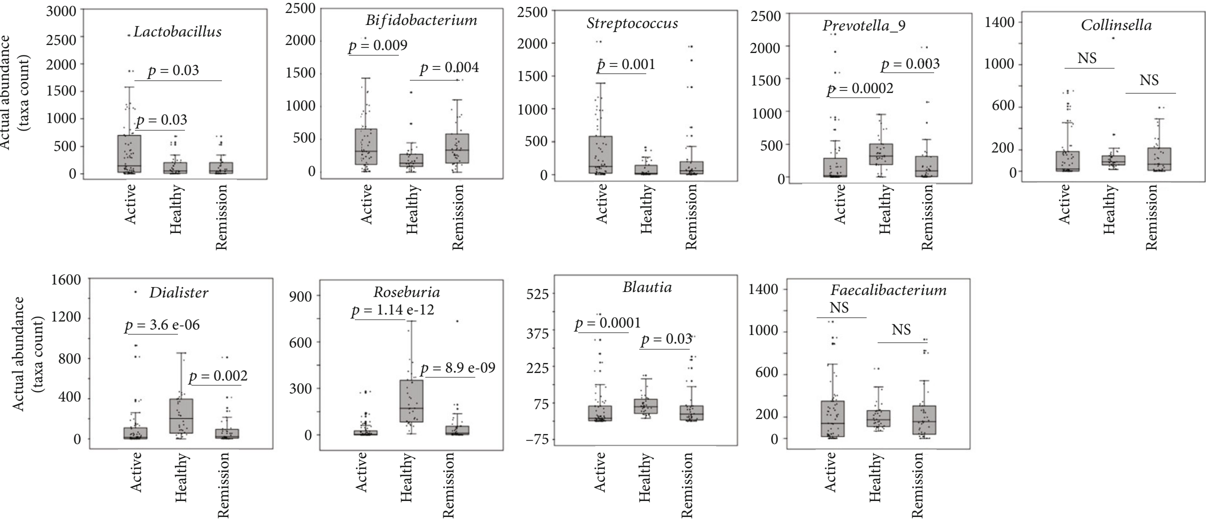

Figure 5: Median taxonomic abundance of common differentially abundant genera between active disease, remission, and healthy controls. NS: not significant. The calculated p value is Mann–Whitney with Bonferroni correction.

reducing bacteria in UC patients compared to healthy controls (SI-Figure-1) implying that persistent inflammation in UC patients accumulates nonfermentable nitrate. A recent study has revealed the ability of *Bifidobacterium* and *Lactobacillus* to utilize nonfermentable forms of nitrate [49]. This may explain the growth advantage exhibited by both of these

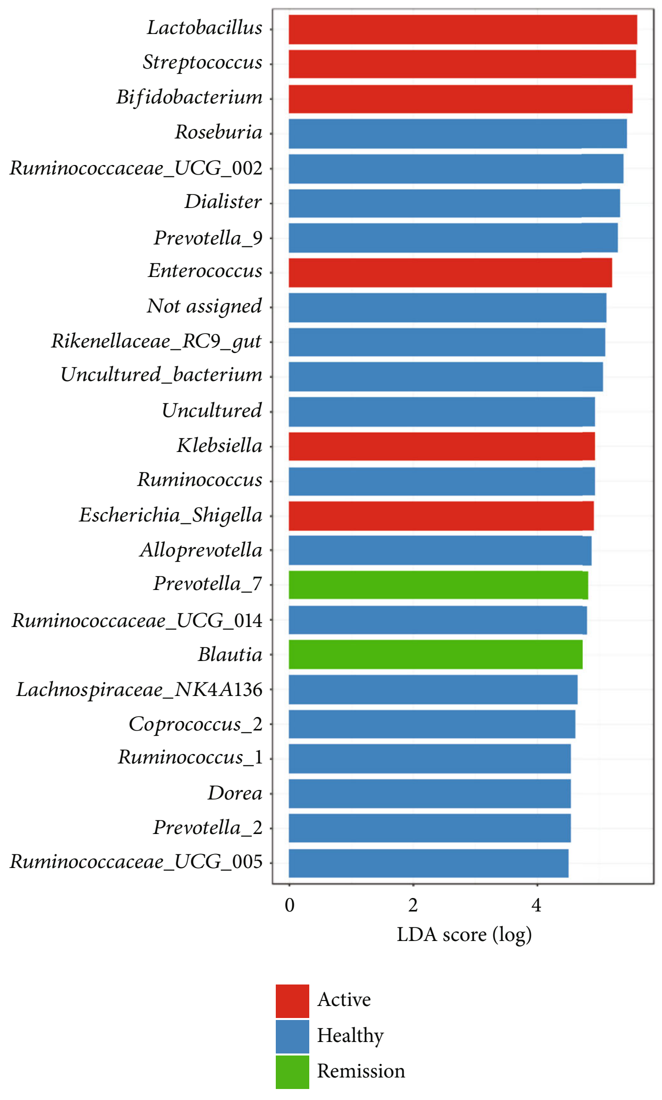

Figure 6: LEfSe analysis of fecal microbiome of UC patients (with active disease and in remission) and healthy controls.

genera over others, attributed to the utilization of nonfermentable nitrate as an energy source through respiration. Interestingly, metabolic pathway analysis also revealed an increase in (a) nitrate reduction I (DENITRIFICATION-PWY) and (b) lactose degradation-1 (LACTOSECAT-PWY) that were shared between UC patients with active disease and those in remission. This further supports the dominance of LABs (lactic acid bacteria) in the gut microbial diversity of UC patients, as they thrive under favorable growth conditions caused by inflammation.

Further, it has also been reported that increased accumulation of nonfermentable nitrate further inhibits the growth of short-chain fatty acid- (SCFA-) producing microbes, as they are unable to utilize this substrate [51, 52]. This was evidenced by the decrease in the relative abundance of SCFA-producing microbes and the reduction in the number of metabolic pathways involved in SCFA production (PWY-6590 and CENTFERM-PWY). However, loss of SCFA-producing microbes is not unique to our populations, and the loss of SCFA-producing bacteria in UC patients has been reported in previous studies from European and Asian populations as well (Table 2) [22, 23, 27, 52–55]. SCFAs are critical signalling molecules that regulate different biological processes, including promotion of gut integrity, regulation of immune response, reduction of pathogenic bacterial population, and prevention of unwanted infiltration of bacteria from lumen to lamina propria [52, 56–59]. A significant loss of SCFA-producing microbes and the concurrent increase in nitrate-reducing bacteria in UC patients favor a shift towards a proinflammatory microbiome, thereby enhancing host inflammation and modifying the disease behaviour. This furthers our argument that the enrichment of lactic acid-producing bacteria is a consequence of increased inflammation.

Two more observations emerge from our study. Firstly, contrary to earlier reports, no significant loss of *Faecalibacterium* genera was observed in UC patients compared to healthy controls (Table 2) [54, 60, 61]. Surprisingly, there

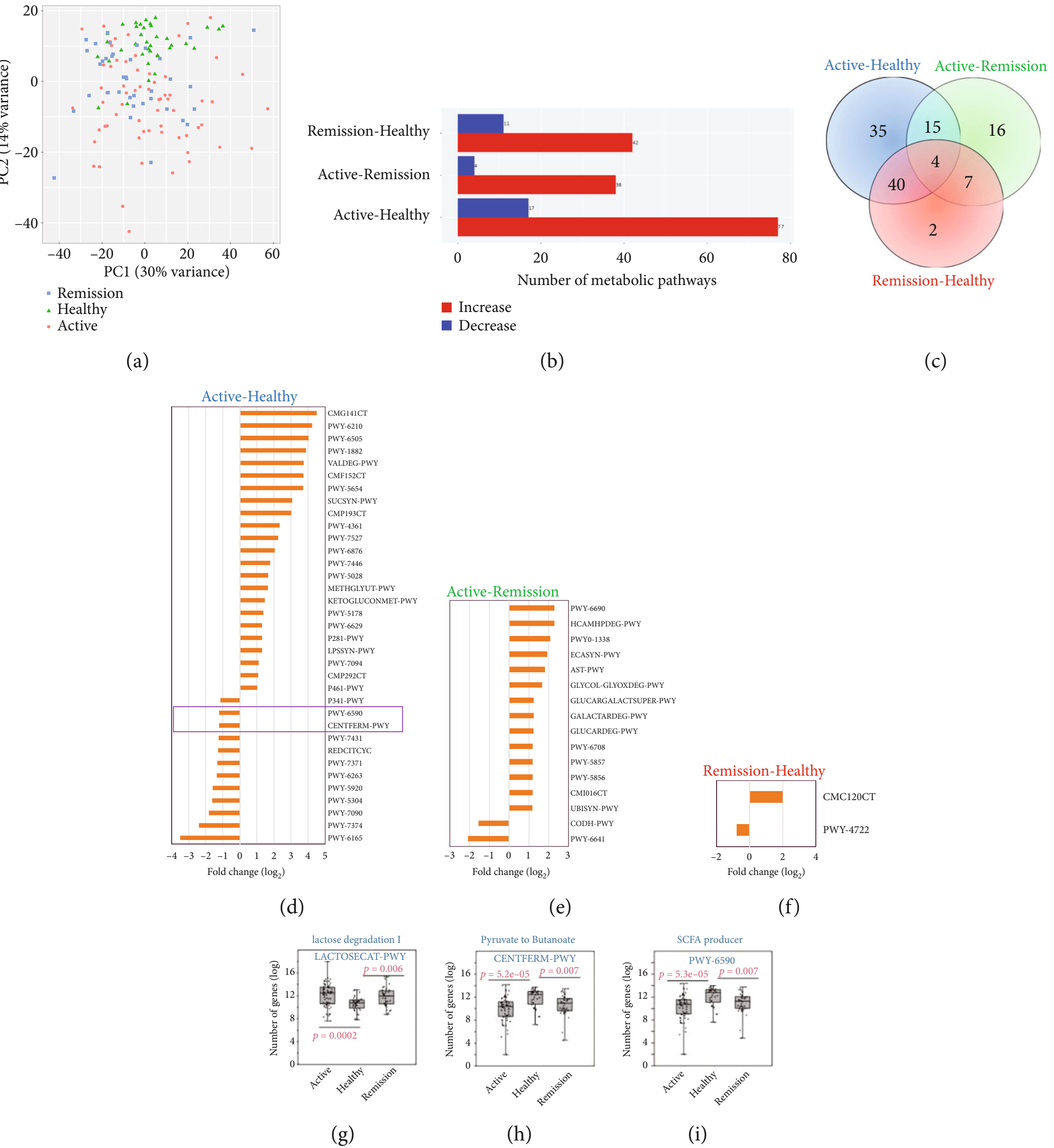

FIGURE 7: Differential metabolic pathways observed between UC subgroups and healthy controls. (a) PCA plot based upon metabolic pathways enrichment. (b) Total number up (increase) and down (decrease) regulated metabolic pathways. (c) Venn diagram representing overlap and different metabolic pathways. (d–f) Log fold change in the unique metabolic pathway observed between comparative groups. (g) Relative abundance of selected shared metabolic pathways (lactose degradation) between active and healthy and remission and healthy. (h, i) Relative abundance of selected downregulated metabolic pathways (pyruvate fermentation to butanoate and SCFA producer) between active and healthy. p value is Mann–Whitney with Bonferroni correction.

was even a slight increase in the abundance of *Faecalibacterium* in UC patients, although this increase was not statistically significant. We propose that this increase could be attributed to the metabolic cross-feeding pattern between *Bifidobacterium* and *Faecalibacterium* strains, as previously reported [62]. The second interesting finding of our study is the absence of significant differences in the relative abundance of microbial composition between patients with active ulcerative colitis (UC) and those in remission, which contrasts with previous studies. Similar observation has been reported in a recent large intercontinental study [10]. A linear discriminant analysis effect size (LEfSe) using the microbial taxa abundance revealed an association of *Prevotella_7* and *Blautia* with patients in remission compared to those with active disease and healthy controls. Further, differential analysis of predicted metabolic pathways (based upon microbial taxa abundance and composition) revealed 16 unique metabolic pathways, of which only two were increased in patients in remission. These pathways included the reductive acetyl coenzyme A pathway I, which is associated with homoacetogenic bacteria, and the superpathway of sulfolactate degradation involved in the catabolism of aliphatic C3 sulfonates. Our findings highlight that in order to identify robust differentiating microbial signatures or metabolites associated with UC disease activity, it is imperative to increase the sample size as well as validate these findings in ethnicity-matched independent cohorts.

Table 3: Differential metabolic pathways.

Names	Number	Pathway name
Active vs. remission active vs. healthy remission vs. healthy	4	PWY-6470, PWY-6396, P125-PWY, GALLATE-DEGRADATION-II-PWY
Active vs. healthy remission vs. healthy	40	PWY-6338, CMP352CT, PWY-5177, DHGLUCONATE-PYR-CAT-PWY, PWY-6383, PWY-5655, PWY-7209, CHLOROPHYLL-SYN, PWY-5910, PWY-7347, PWY1G-0, CMT174CT, PWY-5420, PWY-5005, PWY-5419, LACTOSECAT-PWY, CRNFORCAT-PWY, PWY-6339, DENITRIFICATION-PWY, PWY-7097, PWY0-42, PWY-5529, PWY-922, PWY-5183, PWY-5430, POLYAMINSYN3-PWY, P381-PWY, CMB025CT, PWY-6397, P101-PWY, NADSYN-PWY, P184-PWY, PWY-7376, PWY-7159, PWY-7007, PWY-6944, PWY-7098, PWY-7616, PWY-5747, CMC137CT
Active vs. remission active vs. healthy	15	GOLPDLCAT-PWY, XYPHENYLACETATE-DEGRADATION-PWY, 3-HYDRO, CMT552CT, METHYLGALLATE-DEGRADATION-PWY, PWY-5417, TYRFUMCAT-PWY, CMS125CT, PWY-6182, CATECHOL-ORTHO-CLEAVAGE-PWY, PWY-5181, GALLATE-DEGRADATION-I-PWY, PWY-5431, PWY-5415, PWY-6185, PROTOCATECHUATE-ORTHO-CLEAVAGE-PWY
Active vs. remission vs. healthy	7	CMN129CT, ORNARGDEG-PWY, THREOCAT-PWY, PWY0-321, PWY-6071, ARGDEG-PWY, PWY0-1277
Active vs. healthy	35	PWY-6165, CMP292CT, PWY-6210, PWY-7431, SUCSYN-PWY, P341-PWY, PWY-7090, PWY-5920, PWY-5178, P281-PWY, CMF152CT, PWY-5028, PWY-7094, LPSSYN-PWY, PWY-7446, PWY-6876, PWY-7371, PWY-6505, REDCITCYC, PWY-6263, PWY-4361, PWY-5304, PWY-6629, PWY-5654, PWY-1882, CMG141CT, METHGLYUT-PWY, PWY-7374, PWY-6590, CMP193CT, P461-PWY, CENTFERM-PWY, VALDEG-PWYKETOGLUCONMET-PWY
Remission vs. healthy	2	PWY-722, CMC120CT
Active vs. remission	16	CODH-PWY, HCAMHPDEG-PWYGLUCARDEG-PWY, CMI016CT, UBISYN-PWY, AST-PWY,PWY-5856, PWY-6641, PWY-6690, PWY0-1338, GLUCARGALACTSUPER-PWY, ECASYN-PWY, PWY-5857, GALACTARDEG-PWY, PWY-6708, GLYCOL-GLYOXDEG-PWY

5. Conclusions

In our study of a North Indian UC cohort using 16S rRNA gene sequencing-based fecal microbiome analysis, we demonstrated an increased abundance of *Bifidobacterium*, *Lactobacillus*, and *Streptococcus*, as well as depletion of SCFA-producing bacteria such as *Prevotella*, *Roseburia*, *Lachnospiraceae*, and *Ruminococcaceae*. Additionally, our findings demonstrated a significant enrichment of nitrate-respiration-proficient bacteria in UC patients, supporting that observed microbial dysbiosis is driven by persistent inflammation. Interestingly, our findings imply that *Prevotella_7* and *Blautia* taxa abundance, as well as two metabolic pathways, might serve as a discriminating signature between north Indian UC patients with active disease and those in remission. In summary, our study cautions towards using *Bifidobacterium* and *Lactobacillus* as probiotics in North Indian populations. Instead, we propose the restoration of butyrate-producing bacteria as a promising alternative therapeutic approach.

Data Availability

The data that support the findings of this study are available from the corresponding author (GJ) upon reasonable request.

Disclosure

This study has previously been presented as a preprint entitled "Correlation between faecal microbial taxa and ulcerative colitis in different phases of disease activity in a north Indian cohort" on medRxiV [63]. Vijay Verma current address is North Florida Research and Education Centre, Institute of Food and Agricultural Sciences, University of Florida, Quincy 32351, USA. Rakesh Bhatnagar current address is School of Biotechnology, Jawaharlal Nehru University, New Delhi, India. Mohan C. Joshi current address is Multidisciplinary Centre for Advance Research and Studies (MCARS), Jamia Millia Islamia, New Delhi, India.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Authors' Contributions

GJ, AjS, and VM conceptualized the study. GJ, AjS, and VM contributed to the methodology. VV was responsible for the software. AjS, VM, AS, and VV validated the study. GJ and MCJ contributed to formal analysis. GJ was responsible for the investigation. GJ, AjS, VM, and RB provided the resources. AS and DS contributed to the data curation. GJ

wrote the original draft. GJ, AjS, VM, AS, DS, RM, VV, RB, and MCJ wrote, reviewed, and edited the manuscript. GJ, AjS, VM, AS, and MCJ visualized the study. GJ, AjS, VM, and RB supervised the study. GJ, AjS, VM, and RB were responsible for project administration. GJ contributed to funding acquisition.

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Supplementary Materials

Supplementary Figure 1: whisker box plot illustrates relative absorbance (620 nm) in per gram of feces observed in nitrate broth of healthy controls and patient's active disease and disease in remission. Each dot represents relative absorbance in a sample. The bottom, middle, and top boundaries of each box represent the first, second (median), and third quartiles of the relative absorbance. The whiskers (lines extending from the top and bottom of the box and ending in horizontal cap) extend to points within 1.5 times the interquartile range. The points extending above the whiskers are outliers. p value is Mann–Whitney Bonferroni-corrected p value. Supplementary Figure 2: heatmap representing clustering across samples (UC patient subgroups and healthy control) based on differential abundance of metabolic pathway generated using iDEP. *(Supplementary Materials)*

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