RESEARCH PAPER

Proteolytic Colonic Microbiota Is Associated with Increased Clinical and Endoscopic Disease Activity in Ulcerative Colitis Running head : Microbiota and disease activity in ulcerative colitis

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ABSTRACT

Ulcerative colitis (UC) is a chronic inflammatory bowel disease with an increasing burden in Asia. Although gut microbiota alterations have been widely reported in UC, evidence linking microbiota patterns with both clinical and endoscopic disease activity remains limited, particularly in Indonesian populations. This study aimed to evaluate the association between colonic microbiota patterns and clinical and endoscopic disease activity in patients with UC. This cross-sectional study was conducted at a tertiary referral hospital in Makassar, Indonesia. Adult patients with UC undergoing lower gastrointestinal endoscopy were consecutively recruited. Colonic samples were collected during endoscopy and analyzed using 16S rRNA sequencing targeting the V3–V5 region. Microbiota were classified as saccharolytic (Firmicutes, Bacteroidetes) or proteolytic (Proteobacteria, Actinobacteria, Euryarchaeota). Disease activity was assessed using the Mayo Clinical Score (MCS) and the Ulcerative Colitis Endoscopic Index of Severity (UCEIS). A total of 53 patients were included. Proteobacteria was the dominant phylum in 62.3% of patients, and 66.0% exhibited a proteolytic microbiota pattern. Moderate–severe disease activity was observed in 37.7% of patients based on MCS and 32.1% based on UCEIS. A proteolytic microbiota pattern was significantly associated with moderate–severe clinical disease activity (OR 8.47; 95% CI 1.69–42.48; p=0.009) and moderate–severe endoscopic disease activity (OR 6.00; 95% CI 1.19–30.17; p=0.030). A proteolytic colonic microbiota pattern was significantly associated with increased clinical and endoscopic disease activity in UC, suggesting that microbiota composition may reflect inflammatory burden. Further longitudinal studies are required to confirm these associations..

Keywords: ulcerative colitis, gut microbiota, proteolytic microbiota, disease activity, UCEIS

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INTRODUCTION

Inflammatory bowel disease (IBD), comprising Crohn's disease and ulcerative colitis (UC), is a chronic relapsing inflammatory disorder with a rapidly increasing burden in Asia [1]. Asia accounts for nearly 60% of the global population, underscoring the substantial public health implications of rising IBD incidence in this region [2]. Epidemiological data demonstrate a growing incidence of UC in Asia, including Indonesia, highlighting the need to identify clinically relevant factors associated with disease activity and progression [3,4]. Disease activity in UC is commonly assessed using clinical indices and endoscopic severity scoring; however, discordance between clinical symptoms and mucosal inflammation is frequently observed, emphasizing the importance of identifying biological correlates that reflect both clinical and endoscopic activity [5,6].

Alterations in gut microbiota composition have been increasingly recognized as a key factor in UC pathogenesis. Dysbiosis, characterized by depletion of beneficial commensal bacteria and enrichment of potentially pathogenic taxa, has been consistently reported in IBD [7]. A reduction in microbial diversity of approximately 25% has been observed in IBD patients compared with healthy controls [8]. At the phylum level, reductions in Firmicutes and Bacteroidetes and enrichment of Proteobacteria have been associated with inflammatory states [9,10]. These changes are often accompanied by reduced abundance of short-chain fatty acid–producing bacteria, which play a critical role in maintaining epithelial integrity and immune homeostasis [11–14].

Beyond mechanistic evidence, gut microbiota composition has also been linked to disease severity and therapeutic response. Patients with severe disease activity tend to show greater dysbiosis and increased abundance of opportunistic pathogens, whereas remission has been associated with partial restoration of commensal microbial profiles [5]. In addition, higher abundance of Faecalibacterium prausnitzii and Bacteroides fragilis has been reported among UC

Author for Correspondence: jessicakwenandar@gmail.com patients achieving remission following infliximab therapy [15], and enrichment of protective Firmicutes has been observed after successful treatment [7].

Despite these advances, evidence linking microbiota patterns with validated clinical and endoscopic disease activity indices remains limited, particularly in Indonesian populations. Population-specific microbiota profiles may differ due to variations in environmental exposures, host characteristics, and treatment practices, highlighting the importance of generating locally derived evidence [16,17]. Therefore, this study aimed to investigate the association between colonic microbiota patterns and clinical and endoscopic disease activity in patients with ulcerative colitis.

Materials and methods

Study design and participants

This observational analytic study used a cross-sectional design and was conducted at a tertiary referral hospital in Makassar, Indonesia, from January to July 2025. Laboratory analyses were performed at a university-based research laboratory. Adult patients (≥18 years) with a confirmed diagnosis of ulcerative colitis who underwent lower gastrointestinal endoscopy during the study period were consecutively recruited from inpatient and outpatient services. The diagnosis of ulcerative colitis was established based on clinical presentation, endoscopic findings, and histopathological confirmation. Patients were excluded if they had received antibiotics, nonsteroidal anti-inflammatory drugs, or immunomodulatory therapy within 30 days prior to enrollment, or if they had active gastrointestinal infection.

Sample size calculation

The minimum required sample size was calculated using a two-proportion comparison formula, assuming proportions of moderate–severe disease activity of 0.70 and remission–mild activity of 0.35, with a significance level of 0.05 and 80% power. The estimated minimum sample size was 51 subjects.

Clinical and endoscopic assessment

Clinical disease activity was assessed using the Mayo Clinical Score (MCS), categorized into remission, mild, moderate, and severe according to standard criteria. Endoscopic severity was evaluated using the Ulcerative Colitis Endoscopic Index of Severity (UCEIS). For analysis, patients were grouped into remission–mild and moderate–severe categories.

Sample collection and DNA sequencing

Colonic samples were obtained during lower gastrointestinal endoscopy under sterile conditions and immediately stored at −80°C until analysis. Microbial DNA was extracted using the Zymo DNA Extraction Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. The extracted DNA underwent polymerase chain reaction amplification targeting the V3–V5 regions of the 16S rRNA gene. Sequencing was performed using the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA).

Microbiota classification

Microbiota were classified based on dominant bacterial phyla identified through sequencing. For analytical purposes, microbiota were categorized into saccharolytic (Firmicutes and Bacteroidetes) and proteolytic (Proteobacteria, Actinobacteria, and Euryarchaeota) patterns, based on their predominant metabolic functions as described in previous studies [18–22].

Statistical analysis

Statistical analyses were performed using SPSS version 30 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean ± standard deviation, and categorical variables as frequencies and percentages. Binary logistic regression analysis was conducted to evaluate the association between microbiota classification (proteolytic vs saccharolytic) and disease activity categories (moderate–severe vs remission–mild), assessed using both the Mayo Clinical Score (MCS) and the Ulcerative Colitis Endoscopic Index of Severity (UCEIS). Both unadjusted and adjusted models were performed, with age and sex included as potential confounders. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. A two-tailed p-value <0.05 was considered statistically significant.

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the institutional ethics committee (approval number: 1091/UN4.6.4.5.31/PP36/2024). Written informed consent was obtained from all participants prior to enrollment.

Results

A total of 61 patients were assessed for eligibility, of whom 8 were excluded due to not meeting inclusion criteria (n=2), recent antibiotic use (n=5), or active infection (n=1). A total of 53 patients were included in the final analysis (Figure 1).

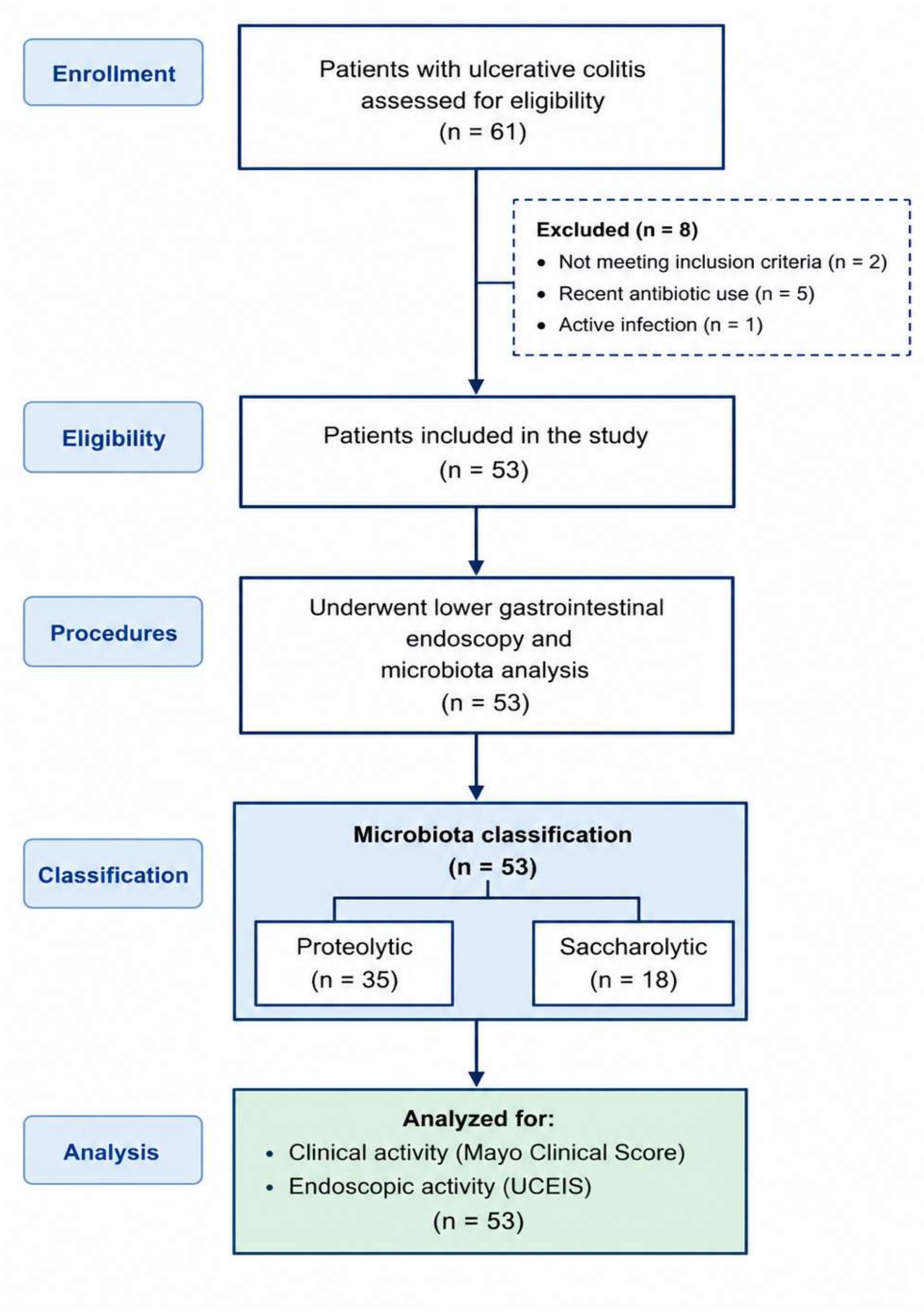

Fig 1. Flowchart of patient selection and analysis.

Patients with ulcerative colitis were consecutively recruited and classified according to microbiota pattern for analysis of clinical (MCS) and endoscopic (UCEIS) disease activity.

The baseline characteristics of the study participants are presented in Table 1. The sex distribution was balanced, and the mean age was 52.20 ± 16.64 years. Most patients had a normal body mass index and were non-smokers. The prevalence of herbal use, probiotic use, and alcohol consumption was low, and comorbidities were present in a minority of patients.

Table 1. Baseline characteristics of patients with ulcerative colitis (n=53)

Variable	Value
Sex	
Male	27 (50.9%)
Female	26 (49.1%)
Age (years)	52.20 ± 16.64
< 60 years	32 (60.4%)
≥ 60 years	21 (39.6%)
Body Mass Index (kg/m²)	21.78 ± 2.49
Underweight	5 (9.4%)
Normal	32 (60.4%)
Overweight	13 (24.5%)
Obese	3 (5.7%)
Smoking	
Yes	10 (18.9%)
No	43 (81.1%)
Herbal use	
Yes	3 (5.7%)
No	50 (94.3%)
Probiotic use	
Yes	1 (1.9%)
No	52 (98.1%)
Alcohol consumption	
Yes	1 (1.9%)
No	52 (98.1%)
Comorbidities	
Yes	12 (22.7%)
No	41 (77.3%)

Data are presented as n (%) for categorical variables and mean ± SD for continuous variables.

The distribution of microbiota profiles and disease activity indices is shown in Table 2. Proteobacteria was the predominant phylum (62.3%), and 66.0% of patients exhibited a proteolytic microbiota pattern. Moderate–severe disease activity was observed in 37.7% of patients based on the Mayo Clinical Score (MCS) and 32.1% based on the Ulcerative Colitis Endoscopic Index of Severity (UCEIS).

Table 2. Distribution of microbiota profile and disease activity indices (n=53)

Variable	Value
Dominant Phylum	
Firmicutes	12 (22.6%)
Proteobacteria	33 (62.3%)
Bacteroidetes	6 (11.3%)
Euryarchaeota	1 (1.9%)
Actinobacteria	1 (1.9%)
Microbiota Classification	
Saccharolytic	18 (34.0%)
Proteolytic	35 (66.0%)
Mayo Clinical Score (MCS)	5.25 ± 2.45
Remission–mild	33 (62.3%)
Moderate–severe	20 (37.7%)
Ulcerative Colitis Endoscopic Index of Severity (UCEIS)	3.04 ± 2.15
Remission–mild	36 (67.9%)
Moderate–severe	17 (32.1%)

Data are presented as n (%) for categorical variables and mean ± SD for continuous variables.

The association between microbiota classification and clinical disease activity is presented in Table 3. In the unadjusted analysis, a proteolytic microbiota pattern was significantly associated with moderate–severe clinical disease activity (OR 8.47; 95% CI 1.69–42.48; p=0.009). This association remained significant after adjustment for age and sex (adjusted OR 7.43; 95% CI 1.42–39.04; p=0.018).

Table 3. Association between microbiota classification and moderate–severe clinical disease activity

Microbiota Type	Moderate–Severe n (%)	Remission–Mild n (%)	OR (95% CI)	p-value	Adjusted OR (95% CI)†	p-value
Proteolytic (n=35)	18 (51.4%)	17 (48.6%)	8.47 (1.69–42.48)	0.009	7.43 (1.42–39.04)	0.018
Saccharolytic (n=18)	2 (11.1%)	16 (88.9%)	Reference	—	Reference	—

Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using binary logistic regression. Adjusted for age and sex. Moderate–severe disease activity was defined based on the Mayo Clinical Score (MCS).

The association between microbiota classification and endoscopic disease activity is shown in Table 4. In the unadjusted analysis, a proteolytic microbiota pattern was significantly associated with moderate–severe endoscopic disease activity (OR 6.00; 95% CI 1.19–30.17; p=0.030). However, this association was not statistically significant after adjustment for age and sex (adjusted OR 4.55; 95% CI 0.82–25.27; p=0.084).

Table 4. Association between microbiota classification and moderate–severe endoscopic disease activity

Microbiota Type	Moderate–Severe n (%)	Remission–Mild n (%)	OR (95% CI)	p-value	Adjusted OR (95% CI)†	p-value
Proteolytic (n=35)	15 (42.9%)	20 (57.1%)	6.00 (1.19–30.17)	0.030	4.55 (0.82–25.27)	0.084
Saccharolytic (n=18)	2 (11.1%)	16 (88.9%)	Reference	—	Reference	—

Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using binary logistic regression. Adjusted for age and sex. Moderate–severe disease activity was defined based on the Ulcerative Colitis Endoscopic Index of Severity (UCEIS).

Discussion

This study demonstrates that colonic microbiota composition is associated with disease activity in patients with ulcerative colitis (UC), with a stronger and independent association observed for clinical disease activity than for endoscopic activity. A proteolytic microbiota pattern was predominant in this cohort and remained significantly associated with moderate–severe clinical disease activity after adjustment for age and sex, whereas its association with endoscopic disease activity was attenuated after adjustment. This pattern suggests that microbiota composition may be more closely related to clinical inflammatory burden than to endoscopic severity alone.

The predominance of Proteobacteria observed in this study is consistent with previous reports identifying this phylum as a key microbial signature of dysbiosis in inflammatory bowel disease, particularly during active intestinal inflammation [18,23,24]. Proteobacteria includes several opportunistic taxa capable of thriving in inflammatory environments due to their ability to utilize host-derived substrates and tolerate oxidative stress [18,25]. Therefore, the enrichment of Proteobacteria likely reflects a microbial ecosystem shaped by chronic inflammation rather than a single causative organism.

The relatively lower abundance of Firmicutes and Bacteroidetes further supports the presence of dysbiosis, as these phyla are frequently associated with beneficial metabolic activity, including fermentation of dietary fiber and production of short-chain fatty acids (SCFAs) that support epithelial integrity and immune regulation [19,20]. Although SCFA levels were not measured in the present study, the observed microbiota profile is consistent with the concept that UC is accompanied by functional shifts in microbial metabolism and loss of protective commensal communities [21,22].

A key finding of this study is the significant association between a proteolytic microbiota pattern and clinical disease activity. Patients with a proteolytic microbiota pattern were more likely to exhibit moderate–severe clinical activity based on MCS. This observation is consistent with evidence suggesting that dysbiosis contributes to epithelial barrier dysfunction and immune dysregulation, thereby exacerbating symptom burden such as diarrhea, rectal bleeding, and abdominal pain [21,26]. From a mechanistic perspective, proteolytic microbiota dominance may reflect an intestinal environment characterized by increased abundance of pathobionts and reduced commensal SCFA-producing taxa. SCFAs, particularly butyrate, are known to maintain epithelial barrier integrity and promote immune tolerance by supporting regulatory T-cell function and anti-inflammatory cytokine production [27–30]. In contrast, depletion of SCFA-producing bacteria may weaken epithelial resilience and amplify inflammatory signaling, thereby sustaining chronic disease activity. Although causality cannot be inferred because of the cross-sectional design, the observed association suggests that microbiota classification may reflect clinically meaningful inflammatory states.

In the unadjusted analysis, a proteolytic microbiota pattern was also associated with moderate–severe mucosal inflammation assessed by UCEIS. Endoscopic disease activity is considered a more objective indicator of inflammatory burden than clinical symptoms alone, and persistent endoscopic inflammation has been linked to higher risks of relapse and long-term complications in UC [6,31]. However, this association was attenuated after adjustment for age and sex, indicating that the relationship between microbiota composition and mucosal injury may be influenced by additional host-related factors. This finding suggests that microbiota composition alone may not independently explain endoscopic severity, although it remains biologically relevant.

The relationship between dysbiosis and endoscopic severity may be interpreted through several biologically plausible pathways. Inflammatory conditions in the colon promote disruption of tight junction integrity and mucosal barrier permeability, allowing increased bacterial translocation and sustained immune activation [32–34]. Dysbiotic microbial communities may further aggravate this process through expansion of opportunistic organisms and biofilm formation, which has been associated with epithelial invasion and mucosal injury in inflammatory bowel disease [35,36]. In turn, increased activation of pattern recognition receptors such as Toll-like receptors may perpetuate cytokine-mediated inflammation, thereby reinforcing the cycle of mucosal damage [37,38]. Taken together, these mechanisms support the biological plausibility of an association between proteolytic microbiota and endoscopic activity, even though the adjusted association in the present study was not statistically significant.

From a clinical perspective, these findings highlight the potential role of microbiota composition as a marker of inflammatory burden in UC. While endoscopy remains the gold standard for assessing mucosal inflammation, it is invasive, resource-intensive, and not always feasible for frequent monitoring. Identifying microbial patterns associated with disease activity may contribute to future strategies for risk stratification and less invasive monitoring, although further validation is required [39–41]. Additionally, the high prevalence of a proteolytic microbiota pattern in this cohort suggests that dysbiosis may be common among UC patients in this setting. These findings provide a rationale for further research to explore whether microbiota modulation strategies are associated with changes in disease activity [22,42]. However, the present study did not assess treatment response; therefore, direct clinical recommendations regarding microbiota-targeted therapy cannot yet be made.

This study has several strengths. First, it integrates both clinical (MCS) and endoscopic (UCEIS) measures, allowing assessment across two complementary domains of disease activity. Second, microbiota characterization was based on colonic samples obtained during endoscopy, which may better reflect mucosal microbial communities than fecal samples in certain contexts [43,44]. Third, this study provides population-specific evidence from Indonesia, where microbiota data in UC remain limited.

Nevertheless, several limitations should be considered. First, the cross-sectional design does not allow causal inference regarding whether dysbiosis contributes to increased disease activity or arises as a consequence of active inflammation. Second, the classification of microbiota into proteolytic and saccharolytic patterns, although biologically plausible and useful for clinical interpretation, represents a simplified framework that does not capture species-level composition, functional metabolic pathways, or microbial metabolites. Third, this study did not assess alpha diversity indices such as Shannon or Simpson indices [12], nor did it include inflammatory biomarkers such as fecal calprotectin or C-reactive protein, which could have strengthened biological interpretation [45]. Finally, the sample size was relatively modest and derived from a single center, which may limit generalizability. The wide confidence intervals observed in the regression analyses also indicate limited statistical precision, and the magnitude of the associations should therefore be interpreted with caution.

Future studies should use longitudinal designs to clarify temporal relationships between microbiota alterations and disease activity and should incorporate functional, metabolomic, and biomarker-based approaches to better characterize microbial activity and inflammatory burden. Further work is also needed to determine whether microbiota-based classification can improve disease monitoring or inform therapeutic strategies in UC.

Conclusion

A proteolytic colonic microbiota pattern was associated with increased clinical disease activity in patients with ulcerative colitis and remained significant after adjustment for age and sex. In contrast, its association with endoscopic disease activity was not independent after adjustment, suggesting that microbiota composition may more closely reflect clinical inflammatory burden than mucosal injury alone. These findings highlight the potential role of microbiota patterns as a complementary marker of disease activity. However, given the cross-sectional design and limited sample size, these findings should be interpreted cautiously. Further longitudinal and mechanistic studies are needed to clarify the role of microbiota composition in disease progression and its potential clinical applications.

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Data Availability Statement

All relevant data are within the manuscript and its Supporting Information files.

Competing interests

The authors declare that no competing interests exist.

Author Contributions

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Formal analysis: Jessica Kwenandar, Arifin Seweng.
Investigation: Jessica Kwenandar, Femi Syahriani, Syakib Bakri, Dimas Bayu.
Methodology: Jessica Kwenandar, A.M. Luthfi Parewangi, Femi Syahriani.
Supervision: Syakib Bakri, Dimas Bayu, Arifin Seweng.
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Writing – original draft: Jessica Kwenandar, A.M. Luthfi Parewangi, Femi Syahriani.
Writing – review & editing: Jessica Kwenandar, A.M. Luthfi Parewangi, Femi Syahriani, Syakib Bakri, Dimas Bayu, Arifin Seweng.

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