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RESEARCH

# Dark-purple rice extract modulates gut microbiota composition in acetic acid– and indomethacin-induced inflammatory bowel disease in rats

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## Abstract

Ulcerative colitis (UC) and Crohn's disease (CD) are two major forms of inflammatory bowel disease (IBD). The disease has been linked with gut microbiota dysbiosis in which the balance of commensal communities is disrupted. Accumulating evidence demonstrates that treatment with biologically active compounds can modulate gut microbiota composition in animal models. Our previous work has also shown the beneficial effect of Luem Pua (LP) rice extract, which is rich in anthocyanins, on inflammation. However, its effect on gut microbiota is yet to be explored. In this study, we profiled fecal microbiota of acetic acid (AA)–induced UC and indomethacin (ID)–induced CD rat models with and without pretreatment with LP rice extract by 16S rRNA gene sequencing. The results showed that gut microbiota communities of rats were altered by both AA-induced UC and ID-induced CD. The relative abundances of beneficial bacteria, especially the *Lachnospiraceae* NK4A136 group and *Lactobacillus*, were decreased in the AA-induced UC model, while some opportunistic pathogens (*Bacteroides*, *Escherichia/Shigella*, *Fusobacterium*, and *Veillonella*) were raised by ID-induced CD. Interestingly, pretreatment with LP rice extract before AA-inducing UC in rats increased the proportion of the butyrate-producing bacteria (*Lachnospiraceae* NK4A136 group). The abundances of these beneficial bacteria and other SCFA-producing bacteria were unaffected by the indomethacin treatment with LP. Overall, our study revealed different impacts of AA-induced UC and ID-induced CD on changes in community composition and hinted at how LP may protect against UC by modifying the gut microbiota.

**Keywords** LP rice extract · Gut microbiota · Inflammatory bowel disease · Rats

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## Abbreviations

| | |
|---|---|
| CD | Crohn's disease |
| IBD | Inflammatory bowel disease |
| LP | Luem Pua rice extract |
| UC | Ulcerative colitis |
| AA-induce UC | Acetic acid–induced ulcerative colitis |
| D0C | Control group on day 0 of acetic acid–induced ulcerative colitis |
| D7C | Control group on day 7 of acetic acid–induced ulcerative colitis |
| D7AA | Acute acetic acid–induced ulcerative colitis |
| D7AALP | Acute acetic acid–induced ulcerative colitis pretreated with Luem Pua rice extract |
| ID-induce CD | Indomethacin-induced Crohn's disease |
| D0DW | Control group on day 0 of chronic indomethacin-induced Crohn's disease |

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|---|---|
| D11DW | Control group on day 11 of chronic indomethacin-induced Crohn's disease |
| D11ID | Chronic indomethacin-induced Crohn's disease |
| D11IDLP | Chronic indomethacin-induced Crohn's disease pretreated with Luem Pua rice extract |

## Introduction

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a group of intestinal disorders characterized by chronic or relapsing inflammation in parts of the small intestine and/or colon. The pathophysiology of CD is characterized by transmural inflammation and can affect any region of the intestine in a discontinuous pattern. In contrast, UC is a superficial inflammation and restricted to the mucosal layers of the distal colon and rectum (Li et al. 2019). CD and UC are commonly found in industrialized countries, especially North America and Europe, which show the highest incidence and prevalence of IBD (Mak et al. 2020). Recently, IBD has also increased in developing countries such as Brazil, Taiwan, South Korea, China, and Thailand (Ng et al. 2017; Khan et al. 2019; Guan 2019). IBD also increases the risk of colorectal cancer (Khor et al. 2011). Taken together, IBD has been considered a global public health problem. The exact etiology and pathogenesis of CD and UC involve various factors, such as genetics, epithelial barrier dysfunction, and alteration of gut microbial composition, that can lead to the development of chronic intestinal inflammation (Thomas et al. 2017).

The gut microbiota are a large and diverse community of commensal bacteria that are associated with human health and many diseases such as IBD. Well-balanced gut microbiota can protect the intestinal epithelium from the harmful effects of pathogens by inhibiting the colonization of pathogenic bacteria through mucus production, producing antimicrobial compounds, and enhancing epithelial cell barrier function to homeostasis (Schroeder 2019). Imbalance of gut microbiota, known as gut dysbiosis, has been suggested as one of the important factors associated with the initiation of intestinal inflammation and development of IBD (Glassner et al. 2020). However, whether the alterations of gut microbiota are a cause or a consequence of IBD remains unclear. To help clarify potential mechanisms through which the microbiota may drive IBD, researchers frequently turn to animal models of IBD (Thomas et al. 2017). Previous studies have confirmed that many chemical-induced models of IBDs, such as acetic acid–induced UC and indomethacin-induced CD, are useful for the study of IBD given that they elicit pathologies and therapeutic responses that are similar to human IBD (Pawar et al. 2011; El-Akabawy and El-Sherif 2019). These models, especially when considered alongside clinical data, have helped to clarify the potential interactions between gut microbiota and IBD. For example, research has revealed the existence of significant differences in gut microbiota composition that associate with IBD, particularly for Bacteroidetes (*Bacteroides*) and Firmicutes (*Lactobacillus*, *Bifidobacterium*, *Faecalibacterium*, *Eubacterium rectale*, and *Roseburia*), which negatively associate with IBD (Khan et al. 2019). Additionally, Proteobacteria, especially *Enterobacteriaceae* and *Fusobacterium*, tend to increase in IBD-afflicted individuals. Moreover, reduction of SCFA-producing bacteria is consistent across studies in both animal models of IBD (Xu et al. 2021) and humans suffering from IBD (Venegas et al. 2019). Recently, natural/alternative treatments have been suggested to restore the composition of the gut microbiota in colitis mouse models in which the abundances of beneficial taxa were improved (e.g., *Bifidobacterium*, *Lactobacillus*, *Lachnospiraceae*, and *Ruminococcaceae*) (Yeom et al. 2016; Han et al. 2019). Thus, modulation of gut microbiota influenced by natural compounds might provide a protective effect against IBD.

Thai dark-purple glutinous rice variety Luem Pua (LP) is a special variety of glutinous rice having dark-purple pericarp. LP is rich in polyphenolic compounds and exhibits a higher level of anthocyanin pigment and total antioxidant activity than other black rice (Suwannalert and Rattanachitthawat 2011). Previous studies also demonstrated that the aqueous extract of LP possessed significant radical scavenging as well as significantly reduced the severity of IBD in rat models, as assessed by disease activity index (DAI) scores, colon length, and the enlargement of spleen weight (Srisuwan et al. 2015; Thipart et al. 2020, 2021). These findings suggested a promising anti-inflammatory effect of LP rice extract against colonic inflammation. However, there is limited evidence concerning the effect of the aqueous extract of LP on gut microbiota profiles. Therefore, the present study aimed to evaluate whether the aqueous LP extract can modulate the composition of gut microbiota in experimental rat models of acetic acid– and indomethacin-induced IBD using the 16S rRNA gene sequencing analysis.

## Materials and methods

### Experimental animals

Male Wistar rats (6–8 weeks old, the average weight of 250 g) were received from Nomura Siam International Co., Ltd., Pathumwan, Bangkok, Thailand. Animals were housed in stainless steel cages and maintained in an air-conditioned room (25 ± 1 °C) with a 12-h light:12-h dark cycle. Food and water were available ad libitum. Rats were acclimatized to the laboratory room and handled for 1 week to confirm

their health status before being randomized into three groups ($n = 6$/group).

### Preparations of LP rice extract

The aqueous LP extract was prepared as described earlier (Thipart et al. 2020). In brief, LP rice, authenticated by the Phitsanulok Rice Research Center, Wang Thong, Phitsanulok, Thailand, was soaked with hot water for 5 min, filtered, and lyophilized. The percentage yield of the LP extract was calculated to be 2.16%. Dried LP extract was stored at 4 °C and freshly dissolved in distilled water before being used.

### Induction of acetic acid–induced UC in rats

UC was induced in all groups by the transrectal treatment of 4% acetic acid (AA) once on day 6 except for the control group as previously described (Dey et al. 2017). In experimental rat groupings (Fig. S1a), the rats were categorized into three groups: (1) control group (daily oral gavage infused with distilled water for 7 consecutive days), (2) AA group (daily oral gavage of distilled water for 7 consecutive days; on day 6, transrectal administration of 2 mL of 4% AA), and (3) AA + LP group (daily oral gavage of 5 g/kg of LP extract for 7 consecutive days; on day 6, transrectal administration of 2 mL of 4% AA). Fecal samples were collected in a sterilized container before treatment (day 0) as a control group (D0C ($n = 6$)) and after treatment (day 7) as a treatment group (D7C ($n = 6$), D7AA ($n = 6$), and D7AALP ($n = 6$)). Fecal samples from each group were then pooled and divided into three subgroups (D0C1-D0C3, D7C1-D7C3, D7AA1-D7AA3, and D7AALP1-D7AALP3). All samples were immediately stored at − 80 °C until DNA extraction.

### Induction of indomethacin-induced CD in rats

Indomethacin-induced IBD model was done by using indomethacin which is a non-selective COX inhibitor that produces enterocolitis progression (Pawar et al. 2011). Rats were randomly divided into three groups (Fig. S1b): (1) control group (daily oral gavage of distilled water for 11 consecutive days), (2) indomethacin (ID) group (daily oral gavage of distilled water for 11 consecutive days; on day 8 and day 9 of treatment, rats were injected subcutaneously with 7.5 mg/kg of ID), and (3) ID + LP group (daily oral gavage of 5 g/kg of LP extract for 11 consecutive days; on day 8 and day 9 of treatment, rats were injected subcutaneously with 7.5 mg/kg of ID). Fecal samples were collected in a sterilized container before treatment (day 0) as a control group (D0DW ($n = 6$)) and after treatment (day 11) as a treatment group (D11DW ($n = 6$), D11ID ($n = 6$), and D11IDLP ($n = 6$)). Fecal samples from each group were then pooled and divided into three subgroups (D0DW1-D0DW3, D11DW1-D11DW3, D11ID1-D11ID3, and D11IDLP1-D11IDLP3). All samples were immediately stored at − 80 °C until DNA extraction.

### Fecal DNA extraction

Fecal samples were stored at − 80 °C once being collected from rats in each group until analysis. Bacterial DNA was extracted from 180 to 220 mg of feces using the Qiagen QIAamp DNA Stool Minikit (QIAGEN, Hilden, Germany) following the instructions of the manufacturer. Briefly, the procedure involved lysis of the bacterial cells within the fecal samples in ASL buffer, adsorption of impurities to InhibitEX reagent, and purification of the DNA on a spin column. ASL buffer has been specially developed to remove inhibitory substances from fecal samples. The DNA was eluted to the final volume of 200 µL. The concentration and quality of DNA were measured by a NanoDrop™ spectrophotometer (Thermo Fisher Scientific). The extracted total DNA was stored at − 20 °C.

### Library preparation 16S rRNA gene sequencing

The hypervariable regions V3–V4 of the 16S rRNA gene were amplified using specific primers: forward primers (5′–CCTACGGRRBGCASCAGKVRVGAAT–3′) and reverse primers (5′–GGACTACNVGGGTWTCTAATC C–3′) (Klindworth et al. 2013). PCR was performed using 20–30 ng template DNA and Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Beverly, MA, USA). Amplified products of 400 bp to 450 bp were detected by a 2% agarose gel electrophoresis and selected for further processes. Next-generation sequencing (NGS) library preparations and Illumina MiSeq sequencing were conducted at GENEWIZ, Inc. (Suzhou, China) and quantified using a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). DNA libraries were loaded on the Illumina MiSeq platform (Illumina, San Diego, CA, USA). Sequencing was performed using a 2 × 250 paired-end (PE) method conducted by the MiSeq Control Software (MCS) embedded in the MiSeq instrument.

### Sequencing data analysis

Raw DNA sequencing data was analyzed using the QIIME platform (version 1.9.1) (Caporaso et al. 2010). Reads obtained from high-throughput sequencing were quality filtered to remove barcodes and primer sequences under specific parameters (Bokulich et al. 2013) according to the QIIME quality-controlled process (version 1.9.1, http://qiime.org/index.html) (Caporaso et al. 2012). Subsequently, chimera detection and removal were achieved by using the UCHIME algorithm (UCHIME Algorithm, http://www.drive5.com/usearch/

manual/uchime_algo.html) (Edgar et al. 2011). The effective 16S reads were then clustered into operational taxonomic units (OTUs) using the clustering program VSEARCH (version 1.9.6) at 97% sequence identity. OTUs were classified into six taxonomic ranks (phylum, class, order, family, genus, and species) at a confidence threshold of 0.8 (Wang et al. 2007) using Ribosomal Database Program (RDP) classifiers (version 2.2). The SILVA reference database (version 128, http://www.arb-silva.de/) was used for OTU annotation (Quast et al. 2013). The raw sequence data is available in the NCBI Sequence Read Archive (SRA) repository under the BioProject accession number PRJNA785748 (BioSample accession numbers SAMN23575674–SAMN23575697).

### Microbiome data analysis

Analysis of the gut microbiota community was subsequently processed in R software version 4.0.3 (R Core Team 2020). The phyloseq package (version 1.32.0) was used to generate a phyloseq object through a combination of an OTU table (.biom), sample data, and phylogenetic tree of OTUs (.tre) (McMurdie and Holmes 2013). An ambiguous kingdom annotation was removed. OTUs classified as "Cyanobacteria" (presented in only 1 sample out of 12 samples) and "Mitochondria" were also discarded from the indomethacin-induced CD model. "Cyanobacteria" was detected in all samples of the acetic acid–induced UC model except the D0C group, while "Mitochrondria" was absent in this experimental model. The acetic acid–induced UC and indomethacin-induced CD models contained a set of 313 and 237 bacterial OTUs, respectively. Reads from both models were rarefied to the minimum depth of 68,082 and 111,052 reads, respectively. Alpha diversity was calculated using five indices (ACE, Chao1, observed species, Shannon, and Simpson) and visualized with ggplot2 (Wickham 2009). Beta diversity was analyzed based on weighted UniFrac and unweighted UniFrac distance matrices calculated using the ordinate function in the phyloseq package (version 1.32.0) (McMurdie and Holmes 2013). Principal coordinates analysis (PCoA) plots were generated and visualized using the plot_ordination function in the phyloseq package (version 1.32.0) (McMurdie and Holmes 2013). Differences in community structure and composition among groups were determined using performed permutational multivariate analysis of variance (PERMANOVA, with 1000 permutations) based on weighted UniFrac and unweighted UniFrac distances using the Adonis function in the vegan package (version 2.5–6) (Oksanen et al. 2016). Homogeneity of dispersion among groups (Anderson 2006; Anderson et al. 2006) was tested using the betadisper and a permutation function (1000 permutations) (the vegan R package version 2.5–6) (Oksanen et al. 2016). Bar plots of the relative abundance of bacterial taxa were constructed using ggplot2 (Wickham 2009). Differentially abundant genera that characterized each group were identified using linear discriminant analysis (LDA) effect size (LEfSe) analysis (https://huttenhower.sph.harvard.edu/lefse/) (Segata et al. 2011).

### Statistical analysis

Statistical analyses were performed using R software version 4.3 (R Core Team 2020). Data assumptions for normal distribution and homogeneity of variance were assessed by the Shapiro–Wilk test (the stat R package) and Levene's test (the car R package, version 3.0–10) (Fox and Weisberg 2019), respectively. Comparisons of means of alpha diversity indices, distance matrices (weighted UniFrac and unweighted UniFrac) on both axes (PCoA1 and PCoA2), and bacterial relative abundances between groups were assessed by Tukey's HSD and Dunn's tests following significant one-way ANOVA (normally distributed data) and Kruskal–Wallis (non-normally distributed data) tests, respectively. Multiple comparisons were adjusted using the Benjamini–Hochberg method (adjusted $p$-value $< 0.05$, hereafter referred to as $q$-value).

## Results

### Sequencing results of fecal samples from experimental rat models of acetic acid–induced UC and indomethacin-induced CD

We sequenced fecal samples of the rats from both experimental models of acetic acid–induced UC and indomethacin-induced CD using an Illumina MiSeq high-throughput sequencing platform targeting V3–V4 regions of bacterial 16S rRNA genes. For the model of acetic acid–induced UC, a total of 1,627,993 clean reads were obtained with an average of 135,666 reads per sample and an average sequence length of 449 bps (Supplementary File 1). The gut microbiota of all samples in D0C, D7C, D7AA, and D7AALP groups was classified into 313 OTUs, 92 genera, 37 families, 19 orders, 14 classes, and 9 phyla. For the model of indomethacin-induced CD, a total of 1,826,407 clean reads were obtained with an average of 152,200 reads per sample and an average sequence length of 442 bps (Supplementary File 2). These filtered reads from all samples in D0DW, D11DW, D11ID, and D11IDLP groups were clustered into 237 OTUs, 68 genera, 34 families, 21 orders, 15 classes, and 9 phyla.

### Diversity of gut microbiota in experimental rat models of acetic acid–induced UC and indomethacin-induced CD

#### Significant differences in the gut microbial diversity of the acetic acid–induced UC model between day 0 control (D0C) and treatment groups (D7C, D7AA, and D7AALP)

The diversity of rat microbiota was estimated at a minimum depth of 68,082 reads (Fig. S2a; Table S1). Microbial

diversity estimators for twelve samples reached the plateau phase, confirming that most microbial OTUs had been detected in all samples. The total number of OTUs presented in the D0C, D7C, D7AA, and D7AALP groups was 153, 293, 299, and 303, respectively (Fig. S2b), wherein 47% of the observed OTUs (147 OTUs) were shared between D7AA and D7AALP (Supplementary File 3). Alpha diversity measured by ACE, Chao1, observed OTUs, Shannon, and Simpson indices revealed a significant difference in gut microbial diversity between control (D0C) and rats pretreated with LP extract for 1 week prior to colitis induction with acetic acid (D7AALP). D7AALP showed a significant increase in all indices compared to D0C ($q < 0.05$) (Fig. S3a), while the alpha diversity of D7AA and D7C was not significantly different from that of D0C. However, the trend in species richness was likely to increase among treatment groups, in which the gut community of D7AALP exhibited the highest species richness, as indicated by Shannon and Simpson indices. It seems that the intervention of LP rice extract for 7 days might be able to modulate the diversity of gut microbiota in rats, although no significant difference was observed among these three groups (D7C, D7AA, and D7AALP).

Beta diversity analysis revealed clear differences in gut microbial community composition and dispersion between control and treatment groups. PERMANOVA indicated significant differences in community structure and composition among four groups based on unweighted ($p < 0.001$) and weighted UniFrac distance matrices ($p < 0.001$) (Fig. S3b). However, non-homogeneous dispersion among groups was detected ($\text{Permutest}_{\text{unweighted UniFrac}}$, $p < 0.001$) when comparing D0C to treatment groups ($p = 0.02$ (vs D7C), $p < 0.01$ (vs D7AA), $p < 0.01$ (vs D7AALP)) and D7C to D7AALP ($p = 0.01$), indicating high variability in the community structure within the D0C group. For the weighted UniFrac with homogeneous dispersion ($\text{Permutest}_{\text{weighted UniFrac}}$, $p = 0.67$), an even stronger separation was observed where each group represented its unique cluster (PCoA1 = 70.8% and PCoA2 = 23.7%). Altogether, the analyses suggested that both AA and AALP treatments affected the composition of gut microbial communities, while the microbial community structures of D7AA and D7AALP were more similar to that of D7C than that of D0C.

### Significant differences in the gut microbial diversity of the indomethacin-induced CD model between day 0 control (D0DW) and treatment groups (D11DW, D11ID, and D11IDLP)

Estimation of gut microbiota diversity in the rat model of indomethacin-induced CD at a minimum depth of 111,052 reads (Fig. S4a, Table S2) showed that 151 (64%) OTUs were shared among four groups, whereas no common OTUs were found between D0DW and D11DW, between D11DW and D11IDLP, and among D0DW, D11DW, and D11IDLP groups (Fig. S4b, Supplementary File 4). Comparisons of five alpha diversity indices revealed that the diversity of gut microbiota was significantly higher in rats with indomethacin-induced CD (D11ID) than that in day 11 control (D11DW), after multiple testing corrections ($q < 0.001$ (Shannon), $q < 0.01$ (observed OTUs and Simpson), $q < 0.05$ (ACE and Chao1); Fig. S5a). Microbial community richness (Shannon) and evenness (Simpson) also significantly increased in D11ID compared to day 0 control (D0DW) ($q < 0.01$ (Shannon), $q < 0.05$ (Simpson)) and in D11IDLP compared to D11DW ($q < 0.01$). No significant differences in alpha diversity were detected between rats with indomethacin-induced CD pretreated with (D11IDLP) and without (D11ID) LP rice extract.

Beta diversity analysis showed a distinct separation of the gut microbiota community across four groups based on unweighted and weighted (Fig. S5b) UniFrac distances. Bacterial community structure and composition across D0DW, D11DW, D11ID, and D11IDLP groups were significantly different ($\text{PERMANOVA}_{\text{unweighted UniFrac}}$, $p < 0.001$; $\text{PERMANOVA}_{\text{weighted UniFrac}}$, $p < 0.001$). When considering a dispersion effect, all groups that clustered apart had no significant difference in dispersion ($\text{Permutest}_{\text{unweighted UniFrac}}$, $p = 0.09$; $\text{Permutest}_{\text{weighted UniFrac}}$, $p = 0.64$). In summary, two different beta diversity metrics clearly illustrated that both indomethacin-induced CD and pretreatment with LP rice extract had significant associations with rat gut microbiota variation in terms of structure and composition.

## Significant differences in abundances of gut microbiota across groups of experimental rat models of acetic acid–induced UC and indomethacin-induced CD

### Gut microbiota associated with the rat model of acetic acid–induced UC (D7AA) and acetic acid–induced UC pretreated with LP rice extract (D7AALP)

Gut microbiota composition varied greatly across groups. At the phylum level (Fig. 1a), Firmicutes, Patescibacteria, and Bacteroidetes comprised the most abundant phyla across treatment groups (accounting for ~95% of total abundance), whereas Firmicutes and Patescibacteria were the top 99% of phyla in day 0 control (D0C). The abundance of Patescibacteria was significantly higher in D0C compared to D7C ($q < 0.05$; Fig. 1b). Firmicutes increased upon the rat models of AA and AALP treatments, which was over-represented in D7AALP (Fig. 1c; Table S3). The gut community of D7C exhibited a higher abundance of Bacteroidetes than that of D0C ($q < 0.05$; Fig. 1d); however, no significant differences were found when compared to D7AA and D7AALP.

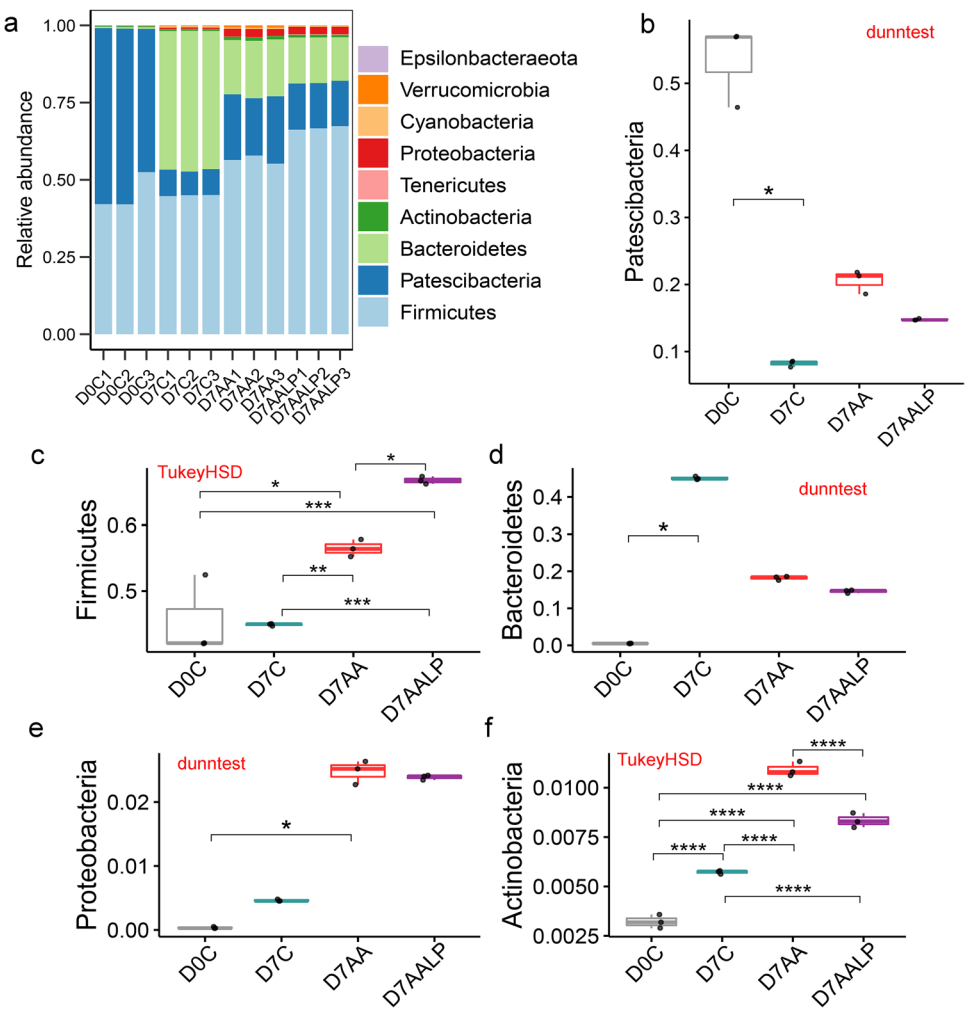


**Fig. 1** Significant differences in the relative abundance of gut microbiota among treatment groups. **a** Bar plot of the relative abundance of top ten phyla. **b–f** Boxplots of the relative abundance of gut microbiota at the phylum level. The paired comparisons were determined using Dunn's test with the Benjamini-Hochberg (BH) *p*-value correction method following a significant Kruskal–Wallis test and Tukey's HSD test following a significant ANOVA test. Asterisks indicate a significant difference between groups (****$q < 0.0001$, ***$q < 0.001$, **$q < 0.01$, *$q < 0.05$). D0C, control group on day 0; D7C, control group on day 7; D7AA, acute AA-induced UC; D7AALP, acute AA-induced UC pretreated with LP rice extract

Proteobacteria was significantly more abundant in D7AA than in D0C ($q < 0.05$; Fig. 1e). Furthermore, Actinobacteria that was enriched in D7AA was found to decrease in D7AALP ($q < 0.0001$; Fig. 1f). Its abundance was also lower in D0C and D7C ($q < 0.0001$).

At the family level (Fig. S6), significant differences in the relative abundance of rat gut microbiota across groups were detected. *Peptostreptococcaceae* ($q < 0.05$) and *Burkholderiaceae* ($q < 0.05$) were found to be significantly increased in D7AA compared to D0C. *Muribaculaceae* also increased in D7AA. *Lachnospiraceae* was significantly higher in D7AALP compared to D7C ($q < 0.05$). The abundance of *Ruminococcaceae* was significantly higher in both D7AA and D7AALP than in D7C ($q < 0.05$). Out of the 10 families, two families, namely *Bacteroidaceae* and *Prevotellaceae*, were more abundant in D7C compared to D0C ($q < 0.05$). The D7C group also harbored the highest level of *Lactobacillaceae* and *Muribaculaceae* followed by D7AA and D7AALP ($q < 0.0001$). The abundance of *Saccharimonadaceae* was not significantly different among treatment groups; however, this gut bacterial family was enriched in D0C.

At the genus level (Fig. S7), comparisons of top 30 genera also demonstrated different abundances among four groups (Table S4). AA treatment with and without LP extract decreased the abundances of *Lactobacillus* compared with that in the control group on day 7 (D7C) ($q < 0.0001$), whereas *Ruminococcaceae* UCG.014, *Romboutsia*, *Roseburia*, *Ruminococcaceae* UCG.005, *Turicibacter*, *Oligella*, *Staphylococcus*, and *Akkermansia* increased in the gut of AA-treated rats. The LEfSe analysis (LDA score > 2, $p < 0.05$) further indicated distinct enrichment of these bacterial genera in the gut of the control and treated rats (Fig. 2). While *Lactobacillus* was highly associated with D7C (Fig. S8b), *Ruminococcaceae* UCG.014 and *Romboutsia* dominated the gut of AA-treated rats (Fig. S9). Moreover, the *Lachnospiraceae* NK4A136 group that was higher in D7AALP than in D7C ($q = 0.01$) was markedly increased by AALP treatment (Fig. S10b). It is remarkable that pretreating rats with LP extract prior to colitis induction with acetic acid significantly enhanced the accumulation of this

butyrate producer; its abundance was nearly 70 times and 21 times higher than that of D7C and D7AA, respectively. Other genera that characterized D0C included *Candidatus Saccharimonas* and *Ruminococcaceae* UCG.013 (Figs. S8a and S10a). In summary, these results indicated that transrectal administration of acetic acid in rats affected the gut microbiota composition in AA-induced UC models with and without LP extract. However, it is interesting to note that oral administration of LP rice extract for 1 week in the D7AALP group considerably promoted the abundance of butyrate-producing bacteria.

### Gut microbiota associated with the rat model of indomethacin-induced CD (D11ID) and indomethacin-induced CD pretreated with LP rice extract (D11IDLP)

Firmicutes and Patescibacteria were the most abundant phyla in all samples of D0DW, D11DW, D11ID, and D11IDLP groups, which represented about 94%, 99%, 85%, and 90% of the community in each group (respectively) (Fig. 3a). Although these phyla were shared across groups, their abundances significantly varied (Table S5). The abundance of Firmicutes was significantly decreased among the indomethacin-induced CD groups with and without pretreatment with LP rice extract (D11ID and D11IDLP) and day 11 control compared to the day 0 control (D0DW) ($q < 0.01$; Fig. 3b). The level of Patescibacteria decreased in D11ID ($q < 0.01$) and D11IDLP ($q = 0.02$) groups compared to D11DW (Fig. 3c). The abundance of this bacterial phylum was also lower in D0DW compared to that in D11DW ($q < 0.001$) and D11IDLP ($q < 0.05$). Bacteroidetes dominated the gut of indomethacin-treated rats (D11ID), while the gut of D11DW harbored the lowest proportion of this phylum (less than 1%) (Fig. 3d). Moreover, the D11IDLP group had the highest level of Proteobacteria compared to the rest of the groups (Fig. 3e). Interestingly, Fusobacteria was abundant in D11ID and D11IDLP, but it was completely absent in both day 0

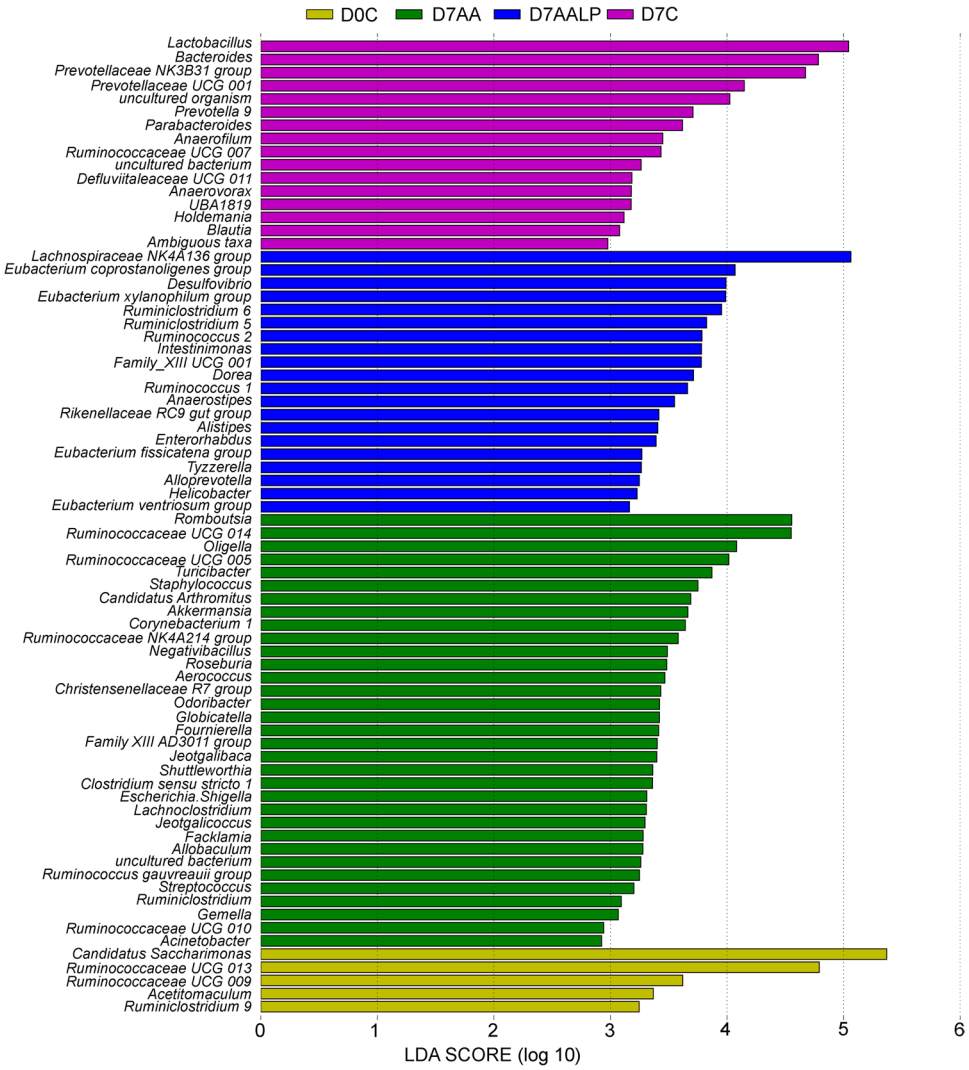


**Fig. 2** Histogram of LDA scores (log10) showing discriminatory features (gut microbiota at the genus level) across treatment groups. The features (colored LEfSe bars) with LDA scores > 2 ($p < 0.05$, pairwise Wilcoxon tests following a significant Kruskal–Wallis test) were considered to be significantly different. D0C (yellow), control group on day 0; D7C (purple), control group on day 7; D7AA (green), acute AA-induced UC; D7AALP (blue), acute AA-induced UC pretreated with LP rice extract

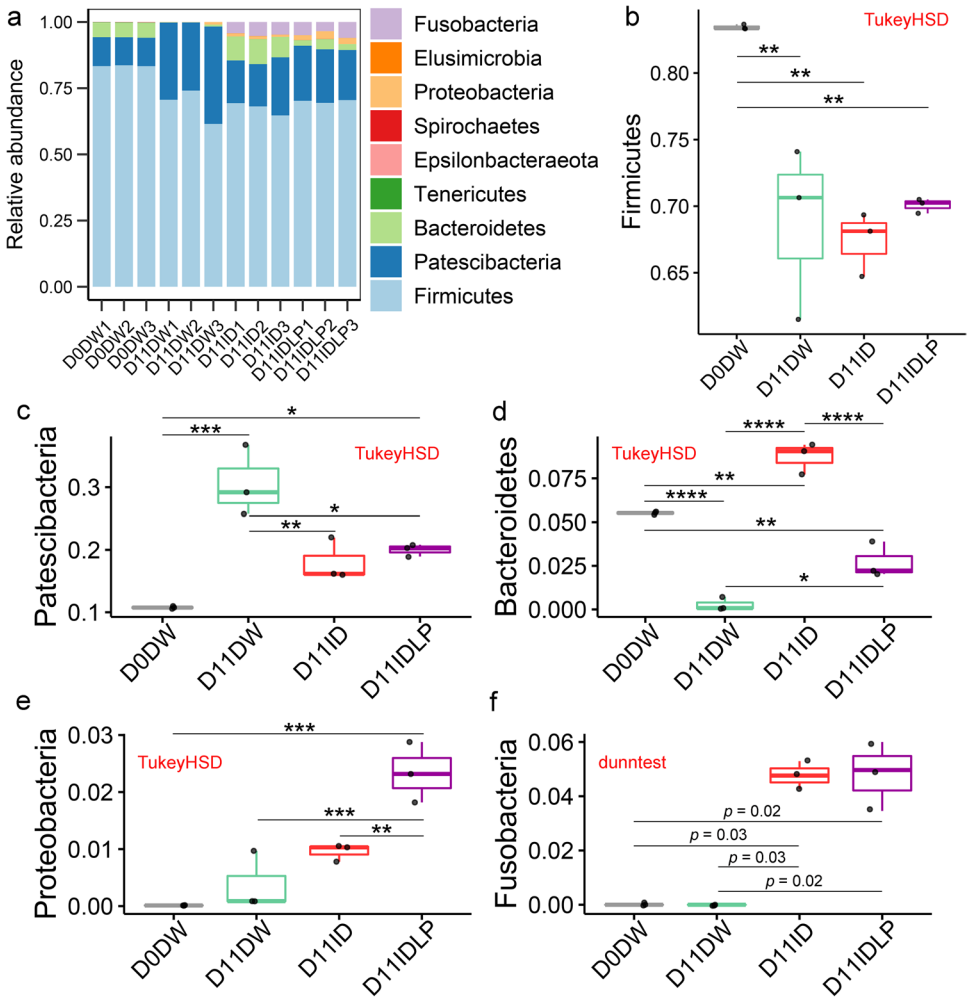


**Fig. 3** Significant differences in the relative abundance of gut microbiota among treatment groups. **a** Bar plot of the relative abundance of top ten phyla. **b–f** Boxplots of the relative abundance of gut microbiota at the phylum level. The paired comparisons were determined using Dunn's test with the Benjamini-Hochberg (BH) *p*-value correction method following a significant Kruskal–Wallis test and Tukey's HSD test following a significant ANOVA test. The *p*-values indicate significant differences in the mean relative abundances of Fusobacteria between groups without multiple comparison adjustments. Asterisks indicate a significant difference between groups (****$q < 0.0001$, ***$q < 0.001$, **$q < 0.01$, *$q < 0.05$). D0DW, control group on day 0; D11DW, control group on day 11; D11ID, chronic ID-induced CD; D11IDLP, chronic ID-induced CD pretreated with LP rice extract

and day 11 control groups (D0DW and D11DW) (Fig. 3f). Pairwise comparisons showed significant differences in the mean relative abundances of these phyla between groups; however, no significant differences were detected after multiple comparison adjustments.

At the family level, the composition of gut microbiota was differentially associated with different groups (Fig. S11). *Ruminococcaceae* were more abundant in D11IDLP than in D11ID ($q < 0.05$) and in D11DW groups ($q < 0.01$). This bacterial taxon was over-represented in D0DW. *Sacchari-monadaceae* was significantly decreased in D11ID ($q < 0.01$), D11IDLP ($q < 0.05$), and D0DW ($q < 0.001$) as compared to D11DW. Significant differences in the abundance were observed for two families from the phylum Bacteroidetes, namely *Prevotellaceae* and *Bacteroidaceae*. The former was higher in D11ID than in D11DW ($q < 0.05$) and the latter was also more represented in D11ID than the rest of the groups. While *Lactobacillaceae* increased ($q < 0.05$), *Rikenellaceae* decreased in D11DW ($q < 0.05$) compared to D0DW. The proportions of the other two families were found to be different between indomethacin-treated and control groups: *Acidaminococcaceae* belonging to the phylum Proteobacteria was significantly increased in D11ID compared to D11DW ($q < 0.05$), while *Moraxellaceae* from the phylum Firmicutes was more abundant in D11IDLP than in D0DW ($q < 0.05$).

At the genus level, an alteration in the abundance of gut microbiota was observed between indomethacin-treated and control groups (Fig. S12). Out of 30 most abundant bacterial genera, 19 taxa were found to be significantly different between groups (Table S6). The abundance of *Ruminococcaceae* UCG.014 was significantly decreased in D11ID ($q < 0.01$), D11IDLP ($q < 0.05$), and D11DW ($q < 0.001$). We observed higher abundances of three genera from the phylum Firmicutes, namely *Lachnospiraceae* NK4A136 group ($q < 0.05$), *Coprococcus* 2 ($q < 0.05$), and *Turicibacter* ($q < 0.05$), in D11ID than those in D0DW. A significant increase in the abundances of *Bacteroides* and *Phascolarctobacterium* (belonging to the phylum Firmicutes) was found in D11ID as compared to D11DW ($q = 0.01$). Moreover, the LEfSe analysis (LDA score > 2, $p < 0.05$) indicated SCFA-producing bacteria differentially associated with indomethacin-treated rats (D11ID), which included *Lachnospiraceae*

NK4A136 group, *Coprococcus* 2, and *Phascolarctobacterium* (Fig. 4; see also Fig. S13b). A high abundance of *Bacteroides* also characterized the D11ID group (Fig. S14a). Treating rats (for 11 days) with LP rice extract before and during induction with indomethacin was highly associated with an increase in the abundances of two bacterial genera, namely *Acinetobacter* and *Escherichia/Shigella*, from the phylum Proteobacteria (Figs. S14b, S14c). For the control groups, *Candidatus Saccharimonas* and *Lactobacillus* were enriched in D11DW (Figs. S15a, S15b), while *Ruminococcaceae* UCG.014 was predominant in D0DW (Fig. S15c). Although no significant differences between groups were observed for *Fusobacterium* and *Veillonella* after multiple comparison adjustments, both bacterial genera were completely absent in the control groups (D0DW and D11DW) (Fig. S16). Altogether, these results demonstrated that the inflammatory condition (CD induction with indomethacin) led to significant changes in rat gut microbiota composition, affecting the balance of beneficial bacteria. Moreover, treating rats (for 11 days) with LP rice extract before and during induction with indomethacin raised the levels of the members of Proteobacteria.

## Discussion

In this study, we demonstrated the effects of acetic acid– and indomethacin-induced inflammatory bowel diseases on rat gut microbiome as well as highlighted a protective effect of LP rice extract on gut microbiota dysbiosis. Our results indicated significant alterations in gut microbiota communities in both AA-induced UC and ID-induced CD models. LP rice extract treatment also promoted the abundance of butyrate-producing bacteria in AALP-treated rats and Proteobacteria in IDLP-treated rats. The latter bacterial phylum is associated with IBD in humans (Guo et al. 2022). Our recent work has shown that pretreatment with LP rice extract in AA- and ID-induced IBD models ameliorated disease severity as characterized by a decrease in disease activity index (DAI) (by the scores of body weight loss, stool consistency, stool blood), macroscopic inflammation, spleen enlargement, and colon length reduction (Thipart et al. 2020). However, an increased abundance of Proteobacteria after treatment (D11IDLP) suggests that the concentration of LP extract at 5 g/kg might not be able to antagonize the effect of indomethacin in this study. Furthermore, as previously established, the pathophysiology of indomethacin-induced CD is more severe than that of acetic acid–induced UC in terms of its involvement in fistula formation throughout the rat's gastrointestinal (GI) tract (Thipart et al. 2020). In this regard, additional research should be conducted to modify the concentration of LP extract and extend the experimental duration in order to determine the effect of LP rice extract on Proteobacteria.

IBD has been frequently shown to be associated with a decrease in the diversity of fecal microbiota (Zuo and Ng 2018; Khan et al. 2019). Recent studies in the colitis

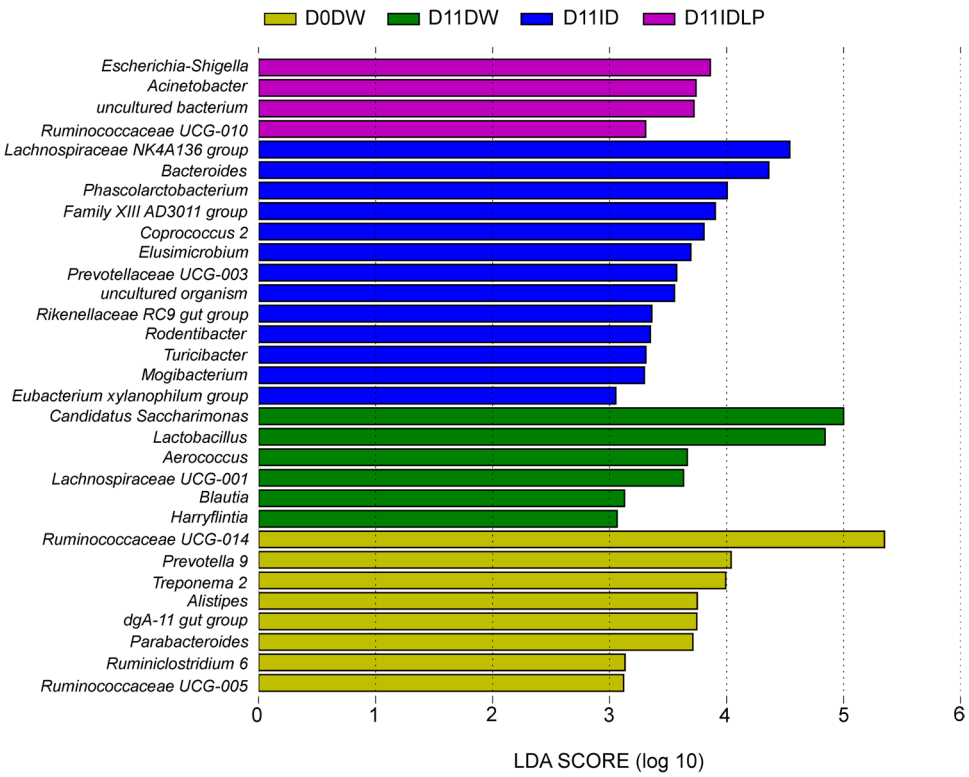


**Fig. 4** Histogram of LDA scores (log10) showing discriminatory features (gut microbiota at the genus level) across treatment groups. The features (colored LEfSe bars) with LDA scores > 2 ($p < 0.05$, pairwise Wilcoxon tests following a significant Kruskal–Wallis test) were considered to be significantly different. D0DW (yellow), control group on day 0; D11DW (green), control group on day 11; D11ID (blue), chronic ID-induced CD; D11IDLP (purple), chronic ID-induced CD pretreated with LP rice extract

mouse model also showed a decrease in richness diversity (Shang et al. 2021; Huangfu et al. 2021), whereas we found an increasing trend in our AA-induced UC rats (D7AA). We suspected that the variability in results might be caused by differences in experimental design (e.g., administration methods for colitis induction, treatment period). Future investigations are needed to resolve the nature of these differences and validate study outcomes. Furthermore, in the rat model of AA-induced UC, we found no significant differences in alpha diversity among the control (D7C), AA-treated, and AALP-treated groups. However, an increasing trend in species richness (Shannon) and evenness (Simpson) was noted in the D7AALP group. A similar pattern has been observed in previous studies, showing an increased diversity of gut microbiota in both AA- (Lee et al. 2018) and DSS-induced (Han et al. 2019) colitis models supplemented with natural compounds with antioxidant abilities. LP rice extract used in our study is also rich in anthocyanins, especially cyanidin-3-glucoside (C3G) (accounts for up to 86% of the total active polyphenolic compounds) which has antioxidant powers (Thipart et al. 2020). Taken together, these observations raise the question of whether natural products including the LP rice extract used in our study could counteract AA-induced UC and enhance microbial diversity in the rat model.

In contrast with the AA-induced UC model, ID-induced CD significantly increased diversity in rats (D11I D) and it was more prominent than in the ID-induced CD group pretreated with LP rice extract (D11IDLP). However, considering fluctuations of diversity among control (D0DW and D11DW), ID-treated, and IDLP-treated groups, a further experiment that includes control rats treated with LP rice extract is required to validate whether changes in diversity patterns are due to induction of CD by indomethacin with and without pretreatment with LP rice extract, or individual variations.

Compositional shift in the rat gut microbiota was associated with IBD induction in which the effect of treatments was stronger in AA-induced colitis than in ID-induced CD. Acute induction with 4% AA significantly increased the *Ruminococcaceae* UCG.014, *Ruminococcaceae* UCG.005, and *Ruminococcaceae* NK4A214 group, whereas *Lactobacillus* decreased in both AA and AALP treatments. Other studies also indicated *Ruminococcaceae* UCG.014 enriched and a reduction of beneficial bacteria including *Lactobacillus* in UC model (Huang et al. 2019; Huangfu et al. 2021). Interestingly, pretreatment with LP rice extract was able to reduce abundances of those members of the *Ruminococcaceae* family and increase the level of the *Lachnospiraceae* NK4A136 group in the AALP group. The latter genus is one of the main butyrate producers, whose essential role and metabolic products have been proposed to strengthen intestinal integrity (Hu et al. 2019; Venegas et al. 2019). In this case, the favorable effect of LP rice extract on the increase of the *Lachnospiraceae* NK4A136 group in AA-induced rats might imply a positive influence on promoting the growth of beneficial bacteria in colonic inflammation.

Although the family *Lachnospiraceae* is likely to be disrupted in both animal models and humans experiencing IBD (Khan et al. 2019; Xu et al. 2021), several SCFA-producing bacteria including the *Lachnospiraceae* NK4A136 group, *Coprococcus* 2, and *Phascolarctobacterium* were associated with ID-induced CD in this present work. The abundance of *Phascolarctobacterium*, which was reduced in IBD patients (Morgan et al. 2012), was even stimulated by the ID treatment. Moreover, the abundances of the two butyrate producers (*Lachnospiraceae* NK4A136 group and *Coprococcus* 2) in ID-treated rats were not significantly different from those in the day 11 control (D11DW) and the D11IDLP groups. Such observed results demonstrated that these bacteria involved in the SCFA production were not affected by chronic induction, suggesting the expansion of the SCFA-producing bacterial group for their survival under the inflammatory condition in the ID model.

In addition, *Bacteroides* also characterized the D11ID group, as indicated by LEfSe. This is consistent with the previous finding that rats with indomethacin-induced inflammation showed an increase in the number of *Bacteroides* spp. (Terán-Ventura et al. 2014). Other genera that were found to be increased after ID treatment included *Fusobacterium* and *Veillonella*. The absence of these bacteria in the control groups (D0DW and D11DW) emphasizes their associations with intestinal disorders, particularly IBD (Zou et al. 2018). Furthermore, ID induction in rats significantly promoted the accumulation of the genera *Escherichia/Shigella* with respect to controls. Their positive associations with IBD are also obvious (Morgan et al. 2012). Disruption of the epithelial barrier integrity in this CD-like model might exert proinflammatory properties of the genus (Pawłowska and Prof. Sobieszczańska B 2017). Thus, our findings support the concept of the inflamed gut which is conducive to blooms of these pathogenic bacteria (Morgan et al. 2012; Zuo and Ng 2018).

Our study has elucidated that both AA-induced UC and ID-induced CD were associated with alterations in gut microbiota communities in rat models. The effect of chemical induction was even stronger in rats with acute colitis than in the chronic model in which the abundances of beneficial bacteria, especially the *Lachnospiraceae* NK4A136 group and *Lactobacillus*, were strongly disrupted. Some opportunistic pathogens (*Bacteroides*, *Escherichia/Shigella*, *Fusobacterium*, and *Veillonella*), however, were raised by ID-induced CD. These differences in outcomes between two chemical-induced colitis models could be attributed to induction and dosing strategies. It is important to note that pretreatment with LP rice extract in the acute UC model exhibited an anti-inflammatory property by elevating the proportion of the butyrate-producing bacteria. Its protective effect is worth further investigation. Moreover, sustained abundances of SCFA-producing bacteria in the chronic ID-induced CD might reflect the degree of

change in gut microbiota composition during disease progression that may take a longer period than our modeling duration (11 days). As regards the limitations of this study (small sample size and a lack of correlations between gut microbiota and inflammatory records (e.g., disease activity index, colon length) together with measurement of SCFA), it remains to be concluded whether shifts in composition among these bacteria are associated with inflammatory indicators omitted in these experimental models. Such key points should be further validated to support the results of the present study.

## Conclusions

Our study of the effects of acetic acid–induced UC, indomethacin-induced CD, and pretreatment with LP rice extract (in both models) on rat gut microbiota community revealed that chemical-induced IBD significantly altered the composition of gut microbiota in experimental rat models. This dysbiosis state was associated with increased abundances of some *Ruminococcaceae* taxa (i.e., *Ruminococcaceae* UCG.014, *Ruminococcaceae* UCG.005, and *Ruminococcaceae* NK4A214 group) in the acute colitis model and potential pathogens (*Bacteroides*, *Fusobacterium*, *Veillonella*, and *Escherichia/Shigella*) in the chronic CD model. Pretreatment with LP rice extract induced the butyrate-producing bacteria especially the *Lachnospiraceae* NK4A136 group in the UC-induced model, while the abundance of these beneficial bacteria was unaffected by the indomethacin treatment or the presence of a natural compound. Altogether, these observations suggested different effects of AA-induced UC and ID-induced CD on rat gut microbiota. LP rice extract might have a preventive implication in UC. Its anti-inflammatory effect against inflammatory conditions warrants further investigation.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10123-022-00309-x.

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**Author contribution** Kornsuda Thipart, Kutcharin Phunikhom, Jintana Sattayasai, and Siam Popluechai conceived and designed the study. Kornsuda Thipart performed the experiments. Lucsame Gruneck analyzed the data and prepared the figures. Kornsuda Thipart, Lucsame Gruneck, and Siam Popluechai wrote the original draft. Kutcharin Phunikhom, Thomas J. Sharpton, Jintana Sattayasai, Lucsame Gruneck, and Siam Popluechai reviewed and edited the manuscript. All authors read and approved the final manuscript.

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**Data availability** The raw sequence data generated during the current study are available in the NCBI Sequence Read Archive (SRA) repository under the BioProject accession number PRJNA785748 (BioSample accession numbers SAMN23575674–SAMN23575697) (https://www.ncbi.nlm.nih. gov/biopro ject/PRJNA785748).

## Declarations

**Ethics approval** All procedures were complied with the standards for the care and use of experimental animals and approved by the Animal Ethics Committee of Khon Kaen University according to the Ethics of Animal Experimentation of National Research Council of Thailand (Ethics Registry: ACUC-KKU-21/2560). Animal use was constrained to abide with the Buddhist moral of the country.

**Conflict of interest** The authors declare no competing interests.

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