Lacidophilin Modulated Gut Microbiota and Ameliorated Dextran Sulfate Sodium-Induced Mouse Colitis

Yu Jingting  
State Key Laboratory for the Modernization of Classical and Famous Prescriptions of Chinese Medicine

Cheng Xiaoying  
State Key Laboratory for the Modernization of Classical and Famous Prescriptions of Chinese Medicine

Zhan Yang  
State Key Laboratory for the Modernization of Classical and Famous Prescriptions of Chinese Medicine

Zhang Jingwen  
State Key Laboratory for the Modernization of Classical and Famous Prescriptions of Chinese Medicine

Li Yingmeng  
State Key Laboratory for the Modernization of Classical and Famous Prescriptions of Chinese Medicine

Sun Denglong  
State Key Laboratory for the Modernization of Classical and Famous Prescriptions of Chinese Medicine

Zheng Longjin  
State Key Laboratory for the Modernization of Classical and Famous Prescriptions of Chinese Medicine

Liu Wenjun  

Article

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Abstract

The prevalence of inflammatory bowel disease (IBD) has been rising significantly in recent years. It is widely accepted that gut microbes play an essential role in the development of IBD. Lacidophilin is a product of milk fermentation by lactobacillus acidophilus. The aim of this study was to investigate the effect of Lacidophilin on colitis induced by dextran sulfate sodium (DSS). 16s RNA sequencing was performed to determine the changes of species composition and community structure of the intestinal microflora, and transcriptome sequencing was conducted to find out the gene or protein which may be affected by Lactobacillus on colitis development potentially. It was observed that the 7 days administration of Lacidophilin protected the intestinal mucosal barrier from damage, and thereby enabled the remission of colitis severity. Compared to the model group, Lacidophilin could restore the shortened colon length and marked decrease levels of TNF-α and IL-6 in serum. More importantly, Lacidophilin significantly increased the abundance of beneficial bacteria such as *Lactobacillus*, *Bifidobacterium* and *Lachnospiraceae_NK4A136_group*, decreased the abundance of harmful bacteria such as *Escherichia-Shigella* and Parvibacter. Transcriptomic analysis shows that IL-17 signaling pathway, BCR signaling pathway, Toll-like receptor signaling pathway, and TNF signaling pathway was enriched, and we found that Lcn2, Ccl3, Mmp8, Slc11a1, Spp1, and Serpine1 might be potential targets of Lacidophilin treatment. These studies indicate that Lacidophilin can ameliorate colitis in mice through maintaining the integrity of intestinal structure and improving intestinal microbiota, and its mechanism may be involved in immune-related proteins and pathways.

Introduction

Inflammatory bowel disease (IBD) is a chronic, recurrent, long-term inflammatory disease that eventually leads to colon and rectal ulcers, including Crohn's disease (CD) and Ulcerative colitis (UC)[1]. The difference between UC and CD lies in the location of inflammation. Ulcerative colitis is a persistent inflammation of the mucosa that begins in the rectum and extends proximally[2], but in Crohn's disease, inflammation is widespread throughout the gastrointestinal tract[3]. The pathogenesis of UC involves genetic susceptibility components and environmental factors, but its specific pathogenesis remains unclear.

Recent reports have documented the number of individuals who suffer from IBD has increased from 3.7 million to more than 6.8 million during the past three decades, suggesting the prevalence of IBD has risen sharply worldwide[4]. Up to now, first-line medications for IBD include anti-inflammatory drugs, corticosteroids and immunosuppressants, whereas these medications are effective in the early stages of the disease with coming at the cost of severe side effects[5]. Searching for IBD effective treatments with minimal side effects, therefore, has generated considerable recent research interest.

The intestinal microbiome is a complex community of microorganisms that influence human nutrient metabolism, physical development, immune defense and oncogenesis[6]. Numerous experiments have so far revealed the associations between microflora with intestinal health and disorders, especially IBD.
Research have shown a significant decrease of microbial diversity as well as an imbalance of microbial composition observed in IBD patients, often referred to as dysbiosis, mainly characterized by a reduction of beneficial bacteria and an increase of harmful bacteria\cite{7,8}. Therefore, much research in recent years has focused on maintaining gut homeostasis to improve IBD. For example, it was shown that the role in improving UC of *Panax quinquefolius* polysaccharides was conducted by regulating the structure of gut microbiota\cite{9}. In contrast, when intestinal mucosal was disrupted, the colonic homeostasis would be perturbed by *E. coli* capable of producing colistin\cite{10}. Apart from intestinal microflora itself, its metabolites, short-chain fatty-acid (SCFA), also play a key role in maintaining the balance of gut homeostasis, including downregulate the levels of proinflammatory cytokines\cite{11}, regulate intestinal barrier\cite{12} as well as control gut homeostasis\cite{13}. Also, the intestinal microbiota is involved in regulating the integrity and function of the intestinal barrier for maintaining homeostatic balance.

Probiotic activity produced by lactic acid bacteria (LAB) has been expected to improve the intestinal environment, which can prevent some diseases such as cancers\cite{14} and intestinal inflammation\cite{15}. *Lactobacillus acidophilus* is an important member of the lactic acid bacteria family, and there is extensive literature on its amelioration of ulcerative colitis\cite{16-19}. However, it takes a significant risk of bacteremia or other probiotic infections in direct ingesting live bacteria\cite{20}, especially for ulcerative colitis patients whose intestinal barrier was impaired\cite{21,22}. Lacidophilin (LP) is derived from the fermentation of skim milk by lactobacillus acidophilus. Its composition is relatively complex, containing lactic acid, antimicrobial substances, amino acids several trace elements. Different from probiotics which are live microorganisms, LP is a collection of metabolites secreted by live bacteria which is non-viable. Therefore, the safety of LP is more predictable than live *Lactobacillus acidophilus*.

As early as 1930, Okayasu began introducing the model of DSS (dextran sulfate sodium) to induce colitis in mice, since its morphological damage and symptoms of intestinal epithelium were similar to those of UC in humans\cite{23}. In this paper, we applied the DSS-induced acute ulcer model in mice to investigate the effect of LP on UC, and performed 16S rRNA and transcriptome sequencing to find out its possible action mechanism.

### Materials and Methods

#### 2.1 Materials

Lacidophilin was purchased from Jiangzhong Pharmaceutical Co., Ltd. (Jiangxi, China); Dextran sulfate sodium (DSS) was purchased from MP Biomedicals (California, USA).

#### 2.2 Mice

Male C57 mice (6–8 weeks) were provided by Gempharmatech Co., Ltd (Jiangsu, China). All mice (four per cage) housed under the conditions of a controlled temperature of 24 ± 2°C and relative humidity of 45–65% with 12 hours of light/dark cycle. Mice had free access to food and water throughout the
experiment. The experiment protocol was approved by the Jiangzhong Pharmaceutical Co., Ltd. Ethics Committee.

2.3 DSS-induced experimental colitis

After one week of adaptive feeding, the mice were randomly divided into four groups (n = 8): (1) Control group; (2) Model group; (3) Low dose lactobacillus group (LPL). (4) High dose lactobacillus group (LPH). The mice in the Model, LPL, and LPH group were given water containing 3% DSS (w/v) for 5 days, followed by 2 days of sterile water. Meanwhile, the mice in the Control groups only received sterile water for 7 days. The LPL group was orally administrated with 0.6g/kg Lacidophilin for 7 consecutive days, while the LPH group was given 1.2g/kg Lacidophilin, and the mice in the Control and Model groups were given the equivalent volume of saline. At day 8, all mice were euthanized using the method of cervical dislocation (Fig. 1A).

At the endpoint of experiment, blood samples were collected and then serum was stored at -80°C after centrifugation at 3000 rpm for 10 min. Mice were sacrificed by cervical dislocation under anaesthesia, then the colon tissue was removed as soon as possible. The colon tissue was unfolded without forceful pulling, a straightedge was placed underneath the colon and photographed under a stereomicroscope at the same height, light and magnification. Meanwhile, the length of the colon was recorded, and then the proximal colon was stored in 4% paraformaldehyde for histopathological examination. The cecum contents and residual colon tissue were frozen in liquid nitrogen for 16sRNA sequencing and transcriptome sequencing separately.

2.4 Histopathological, immunohistochemistry and immunofluorescence staining

The colons were fixed in 4% paraformaldehyde for paraffin preparation, or embedded in tissue OCT freezing medium (Leica, Germany) for frozen block preparation. Paraffin-embedded colons were cut by ultra-thin semiautomatic microtome (Leica, Germany) to prepared 5µm tissue sections which were used for H&E or immunohistochemical staining of occludin and claudin-4. Hematoxylin counterstain was applied to visualize nuclei. The sections were observed under a light microscope (Leica, Germany). Frozen tissue sections with a thickness of 5µm were prepared with a frozen slicer (Leica, Germany) and subsequently stained with MUC-2 antibodies. DAPI counterstain was applied to visualize nuclei. The sections were observed under a fluorescence microscope (Leica, Germany).

The expression of occludin, claudin-4, and MUC-2 was calculated using the 4 random view fields in each colon sample (n = 3). The fields were analyzed using ImageJ (The University of Nottingham, UK). The area was used to represent the protein expression, and the results of occludin and claudin-4 expression in each group were compared with the control group, while the results of MUC-2 were represented as the ratio of MUC-2 to DAPI positive area.

2.5 Enzyme-Linked Immunosorbent Assay (ELISA)
The concentrations of inflammatory factors were detected by mouse immunoassay kits according to the manufacturer’s instructions. ELISA was used to assay the levels of TNF-α and IL-6 in the serum by commercial ELISA kits (NanJing JianCheng Bioengineering Institute, China).

### 2.6 16sRNA sequencing

The contents of the cecum were taken for total microbiota DNA extraction and PCR amplification of the V3-V4 region of the 16S rRNA gene. The PCR products were detected and quantified by QuantiFluoTM-ST blue fluorescence quantitative system (Promega, USA), and then mixed in proportion according to the sequencing requirements of each sample. Then, Illumina library was constructed using TruSeqTM DNA Sample Prep Kit (Illumina, USA). Sequencing was performed using Illumina's MiseqPE300 platform (Illumina, USA).

### 2.7 Transcriptome sequencing

Total RNA was extracted from the colons using MJZol total RNA extraction kit (Majorbio, China) following the manufacturer’s instructions. All mRNA extracted from colon tissue were sequenced, and the library was constructed according to Illumina® Stranded mRNA Prep, Ligation (Illumina, USA) in the sequencing experiment. According to Illumina’s library construction protocol, the adaptor was connected to the fragments, then the products were purified, sorted and amplified to purify and enrich the library. The amplicons were quantified and then mixed in proportions, and paired-end sequencing was conducted on NovaSeq 6000 sequencer (Illumina, USA).

### 2.8 Statistics

Results were shown as mean ± standard error of the mean (SEM). Differences in expression of colonic length, inflammatory factor and Chao, ACE, Shannon, Simpson indexes were analyzed by one-way analysis of variance (ANOVA), as differences of weight, food intake and water intake were analyzed by two-way ANOVA using GraphPad Prism 8.0 software. P < 0.05 is considered as statistically significant for the results, while P ≥ 0.05 represents no significant difference (NS).

Sequencing data analysis and plotting were analyzed on the online platform of Majorbio Cloud Platform. (https://cloud.majorbio.com/).

### Results

#### 3.1 Lacidophilin relieved DSS-induced ulcerative colitis

To investigate the efficacy and action mechanism of Lacidophilin on UC, DSS-induced colitis mice were administered different dose Lacidophilin or normal saline. Figure 1A shows the experimental timeline. In the present study, the body weight (Fig. 1B) of DSS-induced colitis mice considerably reduced from day 6 to day 8 of the experiment, while this loss was alleviated by LPH group to a greater extent than model group. The food intake (Fig. 1C) and water intake (Fig. 1D) of mice had been recorded within seven days after administrated with DSS. Intake of DSS decreased food intake (P < 0.01) and water intake (NS) of
mice compared with control group, however, there was no significant difference between those group which was interfered by DSS.

After treatment with different dose Lacidophilin, effective reversal of the significant changes in colon length and the level of TNF-α and IL-6 in serum of mice with UC was achieved. Compared with control group, the model group had significantly shorter colonic length, while it was significantly higher in the LPH group than in the model group (Fig. 2A and 2B). The concentrations of TNF-α and IL-6 in the serum were measured by ELISA to assess the effects of Lacidophilin on inflammatory cytokines. We found that DSS-induced upregulations of TNF-α (Fig. 2C) and IL-6 (Fig. 2D) were significantly downregulated by Lacidophilin. H&E staining was performed to evaluate the histopathology of colon injury. Pathological analysis suggested that there were no ulcer on the surface of the mucosa and the structure of the crypt was regular and clear without obvious inflammatory infiltration in the control group, while the mucosal structure of the model group was disorganized, with abnormal crypt and a large number of lymphocyte infiltration. Compared with the model group, the inflammatory reaction of the LPL and LPH groups were reduced, and the mucosal surface was more intact, which tended to be normal (Fig. 2E).

3.2 Lacidophilin improved gut barrier integrity in ulcerative colitis mouse

Occludin and Claudin-4 are important components of tight junctions. Once the function of tight junction proteins is dysfunctional, it weakens intercellular adhesion, promotes intestinal permeability and inflammation. Immunolocalization of Occludin and Claudin-4 were investigated using immunohistochemical staining. Occludin was localized at mucosa layer and the whole crypts, while Claudin-4 was predominantly distributed at the surface and tip of crypts. Intense Occludin and claudin-4 immunostaining was observed in controls, whereas reduced in models. Compared with the model group, LPH group has more intense immunostaining, whereas there appeared to be little change in the LPL group (Fig. 3A, B). Using ImageJ to scan the slides, the staining intensity of Occludin and Claudin-4, expressed as the ratio of the positively stained area of the entire scanned specimen, were higher in the treatment group than model group, especially in LPH group (p < 0.001). (Fig. 3C, D).

Loss of goblet cells characterizes mice with UC. As the most abundant mucins, MUC-2 is which is used as a marker of goblet cell homeostasis. Therefore, we further performed Immunofluorescence staining to examine muc-2 expression in the colon of mice. We observed a significant decrease in muc-2 positive cells in the colon of model group compared to control, while treatment with high dose Lacidophilin moderately enhanced MUC-2 immunostaining (Model: 32.1% positive, LPH: 47.5. p < 0.05) (Fig. 4).

3.3 Lacidophilin improved gut microbial dysbiosis induced by ulcerative colitis

The intestinal microbiota plays an essential role in maintaining gut homeostasis to shield us from diseases associated with dysbiosis. To determine changes in the intestinal microbial community, 16S
rRNA gene sequencing was used to evaluate the microbiota in cecal contents in the control, model and LPH group.

The species abundance was evaluated on the number of OTUs. The OTUs from the samples of control, model and LPH group are shown with a Venn diagram (Fig. 5A). The model group has fewer OTUs than the controls, while the LPH group has more OTUs than the models, which indicated Lacidophilin could be able to increase the abundance of intestinal flora in mice with ulcerative colitis. Moreover, the curve of the pan/core analysis on OTU level implies sufficient samples and reliable sequencing results in the study (Fig. 5B). Diversity of the gut flora was estimated using the Chao\textsubscript{1}/ACE\textsubscript{1}/Shannon and Shannon indices(Fig. 5C). Microbial community diversity was significantly altered by Lacidophilin treatment on Chao\textsubscript{1}/ACE and Shannon indices. Beta diversity of each group was calculated through PCoA. PCoA based on the relative abundance of OTUs revealed a separation of the control\textemdash model\textemdash LPH group(Fig. 5D), suggesting that ulcerative colitis and Lacidophilin may be the important factors accounting for the change in structure of microbial community. Then, Partial Least Squares Discriminant Analysis (PLS-DA) was performed for the further evidence of significant differences between the bacterial communities in the groups(Fig. 5E). The bacterial communities in the model samples and the matched controls as well as the LPH group clustered separately, indicating the overall structures of the microbial communities in the groups were remarkable different.

The gut flora were analyzed at the phylum (Fig. 6A) and genus (Fig. 6C) levels in the groups, as well as differences in the relative abundance of at the phylum (Fig. 6B) and genus (Fig. 6D) levels among groups. The top six most abundant phyla are Firmicutes\textemdash Bacteroidetes\textemdash Verrucomicrobiota\textemdash Actinobacteriota\textemdash Proteobacteria and Desulfovibacterota, and Firmicutes and Bacteroidetes, the dominant phylum in the gut, comprised more than 67% of all sequences. In addition, Proteobacteria, referred to as detrimental bacteria, were observed an increase relative abundance in the model compared to the control group, but a decrease in the LPH group. The relative abundance of Actinobacteriota was significantly lower in the model group than in the control group, while it was higher in the LPH group. At the genus level, *Lactobacillus*\textemdash *Dubosiella*\textemdash *Lachnospiraceae_NK4A136_group*\textemdash *Bifidobacterium*\textemdash *norank-f-norank-o-RF39*\textemdash *norank-f-Lachnospiraceae* and *norank_f_norank_o_Gastranaerophilales* were of decreased abundance in model group, whereas Lacidophilin treatment showed the opposite change. Notably, Lacidophilin-treated mice showed a significant contraction of *Escherichia-Shigella* and *Parvibacter* in the gut microbiota.

### 3.4 Transcriptional changes induced by Lacidophilin treatment in ulcerative colitis mouse

To deeply investigate the underlying mechanisms responsible for Lacidophilin on ulcerative colitis, the transcriptome was conducted. As shown in Fig. 7A, we found that the distribution of samples from the model group and the controls were clearly separated by applying principal component analysis (PCA), so did the control group and LPH group. Then, we defined 1393 significantly differentially expressed genes by setting absolute fold change $\geq 2$ and $P < 0.05$ as thresholds, in which 687 upregulated genes and 706
downregulated genes were identified in the model group relative to control. As for the LPH group versus the models, 67 significantly differentially expressed were determined, among which 63 genes were downregulated and 4 genes were upregulated (Fig. 7B). The Venn diagram shown in Fig. 7C indicated that there occurred significant discrepancy of 50 genes between control vs model group and model vs LPH group. Based on these differentially expressed genes (DEGs), a clustering analysis was performed. As shown in Fig. 7D, the control and LPH groups showed similar trends in DEGs, while those in the model group showed the reverse trend.

Then, to gain a better understanding of how Lacidophilin effect on UC, Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation analysis showed that these 50 genes were associated with immune system (Fig. 7E). Further KEGG pathway enrichment analysis of identified DEGs was performed, and the 20 most significantly enriched pathways were presented in Fig. 7F. Remarkably, cytokine-cytokine receptor interaction, IL-17 signaling pathway, complement and coagulation cascades, B cell receptor signaling pathway, Toll-like receptor signaling pathway, and TNF signaling pathway were clearly enriched. Moreover, these 50 DEGs were used for the protein-protein interactions network analysis (Fig. 7G). We noticed greater degree and more edge connections in the nodes of Ccl3, Lcn2, SPP1 and Serpine1, which involved in immune functions, indicating greater importance in these protein in the network.

**Discussion**

In this study, mice were induced acute ulcerative colitis by DSS, while Lacidophilin was administered for 7 days. We observed that Lacidophilin can prevent the changes of the body weight, colonic length, serum inflammatory factor levels, and histopathological damage in colitis mice effectively. In addition, tight junctions and mucin showed enhanced expression following Lacidophilin treatment compared to controls.

Then we further investigated the action mechanism of Lacidophilin in alleviating colitis. Patients with IBD suffer from dysbacteriosis which are defined as decreased diversity and abundance of gut microbial, with an imbalance between commensal and potentially pathogenic microorganisms[24]. We performed 16S ribosomal RNA tagged sequencing to identify alterations of intestinal microflora. At the phylum level, compared with the control group, the abundance of Bacteroidetes and Proteobacteria was decreased in the model group, whereas that Actinobacteriota of increased. However, Lacidophilin administration reversed this trend, aligning with the research of Zhu[25] and Zhou[26]. In addition, we revealed that *Lactobacillus*, *Dubosiella*, *Lachnospiraceae_NK4A136_group*, *Bifidobacterium*, *norank-f*, *norank-o-RF39*, *norank-f-Lachnospiraceae* and *norank_f_norank_o_Gastranaerophilales* was significantly increased in UC mice with treatment of Lacidophilin. *Lactobacillus*, *Bifidobacterium* and *Lachnospiraceae_NK4A136_group* have potential benefits in improving IBD due to their ability to produce SCFA[26,27]. Moreover, it had been reported that *Lachnospiraceae_NK4A136_group* were capable of maintaining epithelial health and immune balance of the intestine[27].
Toll-like receptors (TLRs) activate inflammatory cells and release pro-inflammatory cytokines, which are the key mediators of inflammatory routes in the intestine\[^{28, 29}\]. It has been shown that norank-f-norank-o-RF39 were negatively correlated with TLR2 receptor expression and proinflammatory factors IL-4 and IL-17 levels, but positively correlated with anti-inflammatory factors IL-10 levels\[^{30}\], which means these intestinal flora might be beneficial to attenuate intestinal inflammation of DSS colitis. Dubosiella thought to be improved colitis symptoms have been explored in several studies\[^{31-33}\]. Nevertheless, some contrary research was also observed with more prominent relative abundance in colitis mice than the normal ones\[^{34, 35}\], which is inconsistent with our results. Hence a further study of Dubosiella function on colitis in vivo will be required in vivo. Conversely, the abundance of \textit{Escherichia-Shigella} and \textit{Parvibacter} was significantly decreased in the LPH group than in UC mice. An increase in the abundance of \textit{Escherichia-Shigella} was observed in the mice accompanied with aggravating DSS-induced colitis\[^{36, 37}\]. Moreover, it is demonstrated that \textit{Parvibacter} might have potential benefit effects on colitis cause its positive correlation with IL-10\[^{38}\], whereas, some research had demonstrated that DSS induced a significant reduction of \textit{Parvibacter} in mice with colitis\[^{39}\]. Therefore, whether it is beneficial for colitis remains further evidence. Taken together, Lacidophilin might restrain the growth of potentially harmful bacteria and enrich potentially beneficial bacteria to improve DSS-induced ulcerative colitis.

To deeply investigate the molecular mechanism of Lacidophilin to UC, transcriptome of mice colon was performed after high-dose Lacidophilin treatment. In the present study, 50 significantly changed genes were observed in Control versus Model and Model versus LPH groups. It is worth mentioning that immune system dominated KEGG pathway, suggesting its mechanism was mainly correlated with immunity. The IL-17 signaling pathway which could mediate the promotion of immune response and activation of various inflammatory pathways\[^{40}\], was originally described in the autoimmune disease, and it has been a drug target for many autoimmune and chronic inflammatory diseases. There is evidence for IL-17 signaling pathway in promoting inflammation that damages the gut mucosa, while it also plays protective roles in regulating intestinal flora\[^{41}\]. The B cell receptor (BCR) signaling pathway is a key signaling pathway for the development and maturation of B cells\[^{42}\]. Most studies in the field of B cell receptor signaling pathway have only focused on lymphoma\[^{43, 44}\], but there was little research on B-cell receptor pathways and UC. Thus, our experiment might provide more evidence for the relationship between the B-cell receptor pathway and UC. Moreover, following our research, Toll-like receptor signaling pathway and TNF signaling pathway which were involved in driving inflammation might be valuable for the molecular mechanisms of Lacidophilin improving UC.

PPI network have demonstrated that the protein, Lcn2, Ccl3, Mmp8, Slc11a1, Spp1 and Serpine1, are involved in mediating multiple pathways to improve DSS-induced intestinal injury. Lipocalin 2 (Lcn2), a multifunctional immune protein, was closely related to the intestinal inflammation. Reports are rather controversial, and there is no general agreement about whether Lcn2 mediate anti-inflammatory or pro-inflammatory functions. Researchers reported that Lcn2 released in a partial MyD88-dependent manner, and UC would be aggravated significantly in Lcn2 knockout mice\[^{45}\]. Lcn2 deletion could augment pro-
inflammatory response in the model challenged with LPS\textsuperscript{[46]}. However, other authors question the function of Lcn2, who suggested that Lcn2 with the activation of NF-κB pathway while enhancing inflammasome assembly and IL-1β secretion, could lead to more severe inflammation\textsuperscript{[47]}. Our results show that an elevated level of Lcn2 in colon tissue of mice with UC, while it declined in mice administrated with Lacidophilin, consistent with increase of intestinal mucosal Lcn2 in patients with IBD\textsuperscript{[48]}.

Notably, Ccl3, belongs to chemokines family, which could induce macrophages and granulocytes migration to sites of acute inflammation\textsuperscript{[49]}, has up-regulated during intestinal inflammation, while oral administration of Lacidophilin could significantly reverse its increase. Consistent with our findings, several studies have shown a strongly up-regulation of Ccl3 expression was observed in IL-10\textsuperscript{-/-} mice\textsuperscript{[50]}, TNBS model\textsuperscript{[51]}, as well as DSS model\textsuperscript{[52]}, all of which are known as common animal models for IBD. It would seem to suggest that Ccl3 was a possible biomarkers and potential therapeutic target for IBD, and our results might provide a rationale for further studies.

It has been accepted that regulated expression of matrix metalloproteinases (MMPs) plays multifaced roles in the pathogenesis of IBD. Previous findings showing that MMP8, which is predominantly expressed by macrophages, led to increased inflammatory cells infiltration after mice exposure to DSS\textsuperscript{[53]}, are compatible with our results. The latest research pointed to a significant role of the Slc11a1 gene (formerly NRAMP1) on the DSS-induced colitis phenotype\textsuperscript{[54]}. In addition, a strong relationship between the secreted phosphoprotein 1 (Spp1) and CD susceptibility was observed\textsuperscript{[55]}. The Serpine1 encoding a protein called plasminogen activator inhibitor-1 (PAI-1) which can promote peripheral angiogenesis\textsuperscript{[56]}, was elevated during the disease activity cycle in the inflamed colon, contributing to an aggravation of mucosal damage in colitis\textsuperscript{[57]}.

Taken together, our findings suggest the role of Lacidophilin in alleviating DSS-induced colitis, identifying the enhancement of colon barrier integrity and improvement of intestinal microflora as a possible action mechanism. Lacidophilin could drive the immune system, reduce the expression of inflammatory cytokines, and decrease the protein expression of Lcn2, Ccl3, Mmp8, Slc11a1, Spp1 and Serpine1, which were associated with immune response or neo-angiogenesis. However, this study was limited by the absence the experimental methods of polymerase chain reaction (PCR) and western blot (WB) to verify its changes at molecular and protein levels, and a more precise mechanism how Lacidophilin mediate immune system in colitis remains to be elucidated. In spite of its limitations, this work offers guidance on future research and clinical application of postbiotics to improve acute ulcerative colitis.

**Declarations**

**Ethics Statement**

The animal study was reviewed and approved by the Experimental Animal Ethics Committee of Jiangzhong Pharmaceutical Co., Ltd. The Ethical Committee protocol number was 20220407. The
reporting in this study follows the recommendations in the ARRIVE guidelines. We have strictly abided by the international animal welfare and ethical standards, carry out the relevant laws, regulations and policies on the management of laboratory animals, and care laboratory animals.

Data availability

Sequence data that support the findings of this study have been deposited in the National Center for Biotechnology Information Archive with the link of http://www.ncbi.nlm.nih.gov/bioproject/1130646 and the China National Gene Bank Nucleotide Sequence Archive with the link of http://db.cngb.org/cnsa/project/CNP0005936_92303499/reviewlink/.

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Author Contributions

WJ-L conceived and designed the experiments. JT-Y, XY-C and ZY performed the experiments. WJ-L, YM-L, LJ-Z contributed reagents/materials/analytical tools. JT-Y, JW-Z and DL-S analyzed the data. JT-Y wrote the manuscript.

References


Figures
Figure 1

The appearance index of Lacidophilin on DSS-induced colitis in mice. Lacidophilin influenced appearance index in mice with colitis. (A) Experimental timeline for DSS-induced ulcerative colitis mouse models of with interventional administration of the Lacidophilin. (B) Body weight evolution (n=9). (C) Food intake evolution (n=3). (D) Water intake evolution (n=3). Data are expressed as mean ± SEM. *P < 0.05, **P < 0.01, compared to the Control group.
Figure 2

Therapeutic evaluation of Lacidophilin on DSS-induced colitis in mice. (A) Changes in colonic length by naked eye. (B) Statistical graph for colonic length (n=9). (C) The level of TNF-α in the serum (n=8). (D) The level of IL-6 in the serum (n=8). (E) Hematoxylin and eosin staining of the colons. Data are representative images or expressed as mean ± SEM. *P < 0.05, ***P < 0.001, compared to the Control group. # P < 0.05, ## P < 0.01, ### P < 0.001 compared to the Model group.
Figure 3

Immunohistochemistry staining for tight junctions in the colon of healthy (Control) and ulcerative colitis (Model) and Treated LPL LPH mice. (A) Occludin expression (200X). (B) Claudin-4 expression (200X). (C) Quantification of Occludin expression. (D) Quantification of Claudin-4 expression.
Figure 4

Immunofluorescence staining for intestinal mucin in the colon of healthy (Control) and ulcerative colitis (Model) and Treated LPL, LPH mice (200X). (A) MUC-2 expression. (B) Quantification of MUC-2 expression.
Figure 5

Comparison of intestinal microbiota structures in the colon of healthy (Control) and acute colitis (Model) and Treated LPH mice. (A) Venn diagram on OTU level. (B) Pan/Core curve on OTU level. (C) Alpha diversities comparisons of microbial communities, Chao, ACE, Shannon, Simpson index (n=8). (D) Principal coordinate analysis of bacterial community composition (beta diversity). (E) Partial least square discriminant score plot of cecal microbiota. *P < 0.05, **P < 0.01, ***P < 0.001, compared to the Control group. # P < 0.05, ## P < 0.01, ### P < 0.001 compared to the Model group.
Figure 6

Lacidophphilin alters the community composition of intestinal microbiota in models of ulcerative colitis. (A) Bacterial composition of the different communities at the phylum level. (B) Relative abundance of intestinal microbiota communities at the phylum level. (C) Bacterial composition of the different communities at the genus level. (D) Relative abundance of intestinal microbiota communities at the genus level.
Figure 7

RNA-seq analysis in the colon of healthy (Control) and acute colitis (Model) and Treated LPH mice. (A) The PCA plot shows a distinct separation based on the gene expression profiles among the Control vs Model group and Model vs LPH groups. (B) Volcano plot showing the differentially expressed genes (DEGs) in the Control vs Model group and Model vs LPH groups. (P ≤ 0.05, Fold change ≥ 2). (C) Venn diagram analyze of gene or transcript expression among the Control vs Model group and Model vs LPH.
groups. (D) DEGs heatmap analysis. Blue indicates downregulated genes. Red indicates upregulated genes. (E) KEGG functional annotation analysis among DEGs. (F) KEGG enrichment functional analysis reveals the biological functions that are enriched in the DEGs. (G) The protein–protein interaction (PPI) network based on DEGs.

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