Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2020, Article ID 8097021, 10 pages
https://doi.org/10.1155/2020/8097021

*Research Article*

# *Lycium* Berry Polysaccharides Strengthen Gut Microenvironment and Modulate Gut Microbiota of the Mice

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Received 23 July 2020; Revised 24 August 2020; Accepted 16 September 2020; Published 5 October 2020

Academic Editor: Jianping Chen



*Lycium barbarum* polysaccharides (LBP) are the main active components of *Lycium barbarum* with many cited health effects. We hypothesize that gut microbiota and their metabolites might contribute to the health benefits of LBP. To test this hypothesis, C57BL/6J mice were gavaged with LBP, and the impact on the gut microbiota was investigated. The results showed that LBP facilitates the shifting of the epithelial immunity from the pro- to the anti-inflammatory microenvironment. We further showed a significant reduction of the potentially pathogenic bacteria in mice treated with LBP based on the 16S sequencing of mouse fecal DNAs. Importantly, LBP treatment enriched the xylan/fiber degrading bacteria and the short-chain fatty acids– (SCFAs-) producing bacteria. Associated with the treatment was the increase of serum SCFAs detected by UHPLC-QTOF/MS analysis as well as the upregulation of the SCFA-sensing receptors, GPRs 41, 43, and 109a. These findings strongly suggest that the health benefits of LBP might act through the modulation of gut microbiota.

## 1. Introduction

Gut microbiota (GM), together with the gut epithelial layer and the local immune system, plays an essential role in maintaining the homeostasis of the gut microenvironment. More and more evidence showed that GM participates in many metabolic processes, such as food digestion and nutrient absorption, as well as drug metabolism [1–3]. On the other hand, studies also indicated that the disturbed GM composition is associated with the development of many diseases, e.g., autism, Alzheimer's disease, irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and cancer [2]. Thus, seeking therapies to treat the disturbed GM has become a current hot topic in medical research, hence the role of prebiotics. In the past decade, merging evidence supports the notion that prebiotics, such as polyphenol, chitin, and dietary fibers, could significantly modulate the GM composition and regulate the host immune system to maintain the balance of gut microenvironment [4, 5].

Polysaccharides derived from herbs and foods are the primary sources of prebiotics. Many such herbal polysaccharides have been pharmaceutically evaluated for anticancer, immunomodulation, antiaging, and anti-obesity properties [6, 7]. However, the mechanisms of these bioactivities remain obscure. The recent advancement of GM research has opened up an essential avenue to explore the health benefits of herbal medicines that fall into the category of prebiotics. Although it is not fully understood, studies revealed that GM modulation plays an integral part in the health promotion effects of dietary polysaccharides [8, 9]. In our recent study, we demonstrated that mushroom polysaccharides present prebiotic properties and promote the health of the treated animals [10].

*Lycium barbarum* (wolfberry) has been documented in numerous Chinese medicinal studies for its tonic effects. It is a common component in many Chinese medicinal formulae as well as a popular daily food supplement in East Asian

countries. The polysaccharide from *Lycium barbarum* (LBP) is the primary active constituent that possesses immunomodulation, antioxidant, anti-inflammation, neuroprotection, and anticancer activities in various cellular and animal studies [11–14]. A report showed that LBP was mainly composed of fucose, ribose, rhamnose, arabinose, xylose, mannose, galactose, and glucose [15]. Interestingly, a recent *in vitro* study showed that LBP could promote the growth of *Bifidobacterium* and *Lactobacillus*, suggesting that LBP might possess prebiotic properties [16]. Such suggestion was reinforced by later research showing that LBP could alleviate colitis through the modulation of GM in IL-10 deficient mice [17]. A recent study also showed that LBP treatment could enhance the growth of probiotic bacteria in Kunming mice [9]. However, most of these studies were performed in diseased mouse models. Considering that LBP is mainly consumed as daily food and health supplements, it is vital to systemically evaluate the impact of LBP on GM in healthy individuals. Thus, in this study, we assessed the interplay between LBP and GM in normal C57BL/6J mice. LBP was daily administered (750 mg/kg) to 6–8 week age mice for 15 days. Fecal DNAs were used to evaluate the GM composition using ERIC-PCR as well as 16S amplicon sequencing. The LBP effects on the mucosal inflammatory mediators, the microbial metabolites, and the host G-protein-coupled receptors were assessed.

## 2. Materials and Methods

*2.1. Animals and Treatments.* C57BL/6J mice (6–8 weeks old) were purchased from the Chinese University of Hong Kong. The animal welfare and experimental procedures were strictly carried out according to the procedures approved by the Ethics Review Committee of Macau University of Science and Technology. The mice were housed in a 12 h light-dark cycle facility and fed with PicoLab® Rodent Diet 20–5053 (LabDiet, USA). All mice had free access to food and water. LBP (30% purity) was purchased from Jiangsu Goodex Mushroom Biotech Co. Ltd. (Yancheng, Jiangsu, China). The purity and quantitation of the polysaccharides were performed as previously described [10]. A total of 16 mice were equally divided into control and LBP treatment groups and administered daily by gavage with 750 mg/kg of polysaccharides or Milli-Q water for 15 consecutive days. The body weight and food and water consumption of the mice were recorded on days 0, 5, 10, and 15. At the end of the experiment, the mice were euthanized with pentobarbital sodium. Blood and gut mucosal samples were collected for later usage.

*2.2. Fecal Sample Collection.* Fecal samples were collected from individual mice on days 0 and 15 and stored at −80°C for later experiments. The schematic experimental design is shown in Figure 1(a).

*2.3. Total RNA Preparation and RNA Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR).* Total RNA was isolated from mucosa samples using RNeasy Mini Kit (Qiagen, Germany) following the manufacturer's instruction. The qRT-PCR was conducted using Applied Biosystems ViiA™ 7 PCR system (Carlsbad, CA, USA), according to our previous study [18]. The $2^{-\Delta\Delta Ct}$ method was applied to calculate the fold change of relative gene expression: $\Delta\Delta Ct = (Ct_{treatment\_target\ gene} - Ct_{treatment\_reference\ gene}) - (Ct_{control\_target\ gene} - Ct_{control\_reference\ gene})$. The sequences of the primers are listed in Table S1.

*2.4. Enterobacterial Repetitive Intergenic Consensus–PCR (ERIC-PCR) Analysis.* Total microbial gDNA was extracted from feces using QIAamp DNA Stool Mini Kit (QIAGEN) following the manufacturer's manual. The extracted DNA was analyzed for highly conserved ERIC regions with a pair of ERIC primers sequences: ERIC1 (5′-ATG-TAAGCTCCTGGGGATTCAC-3′) and ERIC2 (5′-AAG-TAAGTGACTGGGGTGAGCG-3′) [19]. PCR conditions were set as previously described [20]. Obtained ERIC-PCR products were separated in 2% agarose gel at 100V for 45 minutes. Bands were visualized with Gel Doc XR+ system and digitized for microbial clustering analysis using SIMCA-P 14.0 tool (Umetrics, Umea, Sweden) with confidence level 95% ($p < 0.05$).

*2.5. 16S rRNA Gene Sequencing.* DNA samples were sequenced for 16S rRNA genes using Illumina MiSeq (Illumina, San Diego), targeting the V3–V4 region with barcoded 515F and 806R universal primers, and processed as previously described [21]. The detailed experimental procedures were conducted as previously described [10].

*2.6. Measurement of Serum SCFAs.* 5 $\mu$l of 4-Cl-phenylalanine (0.3 mg/mL) was added to the serum samples (50 $\mu$l serum), and then 50 $\mu$l of the mixture was added to 200 $\mu$l of the cold MeOH. The mixture was centrifuged at 13,000 rpm for 5 min at 4°C. The supernatant was extracted twice, then dried under the nitrogen stream, derivatized, and analyzed using UHPLC-Q-TOF/MS as previously described [22].

*2.7. Statistical Analysis.* SPSS version 22 was used for statistical analysis. Bacterial taxa data normality was ascertain with Kolmogorov–Smirnov test. One-way ANOVA (for parametric data) and Kruskal–Wallis tests (for nonnormal data distribution) were performed to observe significantly different bacterial taxa among the groups. PERMANOVA test (Bray method) was performed for the intergroup dispersion. Before PERMANOVA analysis, OTUs abundance was rarefied to even depth and then subjected to PERMDISP test (Jaccard method). Alpha values were set to 0.05, whereas the threshold on the logarithmic score of linear discriminant analysis was ≥2.0. Partial least squares discriminant analysis (PLS-DA) was performed to visualize the changes of microbial communities before and after polysaccharides treatments using SIMCA-P 14.0 tool (Umetrics, Umea, Sweden) for which the confidence level was set at 95% ($P < 0.05$). Weight changes and diet and water consumption data

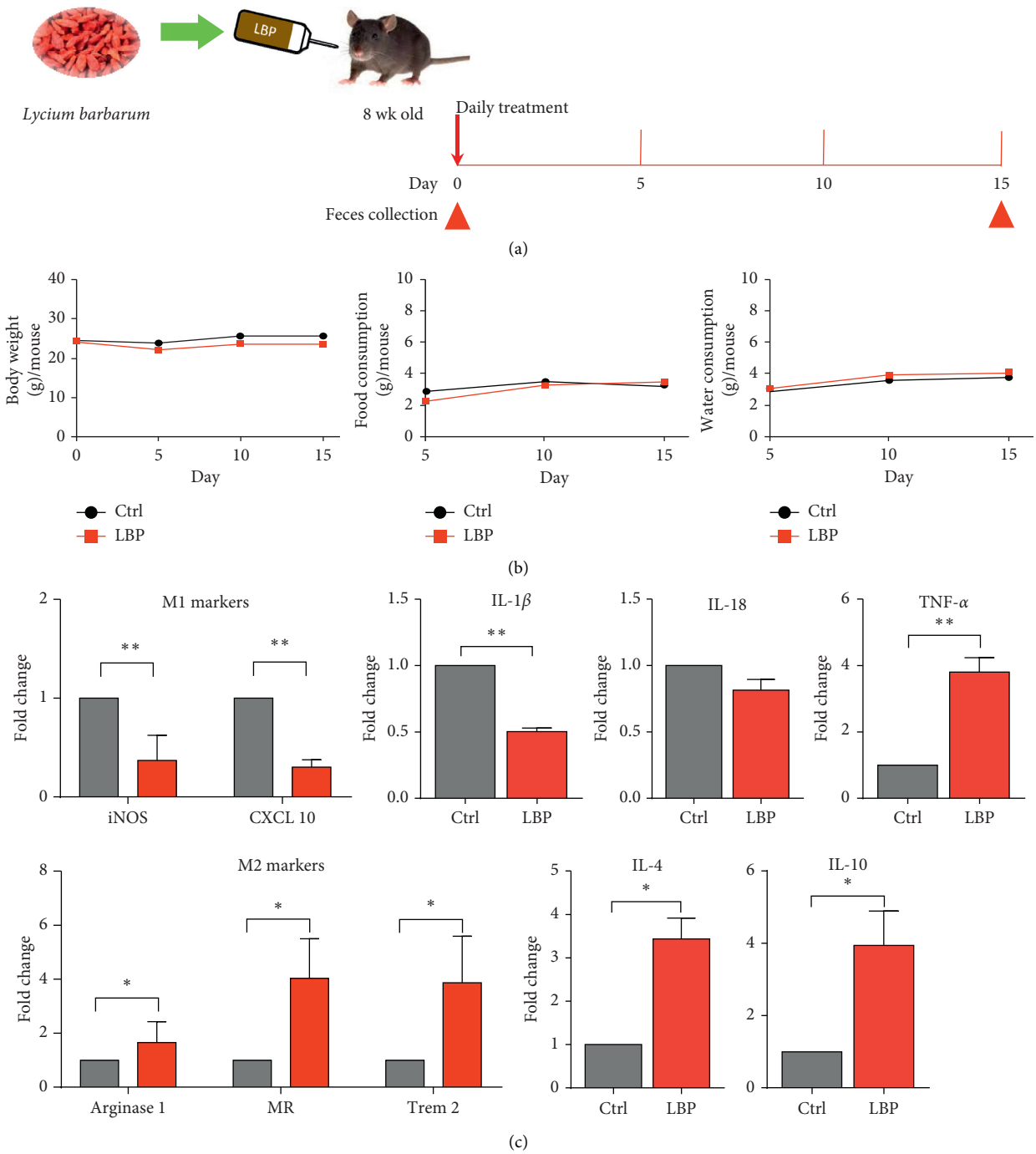


Figure 1: The immunological effects of GBP treatment on C57BL/6j mice. (a) Schematic diagram of experimental design. (b) The profiles of body weight, diet, and water consumption of the control and the GBP-treated mice. (c) Comparisons of the gut epithelial inflammatory mediators between the treated and the control mice. $N = 3$, $^{*}p \leq 0.05$; $^{**}p \leq 0.01$.

were statistically analyzed using one-way ANOVA and Dunnett's post hoc test using GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA, USA).

## 3. Results

*3.1. LBP Treatment Modulated the Gut Epithelial Inflammatory Mediators.* A total of 16 mice were equally divided into control and treatment groups and administered daily by gavage with 750 mg/kg of LBP or Milli-Q water for 15 consecutive days. After 15 days of treatment with LBP, no apparent differences in food and water consumption, as well as body weight, were detected between treated and untreated groups (Figure 1(b)). As LBP is known as an immune modulator, we first looked into the immune responses in the gut mucosa upon LBP treatment. The qRT-PCR results

showed that LBP significantly reduced the mRNA expressions of M1 macrophage markers, iNOS and CXCL 10, as well as the proinflammatory cytokines IL-1$\beta$ and IL-18. On the contrary, LBP increased the expressions of M2 macrophage markers, arginase 1, MR, and Trem 2. In addition, the anti-inflammatory cytokines IL-4 and IL-10 were significantly upregulated in the LBP-treated mice. We also found a significant increase in TNF-$\alpha$ in the LBP group (Figure 1(c)).

*3.2. LBP Altered the Core GM Composition and Increased GM Diversity.* ERIC-PCR was applied to analyze the similarity of the GM profile in the fecal DNA samples. The PLS-DA plot showed distinct clustering between the treated and untreated groups (Figure 2(a)). Further in-depth analysis of GM composition was performed using 16S rRNA gene amplicon sequencing. Higher OTUs diversity was observed in the LBP group compared to the control based on Chao1 and Shannon index (Figure 2(b)). After 15 days of treatment, the relative abundance of phyla Firmicutes, Candidatus, and Acidobacterium significantly differed between the control and the treatment groups (Figure 2(c)). At the species level, the relative abundance of *Barnesiella* spp., *Bacteroides acidifaciens*, and *Akkermansia muciniphila* was significantly reduced in the LBP group. On the other hand, *Clostridium* sp., *Lachnoclostridium clostridium xylanolyticum, Lachnoclostridium clostridium saccharolyticum,* and *Lactobacillus reuteri* were increased considerably in the LBP group (Figure 2(d), Figure S1).

*3.3. LBP Reduced the Relative Abundance of Potential Pathogens.* To investigate the effect of LBP on the potentially harmful bacteria, we found that LBP reduced the overall relative abundance of few disease-associated pathogens (Figure 3(a), Table S2). Few potential pathogens associated with inflammation/cancer, including *Allobaculum stercoricanis*, *Parasutterella excrementihominis*, and *Tannerella* spp., were downregulated by LBP treatment (Figure 3(b)). A high abundance of *Allobaculum stercoricanis* could increase the risk of the development of sporadic colorectal cancer (CRC) [23]. Another group of bacteria causing infection, including *Anaeroplasma bactoclasticum*, *Citrobacter* spp., and *Spirochaeta* spp., were also reduced in LBP treatment (Figure 3(c)). *Citrobacter* spp. were associated with the infections involving the central nervous system and the gastrointestinal, urinary, and respiratory tracts [24].

*3.4. LBP Enriched Xylan/Fiber Degrading and SCFAs-Producing Bacteria, as well as Enhancing the Microbial Metabolites SCFAs and Their Receptors.* In addition to reducing the potential pathogens, LBP also strongly enhanced the presence of beneficial bacteria (Table S3). Our results showed that LBP enriched the overall relative abundance of beneficial bacteria (Figure 4(a)). Xylan/fiber degrading bacteria, such as *Ruminococcus* sp., *Lachnoclostridium clostridium xylanolyticum*, and *Clostridium sulfatireducens* (Figure 4(b)), are among the beneficial bacteria found to be increased in the LBP-treated mice. This group of bacteria is known to facilitate the degradation of indigestible dietary fiber and then convert it into nutrients for the host. Besides, we also found that certain SCFAs-producing bacteria were increased in the LBP-treated group. These included the species of *Roseburia faecis*, *Prevotella* spp., *Butyricicoccus pullicaecorum*, and *Eubacterium uniforme* (Figure 4(c)). UHPLC-Q-TOF/MS showed that the serum SCFAs, especially the propionic acid and butyric acid, were significantly increased in the LBP-treated mice compared to the control (Figure 4(d)). At the same time, we also detected the remarkable activation of the three primary host SCFA-sensing receptors, GPRs 41, 43, and 109a, assessed by qRT-PCR analysis (Figure 4(e)).

## 4. Discussion

Oligo- and polysaccharides derived from many traditional herbal products have been widely used as functional foods and dietary supplements. Some of these products fall into the definition of prebiotics; namely, they are indigestible, become accessible for the probiotic bacteria in the gut, and exert beneficial effects on the host. *Lycium barbarum* contains 5–8% polysaccharides in the dried fruits and is well recognized for its medicinal properties as inflammatory and immune effects [11–13]. However, the underlying mechanism remains obscure. Two recent reports started to explore whether the beneficial effect of LBP might be through regulating the GM composition in an animal model [9, 17]. In this study, we elaborated on the beneficial impact of LBP in the normal C57BL/6j mouse model. Under a healthy condition, the gut immune system is keeping a moderate immune response to incoming pathogens and maintaining homeostasis of the gut barrier [25]. However, factors such as the age, diet, infection, and drugs could weaken the gut immune system and cause disorders of the gut microenvironment. Our finding showed that, even in the presumed "healthy mice," LBP sharply improved the gut epithelia by boosting the anti-inflammatory M2 macrophages-associated biomarkers, while downregulating the M1 macrophage-associated biomarkers (Figure 1(c)). The anti-inflammatory properties of LBP are further supported by the downregulation of inflammatory cytokines IL-1$\beta$ and IL-18 and the upregulation of anti-inflammatory cytokines IL-4 and IL-10 (Figure 1(c)). Interestingly, in our study, we also found that LBP induced the proinflammatory cytokine TNF-$\alpha$. TNF-$\alpha$ is naturally produced by activated macrophages and monocytes and has pleiotropic effects on normal and malignant cells. Bo et al. also reported that LBP could increase the production of TNF-$\alpha$, and the authors claimed that the upregulated TNF-$\alpha$ was associated with the immunomodulation of LBP [26]. Other reports also showed that TNF-$\alpha$ possesses anti-infection effect which is possibly related to the low relative abundance of the potential pathogens in the LBP-treated group. Macrophages are the main immune cells in the gut epithelial barrier. Facilitating the polarization of M1 to M2 macrophages could ameliorate certain gut diseases, such as IBD and IBS, as well as colorectal cancer [27].


FIGURE 2: LBP induced core changes in GM composition. (a) PLS-DA analysis of the digitized ERIC-PCR results. (b) Alpha diversity analysis of the GM compositions between the control and the LBP groups. (c) Comparison of the relative abundance of the dominant phyla between the control and the LBP groups. (d) Comparison of the relative abundance of dominant species taxa between the control and the LBP groups. $^{*}p \leq 0.05$; $^{**}p \leq 0.01$.

Using prebiotics to improve gut health is not a new idea [28, 29]. Here, in this study, our results showed that LBP effectively boosted the beneficial bacteria, while reducing the potential pathogens. It is noteworthy that *A. muciniphila* was obviously decreased in the LBP group, although this species was usually considered as one of the beneficial bacteria for its

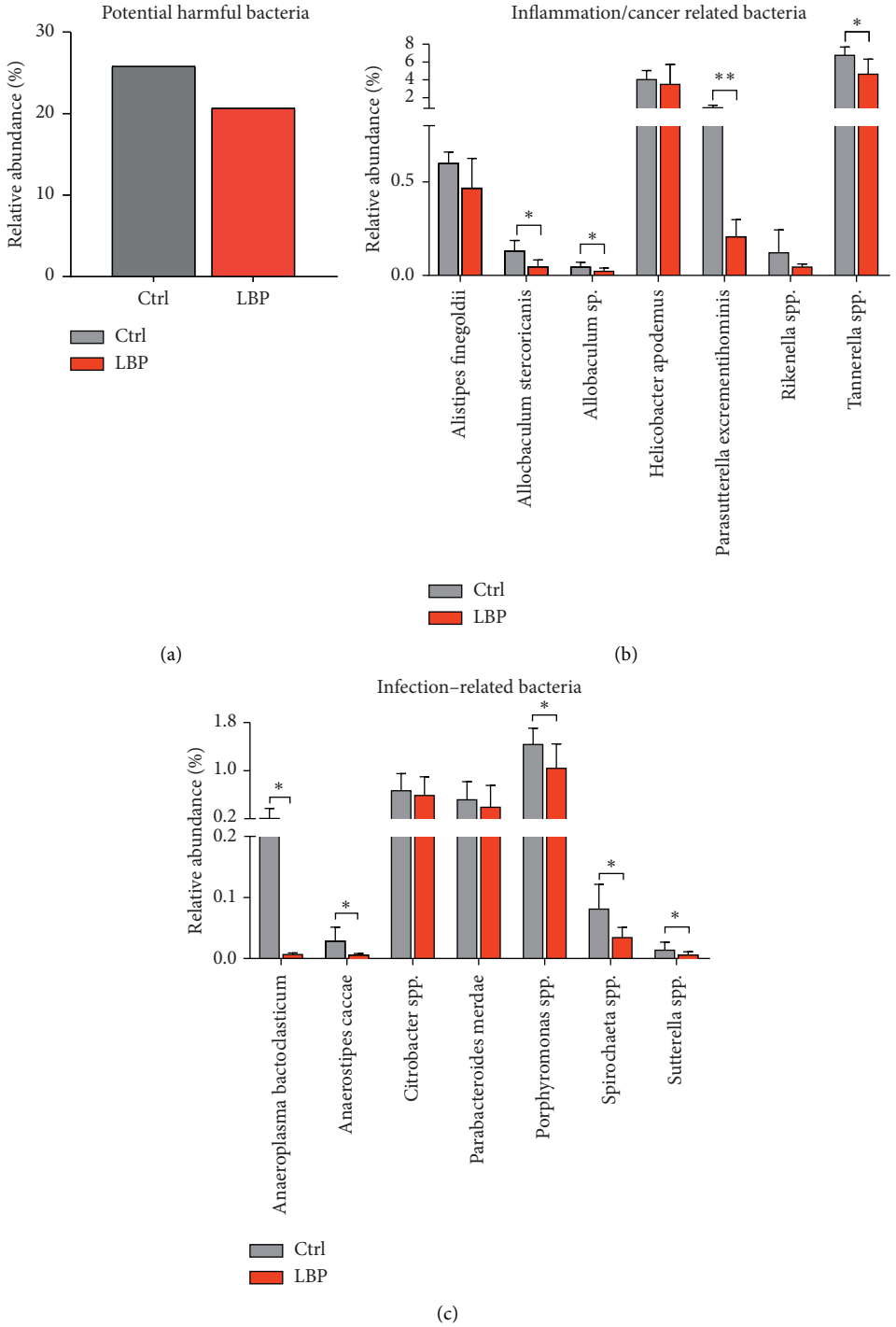


Figure 3: LBP reduced the relative abundance of potential pathogens. The control and the LBP groups were compared for (a) the relative abundance of total potential pathogens; (b) the relative abundance of inflammation/cancer-related bacteria; and (c) the relative abundance of infection-related bacteria. $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$.

role in obesity and diabetes mellitus [30, 31]. However, *A. muciniphila* has been found in many studies as the dominant species in animals with colitis/cancers and in patients with IBD/CRC [32–34]. Xylans and fiber are the main components in many whole plant foods and could be digested by xylanase produced by xylanolytic microbe in the gut. Reports showed that xylan/fiber degradation is a necessary process for maintaining the host gut ecosystems [35]. Here, we showed that LBP treatment enriched the xylan/fiber degrading bacteria, such as *Ruminococcus* sp., *Lachnoclostridium xylanolyticum*, and *Clostridium sulfatireducens* (Figure 4(b)). In our previous study, we found similar growth enhancement of this group of bacteria in the gut of the mice treated with *lingzhi* and *fuling*

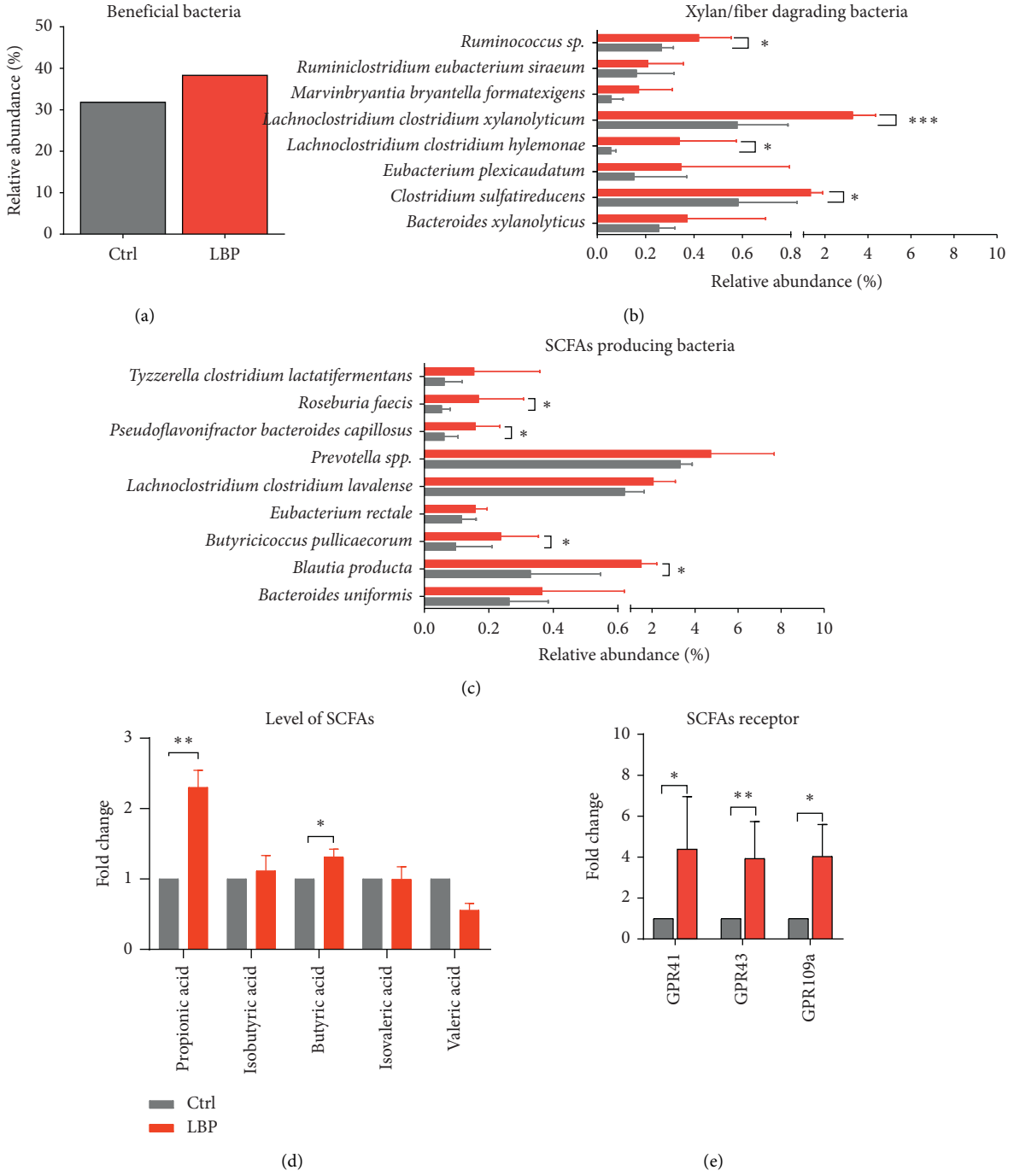


Figure 4: LBP increased the relative abundance of beneficial bacteria. The control and the LBP groups were compared for (a) the relative abundance of total beneficial bacteria; (b) the relative abundance of xylan/fiber degrading bacteria; (c) the relative abundance of SCFAs-producing bacteria; (d) mRNA expression of SCFAs receptor; and (e) the fold change of serum SCFAs. For statistical analysis, ratio data was analyzed with $t$-test using SPSS. $^{*}p \le 0.05$; $^{**}p \le 0.01$; $^{***}p \le 0.001$.

polysaccharides [10]. Some of these bacteria are also found enriched in people on high fiber diet [36].

The group of SCFAs-producing bacteria is another essential probiotic. They convert the ingested fibers and indigestible carbohydrates into SCFAs such as acetate, propionate, and butyrate. The SCFAs are generated in the gut, are quickly absorbed into the blood, and circulate to other organs of the body. These microbial metabolites could affect the epithelial cell proliferation and mucosal immune responses among other physiological impacts beyond the gut microenvironment [37–40]. In this study, LBP significantly enriched the relative abundance of SCFAs-producing bacteria and caused the elevation of serum concentration of propionic acid and butyric acid (Figures 4(c) and 4(e)). Importantly, here we showed that LBP not only modulated the GM composition, but also stimulated strong expression of three main GPCRs, namely, the

GPR 41, 43, and 109a. A similar finding was observed in the mice treated with polysaccharides from *Ganoderma lucidum* in our previous report [41]. Activation of the GPCRs would trigger a series of cellular responses [42–44]. Therefore, the bacteria-derived SCFAs act as signaling molecules through their ability to mimic the host's molecules and interact with the cellular receptors. Such interaction between the SCFAs and the host receptors forms a crucial communication between the commensal bacteria and the host and exerts beneficial effects on the host. One of such interplays between the microbe and the host is through the interactions between the microbial metabolites SCFAs and the specific SCFAs-sensing G-protein-coupled receptors (GPCRs) residing in the gut epithelial cells [45]. Here, we showed that LBP not only modulated the GM composition, but also stimulated strong expression of three key GPCRs, namely the GPR 41, 43, and 109a.

## 5. Conclusion

In summary, treatment with LBP positively modulated gut inflammatory mediators. LBP also effectively down-regulated the relative abundance of potential pathogens and upregulated the relative abundance of beneficial bacteria. Meanwhile, LBP raised the concentration of SCFAs and stimulated the host's SCFAs receptors in the gut barrier. From the prebiotic and food perspective, our study revealed the diverse benefits of LBP to the host health.

## Data Availability

The data used to support the findings of this study are included in the article.

## Ethical Approval

All the procedures used in this study were approved by the Ethics Committee of Animal Experimentation of Macau University of Science and Technology.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

## Authors' Contributions

Wenrui Xia and Guoxin Huang conducted the experiments and composed the manuscript. Imran Khan performed the microbiome analysis. Xiaoang Li, Wai Kit Leong, Lu Su, and Lin Yin carried out animal work. Xiqing Bian made the LC-MS analysis of bacterial metabolites. Jiyan Su exchanged information on herbal polysaccharides. WLW Hsiao coordinated and supervised the project and revised the manuscript.

## Acknowledgments

This work was funded by the Science and Technology Development Fund of Macau Government (grant no. FDCT 0054-2018-A2 to WLW Hsiao) and the Ascent Program for Medical Research of Foshan Government, 2019–2021. This work was also supported by the National Natural Science Foundation of China (no. 31900103 to Guoxin Huang). The authors also thank Dr. Jianlin Wu for his technical advice on the metabolite analysis.

## Supplementary Materials

This supplementary material included all the research results mentioned in the manuscript. Figure S1: The LDA scores for the dominant species in the control and the LBP groups. Table S1: The nucleotide sequences of the primers used to analyze the mRNA expression of the cytokines. Table S2: The selected potential pathogens used to analyze in this study. Table S3: The selected beneficial bacteria used to analyze in this study. (*Supplementary Materials*)

## References

[1] J. R. Marchesi, D. H. Adams, F. Fava et al., "The gut microbiota and host health: a new clinical frontier," *Gut*, vol. 65, no. 2, pp. 330–339, 2016.

[2] I. Sekirov, S. L. Russell, L. C. M. Antunes, and B. B. Finlay, "Gut microbiota in health and disease," *Physiological Reviews*, vol. 90, no. 3, pp. 859–904, 2010.

[3] E. Thursby and N. Juge, "Introduction to the human gut microbiota," *Biochemical Journal*, vol. 474, no. 11, pp. 1823–1836, 2017.

[4] H. D. Holscher, "Dietary fiber and prebiotics and the gastrointestinal microbiota," *Gut Microbes*, vol. 8, no. 2, pp. 172–184, 2017.

[5] M. Roberfroid, G. R. Gibson, L. Hoyles et al., "Prebiotic effects: metabolic and health benefits," *British Journal of Nutrition*, vol. 104, no. S2, pp. S1–S63, 2010.

[6] C.-J. Chang, C.-S. Lin, C.-C. Lu et al., "Ganoderma lucidum reduces obesity in mice by modulating the composition of the gut microbiota," *Nature Communications*, vol. 6, p. 7489, 2015.

[7] S. Wasser, "Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides," *Applied Microbiology and Biotechnology*, vol. 60, no. 3, pp. 258–274, 2002.

[8] D. W. Cockburn and N. M. Koropatkin, "Polysaccharide degradation by the intestinal microbiota and its influence on human health and disease," *Journal of Molecular Biology*, vol. 428, no. 16, pp. 3230–3252, 2016.

[9] W. Zhu, S. Zhou, J. Liu, R. J. C. McLean, and W. Chu, "Prebiotic, immuno-stimulating and gut microbiota-modulating effects of *Lycium barbarum* polysaccharide," *Biomed. Pharmacother*, vol. 121, no. 2019, Article ID 109591, 2020.

[10] I. Khan, G. Huang, X. Li, W. Leong, W. Xia, and W. L. W. Hsiao, "Mushroom polysaccharides from ganoderma lucidum and Poria cocos reveal prebiotic functions," *Journal of Functional Foods*, vol. 41, pp. 191–201, 2018.

[11] W. Chen, X. Cheng, J. Chen et al., "*Lycium barbarum* polysaccharides prevent memory and neurogenesis impairments in scopolamine-treated rats," *PLoS One*, vol. 9, no. 2, 2014.

[12] L. Gan, S. H. Zhang, X. L. Yang, and H. B. Xu, "Immunomodulation and antitumor activity by a polysaccharide-protein complex from *Lycium barbarum*," *International Immunopharmacology*, vol. 4, no. 4, pp. 563–569, 2004.

[13] H. Wu, H. Guo, and R. Zhao, "Effect of *Lycium barbarum* polysaccharide on the improvement of antioxidant ability and DNA damage in NIDDM rats," *Yakugaku Zasshi*, vol. 126, no. 5, pp. 365–371, 2006.

[14] X. Xing, F. Liu, J. Xiao, and K. F. So, "Neuro-protective mechanisms of *Lycium barbarum*," *Neuro Molecular Medicine*, vol. 18, no. 3, pp. 253–263, Sep. 2016.

[15] Q. Zhang, X. Lv, T. Wu et al., "Composition of *Lycium barbarum* polysaccharides and their apoptosis-inducing effect on human hepatoma SMMC-7721 cells," *Food & Nutrition Research*, vol. 59, 2015.

[16] A. M. Rotar, D. C. Vodnar, F. Bunghez et al., "Effect of goji berries and honey on lactic acid bacteria viability and shelf life stability of yoghurt," *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, vol. 43, no. 1, pp. 196–203, 2015.

[17] Y. Kang, G. Yang, S. Zhang, C. F. Ross, and M. J. Zhu, "Goji berry modulates gut microbiota and alleviates colitis in IL-10-deficient mice," *Molecular Nutrition & Food Research*, vol. 62, no. 22, Article ID e1800535, 2018.

[18] G. Huang, I. Khan, X. Li et al., "Ginsenosides Rb3 and Rd reduce polyps formation while reinstate the dysbiotic gut microbiota and the intestinal microenvironment in ApcMin/+ mice," *Scientific Reports*, vol. 7, no. 1, p. 12552, 2017.

[19] J. Versalovic, T. Koeuth, and R. Lupski, "Distribution of repetitive DNA sequences in eubacteria and application to finerpriting of bacterial enomes," *Nucleic Acids Research*, vol. 19, no. 24, pp. 6823–6831, 1991.

[20] L. Chen, W. C. S. S. Tai, M. S. Brar, F. C. C. C. Leung, and W. L. W. W. Hsiao, "Tumor grafting induces changes of gut microbiota in athymic nude mice in the presence and absence of medicinal gynostemma saponins," *PLoS One*, vol. 10, no. 5, 2015.

[21] S. E. Dowd, Y. Sun, R. D. Wolcott, A. Domingo, and J. A. Carroll, "Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) for microbiome studies: bacterial diversity in the ileum of newly weaned salmonella-infected pigs," *Foodborne Pathogens and Disease*, vol. 5, no. 4, pp. 459–472, 2008.

[22] X. Bian, N. Li, B. Tan et al., "Polarity-tuning derivatization-LC-MS approach for probing global carboxyl-containing metabolites in colorectal cancer," *Analytical Chemistry*, vol. 90, no. 19, pp. 11210–11215, 2018.

[23] Y. Zhang, C. Ma, J. Zhao, H. Xu, Q. Hou, and H. Zhang, "Lactobacillus casei Zhang and vitamin K2 prevent intestinal tumorigenesis in mice via adiponectin-elevated different signaling pathways," *Oncotarget*, vol. 8, no. 15, pp. 24719–24727, 2017.

[24] J. P. Lavigne, C. Defez, N. Bouziges, A. Mahamat, and A. Sotto, "Clinical and molecular epidemiology of multidrug-resistant citrobacter spp. infections in a french university hospital," *European Journal of Clinical Microbiology & Infectious Diseases*, vol. 26, no. 6, pp. 439–441, 2007.

[25] J. M. Pickard, M. Y. Zeng, R. Caruso, and G. Núñez, "Gut microbiota: role in pathogen colonization, immune responses, and inflammatory disease," *Immunological Reviews*, vol. 279, no. 1, pp. 70–89, 2017.

[26] R. Bo, Z. Liu, J. Zhang et al., "Mechanism of *Lycium barbarum* polysaccharides liposomes on activating murine dendritic cells," *Carbohydrate Polymers*, vol. 205, pp. 540–549, 2019.

[27] K. Sugihara, T. L. Morhardt, and N. Kamada, "The role of dietary nutrients in inflammatory bowel disease," *Frontiers in Immunology*, vol. 9, p. 3183, 2019.

[28] J. Vulevic, A. Drakoularakou, P. Yaqoob, G. Tzortzis, and G. R. Gibson, "Modulation of the fecal microflora profile and immune function by a novel trans-galactooligosaccharide mixture (B-GOS) in healthy elderly volunteers," *The American Journal of Clinical Nutrition*, vol. 88, no. 5, pp. 1438–1446, 2008.

[29] S. Hooda, B. M. V. Boler, M. C. R. Serao et al., "454 pyrosequencing reveals a shift in fecal microbiota of healthy adult men consuming polydextrose or soluble corn fiber," *Journal of Nutrition*, vol. 142, no. 7, pp. 1259–1265, 2012.

[30] H. Plovier, A. Everard, C. Druart et al., "A purified membrane protein from *Akkermansia muciniphila* or the pasteurized bacterium improves metabolism in obese and diabetic mice," *Nature Medicine*, vol. 23, no. 1, pp. 107–113, 2017.

[31] S. Lukovac, C. Belzer, L. Pellis et al., "Differential modulation by *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* of host peripheral lipid metabolism and histone acetylation in mouse gut organoids," *American Society for Microbiology Journals*, vol. 5, no. 4, p. 10, 2014.

[32] I. Khan, N. Ullah, L. Zha et al., "Alteration of gut microbiota in inflammatory bowel disease ( IBD ): cause or consequence ? IBD treatment targeting the gut microbiome," *Pathogens*, vol. 8, no. 126, pp. 1–28, 2019.

[33] S. S. Seregin, N. Golovchenko, B. Schaf et al., "NLRP6 protects il10 −/− mice from colitis by limiting colonization of *Akkermansia muciniphila*," *Cell Reports*, vol. 19, no. 4, pp. 733–745, 2017.

[34] L. Mira-Pascual, R. Cabrera-Rubio, S. Ocon et al., "Microbial mucosal colonic shifts associated with the development of colorectal cancer reveal the presence of different bacterial and archaeal biomarkers," *Journal of Gastroenterology*, vol. 50, no. 2, pp. 167–179, 2014.

[35] D. Dodd, R. I. Mackie, and I. K. O. Cann, "Xylan degradation, a metabolic property shared by rumen and human colonic bacteroidetes," *Molecular Microbiology*, vol. 79, no. 2, pp. 292–304, 2011.

[36] T. T. T. Tran, F. J. Cousin, D. B. Lynch et al., "Prebiotic supplementation in frail older people affects specific gut microbiota taxa but not global diversity," *Microbiome*, vol. 7, no. 1, p. 39, 2019.

[37] A. Koh, F. De Vadder, P. Kovatcheva-Datchary, and F. Bäckhed, "From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites," *Cell*, vol. 165, no. 6, pp. 1332–1345, 2016.

[38] K. M. Maslowski, A. T. Vieira, N. G. Aylwin et al., "Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43," *Nature*, vol. 461, no. 7268, pp. 1282–1268, 2009.

[39] J. Tan, C. McKenzie, M. Potamitis et al., "The role of short-chain fatty acids in health and disease," *Advances in immunology*, vol. 121, pp. 91–119, 2014.

[40] Y. Furusawa, Y. Obata, S. Fukuda et al., "Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells," *Nature*, vol. 504, p. 446, 2013.

[41] I. Khan, G. Huang, X.-A. Li et al., "Mushroom polysaccharides and jiaogulan saponins exert cancer preventive effects by shaping the gut microbiota and microenvironment in Apc mice," *Pharmacological Research*, vol. 148, Article ID 104448, 2019.

[42] P. Larraufie, J. Doré, N. Lapaque, and H. M. Blottière, "TLR ligands and butyrate increase Pyy expression through two distinct but inter-regulated pathways," *Cellular Microbiology*, vol. 19, no. 2, 2017.

[43] S. D. Chang and M. R. Bruchas, "Functional selectivity at GPCRs: new opportunities in psychiatric drug discovery," *Neuropsychopharmacology*, vol. 39, no. 1, pp. 248-249, 2014.

[44] P. Louis, G. L. Hold, and H. J. Flint, "The gut microbiota, bacterial metabolites and colorectal cancer," *Nature Reviews Microbiology*, vol. 12, no. 10, pp. 661–672, 2014.

[45] M. Sun, W. Wu, Z. Liu, and Y. Cong, "Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases," *Journal of Gastroenterology*, vol. 52, no. 1, p. 8, 2017.