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Original article

Berberine, a potential prebiotic to indirectly promote *Akkermansia* growth through stimulating gut mucin secretion

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ABSTRACT

Background: Akkermansia spp. plays important roles in maintenance of host health. Increasing evidence reveals that berberine (BBR) may exert its pharmacological effects via, at least partially, promotion of *Akkermansia spp*. However, how BBR stimulates *Akkermansia* remains largely unknown.

Purpose: In this study, we investigated the mechanism underlying the *Akkermansia*-promoting effect of BBR.

Materials and methods: The effect of BBR on *Akkermansia* was assessed in BBR-gavaged mice and direct incubation. The influence of BBR on intestinal mucin production was determined by alcian-blue staining and real-time PCR. The feces were analysis by gas chromatography–time-of-flight mass spectrometry (GC-TOF/MS) metabolomics. The role of polyamines in BBR-elicited mucin secretion and *Akkermansia* growth was evaluated by administration of difluoromethylornithine (DFMO) in mice.

Results: Gavage of BBR dose-dependently and time-dependently increased the abundance of *Akkermansia* in mice. However, it did not stimulate *Akkermansia* growth in direct incubation, suggesting that BBR may promote *Akkermansia* in a host-dependent way. Oral administration of BBR significantly increased the transcription of mucin-producing genes and mucin secretion in colon. Untargeted metabolomics analysis showed that BBR increased polyamines production in feces which are known to stimulate goblet cell proliferation and differentiation, but treatment with eukaryotic polyamine synthase inhibitor DFMO did not abolish the stimulating effect of BBR on mucin secretion and *Akkermansia* growth, indicating that the gut bacteria-derived but not the host-derived polyamines may involve in the BBR-promoted *Akkermansia* growth.

Conclusions: Our results reveal that BBR is a promising prebiotic for *Akkermansia*, and it promotes *Akkermansia* growth via stimulating mucin secretion in colon.

1. Introduction

Recently, accumulating studies have demonstrated that gut microbes play important roles in maintaining the health of the host, while gut dysbiosis is closely associated with multiple diseases [1]. Increasing the relative abundance of beneficial bacteria in selectivity has obvious benefits to human health. *Akkermansia muciniphila* is a representative gut microbe favoring for host immunity and metabolism [2,3], which can strengthen intestinal barrier by promoting mucus secretion [4]. Pharmacological studies revealed that the therapeutic effect of metformin on diabetes is partly mediated by promotion the *A. muciniphila* [5]. In addition, increasing *A. muciniphila* may enhance the anti-cancer efficacy of PD-1 antibodies [6,7]. Collectively, *A. muciniphila* is regarded as a therapeutic target for various diseases such as colitis, metabolic syndrome, immune diseases, and cancer [8]. Therefore, finding novel agent capable of promoting *A. muciniphila* growth will provide a new strategy for prevention and treatment of these diseases.

A. muciniphila growth depends on the mucous substrate such as

Abbreviations: BBR, berberine; DFMO, difluoromethylornithine; HFD, high-fat diet; GC-TOF/MS, gas chromatography–time-of-flight mass spectrometry.

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mucin [9], and the first suitable medium is the "mucin medium" containing gastric mucin as the sole carbon and nitrogen source [10]. As mucin can only be obtained from the intestinal mucus layer and is very expensive, it is now cost-inhibitive to achieve large-scale *A. muciniphila* production. Recently, novel prebiotics and small molecules have been intensively investigated for the promoting effect on *A. muciniphila*. In addition to metformin, as a agent found to be able to increase *A. muciniphila* [5]. It has been reported that gavage of oligosaccharides or herbal extract could enhance *A. muciniphila* in high-fat diet (HFD)-induced obese mice [11–13], and proanthocyanidins from blueberry polyphenol extract could increase the number of goblet cells and stimulate mucin secretion, thus enhancing the abundance of *A. muciniphila* [14]. As more *A. muciniphila*-promoting agents are discovered, the clinical utility of this beneficial bacterium and its novel regulatory methods will be greatly extended.

Berberine (BBR), an isoquinoline alkaloid, is widely distributed as a principal constituent in many medicinal plants belonging to *Berberis species* including *B. aristata*, *B. darwinii*, *B. petiolaris* and *B. vulgaris* [15,16]. BBR has been well-proven to be beneficial for various cardiovascular and metabolic diseases, and the gut microbiota has been recently recognized as an important target of BBR. Numerous studies have shown that BBR improves various diseases at least partially by regulating the gut bacteria, especially *A. muciniphila* [17]. The anti-obesity effect of BBR was reported to be associated with the increase of *Butyricimonas*, *Lactobacillus*, *Coprococcus*, *Ruminococcus*, and *Akkermansia* [18]. Another study found the anti-atherosclerotic action of BBR was accompanied with enrichment of *Akkermansia* [17,19]. Therefore, BBR is a potential prebiotic of *A. muciniphila*, which provides a novel insight into the pharmacotherapeutic mechanism. Although a large number of studies have demonstrated the promoting effect of various drugs on *A. muciniphila*, little is known about how BBR and other agents stimulate *A. muciniphila* growth.

In this study, we explored the potential mechanism through which BBR promotes *Akkermansia* growth. The in vivo administration and in vitro incubation experiments showed that BBR increased *Akkermansia* via stimulation of host colon mucin production and secretion. Fecal metabolomics analysis and polyamine inhibitor treatment also revealed that the promoting effect of BBR on *Akkermansia* growth and mucin production was gut microbes-independent.

2. Materials and methods

2.1. Animal experiment for dose-dependent impaction on gut microbes

All the animal experiments were performed in accordance with the National Institutes of Health regulations for the care and use of animals in research. The protocol was approved by the medical ethics committee of Peking Union Medical College. All efforts were made to minimize animal suffering.

Male specific pathogen-free ICR mice (8-week-old, 20–24 g) were obtained from Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). After acclimated for one week on a normal chow diet, mice were randomly divided into five groups with eight animals in each group. The blank control group continued feeding on normal chow (chow group) while other groups were fed a high fat-diet (HFD), containing 65% lipids, 15% proteins and 20% carbohydrates. Animals on HFD were orally administrated with BBR (100, 200 or 300 mg/kg per day) and assigned as BBR-100, BBR-200 or BBR-300 group. Other two groups were assigned as chow and HFD and given distilled water only. Body weight was assessed weekly. Stools for phylogenetic analysis were collected on the day before animals were euthanized. After 5 weeks of HFD feeding, mice were anesthetized in chambers saturated with isoflurane and then sacrificed by cardiac puncture. Blood was drawn in 1.5 mL centrifuge tubes and sera were separated for subsequent biochemical analysis.

2.2. Dynamic modulation of fecal parameters by oral administration of BBR

Male mice (8-week-old, n = 7 for each group) were fed with HFD or HFD+ BBR (200 mg/kg, p.o.) for 14 days. Fecal samples were taken on 0, 3, 7, 10 and 14 day after BBR treatment. Total bacteria number and the relative abundance of *A. muciniphila* were determined by real-time PCR using specific primers as follows: All bacteria, Uni331-F: TCCTACGGGAGGCAGCAGTG, E533-R: TTACCGCGGCTGCTGGCACG; *A. muciniphila*, Akk-F: CAGCACGTGAAGGTGGGGAC, Akk-R: CCTTGCGGTTGGCTTCAGAT.

2.3. The impact of inhibiting polyamine production on gut microbiota and mucin production

Male ICR mice (8-week-old, 20–24 g) were divided into four groups with eight animals in each group. All groups were fed on the HFD as mentioned above. The control group was given distilled water only, while other groups were gavaged with BBR (BBR group, 200 mg/kg per day), difluoromethylornithine (DFMO group, 1% in drinking water), or BBR+DFMO (200 mg/kg BBR + 1% DFMO in drinking water). Stools were collected on the day before animals were euthanized. After a 4-week treatment, mice were anesthetized in chambers saturated with isoflurane and then sacrificed by cardiac puncture. Jejunum, ileum and colon tissues were fixed in 4% formaldehyde, embedded in paraffin and cut at 4 μm. The sections were stained with alcian blue (for jejunum, ileum and colon), and their morphological changes were evaluated. The majority of colon tissue was snap-frozen in liquid nitrogen for subsequent biochemical analysis.

2.4. In vitro incubation

The culture fermentation vessels with 100 mL working volume, previously sterilized, and were filled with 15 mL of the modified Broth medium (Hopebio Ltd., China). Fecal samples obtained from healthy ICR mice were prepared into 5 mL of fecal slurry, prepared as 10% w/v in 0.1 M sterile PBS (pH 7.0), was inoculated into each vessel, respectively. The cultures were run for 24 h in the micro-anaerobic incubation system (INVIVO 400, Ruskin Technologies, UK). BBR was added into the vessels with the final concentration of 5, 10 and 20 μg/mL. After culture, the bacteria were collected by 12,000 rpm centrifugation for 20 min. The cell pellets were used for real-time quantitative PCR analysis to evaluate the direct influence of BBR on the *Akkermansia* growth.

2.5. 16S rRNA-based metagenomics analysis

Fecal microbiota DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, USA) and fecal microbial composition was assessed using Illumina HiSeq sequencing and QIIME-based microbiota analysis. Fecal microbiota DNA extraction, sequencing and microbiota analysis pipeline was performed by Shanghai Majorbio Biopharm Technology Co.,Ltd as previously reported [20].

2.6. Metabolomics analysis

The untargeted metabolomics profiling was performed on XploreMET platform (Metabo-Profile, Shanghai, China). The sample preparation procedures are referred in their previously published methods with minor modifications [21].

2.7. Real-time PCR

The colon tissue was obtained from each mice and used for real-time PCR analysis. Total RNA extraction and first-chain cDNA synthesis were performed by respective commercial kit (Solarbio, China). Real-time quantitative PCR was carried out on ABI7500 PCR platform (Applied

Biosystems, USA). The primers used in this study are provided in Table S1.

2.8. Mucin measure

The fecal content of mucin was determined by alcian blue as previous report[3]. Briefly, 150 mg of feces from each mouse were suspended in 1.5 mL of 0.1 M sodium acetate/acetic acid buffer, pH5.8, containing 25 mM $MgCl_2$. After centrifuged at 1870 g for 30 min, the supernatant (about 450 μL) was transferred into a clean 1.5 mL Eppendorf tube. 150 μL of 0.1% (w/v) Alcian blue solution was added into each sample and equilibrated for 2 h at room temperature. The mucin-Alcian blue complexes were sedimented at 1870 g for 30 min. Supernatant solutions were discarded, and each pellet was washed twice in 40% (v/v) ethanol/0.1 M sodium acetate/acetic acid buffer. Mucin-Alcian blue complexes were dissociated by addition of 1 mL of Manoxol 1B solution and ultrasonication at 50 W for 10 s. Samples were centrifuged as above for 1 min to remove the foam generated during sonication. The absorption at 620 nm (OD620nm) was measured using a Tecan Infinite M1000Pro Microplate Reader (TECAN Group Ltd. Shanghai, China). The relative content of mucin in each animal was calculated based on the OD620nm, with the average OD620nm of chow-fed group as 1 fold.

2.9. Statistics

Data are presented as the means ± sem. SPSS 17.0 software was used for the statistical analysis. The significance of group differences was assessed by one-way ANOVA followed by Newman−Keuls post hoc tests. The significance of the association was evaluated with Spearman's rank correlation test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. BBR dose- and time-dependently increased Akkermansia in mice

Oral administration of BBR significantly suppressed HFD-induced obesity and hyperlipidemia without decreasing food intake (Fig. 1A−D). Simultaneously, BBR shifted the gut microbiota structure in a dose-dependent manner (Fig. 1E and F). The abundance of *Bacteroides*, *Clostridium* clusters XI/XVIII/XIVa, *Blautia*, *Coprobacillus* and *Akkermansia* were significantly increased by BBR (Fig. 1G). Compared with mice treated with 100 or 200 mg/kg BBR, the gut microbiota of animals that received 300 mg/kg BBR was quite different (Fig. 1G), suggesting a much high dosage of BBR may act like a weak antibiotic. Notably, the relative abundance of *Akkermansia* in HFD group was only 0.01%, and it was increased to 5.40%, 4.85% and 10.56% by BBR at 100, 200 and 300 mg/kg, respectively (Fig. 1G). We then performed a time-course experiment to check the time-dependent impact of BBR on *Akkermansia*. BBR did not significantly increased the abundance of *Akkermansia* until treatment for 10 days and thereafter the promotion became more pronounced (Fig. 1H, Table S2). These results suggest that BBR can increase *Akkermansia* in a dose- and time-dependently manner in vivo.

3.2. BBR did not promote Akkermansia when directly incubated in vitro

To verify whether BBR promotes *Akkermansia* via a direct or indirect way, we directly incubated fecal microbiota in vitro with BBR at a concentration of 5, 10 and 20 μg/mL. As shown in Fig. 2, direct incubation with BBR did not increase nor decrease the abundance of *Akkermansia* within 72 h of incubation These results suggest that BBR could not promote the growth of *Akkermansia* directly. However, because this in vitro assay was carried out without a good positive control, further experiments should be performed to confirm this conclusion.

3.3. BBR increased mucin production in mice

As *Akkermansia* uses mucin as an essential nutrient for its survival and proliferation [22], we assessed the effect of BBR on mucin-producing gene expression and mucin secretion. Treatment with BBR significantly increased the transcription of mucin-producing genes (*MUC2*, *MUC3*, *MUC4* and *MUC13*) (Fig. 3A). The most responsive mucin-producing gene was *MUC2* whose mRNA level was enhanced by more than 7 fold after BBR treatment (Fig. 3A). Accordingly, BBR markedly promoted mucin secretion in colon as revealed by alcian blue staining (Fig. 3B). These results suggest that BBR may promote *Akkermansia* growth via stimulation of mucin production in the intestinal lumen.

3.4. BBR increases polyamine content in feces

To explore the potential mechanism through which BBR stimulates mucin production, we performed untargeted metabolomics analysis by gas chromatography-time-of-flight mass spectrometry (GC-TOF/MS) on the fecal samples, to identify the active metabolites (Table S3). Oral administration of BBR significantly changed the gut microbial metabolites in mice (Fig. 4A and B). BBR largely increased polyamine-related metabolites such as cadaverine, putrescine, and 5-aminopentanoic acid, while markedly decreased sphingosine, L-tryptophan and erythrose (Fig. 4C−E). Polyamines were reported to promote colonic mucosal growth [23], implying that polyamines may participate in the stimulating effect of BBR on mucin production and *Akkermansia* growth.

3.5. Inhibition of eukaryotic polyamine production did not abolish the promoting effect of BBR on mucin production and Akkermansia growth

To further elucidate whether BBR promoting the *Akkermansia* growth is induced by promoting polyamines production, we treated HFD-fed mice with polyamine biosynthetic inhibitor DFMO (1% in drinking water) for 4 weeks. DFMO is a mammalian polyamine synthase [24] and previous study has shown that oral DFMO is effective in depleting polyamines in rectal mucosa [25]. Treatment with BBR altered the structure of gut microbiota and supplementary of DFMO did not significantly influence its modulatory effect (Fig. 5A−C). Quantification of *Akkermansia* abundance showed that inhibiting the polyamine production by DFMO did not abolish the enhancing effect of BBR on *Akkermansia* (Fig. 5D). Accordingly, BBR-elicited mucin production in colon was not suppressed by DFMO either (Fig. 5E and F). However, DFMO is a mammalian polyamine synthase [24] and it is possible that there were additional gut bacteria-derived polyamines in the colon cavity of DFMO-treated mice. Therefore, these results could only indicate that the mammal host-derived polyamines may not involve in the *A. muciniphila*-promoting action of BBR. More specific inhibitor for gut bacteria-derived polyamine production should be used in the future to discern whether gut bacteria-produced polyamines involved in the *A. muciniphila*-promoting action of BBR. And further investigations on the effect of gut bacteria-derived polyamines would provide valuable clues.

3.6. BBR decreased the mRNA levels of key genes involved in notch signaling

It has been reported that the notch signaling pathway is a inhibitive factor for the differentiation of goblet cells and mucin production [26]. We therefore tested the effect of BBR on notch signaling in colon by quantitative PCR. Our results showed that HFD feeding increased the mRNA levels of key notch signaling elements such as *notch1*, *jagged1*, and *hes1*, indicating activation of notch pathway (Fig. 6). Treated with BBR significantly decreased the transcription of these genes, suggesting that BBR may inhibit notch signaling in colon tissue and providing a potential mechanism for BBR-induced mucin production and

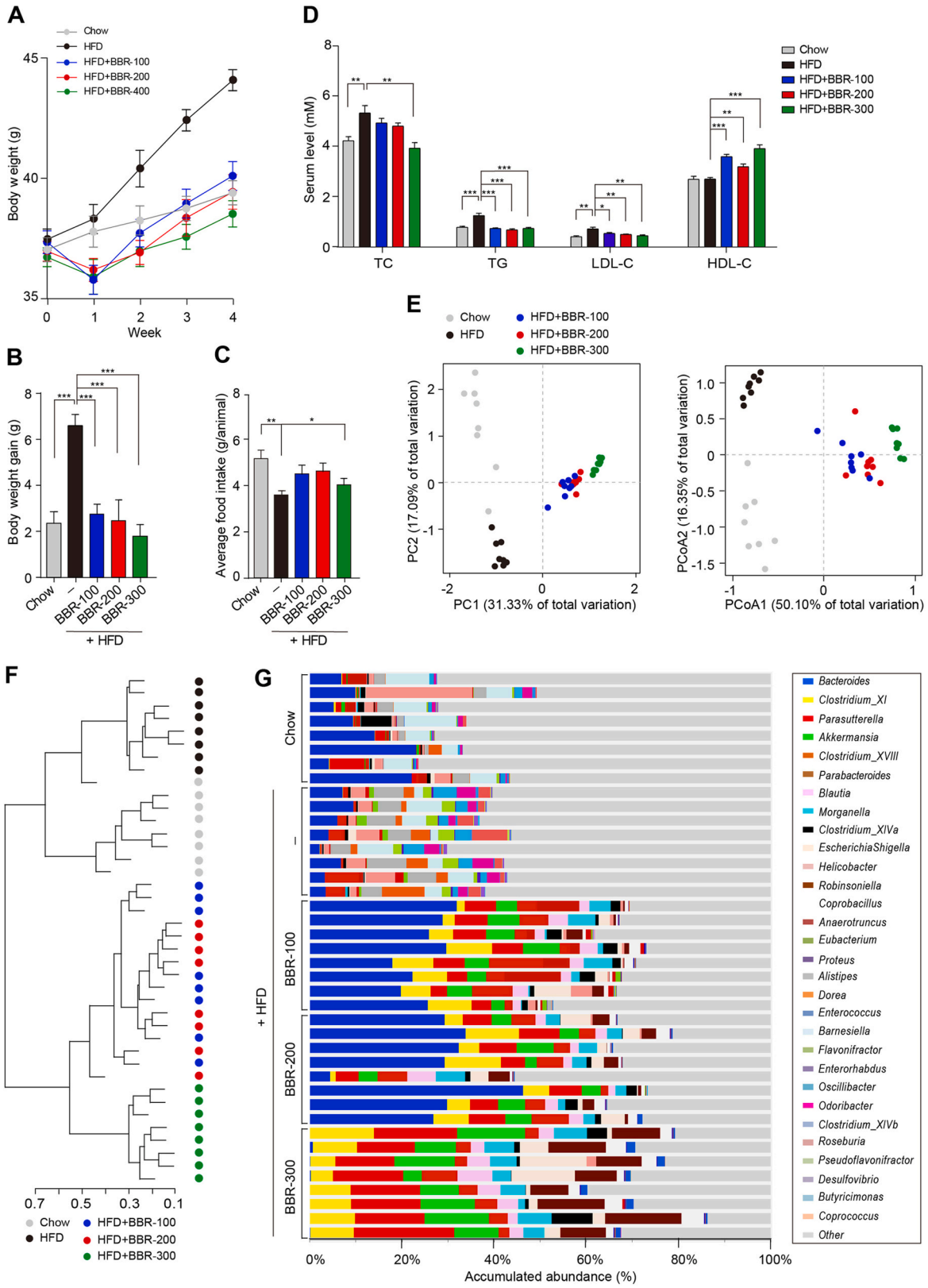

Fig. 1. BBR ameliorated high-fat diet (HFD)-induced hyperlipidemia and modulated gut microbiota in a dose-dependent manner. The time-dependent bodyweight change (A), bodyweight gain (B), average food intake (C), and serum lipid levels (D) are shown. PCoA plots (E) of microbial communities were based on genus composition; each treatment group is represented by different color/symbol combinations. (F) Average clustering of the microbial communities based on Euclidean analysis. (G) Major bacterial genera that were significantly different among the groups. (H) Time-dependent modulation of BBR on abundance of *Akkermansia*. N = 8 for each group. Data are expressed as mean±sem. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

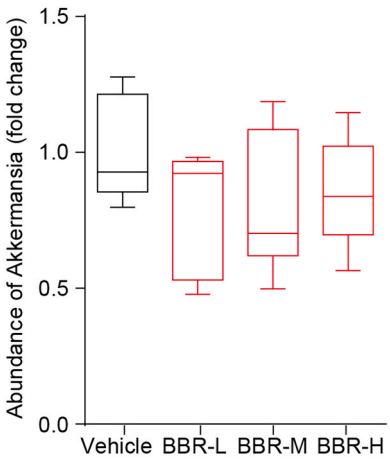

Fig. 2. BBR did not promote *Akkermansia* growth in vitro. The fecal microbiota was incubated for 24 h in the micro-anaerobic incubation system. BBR was added with the final concentration of 5, 10 and 20 μg/mL and indicated as BBR-L, BBR-M and BBR-H, respectively.

Akkermansia growth.

4. Discussion

Akkermansia muciniphila plays critical roles in maintaining human immunity and metabolism and is deemed as a promising therapeutic probiotic [3]. However, its growth highly depends on expensive mucous substrates [9], which prevents it from cost-efficient large-scale cultivation and broad clinical application. Therefore, finding suitable agents and exploring their mechanisms in promoting *A. muciniphila* growth are of great importance. Previous studies have shown that BBR can increase the abundance of *A. muciniphila* [17,19], but the underlying mechanism remains largely unknown. In this work, we demonstrated that BBR is a potent prebiotic of *A. muciniphila*. It can stimulate *A. muciniphila* growth in a dose- and time-dependent manner in vivo but not in vitro. Our results further revealed that the *A. muciniphila*-enhancing action of BBR may be achieved by stimulating mucin production in host intestine, although it needs further verification in Muc2-/- mice. We also found that BBR could increase the fecal contents of polyamines which have been reported to be able to promote goblet cells proliferation and differentiation and thus may increase mucin production. However, inhibition of polyamine production by specific inhibitor for mammal polyamine synthase did not abolish the beneficial effect of BBR on mucin production and *A. muciniphila* growth, indicating that the mammalian host-derived polyamines were not the main source to support BBR promoting mucin production and *A. muciniphila* growth. Further investigations on the effect of gut bacteria-derived polyamines would provide valuable clues.

Berberine (BBR) is a natural alkaloid with multiple pharmacological effects. However, the low oral bioavailability of BBR severely impedes the understanding of its actual mechanism in vivo [27]. An increasing body of investigations has shown that BBR may exert its pharmacotherapeutic effects via modulation of gut microbiota. Specifically, BBR has been reported as a potential promoter for *A. muciniphila* [18]. The anti-obesity and anti-atherosclerosis effects of BBR are closely related to the enrichment of *Akkermansia* [19]. Despite the knowledge about the stimulating role of BBR on *A. muciniphila*, how BBR stimulates *A. muciniphila* growth still remains largely unknown. In this study, we first demonstrated that BBR increases *Akkermansia* in a dose- and time-dependent manner in vivo. However, direct incubation of gut microbiota with BBR in vitro did not promote the growth of *Akkermansia*. Therefore, BBR may stimulate *Akkermansia* growth through an indirect way.

It is well-known that host mucin is an essential nutrient for *Akkermansia* [9]. Previous studies have investigated the modulation of BBR on gut mucin, but their results remain contradictory [28–30]. Our study showed that BBR significantly enhanced the expression of mucin-producing genes and thus stimulated mucin production and secretion in host colon. These results provided solid evidence that BBR may promote *Akkermansia* growth via stimulation of mucin production in host intestinal lumen. Recent studies also demonstrated that BBR could directly increase the expression of mucin genes in IPEC-J2 intestinal epithelial cells [31] and increase mucin release in airway mucin-secreting cells [30], suggesting that BBR can stimulate mucin production in-dependent of bacteria.

We then performed metabolomics analysis on fecal samples to rectify whether BBR-modulated gut microbiota involves in the regulation of host mucin production. BBR markedly altered gut microbial metabolites, and polyamines were the most increased, which is in accordance with previous reports [32]. It has been reported that administration of polyamines could stimulate goblet cell proliferation and differentiation and colonic mucosal growth [23]. However, our study found inhibition of human polyamine production had no influence on the promoting effect of BBR on mucin production and *Akkermansia* growth. These results suggest that depletion of polyamines in rectal mucosa by DFMO [25] does not abolish BBR-elicited mucin secretion and *A. muciniphila* growth. Unfortunately, DFMO is a mammalian polyamine synthase [24] and we cannot exclude the possibility that there were still additional gut bacteria-derived polyamines in the colon cavity of DFMO-treated mice. Therefore, more specific inhibitor for gut bacteria-derived polyamine

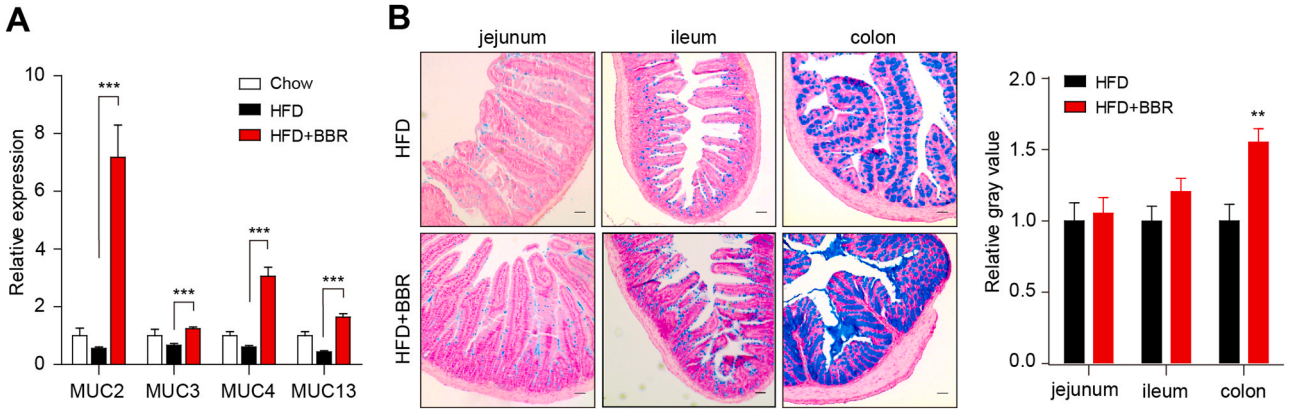

Fig. 3. BBR stimulated mucin-producing gene expression and mucin production in mice. (A) Relative mRNA levels of mucin-generating genes in colon. The total mRNA was extract from the whole colon tissue. (B) Alcian blue staining of colon. Bar = 50 μm. The dose of BBR was 200 mg/kg. Data are expressed as mean±sem. $^{*}P < 0.05$, $^{***}P < 0.001$, HFD+BBR vs HFD control.

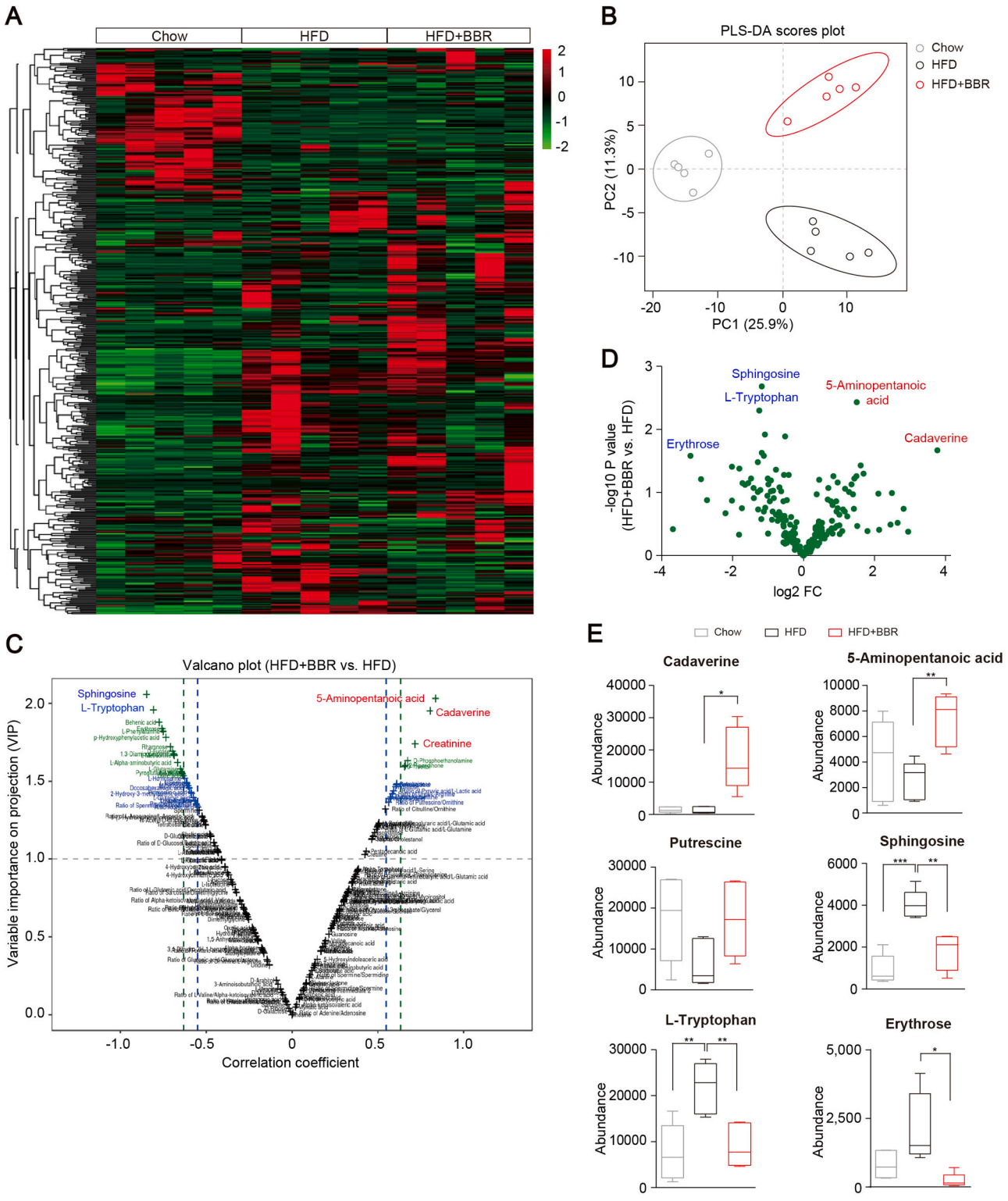

Fig. 4. Untargeted metabolomics analysis of BBR-treated fecal samples. (A) Heatmaps of hierarchically clustered GC-TOF/MS features. (B) PLS-DA scores plot revealing classifications of the subjects. (C) Visualization of differential metabolite profiles using volcano plot. (D) Enhanced volcano plot showing differential metabolite profiles. (E) Top-ranked differential metabolites between the HFD and HFD+BBR groups. The dose of BBR was 200 mg/kg. Data are expressed as mean±sem. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, HFD+BBR vs HFD control.

production should be used in the future to discern whether gut bacteria-produced polyamines involved in the *A. muciniphila*-promoting action of BBR. Further investigations on the effect of gut bacteria-derived polyamines would provide valuable clues.

Investigations have shown that the notch signaling pathway plays key roles in the differentiation of goblet cells [33]. Activation of notch signaling results in decreased differentiation of goblet cells and less mucin production. Previous studies demonstrated that BBR is a potent notch inhibitor [26]. Treatment with BBR significantly inhibited notch activation and signaling in mice [26]. In our study, we also found that

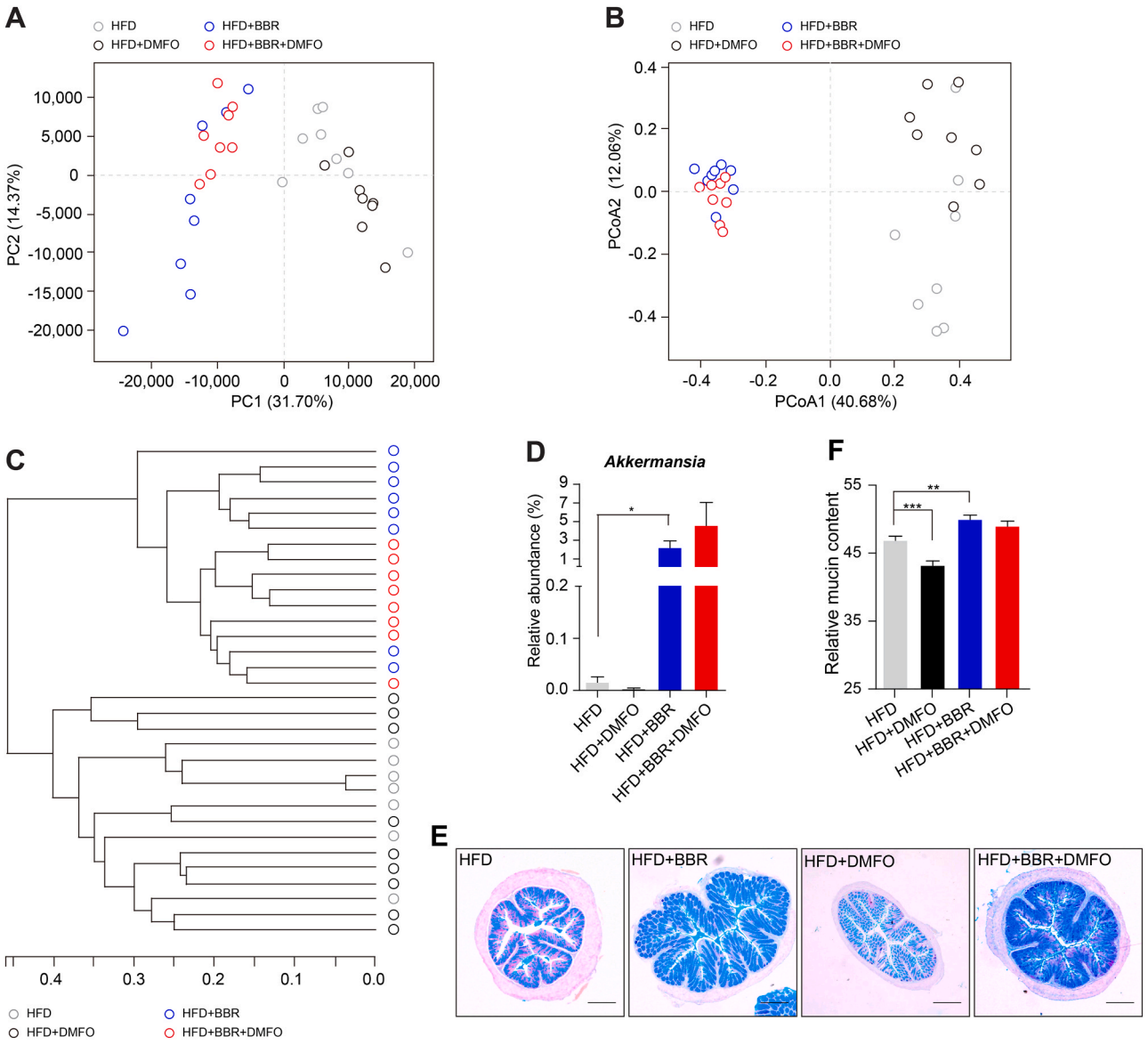

Fig. 5. Inhibition of polyamide production did not disrupt the modulating role of BBR on *Akkermansia* and mucin production. PCA (A) and PCoA (B) plots of microbial communities were based on OTU composition; each treatment group is represented by different color/symbol combinations. (C) Average clustering of the microbial communities based on Euclidean analysis. (D) Relative abundance of *Akkermansia* among groups. (E) Alcian blue staining of colon. (F) Relative mucin content in colonic tissue. The dose of BBR was 200 mg/kg. Data are expressed as mean±sem. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs HFD control.

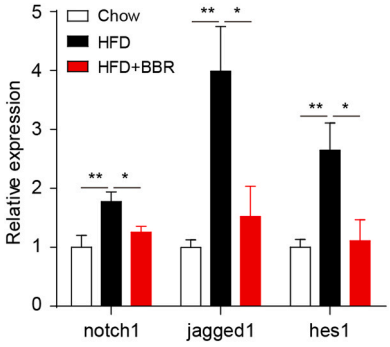

Fig. 6. BBR inhibited mRNA levels of notch signaling genes in colon. The total mRNA was extract from the whole colon tissue. N = 8 for each group. The dose of BBR was 200 mg/kg. Data are expressed as mean±sem. $^{*}P < 0.05$, $^{**}P < 0.01$.

BBR significantly decreased the mRNA levels of key notch signaling elements such as *notch1*, *jagged1*, and *hes1*, suggesting that BBR may promote mucin production via, at least partially, inhibition of notch signaling pathway, thus providing a potential mechanism through which BBR enhances *A. muciniphila* growth.

5. Conclusions

Our results provided solid evidence proving that BBR is a potent promoter for *A. muciniphila*, and it stimulates *A. muciniphila* growth via enhancing mucin production in host intestine. These findings not only provide a novel prebiotic for *A. muciniphila*, but also put forward a novel insight into the modulation of functional gut microbes.

CRediT authorship contribution statement

Chongming Wu conceived the project, designed the experiments. Jiaqi Yu, Yanan Yang and Fang Zhang performed most of the

experiments with the help of Wenquan Su and Qinhua Fan. Chongming Wu, Chaoran Dong and Shengxian Wu analyzed the data and prepared figures. Chaoran Dong and Chongming Wu wrote the manuscript. Jiaqi Yu and Yanan Yang assisted in manuscript preparation.

Conflict of interest statement

There are no conflicts of interest to declare.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2021.111595.

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