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Protective effects of different *Bacteroides vulgatus* strains against lipopolysaccharide-induced acute intestinal injury, and their underlying functional genes

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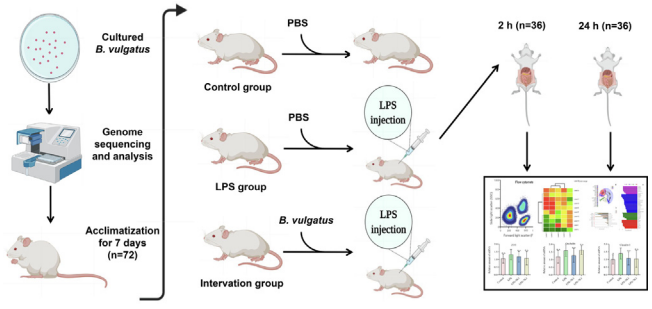
HIGHLIGHTS

- Different *Bacteroides vulgatus* strains have varying effects on inflammatory diseases.
- *B. vulgatus* FTJS7K1 was screened due to its role in alleviating inflammation.
- *B. vulgatus* FTJS7K1 can modulate gut microbial community.
- *B. vulgatus* FTJS7K1 can regulate the levels of related cytokines.
- The genes about SCFAs secretion are responsible for the anti-inflammatory effect.

GRAPHICAL ABSTRACT



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ABSTRACT

Introduction: The roles of *Bacteroides* species in alleviating inflammation and intestinal injury has been widely demonstrated, but few studies have focused on the roles of *Bacteroides vulgatus*.
Objectives: In this study, four *B. vulgatus* strains were selected, based on their genomic characteristics, to assess their ability to alleviate lipopolysaccharide (LPS)-induced acute intestinal injury in C57BL/6J mice.
Methods: Alterations in the intestinal microbiota, intestinal epithelial permeability, cytokine level, short-chain fatty acid (SCFA) concentration, and immune responses were investigated following LPS-induced acute intestinal injury in C57BL/6J mice.
Results: Severe histological damage and a significant change in cytokine expression was observed in the mouse colon tissues 24 h after LPS administration. Oral administration of different *B. vulgatus* strains showed different effects on the assessed parameters of the mice; particularly, only the administration of *B. vulgatus* FTJS7K1 was able to protect the architectural integrity of the intestinal epithelium. *B. vulgatus* FTJS7K1 also negated the LPS-induced changes in cytokine mRNA expression in the colon tissues, and in the proportion of regulatory T cells in the mesenteric lymph node. Compared with the LPS group, the *B. vulgatus* FTJS7K1 group showed significantly increased abundance of *Lactobacillus*, *Akkermansia*, and *Bifidobacterium*, and decreased abundance of *Faecalibaculum*. The *B. vulgatus* FTJS7K1 group also showed significantly increased concentration of SCFAs in fecal samples. The results of genomic analysis

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showed that these protective roles of *B. vulgatus* FTJS7K1 may be mediated through specific genes associated with defense mechanisms and metabolism (e.g., the secretion of SCFAs).
Conclusions: Our findings suggest that the protective role of *B. vulgatus* FTJS7K1 appear to be via modulation of cytokine production in the colon tissue and regulation of the structure of the gut microbiota. These results provide support for the screening of the *Bacteroides* genus for next-generation probiotics.

Introduction

The role of probiotic strains in the treatment of several diseases has been validated [1]. Among these, the *Bacteroides* genus is a primary candidate for next-generation probiotics (NGPs), which have attracted considerable attention due to their relationship with host health and disease. Several studies have shown that the development of some diseases is associated with the ecological status and abundance of some intestinal bacterial species [2,3]. For example, the abundance of the genus *Bacteroides* is significantly decreased in patients with inflammatory bowel disease (IBD) when compared with healthy humans [4–6]. Moreover, the occurrence of colorectal cancer (CRC) has been shown to be positively correlated with the abundance of *B. fragilis* but negatively with that of *B. vulgatus* and *B. uniformis* [7]. In addition, animal experiments have demonstrated the role of certain *Bacteroides* strains in alleviating disease through regulating body metabolism [8] and inhibiting the colonization of pathogenic bacteria [9].

The role of *Bacteroides* in alleviating intestinal inflammation [10] and LPS-induced systemic inflammation [11] is a hot topic. Oral *B. ovatus* ELH-B2 and *B. fragilis* HCK-B3 can relieve LPS-induced inflammation by altering the gut microbiota, regulating cytokine production, and maintaining the regulatory T cell (Treg)/Th-17 balance [11]. Another strain, *B. fragilis* ZY-312 can help to restore intestinal barrier function and induce enterocyte regeneration in rats with antibiotic-associated diarrhea [12]. Given the extraordinary advantages of *Bacteroides* species in alleviating inflammation, ongoing research has focused on screening more *Bacteroides* species with probiotic roles for their scientific and clinical prospects.

The roles of different *Bacteroides* species in alleviating diseases have been demonstrated in a series of studies, including inhibiting tumor [13], protecting against colitis [14], alleviating obesity [15], preventing atherosclerosis [16], etc. Interestingly, different species of *Bacteroides* regulated the human health and diseases through different mechanisms. For example, protective role of oral *B. fragilis* ATCC 25285 in gut integrity was attributed to gut microbial metabolites especially SCFAs [17]. Moreover, capsular polysaccharide A (PSA) could help induce Tregs differentiation in the intestine and thus promote secretion of anti-inflammatory cytokine IL-10 to reduce local inflammation [18,19]. The biosynthesis of PSA of some *Bacteroides* species has been widely considered as a main pathway to alleviate diseases and regulate immune. Moreover, regulating immune response and enhancing phagocytosis of macrophages were also considered as important ways to affect human health. A study by Solis et al. showed that some species of *Bacteroides* can protect against the colonization of *Klebsiella pneumoniae* via IL-36 signaling and enhancing phagocytosis of macrophages, and thus regulate human health [20].

B. vulgatus is a *Bacteroides* species that is common in the human colon [21,22], and is considered to be associated with a healthy host gut [23,24]. Some studies have demonstrated the roles of *B. vulgatus* in protecting against *Escherichia coli* mpk-induced colitis [25], preventing dextran sulfate sodium salt (DSS)-induced acute colitis, and alleviating inflammation and intestinal damage [26]. However, some studies have shown inconsistent results. One such study showed that the abundance of *B. vulgatus* in the total gut microbiota of patients with ulcerative colitis (UC) is higher than that in healthy humans [27], suggesting that the abundance of *B. vulgatus* is associated with the severity of IBD. Indeed, this hypothesis has been proven in some animal experiments, where it has been shown that *B. vulgatus* can induce the expression of pro-inflammatory cytokines [28,29] and adhere to the colonic tissue in patients with UC [30]. These findings suggest that oral administration of different *B. vulgatus* strains has varying effects on inflammatory diseases. Considering these findings, it is important to clarify the complex relationship between *B. vulgatus* and inflammation and establish the reason for the variation in roles between strains.

This study aimed to determine the roles of different strains of *B. vulgatus* in alleviating LPS-induced acute intestinal injury, and to clarify the functional genes that are responsible for the anti-inflammatory effect of selected *B. vulgatus* strains by analyzing the genomic characteristics.

Materials and methods

Bacterial strains and preparation

Seven *B. vulgatus* strains, named FTJS7K1, FTJS5K1, FSDTA11B14, FJSWX62K35, FSDLZ51K1, FBJS10K3 and FGSZY37K4, were isolated from healthy human fecal samples. Among these, *B. vulgatus* FTJS5K1 and FTJS7K1 have been deposited in the Culture Collections of Food Microbiology, Jiangnan University (Wuxi, China). All seven strains were cultured and purified twice by streaking on modified brain heart infusion (BHI) [11] (with 2% agar, pH 7, 37 °C) plates under anaerobic conditions for 48 h. Following incubation, a single colony was selected and cultured in BHI broth (pH 7, 37 °C) for 18 h. The cells were collected by centrifugation at 6000*g* for 10 min, washed three times by using 1 × phosphate buffer saline (PBS), and finally resuspended in 1 × PBS to the final concentration 1 × 10^9 colony-forming unit (CFU).

Genome sequencing, pan-genome analysis, clusters of orthologous group (COG) annotation and phylogenetic tree construction

The genomic DNA of the seven *B. vulgatus* strains was extracted and sequenced on an Illumina Hiseq system (Majorbio) as described previously [31,32]. The genome sequences of the two most studied strains, *B. vulgatus* mpk and *B. vulgatus* ATCC8482 (type strain), were obtained from the National Center for Biotechnology Information (NCBI) database (Table 1). Additionally, a neighbor-joining phylogenetic tree [33] was established by phyML3 to show the relationships between different strains of *B. vulgatus* [34] after alignment of the core genes identified by a graph theory-based Markov clustering algorithm using multiple alignment using fast fourier transform (MAFFT) [35]. Roary software was used for pan-genomic analysis to identify the core genes of *B. vulgatus* [36]. BLASTp software was used to analyze the differences in functional genes to perform clusters of orthologous group (COG) annotation against the COG database [37].

Table 1
B. vulgatus were used in this study.

Strains name	Isolate Location	Isolation Source	Genome Accession Number
ATCC8482	–	–	NC_009614.1
mpk	–	–	CP013020.1
FTJS7K1	Tianjin, China	Human fecal	JACBPY000000000
FTJS5K1	Tianjin, China	Human fecal	JACBPX000000000
FSDTA11B14	Taian, Shandong Province, China	Human fecal	JACBPW000000000
FJSWX62K35	Wuxi, JiangsuProvince, China	Human fecal	JACBPU000000000
FSDLZ51K1	Laizhou, ShandongProvince, China	Human fecal	JACBPV000000000
FBJS10K3	Beijing, China	Human fecal	JACBPS000000000
FGSZY37K4	Zhangye, Gansu Province, China	Human fecal	JACBPT000000000

Note: The genome sequencing of *B. vulgatus* ATCC8482 and *B. vulgatus* mpk were collected from NCBI. The genome sequencing of other 7 *B. vulgatus* strains were completed in this study. *B. vulgatud: Bacteroides vulgatus*

Animal experimental design

Seventy-two 6-week-old male SPF C57BL/6J mice (18–20 g) were purchased from SLAC Laboratory Animal Co., Ltd. (Shanghai, China). The mice were divided into six groups based on their initial weight, with each group containing 12 mice. The mice were housed in an air-conditioned room (21–25 °C) with a relative humidity of 40–60%) and subjected to a 12-h light/dark cycle. The mice were provided with food and water adlibitum, and were allowed a period of 1 week to adapt to the environment of the laboratory. Following acclimatization, the mice in the treatment groups were maintained on their respective diets for 1 week as follows:

1) Control group: Underwent daily gavage with 0.2 ml filter-sterilized phosphate buffered solution (PBS).
2) LPS group: Underwent daily gavage with 0.2 ml PBS for 5 days, followed by an intraperitoneal injection of LPS (0.1 mg/kg in filter-sterilized PBS).
3) *B. vulgatus* intervention groups, including FTJS7K1, FTJS5K1, FSDTA11B14 and FSDLZ51K1, respectively: Underwent daily gavage with the suspension of the corresponding *B. vulgatus* strain (1×10^9 CFU/mL at a dose of 10 ml/kg body weight) for 5 days, followed by an intraperitoneal injection of LPS (0.1 mg/kg in filter-sterilized PBS).

Two hours and 24 h after the intraperitoneal injection of LPS, fecal samples were collected after defecation in sterile fecal collection tubes under aseptic conditions and then frozen at −80 °C until required for genomic DNA extraction. In addition, equal number of mice from each group were sacrificed under isoflurane 2 h (n = 36) or 24 h (n = 36) after the intraperitoneal injection of LPS, and their colons were collected. A part of each colon (1.0 cm × 1.0 cm) was fixed with paraformaldehyde (4%), and the colonic tissues were stored at −80 °C for subsequent RNA extraction.

Ethics statement

All experiments involving animals were conducted according to the ethical policies and procedures approved by the Committee of Ethics in Jiangnan University, China (Approval no. JN. No. 20190915c0921115[224]). All of the procedures involving the use and care of animals complied with the guidelines of the European Community (Directive 2010/63/EU).

Analysis of Foxp3 + Treg cells

Foxp3 + Treg cells were analyzed by flow cytometry using the Mouse Regulatory T Cell staining kit (eBioscience, USA) with a small modification in the manufacturer's instructions [38,39] as follows: 1) Mesenteric lymph nodes (MLNs) were isolated from mice and immediately homogenized in pre-cooled PBS; 2) the homogenate was then filtered to harvest the MLN cells; 3) the cells were centrifuged (300*g* for 5 min, 4 °C), re-suspended in PBS, and stained for CD4, CD25, and Foxp3. A FACSCalibur flow cytometer (BD Biosciences, USA) was used to examine the cell populations, and FlowJo software (Tree Star, USA) was used to analyze the flow cytometry data.

Histological evaluation

To evaluate the histopathological damage induced by LPS, the colon samples were transferred to 70% ethanol for dehydration, followed by clearing and staining with hematoxylin and eosin. A Leica BA410E microscope (Motic China Group Ltd) was used to capture the images. The slides were graded by blinded assessors to assess the severity of tissue damage as described previously [40].

Extraction of total RNA and real-time quantitative polymerase chain reaction (RT-qPCR)

The total RNA of the colon tissue was extracted using a FastPure Cell/Tissue Total RNA Isolation Kit, and the cDNA was synthesized with a RevertAid First Strand cDNA Synthesis Kit (both from Vazyme Biotech Co., Ltd.; Nanjing, China). Gene expression of ZO-1, Occludin, Claudin-1, IL-6, IL-10, and TNF-α were assessed by real-time quantitative polymerase chain reaction (RT-qPCR) [41]. The primers used in this study are shown in Table 2.

Fecal sample collection and genomic DNA extraction

Mouse feces (0.1 g) were used to perform genomic DNA extraction with the Fast DNA SPIN Kit for Feces (MP Biomedicals; Carlsbad, CA, USA). Sequencing of the gut microbiota genomes was performed as described in a previous study [42]. LEfSe software was used to analyze the differences in the gut microbiota between the groups.

Assessment of short-chain fatty acid (SCFA) production

Fecal samples were collected in 2-mL tubes and stored at −80 °C. The concentration of SCFAs was determined by Gas Chromatography-Mass Spectrometer (GC–MS) analysis of 20–50 mg dry weight feces according to a previously described method [1].

Statistical analysis

Data analysis was performed using GraphPad Prism software version v8.0.2.263. The means and standard errors for each group are reported as the mean ± standard error of the mean. *P*-values < 0.05 were taken to indicate statistical significance. When the results of an analysis of variance was statistically significant, Tukey's test was determined to compare the means.

Table 2
Primer sequences used for QPCR analysis.

Gene	Forward	Reverse
β-actin	5′-GGCTGTATTCCCCTCCATCG-3′	5′-CCAGTTGGTAACAATGCCATGT-3′
ZO-1	5′-CTTCTCTTGCTGGCCCTAAAC-3′	5′-TGGCTTCACTTGAGGTTTCTG-3′
Occludin	5′-CACACTTGCTTGGGACAGAG-3′	5′-TAGCCATAGCCTCCATAGCC-3′
Claudin1	5′-GATGTGGATGGCTGTCATTG-3′	5′-CCTGGCCAAATTCATACCTG-3′
IL-6	5′-TACCACTTCACAAGTCGGAGGC-3′	5′-CTGCAAGTGCATCATCGTTGTTC-3′
IL-10	5′-GCTCTTACTGACTGGCATGAG-3′	5′-CGCAGCTCTAGGAGCATGTG-3′
TNF-α	5′-GGTGCCTATGTCTCAGCCTCTT-3′	5′-GCCATAGAACTGATGAGAGGGAG-3′

Note: IL-6: interleukin-6; IL-10: interleukin-10; TNF-α: tumor necrosis factor alpha.

Results

Genetic diversity and evolution of B. vulgatus strains

All *B. vulgatus* strains were found to share 2044 orthologous genes (Fig. 1). Phylogenetic analysis based on the 2044 core genes showed that the nine *B. vulgatus* strains could be divided into different branches. Four *B. vulgatus* strains (FTJS5K1, FTJS7K1, FSDLZ51K1, and FSDTA11B14) from different branches were selected for further assessment of their effects against LPS-induced acute inflammation and intestinal injury in mice.

Effects of B. vulgatus on the physiological indices of LPS-treated mice

Compared to the control group, LPS injection had no significant effect on the spleen index after 2 h of LPS injection, but led to a significant increase in the spleen index after 24 h of LPS injection (Fig. 2). Based on this result, we examined the following indexes 24 h after LPS injection only.

Modulation of Treg cells

LPS injection (after 24 h) significantly increased the number of Treg cells in the MLNs of mice (Fig. 3). Oral gavage of *B. vulgatus* FTJS5K1, *B. vulgatus* FTJS7K1 and *B. vulgatus* FSDTA11B14 could significantly negate the LPS-induced upregulation in the number of Treg cells when compared with the control group.

Effect of B. vulgatus on LPS-inducped colonic tissue injury

Compared with the control group, the colon tissue of LPS-induced (after 24 h) mice showed severe histological damage (Fig. 4). *B. vulgatus* FTJS7K1, but not other strains, could protect the integrity of the intestinal epithelium, significantly inhibit inflammatory cell infiltration, and significantly decrease the DAI score.

Effect of B. vulgatus on intestinal barrier disruption and secretion of inflammatory factors in LPS-treated mice

As shown in Fig. 5, when compared with the control group, *B. vulgatus* FTJS7K1 had no significant effect on the mRNA expression of ZO-1, Claudin-1, and Occludin in the colon tissue of mice injected with LPS. In contrast, orally administered *B. vulgatus* FTJS7K1 significantly upregulated the mRNA expression of the anti-inflammatory cytokine IL-10 ($p < 0.05$), and downregulated the mRNA expression of the pro-inflammatory cytokine TNF-α ($p < 0.05$).

Effect of B. vulgatus on the composition of the bacterial community

At the phylum level, Verrucomicrobia, Firmicutes, Bacteroidetes, and Actinobacteria were the most abundant in fecal samples after 24 h (Fig. 6A). Compared with the control group, LPS injection (after 24 h) could significantly increase the abundance of Actinobacteria and decrease that of Firmicutes. Oral administration of *B. vulgatus* FTJS7K1 could significantly restore the Firmicutes-to-Bacteroidetes ratio. The result of principal co-ordinates analysis (PCoA) showed that the gut microbiota compositions of the control group and the *B. vulgatus* FTJS7K1 group were significantly different from that of the LPS group (Fig. 6B). At the genus level, the abundance of *Dubosiella* and *Faecalibaculum* was significantly reduced in the LPS group, while that of *Dubosiella*, *Lactobacillus*, *Akkermansia*, and *Bifidobacterium* was significantly increased in the *B. vulgatus* group (Fig. 6C).

Effect of B. vulgatus on SCFA concentration in feces

As shown in Fig. 7, there was no significant difference in the concentration of SCFAs between the control group and the LPS group after 24 h. Compared with the LPS group, oral administration of *B. vulgatus* FTJS7K1 could significantly increase the levels of acetate, propionate, isobutyrate, valerate, and isovalerate in fecal samples.

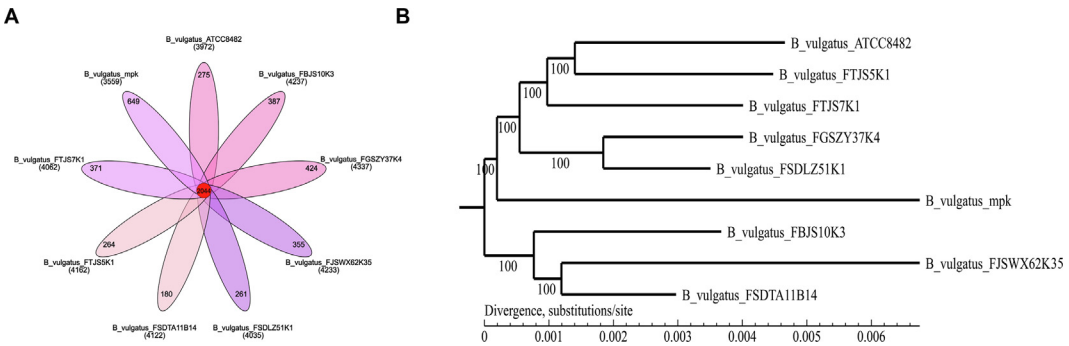


Fig. 1. Core genes and phylogenetic analysis of *Bacteroides vulgatus* strains. (A) Venn diagram of homologous clusters shared among the core genes. (B) Phylogenetic analysis of nine strains of *B. vulgatus*.

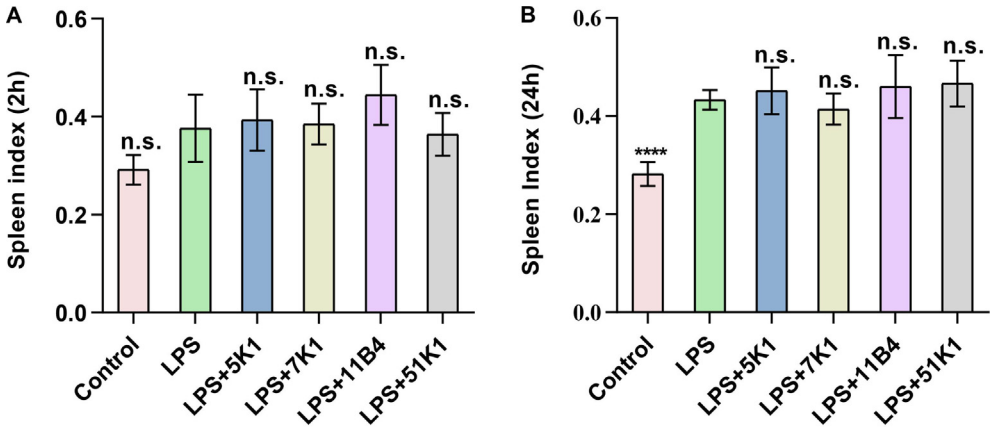


Fig. 2. Effects of *Bacteroides vulgatus* on lipopolysaccharide (LPS)-treated mice. (A) The spleen index after 2 h; (B) the spleen index after 24 h. Note: *Indicates a significant difference when compared with the LPS-treated group. ****$P < 0.0001$; n.s.: No significant difference ($P > 0.05$).

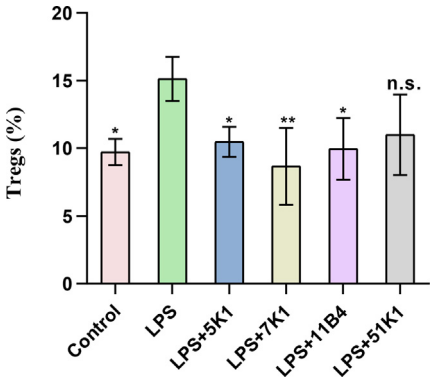


Fig. 3. Effects of the oral administration of different strains of *Bacteroides vulgatus* on the adaptive immune pathways in mesenteric lymph nodes. Note: *Indicates a significant difference when compared with the LPS-treated group. *$P < 0.05$; **$P < 0.01$; n.s.: No significant difference ($P > 0.05$).

Specific genes in the four B. vulgatus strains

Fig. 8A and 8B demonstrate that the relatively high abundance of gene encoding glycoside hydrolases (GH27, GH33, GH43_24, GH105, GH106, and GH141) and glycosyl transferases (GT6) was unique to the *B. vulgatus* FTJS7K1 genome. The *B. vulgatus* genomes were predicted by comparison with the COG database, and seven COG families were found to be unique to the *B. vulgatus* FTJS7K1 genome (Fig. 8C). Moreover, all seven COG families were predicted to be associated with cell wall/membrane biogenesis, amino acid metabolism, translation, replication, and repair.

Discussion

Although a series of studies have shown the relationship between *B. vulgatus* and inflammatory diseases [28–30], the effectiveness of supplementation of *B. vulgatus* strains in alleviating these diseases remains unclear due to the paradoxical results obtained in animal experiments. In the present study, four *B. vulgatus* strains with considerable differences in genomic characteristics were selected to assess their role in alleviating LPS-induced acute inflammation and intestinal injury in mice. We also analyzed the differences in functional genes between these *B. vulgatus* strains to identify those that are responsible for the anti-inflammatory roles of certain strains.

To select the proper dose of LPS, we have searched previously relevant studies. Notably, Guo et al. (2013) have assessed the effects of different LPS concentrations on intestinal barrier function

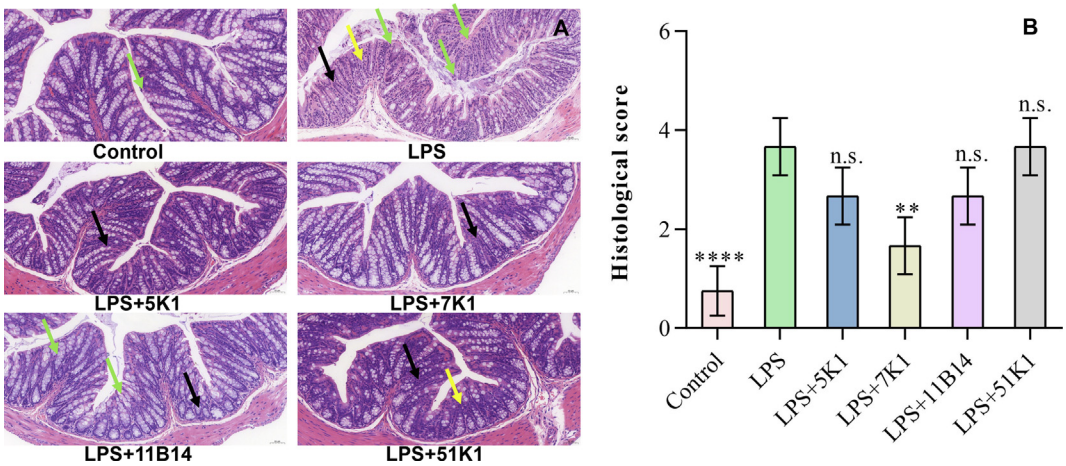


Fig. 4. Effects of the oral administration of different strains of *Bacteroides. vulgatus* on histological alterations in lipopolysaccharide (LPS)-treated mice. Histological images of colonic tissues stained with hematoxylin and eosin for each experimental group (A), and histological scores (B). Note: *Indicates a significant difference when compared with the LPS-treated group. **$P < 0.01$; ****$P < 0.0001$; n.s.: No significant difference ($P > 0.05$); black arrow: depletion of goblet cells; green arrow: erosion or destruction of epithelium; yellow arrow: inflammatory cellular infiltration.

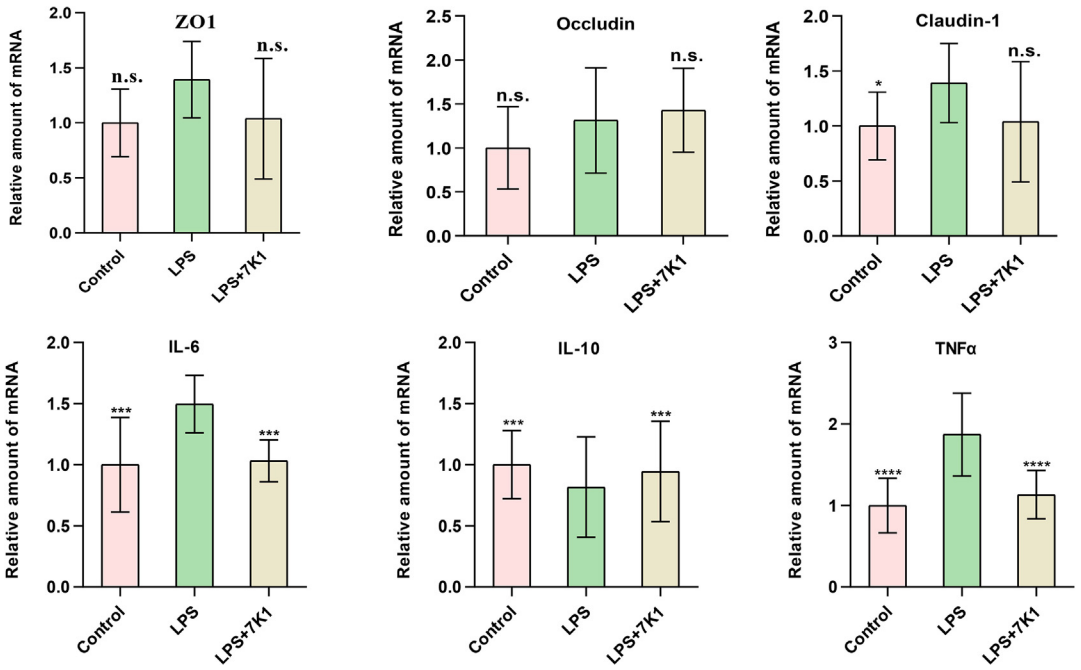


Fig. 5. Effects of the oral administration of different *Bacteroides vulgatus* strains on intestinal barrier disruption in lipopolysaccharide (LPS)-treated mice. mRNA expression of (A) ZO-1, (B) occludin, (C) claudin-1, (D) IL-6, (E) IL-10, and (F) TNFα in the colons of mice. β-actin mRNA expression was considered as an internal control. Note: *Indicates a significant difference when compared with the LPS-treated group. $^{*}P < 0.05$; $^{***}P < 0.001$; $^{****}P < 0.0001$; n.s.: No significant difference ($P > 0.05$).

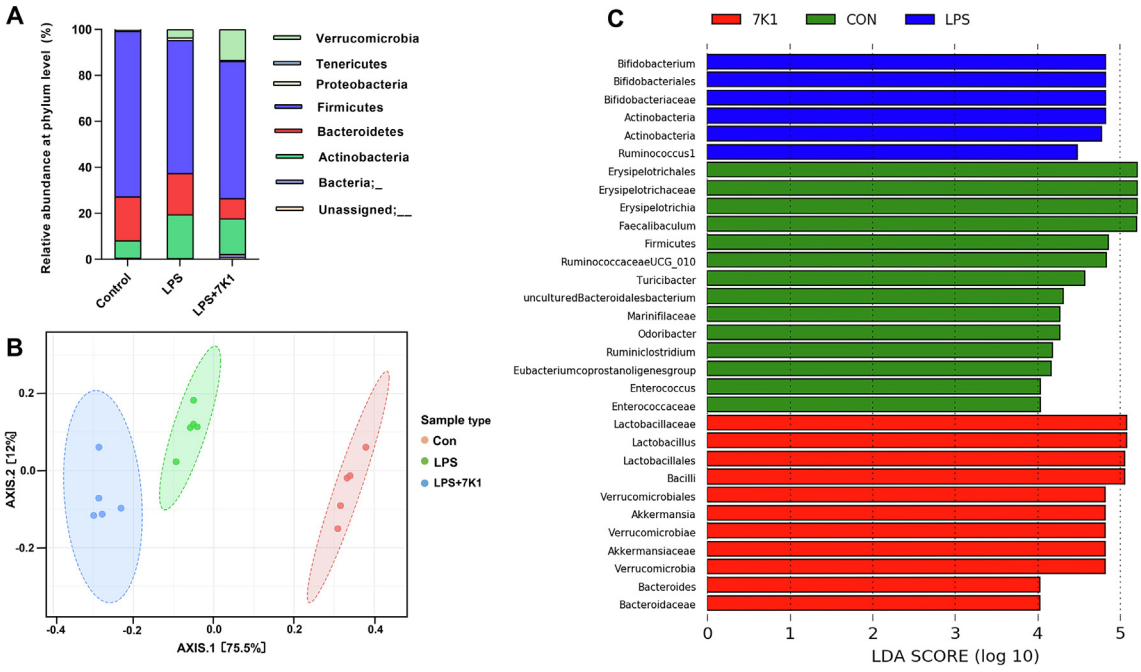


Fig. 6. Impact of *Bacteroides vulgatus* supplementation on the intestinal microbiota of lipopolysaccharide (LPS)-treated mice. (A) The relative abundance of the main phyla. (B) Principal component analysis (PCA) of the gut microbiota. (C) LEfSe analysis of the different groups.

by using mouse model and found that a LPS dose of 0.1 mg/kg could guarantee the effects on intestinal permeability and inflammation development [43]. This result could also be supported by the previous study [11]. Thus, the low dose (0.1 mg/kg) of LPS was selected to induce acute intestinal injury. However, considering the resilience difference against bacterial infection between different vertebrate species [44]. The susceptibility of different strains and sources of mice to LPS should be considered before selecting proper dose of LPS-injection to establish mouse model of acute inflammation and intestinal injury.

The relationship between LPS-induced inflammation and intestine tissue have been demonstrated in a series of studies. It has been demonstrated that LPS could induce inflammation and septic shock [45,46] via intraperitoneal injection. The inflammation and septic shock could thus lead to deleterious functional and structural changes in the gastrointestinal tract [11,47,48]. All of these studies

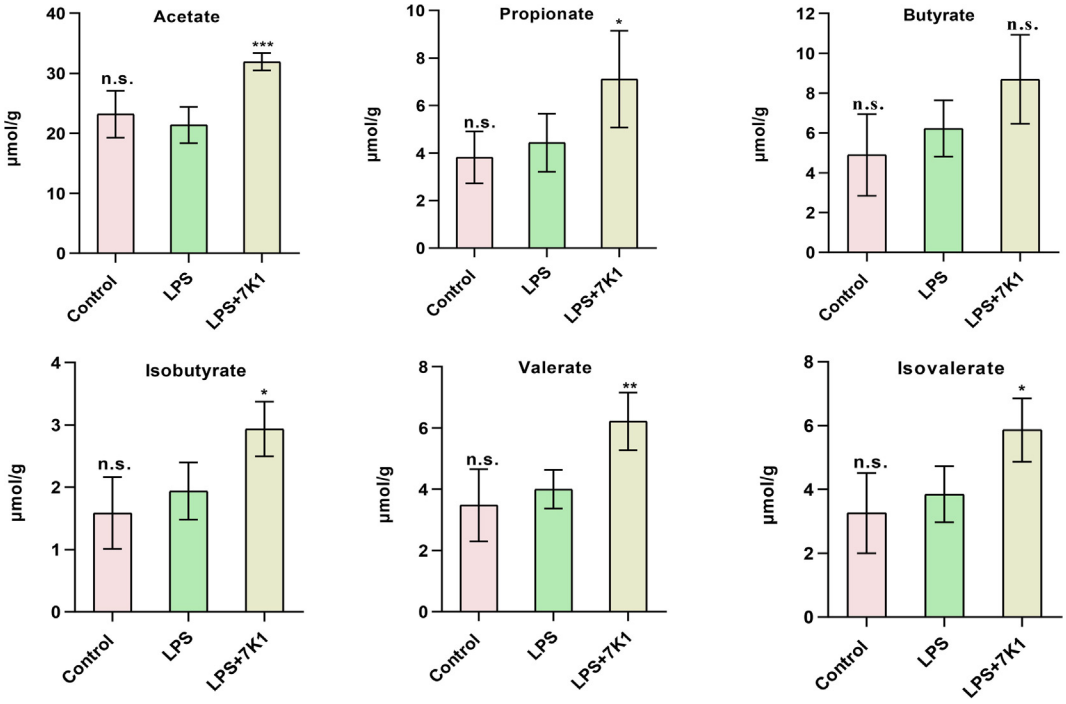
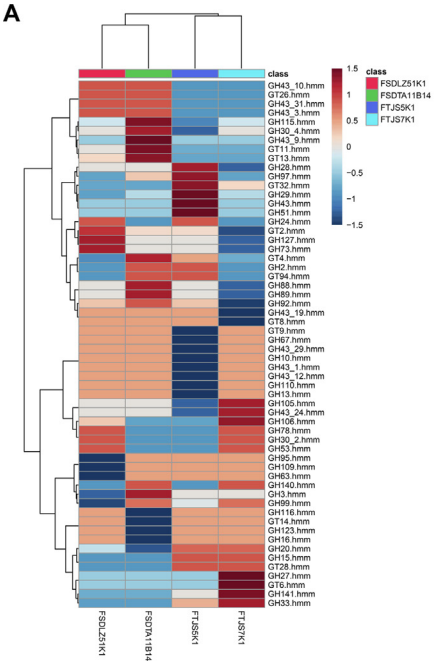


Fig. 7. Concentration of short-chain fatty acids (SCFAs) in the fecal samples of lipopolysaccharide (LPS)-treated mice. The concentrations of (A) acetate, (B) propionate, (C) butyrate, (D) isobutyrate, (E) valerate, and (F) isovalerate in the fecal samples. Note: *Indicates a significant difference when compared with the LPS-treated group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; n.s.: No significant difference ($P > 0.05$).

A



B

CAZY_family	Family_known-activities
GT6	α-1,3-galactosyltransferase & α-1,3 N-acetylgalactosaminyltransferase & α-galactosyltransferase & globoside α-N-acetylgalactosaminyltransferase
GH27	α-galactosidase & α-N-acetylgalactosaminidase & isomalto-dextranase & β-L-arabinopyranosidase & galactan:galactan galactosyltransferase
GH33	sialidase or neuraminidase & trans-sialidase & anhydrosialidase & Kdo hydrolase & 2-keto-3-deoxynononic acid hydrolase/KDNase
GH43_24	β-xylosidase & α-L-arabinofuranosidase & xylanase & α-1,2-L-arabinofuranosidase & exo-α-1,5-L-arabinofuranosidase & [inverting] exo-α-1,5-L-arabinanase & β-1,3-xylosidase & [inverting] exo-α-1,5-L-arabinanase & [inverting] endo-α-1,5-L-arabinanase & exo-β-1,3-galactanase & β-D-galactofuranosidase
GH105	unsaturated rhamnogalacturonyl hydrolase & d-4,5-unsaturated β-glucuronyl hydrolase & d-4,5-unsaturated α-galacturonidase
GH106	α-L-rhamnosidase & rhamnogalacturonan α-L-rhamnohydrolase
GH141	α-L-fucosidase & xylanase

C

COG Num	Functinal Description	Functinal class
COG5206	Glycosylphosphatidylinositol transamidase (GPIT), subunit GPI8	[O]
COG4884	Uncharacterized protein conserved in bacteria	[S]
COG4186	Predicted phosphoesterase or phosphohydrolase	[R]
COG3677	Transposase and inactivated derivatives	[L]
COG3410	Uncharacterized conserved protein	[S]
COG3392	Adenine-specific DNA methylase	[L]
COG3340	Peptidase E	[E]
COG2977	Phosphopantetheinyl transferase component of siderophore synthetase	[Q]
COG2801	Transposase and inactivated derivatives	[L]
COG2378	Predicted transcriptional regulator	[K]
COG2327	Uncharacterized conserved protein	[S]
COG1887	Putative glycosyl/glycerophosphate transferases involved in teichoic acid biosynthesis TagF/TagB/EpsJ/RodC	[M]

Fig. 8. Differential distribution of functional gene categories in *Bacteroides vulgatus* FTJS7K1, FTJS5K1, FSDTA11B14, and FSDLZ51K1. (A) Number of genes associated with CAZY functional categories in *B. vulgatus* FTJS7K1 and the other three strains. (B) Information of the CAZy_family unique to the *B. vulgatus* FTJS7K1 genome. (C) COG categories present in *B. vulgatus* FTJS7K1 only. Note: GHs (glycoside hydrolases): Hydrolysis and/or rearrangement of glycosidic bonds; GTs (glycosyl transferases): Formation of glycosidic bonds; E: Amino acid transport and metabolism; K; Transcription; L; Replication, recombination, and repair; M; Cell wall/membrane/envelope biogenesis; O; Posttranslational modification, protein turnover, chaperones; Q: Secondary metabolite biosynthesis, transport, and catabolism; R: General function prediction only; and S: Function unknown.

reinforced that the intraperitoneal injection of LPS could induce inflammation and even lead to intestinal injury. Thus, the LPS-induced mouse model of intestinal injury could be established by intraperitoneal injection. This result could also be supported by the previous studies [11,48]. Thus, intraperitoneal injection of LPS was used to establish the acute inflammatory and intestinal injury model.

LPS was injected intraperitoneally to produce a mouse model of acute inflammation. Four *B. vulgatus* strains were administered by oral gavage to explore their ability to protect the mice against LPS-induced inflammation. We have analyzed the concentrations of IL-6, IL-10 and TNF-α in serum by enzyme-linked immunosorbent assay (R&D Systems China Co. Ltd.) 2 h and 24 h after LPS administration. The concentrations of IL-6, IL-10 and TNF-α in serum are below the detection limit after 24 h LPS-injection (supplementary Figure S1), which indicate the mice could be difficult to provide us the accurate depiction of physiological alterations for further observation. Thus, all of the mice were sacrificed within 24 h after the intraperitoneal injection of LPS for assessing the roles of different *Bacteroides vulgatus* strains against LPS-induced acute intestinal injury. The results showed that LPS injection (after 24 h) could induced acute inflammation and intestinal injury. Oral gavage of *B. vulgatus* FTJS7K1, but not the other three *B. vulgatus* strains, could not only significantly negate the LPS-induced upregulation of Treg cells in the MLN, but also significantly lower the DAI scores. Additionally, *B. vulgatus* FTJS7K1 could regulate the LPS-induced changes in cytokine mRNA levels in the mouse colon tissue. Moreover, oral supplementation of *B. vulgatus* FTJS7K1 was found to restore the intestinal flora imbalance caused by LPS by increasing the abundance of potential probiotic strains. These results may be attributable to the *B. vulgatus* FTJS7K1-mediated upregulation of SCFAs. These findings provide direct evidence that the intake of *B. vulgatus* FTJS7K1 alleviates the severity of LPS-induced acute inflammation and intestinal injury in mice.

In this study, only *B. vulgatus* FTJS7K1, but not the other three strains, showed a protective role against acute inflammation and intestinal injury, suggesting that there are significant differences in the effects of different *Bacteroides* strains on intestinal diseases. Similar findings have been reported in other studies. Some *B. vulgatus* strains have been shown to play roles in the development of colonic inflammation in mice [49]. In contrast, the strain *B. vulgatus* mpk has been shown to play a significant role in protecting against *E. coli*-induced colitis in gnotobiotic interleukin-2-deficient mice [25] by reducing intestinal inflammation and repairing the intestinal tissue damage [26]. However, few studies have explored the reasons for the different roles of *B. vulgatus* strains in the context of inflammation.

Previous studies have shown that the huge potential of probiotics consumption in alleviating LPS-induced inflammation and injury. These probiotics mainly include *Lactobacillus* sp. [50] and *Bifidobacterium* sp. [51], etc. Some other species, such as *Bacteroides*, have drawn widespread attention because of its roles in human health and diseases. In this study, we found that oral *B. vulgatus* FTJS7K1 can attenuate LPS-induced up-regulation of Treg cells in MLNs. A series of previous studied have shown that MLNs-resident immune cells (such as Treg cells and dendritic cells) have an important role in immune tolerance [52]. Normal mesenteric lymph ameliorated LPS-induced acute kidney injury in mice [53], suggesting that MLNs plays a potential role in regulating LPS-induced inflammation. A similar result can be found in another study which showed that NML treatment could help reduce LPS-induced urea, CD14, TNF-α, and IL-6 levels in mice [53]. Besides, the results of histological evaluation showed that *B. vulgatus* FTJS7K1, but not other *B. vulgatus* strains supplementation, could inhibit LPS-induced proinflammatory cytokines secretion and lower the DAI scores significantly. These results provided direct evidence that *B. vulgatus* FTJS7K1 intake enable efficient access to alleviate the severity of LPS-induced acute intestinal injury in mice.

Accumulating evidence has demonstrated that some species of *Bacteroides* can help protect against inflammation diseases by regulating cytokine levels, such as IL-10 [54] and TNF-α [11]. Among these, TNF-α was considered as an important therapeutic target of IBD because it plays a potential role in triggering the accumulation and activation of leukocytes and inducing inflammation and cell apoptosis [55–57]. Blockade of IL-6 signaling with monoclonal antibodies has also been demonstrated to be able to help reduce chronic intestinal inflammation in mice [58]. IL-10 is a common anti-inflammatory cytokine. *Bacteroides* species could induce Treg cells to secrete IL-10, which is an important way to reduce local inflammation [18,54,59]. Thus, we evaluated the mRNA levels of IL-6, IL-10 and TNF-α in colon tissue to investigate the role of different *B. vulgatus* strains in reducing the severity of LPS-induced inflammation and intestinal injury in mice. The result showed that the supplementation of *B. vulgatus* FTJS7K1 could significantly suppress proinflammatory factor IL-6 and TNF-α mRNA levels, and enhance anti-inflammatory factor IL-10 mRNA levels when compared with LPS groups, suggesting that the regulation of cytokines level from *B. vulgatus* FTJS7K1 could be an important way for helping prevent LPS-induced acute inflammation and intestinal injury.

Intestinal epithelial tight junctions (TJs) proteins, such as ZO-1, occludin, and claudins-1, play a vital role in maintaining the epithelial barrier function to restrict the paracellular movement of harmful substances across intestinal mucosa [60]. The disruption of the TJs barrier could increase dysregulated immune reactions such as the activation of mucosal immune response and the permeation of noxious molecules, and thus inducing gut inflammation [61,62]. In this study, *B. vulgatus* FTJS7K1 has been demonstrated to alleviate LPS-induced inflammatory cell infiltration and goblet cells depletion, and decrease the DAI score. Besides, the result of RT-PCR showed that acute LPS injection has no significant effect on the expression of ZO-1, Claudin-1, and Occludin. Similar result can be found in the previous study of Guo et al. (2013), they found that assessed the mouse intestinal permeability over a 5-day treatment period (LPS injection every 24 h) and showed that intraperitoneal LPS-injection (0.1 mg/kg) did not induce a significant effect on intestinal permeability within 3 days in mouse [43]. All of these results support the ability of *B. vulgatus* FTJS7K1 to maintain intestinal barrier integrity by protecting the integrity of the intestinal epithelial cells but not TJs proteins. Notably, in their study, the effect of intestinal permeability was found after 5 days with in mouse. On the contrary, in the present study, the effect of intestinal injury can be found after 24 h. This result could be attributed to the variable resilience difference against LPS infection between different strains and sources of mice. The susceptibility and resilience of different strains and sources of mice to LPS should be considered before selecting proper dose of LPS-injection to establish mouse model of acute inflammation and intestinal injury.

Accumulating evidence has demonstrated that the variation in genomic characteristics between different probiotic strains is the main reason for their unique functions [63–65]. For example, the fragilysin (*bft*) gene of *B. fragilis* has been shown to encode the [66] *bft* toxin [67], which functions to disrupt the epithelial barrier of the intestine [68]. Moreover, the abundance of genes associated with carbohydrate metabolism in the *B. thetaiotaomicron* genome can effectively help the host to utilize non-digestible polysaccharides [69]. We compared the genome of *B. vulgatus* FTJS7K1, the strain with anti-inflammatory properties, to those of the three inactive strains *B. vulgatus* FTJS5K1, FSDTA11B14, and FSDLZ51K1. The results revealed that 12 genes were specific to the *B. vulgatus* FTJS7K1 genome. Among these specific genes, COG3677, COG3392, COG2801, COG2378, and COG1887 have been shown to be involved in DNA transcription, replication, recombination, repair, and cell wall/membrane/envelope biogenesis, and have been predicted to enhance cell survival [70]. Moreover, COG3340 has been shown to be responsible for amino acid transport and metabolism. This is particularly relevant to the current context given that previous studies have demonstrated that amino acid synthesis could enhance the microbial competitive capacities of

probiotics in the host intestinal tract, in some cases by improving their resistance to bile-salt stress [71,72]. Thus, these specific genes may allow *B. vulgatus* FTJS7K1 to adapt to the survival pressure in the intestinal tract and mediate anti-inflammatory actions.

The biosynthesis and transport of metabolites is the main mechanism by which probiotics play a role in alleviating diseases. For example, siderophores secreted by microorganisms play a role in the absorption and utilization of iron in them [73]. Additionally, the secretion of SCFAs by *Bacteroides* species can prevent the transport of toxins between the gut lumen and blood [74], and colon tumor formation in humans [66]. In this study, a specific gene of *B. vulgatus* FTJS7K1, denoted COG2977, was involved in secondary metabolite biosynthesis, transport, and catabolism. In support of this finding, the analysis of SCFAs showed that supplementation with *B. vulgatus* FTJS7K1 could significantly increase the levels of acetate, propionate, isobutyrate, valerate, and isovalerate in fecal samples. Thus, the secretion of SCFAs from *B. vulgatus* FTJS7K1 could be a mechanism underlying the preventive effect of this strain against LPS-induced acute inflammation and intestinal injury.

The copy numbers of GT6, GH27, GH33, GH141, GH105, GH106, and GH43_24 in *B. vulgatus* FTJS7K1 were higher than those in the other three *B. vulgatus* strains. The GH33 family encodes sialidase or neuraminidase, and sialidases encoded by gut bacteria have been shown to release N-glycolylneuraminic acid (Neu5Gc) from red meat and reduce the risk of inflammatory diseases [75]. The GH27 family encodes α-L-fucosidase, which could play a role of the adaptation of bacterial strains to distinct nutritional environments [76]. Thus, the GH27 family could also help the competitive capacities of *B. vulgatus* FTJS7K1 in the host intestinal tract. The GH43_24, GH105, GH106, and GH141 families encode invertases against carbohydrates, which include arabinan, arabinose, and xylan. Notably, some types of xylans, such as arabinoxylan, can increase the abundance of *Bifidobacterium* [77]. Thus, the existence of these gene families in *B. vulgatus* FTJS7K1 could help to regulate the gut microbiota. This hypothesis was tested in the current study, and the results demonstrated that compared with the control group and the LPS group, the *B. vulgatus* FTJS7K1 group showed a significant increase in the abundance of *Dubosiella*, *Lactobacillus, Akkermansia*, and *Bifidobacterium* (Fig. 6).

It has been demonstrated that the intestinal flora affects the health and disease conditions of the host, which are partly dependent on SCFAs [78,79]. SCFAs can reduce the risk of developing CRC [80], and inhibit inflammation [81,82]. Notably, according to a previous study, some types of xylans encoded by the GH43_24 family can increase the concentration of SCFAs [77]. In the present study, we found that oral administration of *B. vulgatus* FTJS7K1 upregulated the levels of acetate, propionate, isobutyrate, valerate, and isovalerate in mouse feces. Taken together, these results show that *B. vulgatus* FTJS7K1 increased the concentration of SCFAs to protect the mice against LPS-induced acute inflammation and intestinal injury (Fig. 7).

LPS is a common component of the cell wall of Gram-negative bacteria that could enhance the pro-inflammatory cytokines secretion and destruct the intestinal epithelial tight junctions, through focal adhesion kinase (FAK) and Toll-like receptor-4 (TLR4) pathways, and thereby induce different types of intestinal inflammatory disorders [43,48]. Accumulating studies have reinforced the roles of some species of *Bacteroides* in alleviating LPS-induced inflammation through different pathways, including inhibiting enterocyte adherence capacity [83], regulating immune response [84–86], maintaining the diversity of gut microbiota [11].

In the present study, the secretion of SCFAs might be a main way to alleviate LPS-induced colitis. The anti-inflammatory roles of SCFAs have been demonstrated in a series of previous studies [87,88]. In this study, oral administration of *B. vulgatus* FTJS7K1 could significantly increase the levels of acetate, propionate, isobutyrate, valerate, and isovalerate in fecal samples, suggesting *B. vulgatus* FTJS7K1 has a strong ability to produce SCFAs. This has been reinforced by the result of genomic analysis, which suggested that *B. vulgatus* FTJS7K1 might contain specific genes associated with the secretion of SCFAs. Interestingly, SCFAs could regulate the size and function of the colonic Treg pool and protect against colitis in mice [89]. Thus, *B. vulgatus* 7 K1 producing SCFAs could induce Treg cells to secrete IL-10, inhibit IL-6 and TNF-α, and thus provide protection from diseases like colitis.

Conclusion

In conclusion, our results revealed that *B. vulgatus* FTJS7K1 reduces acute inflammation and intestinal injury in mice by modulating the gut microbial community and regulating the levels of related cytokines. Comparative genomics revealed that specific genes present only in the *B. vulgatus* FTJS7K1 genome were responsible for its ability to increase the concentration of colonic SCFAs and its competitive capacities in the host intestinal tract. This may account for the protective role of *B. vulgatus* FTJS7K1 observed against LPS-induced acute inflammation and intestinal injury in the mice. These findings suggest that *B. vulgatus* FTJS7K1 is a potential preventive probiotic against acute intestinal injury. However, further studies are needed to fully determine the efficacy and safety of *B. vulgatus* FTJS7K1 in humans.

Compliance Ethics Requirements

All Institutional and National Guidelines for the care and use of animals (fisheries) were followed.

CRediT authorship contribution statement

Chen Wang: Data curation. **Yue Xiao:** . **Leilei Yu:** Visualization, Investigation. **Fengwei Tian:** Visualization, Investigation. **Jianxin Zhao:** Software, Validation. **Hao Zhang:** Software, Validation. **Wei Chen:** Conceptualization, Methodology, Software. **Qixiao Zhai:** Conceptualization, Methodology, Software.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jare.2021.06.012.

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