



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Ligilactobacillus salivarius AR809 and its exopolysaccharides mitigate antibiotic-induced gut dysbiosis and restore intestinal barrier function

Shen Li^{a,1}, Yao Chen^{a,1}, Yongjun Xia^b, Lianzhong Ai^{ab}, Guangqiang Wang^a, Qixiao Zhai^c, Yijin Yang^{a,*}

^a Shanghai Engineering Research Center of Food Microbiology, School of Health Science and Engineering, University of Shanghai for Science and Technology, Shanghai, 200093, China

^b Shanghai Jiao Tong University, School of Agriculture and Biology, Shanghai, 200240, China

^c State Key Laboratory of Food Science and Resources, Jiangnan University, Wuxi, Jiangsu 214122, China

ABSTRACT: Gut microbiota dysbiosis is closely associated with intestinal barrier dysfunction and immune imbalance. This study evaluated the efficacy of *Ligilactobacillus salivarius* AR809 and its exopolysaccharides (EPS809) in alleviating dysbiosis-associated intestinal dysfunction. In an antibiotic-induced murine model, both viable AR809 cells and EPS809 significantly improved intestinal barrier integrity and immune status. While viable AR809 showed stronger effects, EPS809 also produced significant improvements and similar protective trends, supporting the view that EPS809 may contribute importantly to the overall benefits. Both interventions reduced intestinal permeability, enhanced mucosal immune factors such as secretory IgA, and attenuated histopathological damage. They further helped restore microbiota composition toward that of the healthy state (as evidenced by β -diversity), along with recovery of functional taxa at the family and genus levels. Correlation analyses indicated that these benefits may involve modulation of microbial metabolites, although this remains speculative and warrants future validation. Collectively, the results suggest that AR809 and EPS809 may support recovery of gut microbial balance and intestinal function in antibiotic-challenged mice. Further studies with deeper exopolysaccharide characterization and mechanistic validation are warranted.

Keywords: *Ligilactobacillus salivarius*, Exopolysaccharides, Gut microbiota homeostasis, Intestinal barrier function, Inflammatory responses

1. Introduction

The gut microbiota is a complex microbial community inhabiting the gastrointestinal tract and contributing to essential host functions. As a key interface between the host and the environment, it plays a critical role in maintaining metabolic homeostasis, immune balance, and barrier integrity^[1]. Increasing evidence indicates that gut microbes establish a sophisticated regulatory network through short-chain fatty acids (SCFAs) production, bile acid metabolism, and activation of immune pathways such as Toll-like receptor signaling^[2]. Disruption of this network, known as gut dysbiosis, has been closely linked to

¹ Shen Li and Yao Chen contributed equally to the manuscript.

*Corresponding author
soliaran@163.com

Received 4 October 2025

Received in revised form 14 December 2025

Accepted 20 March 2026

inflammatory bowel diseases, metabolic disorders, and immune-related conditions. Common contributing factors include antibiotic exposure, dietary changes, and environmental stressors, which drive the overgrowth of opportunistic pathogens and depletion of beneficial taxa. Such imbalance increases intestinal permeability, promotes chronic inflammation, and weakens host immunity^[3]. For example, Xu et al. reported that antibiotic-induced dysbiosis downregulated tight junction proteins (ZO-1, Occludin) and impaired the protective function of secretory immunoglobulin A (sIgA), thereby exacerbating barrier dysfunction^[4]. These findings highlight the importance of developing effective strategies to restore gut microbial homeostasis.

Recent studies have emphasized the potential of combining probiotics and prebiotics to achieve synergistic regulation of gut health. Probiotics can strengthen the intestinal barrier through competitive exclusion of pathogens, secretion of antimicrobial peptides, and production of metabolites such as SCFAs. In contrast, prebiotics indirectly modulate host immunity by selectively promoting the growth of beneficial microbes^[5]. For example, Elder et al. showed that exopolysaccharides such as β -glucans enhance Th1/Th2 immune balance by activating dendritic cell-surface Dectin-1 receptors^[6]. Although many studies have described single-target effects of probiotics or their derived exopolysaccharides, systematic understanding of multi-component regulation within dynamic host-microbiota interactions remains limited. This gap hinders the rational development of complex probiotic formulations. Recent advances highlight both opportunities and challenges. Engineered probiotics have been developed to improve bioactive protein delivery^[7]. In addition, co-administration of *Clostridium butyricum* and *Akkermansia muciniphila* has been reported to attenuate the progression of inflammatory bowel disease (IBD) toward colorectal cancer (CRC). This benefit has been linked to modulation of the gut microbiota, suppression of inflammation, and improved immunotherapy efficacy^[8]. These observations illustrate the emerging trend toward precision microbiota-based interventions.

Lactic acid bacteria (LAB), widely recognized for their probiotic properties, have received increasing attention due to the functional roles of their metabolites and structural components. In this context, the study focused on *Ligilactobacillus salivarius* AR809 (*L. salivarius* AR809) and its exopolysaccharide (EPS809). AR809 was selected as the target strain based on its favorable functional profile reported in previous studies^[9]. It shows tolerance to acidic conditions and bile salts, strong adhesion to intestinal epithelial cells, and the ability to inhibit *Staphylococcus aureus* adhesion, and it has also demonstrated protective effects in an *S. aureus*-related inflammation model^[9]. AR809 is an EPS-producing strain, and EPS-related immunomodulatory activities have been reported for LAB, supporting its relevance as a functional component candidate^[10]. Based on these characteristics, we investigated whether viable AR809 and its exopolysaccharides (EPS809) could alleviate antibiotic-induced gut dysbiosis and restore intestinal function. Functional prediction was used to provide preliminary insights into potential metabolic and immune related pathways associated with the observed effects.

This study aimed to systematically evaluate the effects of *L. salivarius* AR809 and EPS809 on antibiotic-induced dysbiosis in mice. A murine model was established using a broad-spectrum antibiotic

cocktail, and treatment groups were designed to assess the effects of viable AR809 cells, EPS809, and relevant controls. Outcomes were examined through microbiota profiling, functional prediction, and host phenotyping (barrier integrity, cytokine levels, sIgA secretion). The primary objective was to investigate the individual effects of AR809 and EPS809 in mitigating dysbiosis and restoring intestinal function. In addition, we sought to examine whether compensatory proliferation of functional taxa, a phenomenon often described as microbial functional redundancy, could contribute to ecological stability under dysbiotic conditions^[11]. These considerations shaped the framework of the present study. This study integrated microbial community analysis with host physiological assessments to explore the potential roles of AR809 and EPS809 in mitigating gut dysbiosis.

2. Materials and methods

2.1 Preparation of EPS809 and basic compositional characterization

2.1.1 Preparation of EPS809

L. salivarius AR809 was activated on MRS agar at 37 °C for 24 h. A single mature colony was inoculated into 1 mL MRS broth and cultured under anaerobic conditions at 37 °C for 12 h. The culture was centrifuged at 4 °C and 8000 × g for 10 min. The supernatant was collected and the cell pellet was discarded to obtain a cell-free fermentation broth. An 80% (w/w) trichloroacetic acid solution was added to the supernatant for protein removal, followed by incubation at 4 °C for 9 h. After centrifugation at 4 °C and 8000 × g for 10 min, the protein precipitate was removed. The supernatant was collected, and ethanol was added to reach a final ethanol volume fraction of 75%. The mixture was kept at 4 °C for 12 h to precipitate crude EPS. The precipitate was collected by centrifugation and redissolved in deionized water. The solution was dialyzed against running water for 72 h, and the retentate was freeze-dried to obtain EPS809 powder.

2.1.2 Total sugar content

Total sugar content of EPS809 was determined using the phenol-sulfuric acid method with glucose as the standard, and absorbance was measured using a microplate reader (SpectraMax i3x, Molecular Devices, USA).

2.1.3 Protein content

Protein content was determined using the Coomassie Brilliant Blue G-250 assay with bovine serum albumin as the standard. The dye reagent was prepared by dissolving 10 mg Coomassie Brilliant Blue G-250 in 5 mL 95% ethanol, followed by addition into 10 mL 85% phosphoric acid, and dilution to 100 mL using ultrapure water. Bovine serum albumin standards were prepared at 0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL. Aliquots (1.0 mL) of standards or EPS809 solution (1.0 mL, 1 mg/mL) were mixed with 5.0 mL dye reagent, incubated at room temperature for 15 min, and measured at 595 nm.

2.1.4 Molecular weight determination

The molecular weight distribution of EPS809 was determined by HPSEC coupled with multi-angle laser light scattering (MALS) and refractive index (RI) detection. Two columns (SB-803 HQ and SB-805 HQ) were connected in series. The mobile phase was 0.1 mol/L NaNO₃. EPS809 was dissolved in the mobile phase to 1 mg/mL prior to analysis. The system temperature was maintained at 40 °C and the flow rate was 0.6 mL/min. Absolute molecular parameters including Mw, Mn,

Mw/Mn, and intrinsic viscosity were calculated from the MALS signals and are summarized in Table S1. The representative chromatogram is shown in Fig. S1a.

2.1.5 FTIR analysis

Fourier transform infrared (FTIR) spectra of EPS809 were recorded using the KBr pellet method. Briefly, 1 mg of freeze-dried EPS809 powder was thoroughly ground with 100 mg of spectroscopic-grade KBr and pressed into a transparent pellet. A blank KBr pellet was used for background collection. Spectra were acquired using a Nicolet FTIR spectrometer (Thermo Fisher Scientific, USA) over 4000–400 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans per sample. The obtained spectra were baseline-corrected and normalized prior to peak assignment and comparison.

2.2 Preparation of fermented milk and analytical measurements

2.2.1 Preparation of fermented milk

High-fat fresh milk (protein 6%, fat 6%) and low-fat fresh milk (protein 6%, fat 0%) were used as the bases for fermented milk preparation. Milk was preheated to 75 °C, sterilized at 95 °C for 10 min, and then cooled to 42 °C. Sucrose was added to a final concentration of 60 g/L, followed by addition of a commercial starter culture at 0.1 g/L. Four fermented milk formulations were prepared according to group allocation. For the control formulations, only sucrose (60 g/L) and starter culture (0.1 g/L) were added to high-fat milk (high-fat fermented milk, Group 1, q0) or low-fat milk (low-fat fermented milk, Group 2, d0). For the AR809 formulation (Group 3, d809), *L. salivarius* AR809 was additionally inoculated into the milk together with sucrose and starter culture. For the EPS formulation (Group 4, d1), EPS809 was added at 0.4 g/L in addition to sucrose and starter culture. All formulations were fermented at 42 °C for 5 h until coagulation. The fermented products were then transferred to 4 °C and stored for post-fermentation maturation for 12 h before use. However, the actual EPS yield in the milk fermentation model was not measured in this study. Future studies will focus on assessing EPS yield in milk fermentation to ensure dose consistency across experimental models.

2.2.2 Texture profile analysis

Fermented milk samples were equilibrated at 4 °C for 12 h before analysis. Texture properties were determined using a Brookfield CT3 texture analyzer (Brookfield Engineering Laboratories, USA) equipped with a TA10 flat-ended cylindrical probe (2.5 cm diameter). Measurements were performed at a test speed of 5.0 mm/s, a compression distance of 10 mm, and a return speed of 5.0 mm/s. Hardness, adhesiveness, and cohesiveness were calculated based on the first and second compression cycles, where cohesiveness was determined by the ratio of the area under the second compression curve (A2) to that under the first compression curve (A1).

The 0.4 g/L EPS809 dose was selected based on texture analysis of fermented milk, which demonstrated favorable effects on sensory attributes such as hardness, adhesiveness, and cohesiveness (Fig. S2). While this dose was selected without *in vivo* metabolism or bioavailability data, it was chosen in line with the concentrations used in similar studies and did not result in any adverse effects on the animals.

2.3 Animals and experimental design

Male C57BL/6J mice (5 weeks old, 20–23 g) were purchased from Shanghai Bikai Keyi Biotechnology Co., Ltd. (Shanghai, China) [license: SCXK (Shanghai) 2023-0009]. All procedures were approved by the Institutional Animal Care and Use Committee of Shidong Hospital, University of Shanghai for Science and Technology (protocol ID: IRB-AF48-V1.0). Mice were maintained under specific pathogen-free (SPF) conditions at 22 ± 2 °C, $50 \pm 10\%$ relative humidity, and a 12 h light/dark cycle, with ad libitum access to standard chow and autoclaved water. After a 7-day acclimatization, animals were randomly allocated to six groups ($n = 8$). Experimental groups were as follows: Control, Model, Group 1-q0, Group 2-d0, Group 3-d809, and Group 4-d1 (Fig. 1). During Week 2, all groups except Control received a broad-spectrum antibiotic cocktail (penicillin 1 mg/mL, streptomycin 1 mg/mL, neomycin 1 mg/mL, and vancomycin 1 mg/mL, freshly prepared and replaced daily) in drinking water for 7 days to induce dysbiosis. In Week 3, interventions were administered once daily by oral gavage at 17:00 (200 μ L per mouse): high-fat fermented milk (q0), low-fat fermented milk (d0), *L. salivarius* AR809 fermented milk (d809, containing 1×10^9 CFU/mL, corresponding to 2×10^8 CFU/mouse), and low-fat fermented milk supplemented with EPS809 (d1). Based on an EPS809 concentration of 0.4 g/L in fermented milk and a gavage volume of 200 μ L, the administered EPS809 dose was 80 μ g EPS/mouse/day. The Model group received sterile water during recovery. Fecal samples were collected at three time points: T1 (post-acclimatization), T2 (after antibiotics), and T3 (after intervention). At the study endpoint, mice were euthanized by cervical dislocation, and tissues were harvested for histological, molecular, and immunological analyses. Humane endpoints were predefined as body-weight loss $>15\%$, severe lethargy, or labored breathing.

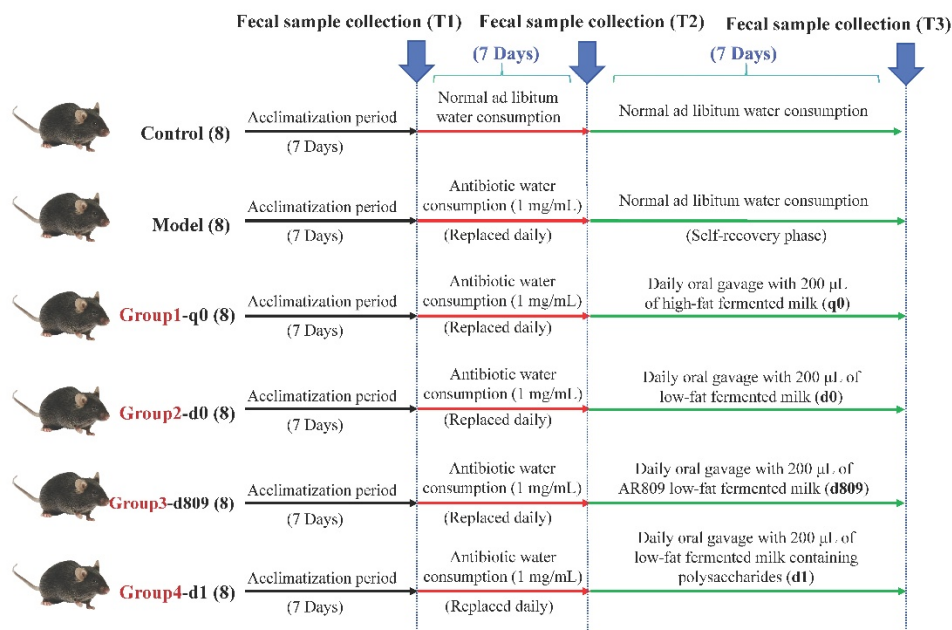


Fig. 1 Experimental design and animal grouping. Mice ($n = 8$ per group) were acclimatized for 7 days and then assigned to six groups: Control, Model, Group1-q0 (high-fat fermented milk), Group2-d0 (low-fat fermented milk), Group3-d809 (low-fat fermented milk with *L. salivarius* AR809), and Group4-d1 (low-fat fermented milk with AR809 exopolysaccharides). During the modeling phase, all groups except Control received antibiotic-containing water (1 mg/mL, replaced daily) for 7 days. In the intervention phase, mice were gavaged daily with 200 μ L of the respective treatments for 7 days, while the Model group underwent self-recovery. Fecal samples were collected at three time points: baseline (T1), post-antibiotic treatment (T2), and post-intervention (T3).

2.4 General health monitoring

Body weight was measured daily. General health status, including diarrhea, fecal characteristics, and mental state, was recorded. The body weight loss fraction was calculated as: Body weight loss fraction = (Initial weight - Measured weight) / Initial weight.

2.5 Serum FITC concentration assay

After the final gavage, mice were fasted for 12 h (water ad libitum). FITC-dextran (4 kDa) was dissolved in PBS (40 mg/mL) and orally gavaged at 0.1 mL/10 g body weight 4 h before blood sampling. Blood was drawn from the retro-orbital sinus into serum collection tubes (without anticoagulant), allowed to clot for 40 min, and centrifuged at $1,500 \times g$ for 15 min. The supernatant (serum) was stored at -80°C . For detection, serum samples were diluted 1:50 with PBS and analyzed using a microplate reader (Excitation: 485 nm, Emission: 528 nm).

2.6 Detection of inflammatory cytokines and intestinal barrier proteins

Total RNA was extracted from colonic tissues using the (Vazyme Biotech Co., Ltd.) RNA extraction kit, following the manufacturer's instructions, and quantified with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). One microgram of RNA was reverse transcribed into cDNA using a (Takara) reverse transcription kit. qPCR was performed using SYBR Green Master Mix (Roche, Italy) on an LightCycler® Systems under standard cycling conditions. GAPDH served as the internal control, and relative expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method. Primers are listed in Table 1. Protein levels of TNF- α , IL-6, IL-10, IL-4, ZO-1, and Occludin were quantified using ELISA kits (Shanghai baipeng Biotechnology Co., Ltd) according to the protocols provided^[12]. Each sample was analyzed in triplicate.

TABLE 1 PRIMER SEQUENCE

GENE	FORWARD SEQUENCE (5'TO3')	REVERSE SEQUENCE (5'TO3')
TNF-A	ACGTGGAAGTGGCAGAAGAG	AGGGTCTGGGCCATAGAACT
IL-6	GAGGATACCACTCCCAACAGACC	AAGTGCATCATCGTTGTTTCATACA
IL-10	GTTTGCCAAGCCTTATCGGA	ACCTGCTCCACTGCCTTGCT
IL-4	TTGAACGAGGTCACAGGAGAAG	CCTTGGAAGCCCTACAGACG
ZO-1	GGGGCCTACACTGATCAAGA	TGGAGATGAGGCTTCTGCTT
OCCUDIN	CTGCAGCTACTGGACTCTAC	GGACTGTCAACTCTTTCCAC

2.7 Determination of MPO activity and sIgA levels

Colonic and small intestinal tissues were harvested, rinsed in cold PBS, and homogenized (1:9, w/v) on ice. Homogenates were centrifuged at $3000 \times g$ for 15 min at 4°C , and the supernatants were collected for analysis. Myeloperoxidase (MPO) activity in colon tissue and secretory immunoglobulin A (sIgA) in small intestinal tissue were quantified using commercial ELISA kits (Shanghai baipeng Biotechnology Co., Ltd) following the manufacturer's protocols. MPO activity was expressed as U/L tissue. sIgA levels were expressed as $\mu\text{g}/\text{mg}$ protein. All assays were performed in duplicate.

2.8 Hematoxylin and eosin (H&E) staining

Colonic tissue segments (5 mm) were fixed in 10% neutral buffered formalin for 24 h, dehydrated in graded ethanol series (75%-100%), cleared in xylene, and embedded in paraffin. Sections (4 μ m thick) were cut, deparaffinized, and stained with hematoxylin for 5 min and eosin for 2 min. Stained sections were scanned using a Panoramic MIDI digital slide scanner (3DHISTECH, Hungary) and examined under a light microscope (Olympus BX53, Japan).

2.9 Intestinal microbiota analysis

DNA was extracted from the mice feces utilizing the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions. The hypervariable regions V3-V4 of the bacterial 16S rRNA gene were amplified with primer pairs 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). The PCR product was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions. Then, the purified PCR products were sequenced on an Illumina MiSeq platform at Majorbio Bio-Pharm Technology Co., Ltd, (Shanghai, China). Further sequence reads processing in the present study was performed by barcodes using a QIIME pipeline and included additional quality trimming, demultiplexing, and taxonomic assignments. Operational taxonomic unit (OTU) classifications were performed using Mothur, with a sequence similarity threshold of 97% for OTU clustering. The classification was conducted against the SILVA database, and the taxonomic assignments were further supported by the RDP classifier with a confidence cutoff of 80%. The final OTUs were selected based on a bootstrap value of $\geq 80\%$. The representative sequences were classified using the RDP classifier method and SILVA database.

2.10 Statistical analysis

Data are presented as mean $\pm$ SD. Statistical analyses were performed using SPSS v22.0 (SPSS Inc., Chicago, IL, USA) and R v4.0. One-way ANOVA followed by Tukey's post hoc test was applied for group comparisons when assumptions were satisfied. Direct pairwise comparisons were conducted between each treatment group and the Model group, and between the Control and Model groups. A two-tailed p value < 0.05 was considered statistically significant. In figures, #, ##, ###, ####, ##### denote comparisons versus the Control group (# $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$, ##### $P < 0.00001$). *, **, ***, **** denote comparisons versus the Model group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). ns indicates not significant. Multiple testing was adjusted using the Benjamini–Hochberg false discovery rate where applicable. For microbiota beta diversity, PERMANOVA (adonis) with 999 permutations was performed.

3. Results and discussion

3.1 Physicochemical characterization of EPS809 and rationale for dose selection

EPS809 was characterized by HPSEC coupled with multi-angle light scattering, refractive index, and differential-pressure detectors. The chromatogram showed a dominant high-molar-mass fraction with strong light-scattering and differential-pressure responses. A later-eluting fraction was mainly detected by the

refractive index trace with minimal light-scattering signal, indicating a minor low-molar-mass component (Fig. S1a). The molecular parameters derived from HPSEC–MALS are summarized in Table S1. EPS809 exhibited a high weight-average molecular weight (M_w) of 1655.00 kDa and a number-average molecular weight (M_n) of 1385.00 kDa, with a polydispersity index of 1.19, indicating a moderately narrow molecular weight distribution (Table S1). The intrinsic viscosity was 840.87 mL/g. In addition, compositional analysis further supported the polysaccharide-dominant nature of EPS809. The total sugar content reached 92.0%, whereas the residual protein content was low (0.60%) (Fig. S1b). The FTIR spectrum of EPS809 showed a broad absorption band at 3416 cm^{-1} , which is attributable to O–H stretching from abundant hydroxyl groups and hydrogen-bonded water, consistent with a carbohydrate-rich polymer (Fig. S1c). A weak band around 2955 cm^{-1} was assigned to C–H stretching vibrations. The signal at 1617 cm^{-1} can be associated with H–O–H bending of bound water and or carboxylate-related vibrations in polysaccharide-containing materials. Together with the low residual protein content measured by the Bradford assay, the FTIR profile supports that EPS809 is predominantly polysaccharide in nature. However, detailed structural analyses such as monosaccharide composition analysis, glycosidic bond type identification, and NMR-based fine structure determination were not performed in this study. These limitations will be addressed in future studies to fully characterize the structure-activity relationship of EPS809 and further elucidate its functional potential.

To define an appropriate fortification level for subsequent animal intervention, EPS809 was further evaluated at different addition levels in fermented milk using texture profile indicators and microstructure observation. Texture analysis showed a concentration-dependent modulation of gel properties (Fig. S2). Increasing EPS809 tended to reduce hardness while enhancing adhesive force and cohesiveness, indicating strengthened internal interactions and improved gel integrity. Notably, the formulation supplemented with 0.4 g/L EPS809 showed the highest cohesiveness and maintained an intermediate hardness, whereas 0.6 g/L further increased adhesive force but produced an overly soft gel with markedly decreased hardness (Fig. S2). Based on these pre-screening results, 0.4 g/L EPS809 was selected as the final supplementation level for Group 4. This corresponds to 0.4 mg/mL EPS809 in fermented milk. With an administration volume of 200 μL per gavage, the actual EPS809 amount delivered was 80 μg per administration. The 0.4 g/L EPS809 dose was selected primarily based on its favorable effects on texture and sensory properties in the fermented milk. This initial dose selection was based on product properties rather than *in vivo* absorption, metabolism, or bioavailability data. Moreover, the polysaccharide content in the fermented milk was not measured, which should be considered a limitation. This is partly because quantifying EPS directly from fermented milk is technically challenging, as milk proteins and other matrix components can interfere with EPS isolation and accurate determination. Therefore, EPS809 was prepared from MRS culture to obtain sufficient material with a defined amount for supplementation, allowing the EPS-only group to serve as a component-validation arm. Future studies will quantify the *in situ* EPS yield during AR809 milk fermentation and evaluate EPS bioavailability and metabolism to improve dose consistency and interpretation.

3.2 AR809 and EPS809 improve intestinal barrier function and mucosal defense

Representative colon images and length measurements were comparable across groups (Fig. 2a), indicating that the short-term antibiotic challenge did not induce marked macroscopic alterations in colon morphology. This observation suggests that antibiotic exposure primarily disturbed microbial and metabolic homeostasis rather than causing overt gross structural changes in the colon^[13,14]. Body weight was monitored throughout the experiment (Fig. 2a). Compared with the Control group, the Model group showed a sustained reduction in body weight during antibiotic induction. During the intervention period, the AR809-fermented milk (Group 3) and EPS809-supplemented fermented milk (Group 4) attenuated body weight loss relative to the Model group, whereas Groups 1 and 2 exhibited weight trajectories broadly similar to the Model group. Notably, Group 2 showed a tendency toward greater weight loss than the Model group, suggesting that the low-fat fermented milk matrix may influence body weight outcomes under antibiotic-challenged conditions. Consistently, the weight loss score (%) was lower in Groups 3 and 4 than in the Model group (Fig. 2b), supporting that AR809 and EPS809 mitigated antibiotic-associated weight loss.

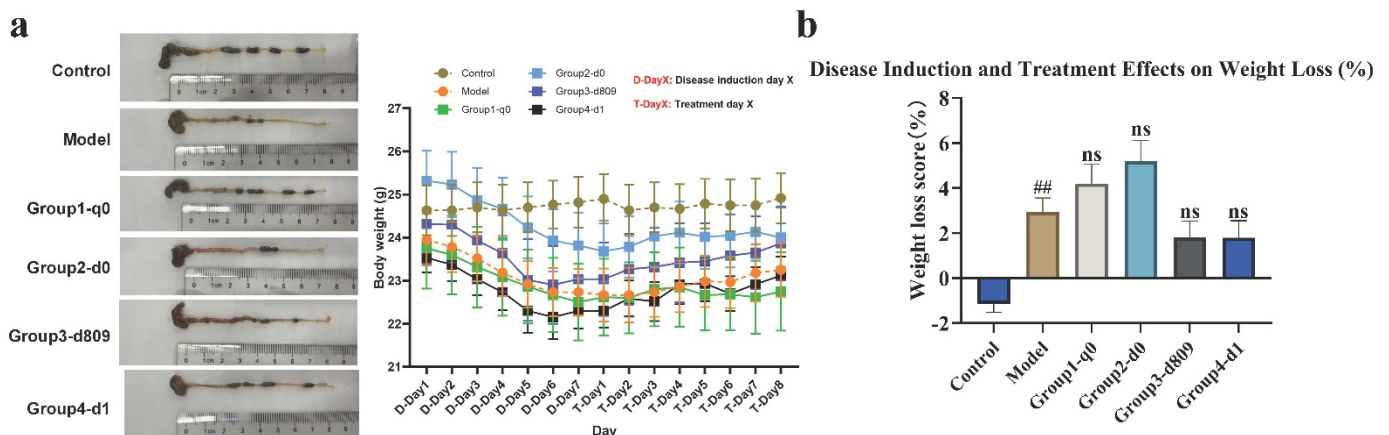


Fig. 2 AR809 and its exopolysaccharides attenuated body weight loss and preserved colon morphology in antibiotic-induced dysbiosis mice. (a) Representative colon images (with length measurement) and body weight changes during disease induction and intervention. (b) Summary of weight loss score (%) across groups. One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. Symbols indicate statistical significance. #, ##, ###, ####, ##### denote comparisons versus the Control group (# $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$). *, **, ***, **** denote comparisons versus the Model group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). ns, not significant.

To evaluate intestinal permeability, mice were orally administered FITC-dextran 4 h before euthanasia. Serum FITC levels in the Model group were dramatically higher than in the Control ($P < 0.001$), confirming that antibiotic treatment impaired barrier function (Fig. 3a). After intervention, FITC levels declined in all treatment groups. Significant reductions were observed in Group 1 (high-fat fermented milk), Group 3 (AR809 fermented milk) and Group 4 (EPS809) compared with the Model group (* $P < 0.05$), whereas Group 2 (low-fat fermented milk) showed no significant difference. Although FITC levels in Groups 3 and 4 remained higher than those in the Control group, the data indicate a partial restoration of barrier integrity. Histological examination by H&E staining supported the permeability results (Fig. 3b). The Model group exhibited disrupted epithelial architecture and inflammatory infiltration relative to the Control. Groups 1 and 2 showed modest amelioration but still had visible mucosal injury. In Groups 3 and 4, the epithelial structure was largely preserved, crypts were aligned, and inflammatory infiltration was reduced, indicating a

morphology closer to normal. These observations suggest that AR809 and EPS809 more effectively ameliorate barrier disruption than milk fat adjustment alone.

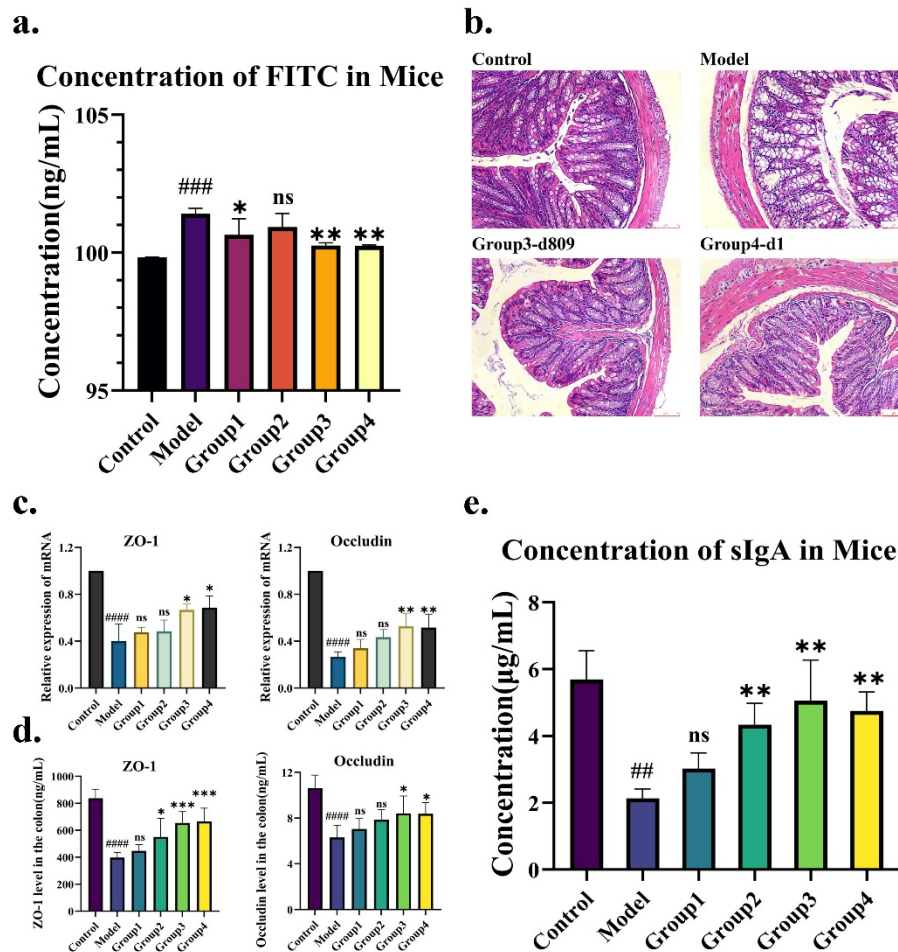


Fig. 3 AR809 improves intestinal barrier integrity in mice with gut dysbiosis. a. Serum FITC concentration. b. Representative H&E-stained colon sections. c, d. mRNA and protein expression of tight junction proteins (ZO-1, Occludin). e. Small intestinal sIgA concentration. One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. Symbols indicate statistical significance. #, ##, ###, ####, ##### denote comparisons versus the Control group (# $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$, ##### $P < 0.00001$). *, **, ***, **** denote comparisons versus the Model group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). ns, not significant.

In addition, an unexpected pattern was observed in the vehicle control, where low-fat fermented milk alone (Group 2) tended to show greater body weight loss than the untreated Model group. This finding suggests that the fermented milk matrix, particularly under antibiotic-challenged conditions, may influence short-term body weight outcomes, potentially through differences in energy density, palatability, or gastrointestinal adaptation^[15-17]. Notably, colon length did not differ among groups, indicating that the body weight phenotype was not accompanied by overt macroscopic colonic injury. Importantly, the effects of the AR809-fermented milk (Group 3) and EPS809-supplemented fermented milk (Group 4) were evaluated against fermented milk controls within the same experimental framework rather than against an unrelated vehicle. Therefore, the attenuation of weight loss in Groups 3 and 4 is more reasonably interpreted as an additional effect associated with AR809 or EPS809 on top of the vehicle background, instead of merely reflecting avoidance of an unfavorable matrix effect. Nonetheless, the potential influence of the vehicle on body weight represents a limitation for interpreting this endpoint.

Relative mRNA expression of tight junction proteins ZO-1 and Occludin was significantly reduced in the Model group compared with the Control group (Fig. 3c). Compared with the Model group, Groups 3 and 4 showed significant increases in ZO-1 and Occludin expression, whereas Group 1 showed only a modest change and Group 2 showed no significant improvement. Protein levels exhibited the same pattern (Fig. 3d). Similarly, small-intestinal sIgA was significantly decreased in the Model group versus the Control group, and was significantly restored in Groups 3 and 4 compared with the Model group, while Groups 1 and 2 remained not significantly different from the Model group (Fig. 3e). These results suggest that AR809 strengthens mucosal defense in addition to improving barrier integrity^[18].

3.3 AR809 and EPS809 alleviate antibiotic-induced inflammation

Colonic myeloperoxidase (MPO) activity, a marker of neutrophil infiltration^[19, 20], was increased in the Model group compared with the Control group (#### $P < 0.0001$, Fig. 4a). MPO activity was reduced in Groups 3 and 4 relative to the Model group ($*P < 0.05$ to $***P < 0.001$), approaching levels observed in the Control group. Compared with the Control group, the Model group showed higher TNF- α and IL-6 and lower IL-4 and IL-10 (#### $P < 0.0001$, Fig. 4b, c). Groups 3 and 4 decreased pro-inflammatory cytokines and increased anti-inflammatory mediators relative to the Model group ($*P < 0.05$ to $****P < 0.0001$). Groups 1 and 2 showed non-significant effects. Overall, these data demonstrate that AR809 and EPS809 effectively mitigate antibiotic-induced intestinal inflammation.

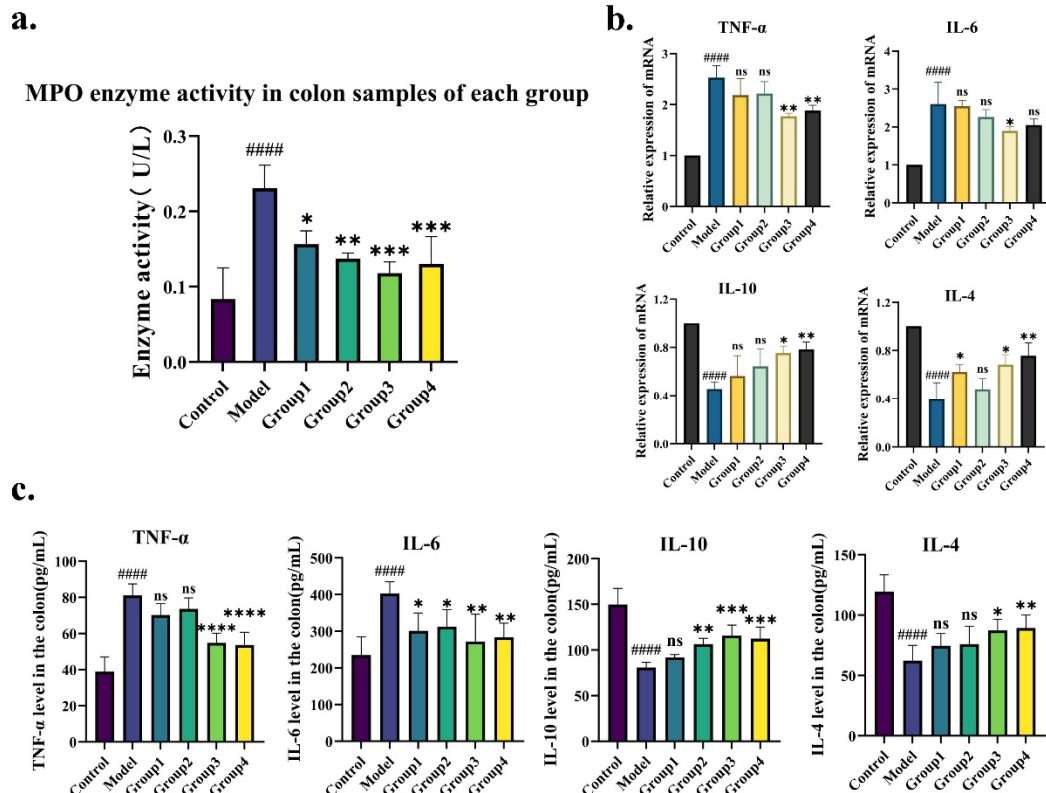


Fig 4. AR809 reduces intestinal inflammation and modulates cytokine expression. a. MPO activity in colon tissues. b. Relative mRNA expression of pro- and anti-inflammatory cytokines. c. Cytokine protein levels measured by ELISA.

One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. Symbols indicate statistical significance. #, ##, ###, #### denote comparisons versus the Control group (# $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$). *, **, ***, **** denote comparisons versus the Model group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). ns, not significant.

3.4 AR809 and EPS809 facilitate partial restoration of gut microbiota composition

To confirm successful dysbiosis induction at T2 prior to intervention, fecal samples collected after the antibiotic challenge were analyzed (Fig. S3). Compared with the Control group, antibiotic-exposed mice showed altered alpha diversity, with the Model group exhibiting reduced ACE richness and Shannon diversity (Fig. S3a, b). PCoA based on Bray-Curtis distances further demonstrated a clear separation between antibiotic-exposed samples and the Control group (Fig. S3c). PERMANOVA confirmed a significant effect of antibiotic exposure ($R^2 = 0.40$, $P < 0.001$). Genus-level profiles also supported dysbiosis induction, showing marked compositional rearrangements relative to the Control group, as illustrated by the stacked bar plot and the heatmap of the top 30 genera (Fig. S3d, e). Collectively, these results verified that the antibiotic cocktail effectively induced gut microbiota dysbiosis at T2.

Following confirmation of dysbiosis induction at T2 prior to intervention, the effects of AR809 and EPS809 on microbial diversity and community structure were subsequently evaluated after supplementation. Alpha diversity analysis revealed pronounced alterations in microbial richness and diversity across groups (Fig. 5a). The Control group displayed the highest ACE and Chao1 indices, whereas the antibiotic-treated Model group showed significant reductions compared with the Control group (Fig. 5a, $\#P < 0.05$), confirming disruption of the gut microbiota. The AR809-fermented milk (Group 3) and EPS809 intervention (Group 4) showed no significant differences in richness or Shannon diversity compared with the Model group. This pattern suggests selective enrichment of specific functional taxa rather than broad recovery of microbial abundance^[21]. Notably, inter-individual variability was reduced in Groups 3 and 4, indicating improved community stability^[22]. A significant difference in gut microbiota community structure among the Control, Model, Group 3, and Group 4 groups was supported by PERMANOVA ($R^2 = 0.34$, $P < 0.001$) and visualized by the PCoA plot (Fig. 5b). The Model group separated clearly from the Control, while Group 3 shifted closer to the Control cluster. Group 4 partially overlapped with both the Control and Model groups. Pairwise PERMANOVA further confirmed that the Model group differed significantly from the Control group ($p = 0.008$) and that Group 4 differed from the Model group ($p = 0.013$). In contrast, the difference between Group 3 and the Model group did not reach statistical significance ($p = 0.061$). Moreover, Group 3 and Group 4 differed significantly ($p = 0.013$), indicating differential modulation of microbiota by viable cells and exopolysaccharides^[23]. Beta diversity analyses supported these observations (Fig. 5c). The Model group exhibited reduced divergence, reflecting antibiotic-induced homogenization, whereas Group 4 displayed stronger compositional shifts than Group 3^[24, 25]. Venn and UpSet analyses illustrated differences in OTU distribution among groups (Fig. 5d, e). The Control group harbored the greatest number of OTUs, whereas the Model group suffered substantial OTU loss after antibiotic treatment. Both interventions partially restored OTU numbers, with Groups 3 and 4 recovering a larger proportion of Control-associated OTUs compared with the Model group. A core set of 295 OTUs was conserved across all groups, reflecting microbial taxa resilient to antibiotic disturbance^[26, 27].

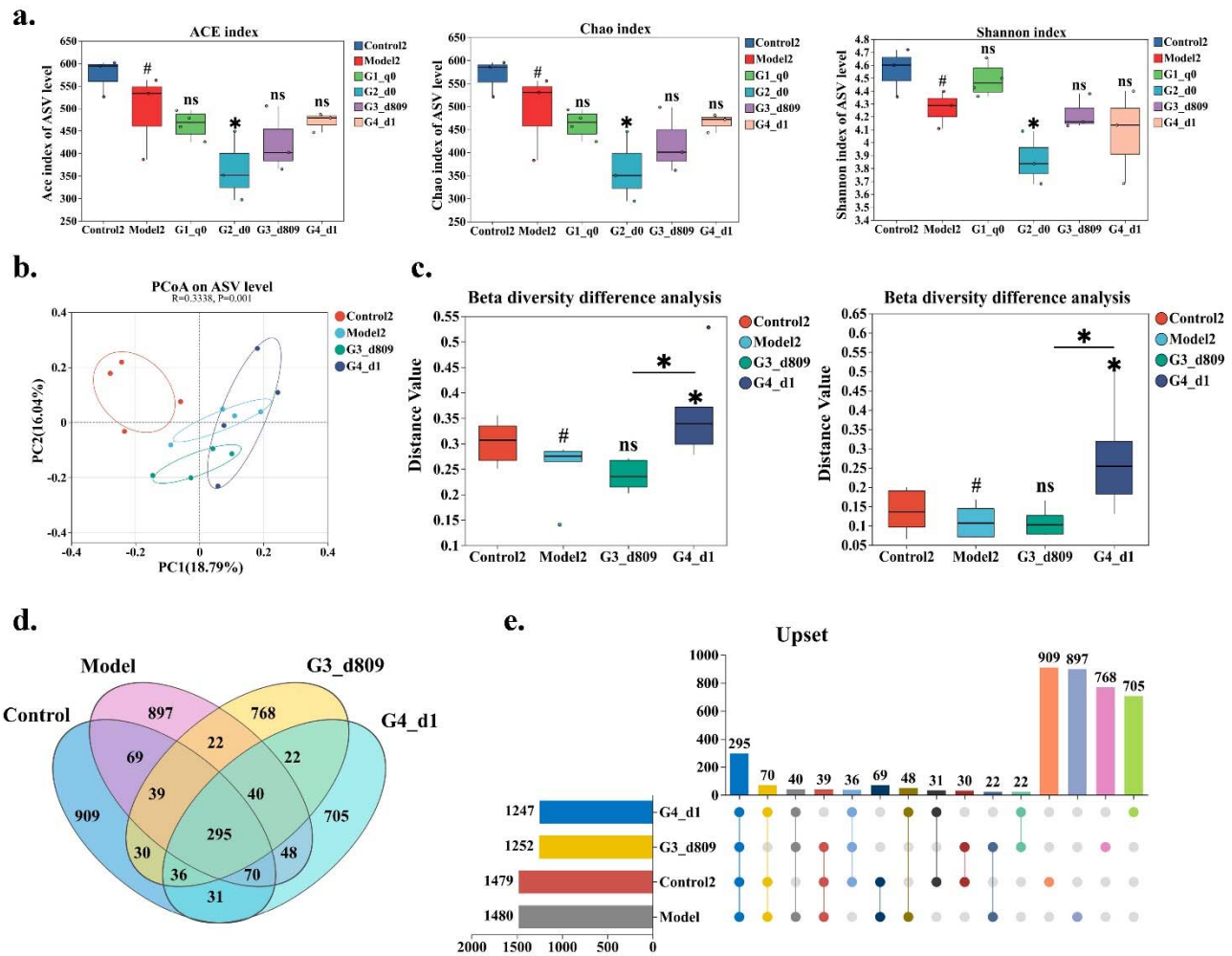


Fig. 5 AR809 modulates gut microbiota diversity and community structure. a. Alpha diversity indices (ACE, Chao1, Shannon). b. PCoA plot of microbial community structure. PERMANOVA (Bray-Curtis, 999 permutations): $R^2 = 0.34$, $P < 0.001$. c. Beta diversity differences at phylum and genus levels. d. Venn diagram of shared OTUs. e. UpSet plot of unique and shared OTU distributions. One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. Symbols indicate statistical significance. #, ##, ###, #### denote comparisons versus the Control group (# $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$). *, **, ***, **** denote comparisons versus the Model group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). ns, not significant.

To further identify taxa driving these compositional differences, LEfSe analysis was conducted (Fig. 6a, b). The Control group was enriched in Oscillospiraceae and Eubacterium_nodatum_group, reflecting a metabolically stable community. In contrast, the Model group was characterized by Clostridiaceae and Clostridium_sensu_stricto_1, consistent with dysbiosis and opportunistic proliferation. Group 3 (AR809 fermented milk) showed enrichment of Erysipelotrichaceae and *Turicibacter*, while Group 4 (EPS809) was associated with Coriobacteriaceae_UCG-002 and *Dubosiella*. Circos analysis highlighted the dominance of Muribaculaceae (Bacteroidetes) and *Lactobacillus* (Firmicutes) as major components of the murine gut microbiota (Fig. 6c). Both Groups 3 and 4 exhibited higher relative abundances of these taxa compared with the Model group. Group 4 showed particularly strong enrichment of Muribaculaceae and *Dubosiella*, whereas Group 3 was more associated with the recovery of *Turicibacter*. These differences suggest that viable cells and exopolysaccharides modulate gut microbiota through partially distinct mechanisms. Heatmap analysis (Fig. 6d) confirmed these distribution patterns. Romboutsia declined sharply after antibiotic treatment and

showed little response to intervention. In contrast, taxa such as *Turicibacter*, *Coriobacteriaceae*_UCG-002, and *Dubosiella* exhibited partial or marked recovery in the intervention groups (Groups 3 and 4), especially in Group 4.

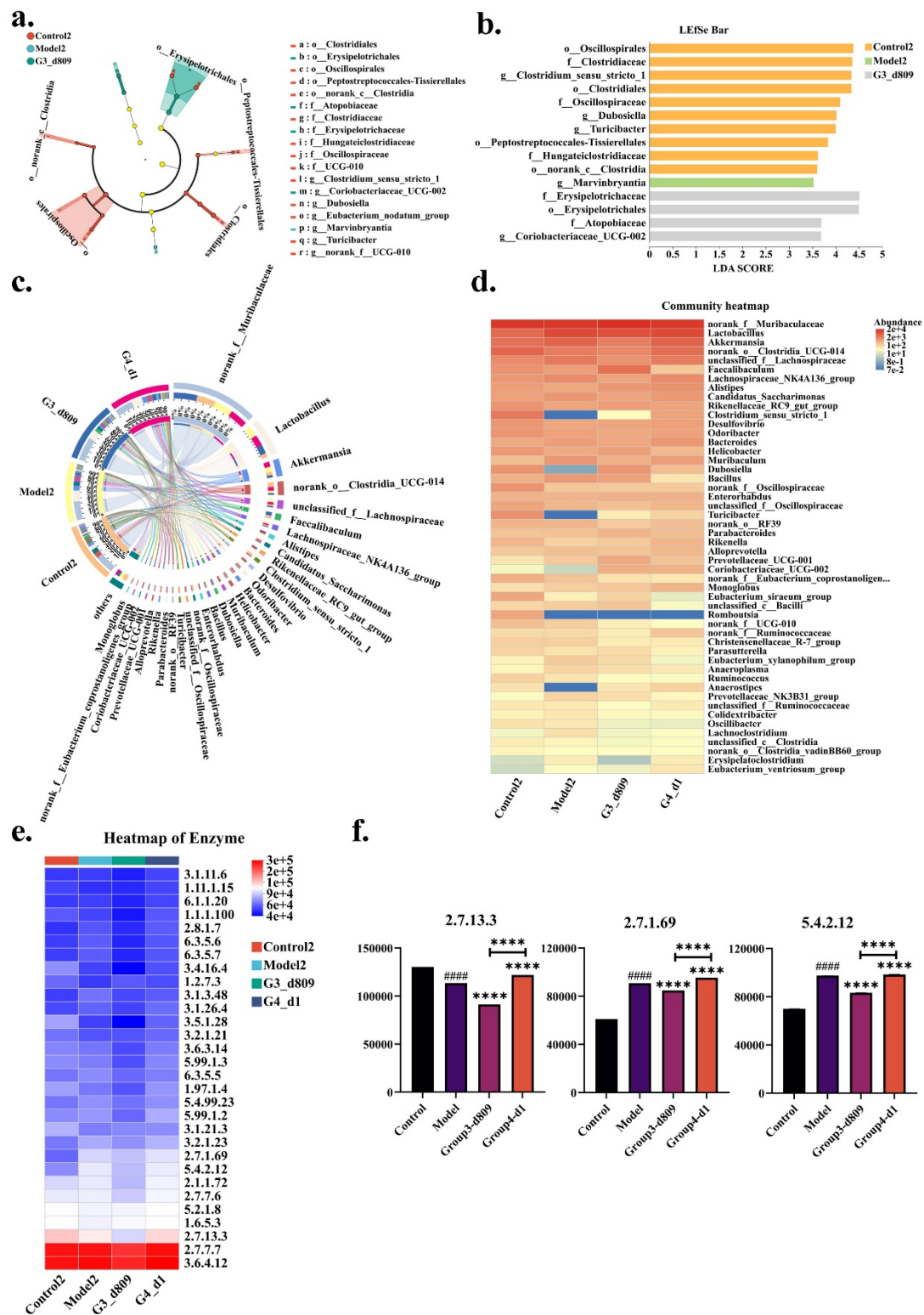


Fig. 6 Effects of viable AR809 and AR809 exopolysaccharides on gut microbiota composition and predicted functions. a. LefSe cladogram. b. LDA score plot. c. Circos plot. d. Heatmap of differentially abundant genera. e, f. PICRUSt2-based functional prediction showing group-wise differences in KEGG pathways. One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. Symbols indicate statistical significance. #, ##, ###, ####, ##### denote comparisons versus the Control group (# $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$). *, **, ***, **** denote comparisons versus the Model group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). ns, not significant.

Overall, viable AR809 cells and EPS809 supplementation promoted partial restoration of microbiota composition, characterized by preservation of a core community and enrichment of beneficial taxa. Viable cells mainly favored *Turicibacter* enrichment, which may be related to bile acid metabolism^[26-29]. In contrast, exopolysaccharides preferentially enhanced Muribaculaceae and *Dubosiella*, consistent with prebiotic-driven effects^[29-31].

3.5 AR809 viable cells and exopolysaccharides differentially regulate microbial metabolism and host signaling

Functional predictions based on PICRUST2 and KEGG annotation were used to explore potential mechanisms underlying AR809-mediated effects (Fig. 6e, f)^[32]. The Model group exhibited clear metabolic disruption, with a notable reduction in several microbial enzymes, including histidine kinase (EC 2.7.13.3). Histidine kinase is associated with the EnvZ/OmpR pathway and may contribute to bacterial osmoregulation and host-microbiota communication. This down-regulation suggests that antibiotic-induced dysbiosis impaired microbial sensing of host signals. AR809 polysaccharide treatment (Group 4) appeared to restore this pathway toward Control levels, which may be associated with improved mucosal immune function such as recovery of sIgA^[33]. In contrast, viable AR809 cells (Group 3) were linked to enhanced microbial metabolic capacity. Enrichment of glycolytic and carbohydrate fermentation-related enzymes, including phosphotransferase (EC 2.7.1.69) and phosphoglycerate mutase (EC 5.4.2.12), indicated a shift toward increased SCFA precursor synthesis. These predictive findings are consistent with the observed improvement in barrier integrity and ZO-1 expression. This consistency suggests that viable cells may promote intestinal recovery through a metabolism-centered route.

Collectively, these findings indicate that AR809 and EPS809 exert beneficial effects through different tendencies. Viable cells may promote intestinal recovery mainly by modulating microbial metabolic functions, while EPS809, as an extracellular polysaccharide, could influence host-microbiota communication pathways, such as the EnvZ/OmpR-associated pathways, thereby supporting intestinal barrier integrity and immune function. In addition to the individual effects of AR809 and EPS809, it is hypothesized that their combined action could generate synergistic benefits. The viable AR809 bacteria contribute to gut microbiota regulation by promoting microbial diversity and metabolic functions, while EPS809 enhances gut barrier integrity and modulates immune responses. This dual action could result in a more comprehensive modulation of gut health than either component alone. The synergy between AR809 and EPS809 could also lead to the development of novel compound probiotic formulations, where the combined benefits of live bacteria and extracellular polysaccharides may offer enhanced protection against dysbiosis and related intestinal dysfunctions. This interpretation suggests that viable cells and EPS809 may act together, supporting further evaluation of AR809-based formulations.

In addition, several limitations should be considered when interpreting these findings. First, EPS809 was characterized by its basic composition (total sugar content and residual protein), molecular weight distribution, and FTIR profile, whereas advanced structural analysis, such as monosaccharide composition,

glycosidic bond type identification, and NMR-based fine-structure determination, was not performed in the current study. This lack of in-depth structural characterization limits our ability to fully explore the structure-activity relationship between EPS809's molecular features and its functional effects. Second, the predicted microbial enzyme activities and pathway changes were derived from 16S rRNA-based inference tools, which provide only indirect approximations and should be validated using targeted metabolomics or metagenomic approaches. Third, microbial metabolites were not directly quantified, limiting the ability to establish causal links between functional predictions and host responses. Fourth, the sample size was relatively modest, which may reduce statistical power and limit generalizability. Fifth, the 7-day acute gut dysbiosis model used in this study may not fully represent the long-term effects of antibiotic-induced dysbiosis and probiotic intervention in clinical settings. Future work should incorporate chronic gut dysbiosis models to better reflect clinically relevant long-term antibiotic use, and more comprehensively evaluate the prolonged therapeutic effects of AR809. Additionally, the 0.4 g/L EPS809 dose, selected based on its effects on texture and sensory properties, has not been validated for bioavailability and metabolism, which remains a limitation. In future studies, we plan to incorporate advanced structural analysis techniques, including monosaccharide composition and glycosidic bond type identification, to further elucidate the structure-activity relationship of EPS809. Combining rigorous material characterization, targeted metabolomics, and functional validation experiments will be crucial to substantiate the predicted pathways and clarify causal relationships.

4. Conclusion

Gut microbiota dysbiosis is a critical contributor to intestinal dysfunction, and probiotic-derived strategies are increasingly explored to support gut homeostasis [35-37]. This study demonstrated that AR809 and EPS809 both alleviated antibiotic-induced gut dysbiosis and promoted the restoration of intestinal function. AR809 exhibited stronger effects, while exopolysaccharides also produced significant improvements and similar protective trends, suggesting that exopolysaccharides may contribute to the benefits associated with AR809. Both interventions improved intestinal barrier integrity, enhanced mucosal immunity, and attenuated inflammatory responses, accompanied by partial recovery of microbial composition and enrichment of beneficial taxa. Predictive analyses suggested potential involvement of microbial metabolic modulation and host-microbiota communication pathways, although these interpretations remain to be validated by targeted experiments. Overall, these findings suggest that AR809 and EPS809 are functionally relevant constituents that may contribute to improving dysbiosis-associated intestinal outcomes in this murine model. Future studies should incorporate targeted metabolomics, gene-level validation, and advanced experimental models, followed by clinical evaluation, to further substantiate these observations.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (U23A20259 and 32372307), the National Science Fund for Distinguished Young Scholars of China (32025029), the

Young Elite Scientist Sponsorship Program of China Association for Science and Technology (YESS20220128), The Shanghai Oriental Talent Youth Project (T2023218), Shanghai Rising-Star Program (24QA2706000), and the Shanghai Research Center for Food Microbiology Engineering (19DZ2281100).

Conflict of interest

We declare that we have no conflict of interest.

Reference

- [1] G. Zhu, L. Yan, L. Fang, et al., Possible immune mechanisms of gut microbiota and its metabolites in the occurrence and development of immune thrombocytopenia, *Front. Microbiol.* 15 (2024). <https://doi.org/10.3389/fmicb.2024.1426911>.
- [2] M.B. Azad, T. Konya, D.S. Guttman, et al., Infant gut microbiota and food sensitization: associations in the first year of life, *Clinical & Experimental Allergy*. 45 (2015) 632-643. <https://doi.org/10.1111/cea.12487>.
- [3] R.B. Panwar, R.P. Sequeira, T.B. Clarke, Microbiota-mediated protection against antibiotic-resistant pathogens, *Genes & Immunity*. 22 (2021) 255-267. <https://doi.org/10.1038/s41435-021-00129-5>.
- [4] B.F. Xu, Z.B. Wang, Y.Q. Wang, et al., Milk-derived *Lactobacillus* with high production of short-chain fatty acids relieves antibiotic-induced diarrhea in mice, *Food & Function*. 15 (2024). <https://doi.org/10.1039/D3FO04706G>.
- [5] L.A. Hesser, A.A. Puente, J. Arnold, et al., A synbiotic of *Anaerostipes caccae* and lactulose prevents and treats food allergy in mice, *Cell Host & Microbe*. 32 (2024) 1163-1176. <https://doi.org/10.1016/j.chom.2024.05.019>.
- [6] M.J. Elder, S.J. Webster, W. Chee, et al., β -Glucan Size Controls Dectin-1-Mediated Immune Responses in Human Dendritic Cells by Regulating IL-1 β Production, 8 (2017). <https://doi.org/10.3389/fimmu.2017.00791>.
- [7] X. Gong, S. Liu, B. Xia, et al., Oral delivery of therapeutic proteins by engineered bacterial type zero secretion system, *Nature Communications*. 16 (2025). <https://doi.org/10.1038/s41467-025-57153-6>.
- [8] D. Hua, Q. Yang, X. Li, et al., The combination of *Clostridium butyricum* and *Akkermansia muciniphila* mitigates DSS-induced colitis and attenuates colitis-associated tumorigenesis by modulating gut microbiota and reducing CD8⁺ T cells in mice, *MSYSTEMS*. 10 (2025). <https://doi.org/10.1128/msystems.01567-24>.
- [9] G.C. Jia, N. Che, Y.J. Xia, et al., Adhesion to pharyngeal epithelium and modulation of immune response: *Lactobacillus salivarius* AR809, a potential probiotic strain isolated from the human oral cavity, *Journal of Dairy Science*. 102 (2019) 6738-6749. <https://doi.org/10.3168/jds.2018-16117>.
- [10] W. Ren, Y. Xia, G. Wang, et al., Bioactive exopolysaccharides from a *S. thermophilus* strain: Screening, purification and characterization, *International Journal of Biological Macromolecules*. 86 (2016) 402-407. <https://doi.org/10.1016/j.ijbiomac.2016.01.085>.
- [11] D.D. Li, J. Wang, Y. Jiang, et al., Quantifying functional redundancy in polysaccharide-degrading prokaryotic communities, *Microbiome*. 12 (2024). <https://doi.org/10.1186/s40168-024-01838-5>.
- [12] Z. Mu, Y. Yang, Y. Xia, et al., Probiotic yeast BR14 ameliorates DSS-induced colitis by restoring the gut barrier and adjusting the intestinal microbiota, *Food & Function*. 12 (2021). <https://doi.org/10.1039/D1FO01314A>.
- [13] L. Mei, Y. Liu, Y. Chen, et al., *Lactobacillus rhamnosus* alters intestinal IgA levels and IgA -microbiota interactions in healthy mice in a strain-specific manner, *Food Bioscience*. 59 (2024). <https://doi.org/10.1016/j.fbio.2024.103936>.
- [14] Q. Lu, T.C.A. Hitch, J.Y. Zhou, et al., A host-adapted auxotrophic gut symbiont induces mucosal immunodeficiency, *Science*. 385 (2024). <https://doi.org/10.1126/science.adk2536>.
- [15] A.L. Unger, A.Astrup, E.L. Feeney, et al., Harnessing the Magic of the Dairy Matrix for Next-Level Health Solutions: A Summary of a Symposium Presented at Nutrition 2022, *Current Developments in Nutrition*. 7 (2023). <https://doi.org/10.1016/j.cdnut.2023.100105>.
- [16] T. Huppertz, B. Shkempi, L. Brader, et al., Dairy Matrix Effects: Physicochemical Properties Underlying a Multifaceted Paradigm, *Nutrients*. 16 (2024). <https://doi.org/10.3390/nu16070943>.

- [17] C. Humblot, P. Alvanoudi, E. Alves, et al., A scoping review of the health effects of fermented foods in specific human populations and their potential role in precision nutrition: current knowledge and gaps, *Frontiers in Nutrition*. 12 (2025). <https://doi.org/10.3389/fnut.2025.1650633>.
- [18] S.S. Zhang, Y.G. Sun, Q.X. Nie, et al., Effects of four food hydrocolloids on colitis and their regulatory effect on gut microbiota, *Carbohydrate Polymers*. 323 (2024). <https://doi.org/10.1016/j.carbpol.2023.121368>.
- [19] X. Zhang, Z. Yuan, J.S. Wu, Y. He et al., An Orally-Administered Nanotherapeutics with Carbon Monoxide Supplying for Inflammatory Bowel Disease Therapy by Scavenging Oxidative Stress and Restoring Gut Immune Homeostasis, *ACS nano*. 17 (2023) 21116-21133. <https://doi.org/10.1021/acsnano.3c04819>.
- [20] J. Yang, H. Kuang, N. Li, et al., The modulation and mechanism of probiotic-derived polysaccharide capsules on the immune response in allergic diseases, *Critical Reviews in Food Science & Nutrition*. 63 (2023) 14. <https://doi.org/10.1080/10408398.2022.2062294>.
- [21] Y.J. Wu, X.Y. Zhang, X.Q. Liu, et al., Galactooligosaccharides and *Limosilactobacillus reuteri* synergistically alleviate gut inflammation and barrier dysfunction by enriching *Bacteroides acidifaciens* for pentadecanoic acid biosynthesis, *Nature Communications*. 15 (2024). <https://doi.org/10.1038/s41467-024-53144-1>.
- [22] X.Z. Wang, X. Li, L.Y. Zhang, et al., Recent progress in plant-derived polysaccharides with prebiotic potential for intestinal health by targeting gut microbiota: a review, *Critical Reviews in Food Science and Nutrition*. 64 (2024) 12242-12271. <https://doi.org/10.1080/10408398.2023.2248631>.
- [23] L. Tian, X.W. Wang, A.K. Wu, et al., Deciphering Functional Redundancy in the Human Microbiome, *Nature Communications*. 11 (2019). <https://doi.org/10.1038/s41467-020-19940-1>.
- [24] L. Liu, Z. Jiang, I. Khan, et al., Ginseng polysaccharides alter the gut microbiota and kynurenine/tryptophan ratio, potentiating the antitumour effect of antiprogrammed cell death 1/programmed cell death ligand 1 (anti-PD-1/PD-L1) immunotherapy, *Gut*. 71 (2022) 734-745. <https://doi.org/10.1136/gutjnl-2020-321031>.
- [25] J.M. Pickard, S. Porwollik, G. Caballero-Flores, et al., Dietary amino acids regulate *Salmonella* colonization via microbiota-dependent mechanisms in the mouse gut, *Nature Communications*. 16 (2025). <https://doi.org/10.1038/s41467-025-59706-1>.
- [26] W.J. Ma, C. Wang, H. Brumer, Bespoke plant glycoconjugates for gut microbiota-mediated drug targeting, *Science*. 388 (2025) 1410-1416. <https://doi.org/10.1126/science.adk7633>.
- [27] J. Lynch, E.L. González, K. Choy, et al., Gut microbiota *Turicibacter* strains differentially modify bile acids and host lipids, *Nature Communications*. 14 (2023). <https://doi.org/10.1038/s41467-023-39403-7>.
- [28] T.H. Won, M. Arifuzzaman, C.N. Parkhurst, et al., Host metabolism balances microbial regulation of bile acid signalling, *Nature*. 638 (2025) 216-224. <https://doi.org/10.1038/s41586-024-08379-9>.
- [29] Cockburn, W. Darrell, Koropatkin, Polysaccharide Degradation by the Intestinal Microbiota and Its Influence on Human Health and Disease, *Journal of Molecular Biology*. 428 (2016) 3230-3252. <https://doi.org/10.1016/j.jmb.2016.06.021>.
- [30] T. Zhang, T.T. Cheng, S. Geng, et al., Synbiotic Combination between *Lactobacillus paracasei* VL8 and Mannan-Oligosaccharide Repairs the Intestinal Barrier in the Dextran Sulfate Sodium-Induced Colitis Model by Regulating the Intestinal Stem Cell Niche, *Journal of Agricultural and Food Chemistry*. 72 (2024) 2214-2228. <https://doi.org/10.1021/acs.jafc.3c08473>.
- [31] Y.K. Zhao, W.X. Zhao, X.J. Chai, et al., Reshaping the gut microbiota: A novel oppinion of *Eucommiae cortex* polysaccharide alleviate learning and memory impairments in Alzheimer's disease, *Journal of Advanced Research*. (2025). <https://doi.org/10.1016/j.jare.2025.04.025>.
- [32] R. Valdés-Mas, A. Leshem, D. Zheng, et al., Metagenome-informed metaproteomics of the human gut microbiome, host, and dietary exposome uncovers signatures of health and inflammatory bowel disease, *Cell*. 188 (2025) 1062-1083. <https://doi.org/10.1016/j.cell.2024.12.016>.
- [33] X.P. Li, Y. Pang, L.Y. Jiang, et al., Two-component system GrpP/GrpQ promotes pathogenicity of uropathogenic *Escherichia coli* CFT073 by upregulating type 1 fimbria, *Nature Communications*. 16 (2025). <https://doi.org/10.1038/s41467-025-55982-z>.
- [34] H.Y. Chen, T.Y. Tong, S.Y. Lu, et al., Urea cycle activation triggered by host-microbiota maladaptation driving colorectal tumorigenesis, *Cell Metabolism*. 35 (2023) 651-666. <https://doi.org/10.1016/j.cmet.2023.03.003>.

- [35] P. Manrique, I. Montero, M. Fernandez-Gosende, et al., Past, present, and future of microbiome-based therapies. *Microbiome Res Rep.* 3 (2024) 23. <http://dx.doi.org/10.20517/mrr.2023.80>.
- [36] C.Y. Sun, J.W. Zhu, X.Y. Sun, et al., Targeting the human gut microbiome: a comparative review of probiotics, prebiotics, synbiotics, and postbiotics. *Journal of Advanced Research.* (2025). <https://doi.org/10.1016/j.jare.2025.12.032>.
- [37] K.S. Tukaram, S.R. Sopanrao, L.O. Kacharu. Unveiling The Therapeutic Potential of Probiotics: A Review. *Journal of Future Foods.* (2025). <https://doi.org/10.1016/j.jfutfo.2025.09.011>.