Exploring the anti-inflammatory effects of postbiotic proteins from Lactobacillus delbrueckii CIDCA 133 on inflammatory bowel disease model

Andria dos Santos Freitas, Fernanda Alvarenga Lima Barroso, Gabriela Munis Campos, Monique Ferrary Américo, Rhayane Cristina dos Santos Viegas, Gabriel Camargos Gomes, Kátia Duarte Vital, Simone Odília Antunes Fernandes, Rodrigo Dias de Oliveira Carvalho, Julien Jardin, et al.

International Journal of Biological Macromolecules 277 (2024) 134216

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Exploring the anti-inflammatory effects of postbiotic proteins from *Lactobacillus delbrueckii* CIDCA 133 on inflammatory bowel disease model

Andria dos Santos Freitas [a], Fernanda Alvarenga Lima Barroso [a], Gabriela Munis Campos [a], Monique Ferrary Américo [a], Rhayane Cristina dos Santos Viegas [a], Gabriel Camargos Gomes [a], Kátia Duarte Vital [b], Simone Odília Antunes Fernandes [b], Rodrigo Dias de Oliveira Carvalho [b], Julien Jardin [c], Ana Paula Gomes dos Santos Miranda [d], Enio Ferreira [d], Flaviano Santos Martins [e], Juliana Guimarães Laguna [a], Gwénaël Jan [c], Vasco Azevedo [a,*,1], Luís Cláudio Lima de Jesus [a,*,1]

[a] Federal University of Minas Gerais, Department of Genetics, Ecology, and Evolution, Belo Horizonte, Minas Gerais, Brazil
[b] Federal University of Minas Gerais, Department of Clinical and Toxicological Analysis, Belo Horizonte, Minas Gerais, Brazil
[c] STLO, INRAE, Institut Agro, Rennes, France
[d] Federal University of Minas Gerais, Department of General Pathology, Belo Horizonte, Minas Gerais, Brazil
[e] Federal University of Minas Gerais, Department of Microbiology, Belo Horizonte, Minas Gerais, Brazil

ARTICLE INFO

Keywords:
Ulcerative colitis
Extractable proteins
Immunomodulation

ABSTRACT

Lactobacillus delbrueckii CIDCA 133 is a promising health-promoting bacterium shown to alleviate intestinal inflammation. However, the specific bacterial components responsible for these effects remain largely unknown. Here, we demonstrated that consuming extractable proteins from the CIDCA 133 strain effectively relieved acute ulcerative colitis in mice. This postbiotic protein fraction reduced the disease activity index and prevented colon shortening in mice. Furthermore, histological analysis revealed colitis prevention with reduced inflammatory cell infiltration into the colon mucosa. Postbiotic consumption also induced an immunomodulatory profile in colitic mice, as evidenced by both mRNA transcript levels (*Tlr2, Nfkb1, Nlpr3, Tnf,* and *Il6*) and cytokines concentration (IL1β, TGFβ, and IL10). Additionally, it enhanced the levels of secretory IgA, upregulated the transcript levels of tight junction proteins (*Hp* and *F11r*), and improved paracellular intestinal permeability. More interestingly, the consumption of postbiotic proteins modulated the gut microbiota (*Bacteroides, Arkkemansia, Dorea,* and *Oscillospira*). Pearson correlation analysis indicated that IL10 and IL1β levels were positively associated with *Bacteroides* and *Arkkemansia_Lactobacillus* abundance. Our study reveals that CIDCA 133-derived proteins possess anti-inflammatory properties in colonic inflammation.

1. Introduction

Ulcerative colitis (UC) is an inflammatory disease with a worldwide increasing incidence, affecting colon tissues [1]. The etiology of UC involves genetic predisposition, altered immunological responses, disruption of epithelial barrier function, and intestinal dysbiosis [2,3], resulting in symptoms such as diarrhea, rectal bleeding, abdominal cramps, and weight loss. UC constitutes a serious risk factor for the development of colorectal cancer [4]. Due to the limited efficacy of available therapeutics agents (e.g., 5-aminosalicylic acid, corticosteroids, antibiotics, and immunomodulators) in treating or alleviating UC [2,5], microbiota regulation-focused strategies have emerged as potential therapies [6].

Traditionally, the consumption of probiotics has been considered a promising therapeutic option for attenuating IBD due to their ability to modulate inflammation and regulate the intestinal microbiota [7,8]. Emerging evidence suggests that health benefits for the host do not intrinsically depend on the viability of probiotics. Instead, these benefits may be mediated, at least in part, by the release of bioactive compounds derived from health-promoting bacteria known as postbiotics (e.g.,

* Corresponding authors.
E-mail addresses: vasco@icb.ufmg.br (V. Azevedo), luiislimma@gmail.com (L.C.L. de Jesus).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ijbiomac.2024.134216
Received 30 April 2024; Received in revised form 22 July 2024; Accepted 25 July 2024
Available online 26 July 2024

cytoplasmic, secreted, or surface proteins, exopolysaccharides, peptidoglycans, short-chain fatty acids, peptides) [9].

Some postbiotic compounds can act as microbe-associated molecular patterns (MAMP), triggering immunomodulatory effects of probiotic microorganisms through interactions with host cell pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) or NOD-like receptors (NLRs) [10,11] as well as G protein-coupled receptors (GPRs), including GPR43 [12]. These interactions can result in the activation or inhibition of inflammation-related signaling pathways, including the nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways [13]. The biological activity of postbiotic compounds, including immunomodulation, has been reported in potentially beneficial microorganisms, such as *Propionibacterium freudenreichii* [14], *Lacticaseibacillus plantarum* [15], *Limosilactobacillus reuteri* [16], *Faecalibacterium prausnitzii* [17], among others.

Promising anti-inflammatory properties have been identified in the potentially health-promoting strain *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133, isolated from raw cow milk in the La Plata region (Argentina) [18,19]. It has been reported that CIDCA 133 controls intestinal inflammation in a mouse model of 5-Fluorouracil (5-FU)-induced mucositis by regulating the epithelial barrier and TLR2/4/MYD88/NF-κB signaling pathway [20]. Furthermore, this strain was able to maintain its anti-inflammatory effects even after being heat-inactivated or through its secreted products (postbiotics preparation), ameliorating histopathological damage and modulating the gene expression of inflammatory markers, such as *Tlr2, Nfkb1*, *Il1b*, *Il17a*, *Tnf* and *Il10* in the context of mucositis [21]. These data suggest that the anti-mucositis effects reported for this strain may be mediated by MAMPs exposed on the surface of the bacteria (e.g., exopolysaccharides, surface proteins, lipoteichoic acid) or secreted proteins, which can activate human immune proteins involved in the inflammatory NF-κB signaling pathway, as proposed by a previous probiogenomics study [22].

Therefore, as the specific molecules of *Lactobacillus delbrueckii* CIDCA 133 responsible for its anti-inflammatory effects remain poorly characterized, this work investigated whether extractable proteins from this strain could mitigate intestinal inflammation and colonic epithelial damage in a mouse model of ulcerative colitis-induced by dextran sulfate sodium (DSS).

2. Material and methods

2.1. Bacterial growth conditions

Lactobacillus delbrueckii subsp. *lactis* CIDCA 133 was obtained from the culture collection of the CIDCA Center (Centro de Investigación y Desarrollo en Criotecnología de Alimentos, Universidad Nacional de LaPlata, Argentina). CIDCA 133 was grown in liquid MRS broth (Acumedia, Lansing, USA) under microaerophilic conditions at 37 °C for 18 h without agitation.

2.2. Proteins extraction

The extraction of CIDCA 133 proteins was performed using Guanidine Hydrochloride (GuHCl) following the method described by Le Maréchal et al. [23], with some modifications. Specifically, 200 mL of CIDCA 133 culture in the final exponential phase ($O.D_{600nm}$ 1.6; 10^9 CFU/mL) were harvested by centrifugation (8000 ×*g* for 10 min, 4 °C) and washed twice with 20 mL of sterile phosphate-buffered saline (PBS) 0.1 M (pH 7.4). The pellet was then resuspended in 10 mL of 5 M Guanidine Hydrochloride (Sigma-Aldrich, St. Louis, USA) and incubated for 15 min at 50 °C. Subsequently, the suspension was centrifuged at room temperature (21.000 ×*g* for 20 min), and the supernatant (guanidine extract) was collected and dialyzed against 2 L of PBS buffer (pH 7.4) at 4 °C with shaking, using the Slide-A-Lyzer™ Dialysis kit (10.000 MWCO) (Thermo Scientific, Rockford, USA). After 3 h of dialysis, the PBS buffer was refreshed once, and the dialysis process continued for an additional 24 h.

Extractable proteins from CIDCA 133 (30 μL) were analyzed using one-dimensional SDS-PAGE (12 %) (Fig. 1a), following dilution in Laemmli sample buffer and denaturation for 10 min at 95 °C, according to a standard protocol [24]. The proteins were then quantified using a Qubit™ fluorometer (Invitrogen, Oregon, USA) and stored at −80 °C until further use in mice administration and proteomics analysis.

2.3. Protein identification by LC-MS/MS

A standardized quantity of 10 μg of proteins was loaded into each well of a homemade 12 % SDS-PAGE gel (Miniprotean II, Bio-Rad). Additionally, one well was exclusively reserved for pre-stained molecular mass markers (Precision Plus Protein™ Kaleidoscope™ Prestained Protein Standards, Bio-Rad). Gel electrophoresis was conducted until proteins migrated approximately 3 mm into the separating 12 % gel, followed by fixation and Coomassie blue staining (Bio-Safe, Bio-Rad). Gel strips containing all the protein samples were subsequently excised and subjected to in-gel trypsinolysis, following established procedures [25]. The resulting peptides were separated via nano-LC using a nano RSLC Dionex U3000 system before MS/MS identification, as detailed previously [26]. Nano-LC was fitted to a Q-Exactive mass spectrometer with a nanoelectrospray ion source (Thermo Scientific, San Jose, USA). Peptide spectra were recorded in full MS mode within a mass range of 250–2000 *m/z*, with a resolution of 70,000 at m/z 200. Peptide identification from the MS/MS spectra was done using X!Tandem pipeline software [27]. The search was conducted against the proteome of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 (downloaded from NCBI.nlm.nih.gov). The search parameters included trypsin cleavage with peptide mass tolerance set at 10 ppm for MS and 0.05 Da for MS/MS. Methionine oxidation was considered a variable modification. Validation of peptide identification required an *E*-value lower than 0.05 for each peptide. A minimum of two peptides per protein was also mandated, resulting in a false discovery rate (FDR) of 0.15 % for protein identification. Proteins with a coverage threshold ≥20 % were selected. The SurfG+ software was utilized to determine the subcellular localization of these proteins [28].

2.4. Mice handling and DSS-induced acute colitis model

Six-week-old C57BL/6 female mice, purchased from the Bioterism Center (CEBIO-UFMG), were housed in sterile ventilated polycarbonate cages under standard conditions (the temperature at 25 ± 2 °C, with a 12-h light/dark cycle-controlled room) with *ad libitum* access to autoclaved water and standard rodent chow for 24 h before experiments. Before the start of the experiment, all mice were acclimatized for fourteen days. The experimental procedures were approved by the Animal Ethics Committee of the Federal University of Minas Gerais (protocol number 116/2023) and adhered to the guidelines outlined by the Brazilian National Council for the Control of Animal Experimentation (CONCEA).

Mice were randomly allocated into three groups ($n = 6$ animals per group): Control (NC), Colitis (DSS), and Colitis treated with postbiotic proteins from CIDCA 133 (PP).

To induce the acute colitis model, 2.5 % DSS (36–50 kDa, MP Biomedicals, Cat:160110, Lot: S5036) was dissolved in drinking water and orally administrated by *continuous feeding (ad libitum)* to mice in the DSS and PP groups for seven consecutive days [29]. The non-colitis group (NC) received only sterile water. The bottles containing DSS or water were changed every 24 h. Mice in the PP group were treated with 300 μL of postbiotic proteins (100 μg) via gavage for seven consecutive days, once daily. Control groups (NC and DSS) received a 0.1 M PBS solution (300 μL) by the same route. On the 8th experimental day, all mice were anesthetized with a xylazine (16 mg/kg)/ketamine (80 mg/kg) mixture (Syntec, Tamboré, Brazil) for blood collection (Fig. 1b). After

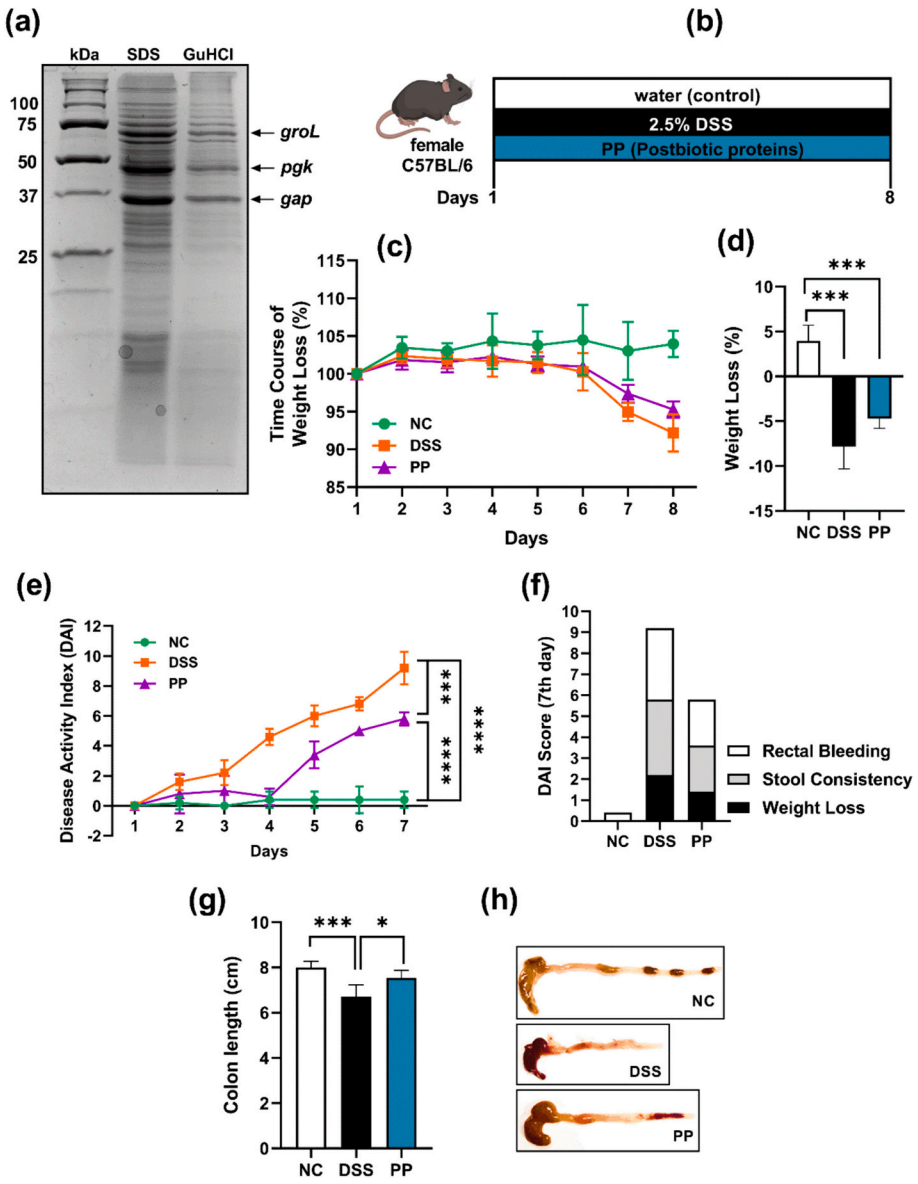

Fig. 1. Postbiotic proteins from *Lactobacillus delbrueckii* CIDCA 133 ameliorate clinical colitis signs. (a) 12 % SDS-PAGE gel of CIDCA 133 proteins extracted with sodium dodecyl sulfate (SDS) and guanidine hydrochloride (GuHCl); (b) Experimental scheme; (c-d) mice weight loss; (e-f) DAI score; (g-h) colon length. Asterisks indicate statistically significant differences ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$) according to the two-way ANOVA plus Tukey's post-test (c, e), or ANOVA plus Tukey's post-test (d, g). NC: negative control (healthy mice); DSS: dextran sodium sulfate (colitic mice); PP: postbiotic proteins (colitic mice consuming postbiotic proteins).

euthanasia, stool and colon tissue samples were collected and stored for subsequent analysis, including 16S rRNA sequencing, hematoxylin and eosin (H&E) and Periodic Acid-Schiff (PAS) staining, measurement of myeloperoxidase (MPO) and *N*-acetylglucosaminidase (NAG) activity, assessment of colonic gene expression, and determination of cytokines levels.

2.5. Disease activity index (DAI)

The severity of colitis was macroscopically assessed using the DAI score [30]. For this, mice were monitored daily, and the DAI score was calculated by summing the scores for the following parameters: weight loss (0: none; 1: 1–5 %; 2: 6–10 %; 3: 11–18 %; 4: > 18 %); rectal bleeding (0: negative hemoccult; 1: negative hemoccult; 2: positive hemoccult; 3: visible blood traces in stool; 4: gross rectal bleeding); and stool consistency (0: normal, 1: soft but still formed; 2: soft; 3: wet, very soft; 4: diarrhea).

2.6. Intestinal permeability

On the euthanasia day, all mice were gavaged with 100 μL of diethylenetriaminepentaacetic acid (DTPA) labeled with 18.5 Mbq of technetium-99m (^{99m}Tc-DTPA) to assess the intestinal permeability. After four hours, all mice were anesthetized, and blood (200 μL) samples were collected from the axial plexus into pre-weighed acrylic test tubes. Subsequently, according to the recommended manufacturer's instructions, blood radioactivity was measured using an automatic gamma radiation counter (ANSR-Abbott, Chicago, USA). The results were expressed as a percentage of the administrated dose of ^{99m}Tc-DTPA per gram of blood (% ID/g) [31].

2.7. Colon histology

The distal colon tissue was collected, and its length was measured. Afterward, the colon was washed with a 0.1 M PBS buffer and opened,

followed by rolling for tissue fixation in 10 % buffered formalin (Synth, São Paulo, Brazil) for 24 h. After fixation, the tissue samples were paraffined and sectioned for H&E and PAS staining. A pathologist performed a double-masked analysis of the colonic histological changes, following the scoring system proposed by Wirtz et al. [29]. A count of mucus-producing goblet cells was performed with ImageJ 1.51j.8 (https://imagej.nih.gov/ij/).

2.8. Myeloperoxidase (MPO) and N-acetyl-β-D-glucosaminidase (NAG) activity measurement

The extension of the infiltrate cellular for neutrophils and macrophages in the colon tissue was performed by MPO and NAG enzyme activities, respectively [32]. Briefly, small sections of the proximal colon were processed and homogenized with a cold buffered solution (1 mL/100 mg) containing NaH_2PO_4 0.02 M, Na_2EDTA 0.015 M, and NaCl 0.1 M (LabSynth, Diadema, Brazil) (pH 4.7), using metal beads in the Precelys® 24 (6500 rpm, 15 s) (Bertin Technologies, Montigny Le Bretonneux, France). The colon homogenates were centrifuged (9.500 ×*g* at 4 °C for 10 min). After that, a hypotonic process [(cold 0.2 % NaCl (500 μL) for 30 s, followed by 500 μL of 1.6 % NaCl (LabSynth, Diadema, Brazil) containing glucose 5 % (Vetec, Rio de Janeiro, Brazil)] was performed. Then, the homogenate aliquots (750 μL each) were harvested for the MPO and NAG enzymatic activity essays.

For MPO, the homogenates were centrifuged (9.500 ×*g* for 10 min at 4 °C). The supernatant was mixed with NaH_2PO_4 (0.05 M) solution (pH 5.4) (1 mL/100 mg) (LabSynth, Diadema, Brazil) containing 0.5 % hexadecyltrimethylammonium bromide (HTAB) (Sigma-Aldrich, St. Louis, USA). Afterward, the supernatant was subjected to three freeze-thaw cycles in liquid nitrogen (lysis process), centrifuged (9500 ×*g* at 4 °C for 15 min), and harvested for colorimetric enzymatic assay. For this, 25 μL of supernatant was mixed with 25 μL of dimethyl sulfoxide (Sigma-Aldrich, St. Louis, USA) containing 1.6 mM 3,3,5,5′-Tetramethylbenzidine (Sigma-Aldrich, St. Louis, USA). After incubation at 37 °C for 5 min, followed by the addition of 100 μL H_2O_2 0.002 % (Vetec, Rio de Janeiro, Brazil) and a new incubation at 37 °C for 5 min, the reactions were stopped with 50 μL of sulphuric acid 1 M (Vetec, Rio de Janeiro, Brazil).

For NAG, the homogenates were centrifuged (9.500 ×*g* at 4 °C for 10 min), and a NaCl 0.9 % (LabSynth, Diadema, Brazil) solution containing Triton X-100 0.1 % (*v*/v) (1 mL/100 mg) (Vetec, Rio de Janeiro, Brazil) was mixed to the supernatant. After centrifugation (850 ×*g* for 10 min at 4 °C), the supernatant was harvested for colorimetric enzymatic assay. Thus, 100 μL of supernatant was added to 100 μL of cold citrate/phosphate buffer (pH 4.5) [(Na_2HPO_4 0.1 M (LabSynth, Diadema, Brazil) plus citric acid 0.1 M (Sigma-Aldrich, St. Louis, USA)] containing the substrate *p*-nitrophenyl-*N*-acetyl-β-D-glucosamine 2.24 mM (Sigma-Aldrich, St. Louis, USA). After incubation for 10 min at 37 °C, the reactions were stopped with 100 μL of glycine 0.2 M buffer [(glycine 0.8 M (Sigma-Aldrich, St. Louis, USA), NaCl 0.8 M (LabSynth, Diadema, Brazil), and NaOH 0.8 M (Vetec, Duque de Caxias, Brazil)] (pH 10.6).

The enzymatic assay absorbance (450 nm for MPO and 400 nm for NAG) was measured on a microplate spectrophotometer (Epoch, BioTek Instruments), and the results represented as arbitrary units of MPO or NAG per milligram of tissue.

2.9. Enzyme-linked immunosorbent assay (ELISA)

The levels of cytokines were measured by ELISA assay. Briefly, small sections of the proximal colon were harvested, weighed, and homogenized (1 mL/100 mg) in a cold PBS 0.1 M buffer containing phenylmethylsulphonyl fluoride (PMSF) 0.1 mM (Sigma- Aldrich, St. Louis, USA), EDTA 10 mM (Synth, São Paulo, Brazil), benzethonium chloride 0.1 mM (Sigma-Aldrich, St. Louis, USA), 0.05 % Tween-20 (Sigma-Aldrich, St. Louis, USA) and aprotinin A 20 KIU/mL (Sigma-Aldrich, St. Louis, USA). The tissue lysis process was performed by metal beads using Precellys® 24 (1 cycle, 6500 rpm, 15 s) (Bertin Technologies, Montigny Le Bretonneux, France). After that, the samples were centrifuged (3000 ×*g* at 4 °C for 10 min), and the resulting supernatants were kept at −80 °C until use. Then, the concentration of cytokines (IFNγ, IL1β, TGFβ, IL10, and IL17) was determined according to the ELISA MAX™ Set (Biolegend®) or DuoSet® ELISA Kit (R&D Systems, Minneapolis, USA), according to the recommended instructions.

2.10. Intestinal secretory IgA (sIgA) levels

Total sIgA levels were determined by ELISA, according to the method described by Barroso et al. [33]. Briefly, the contents of the small intestine were collected, weighed, and suspense with PBS 0.1 M (pH 7.2) (2 mL/500 mg) supplemented with an anti-protease cocktail (PMSF 1 mM; pepstatin 1 μM; aprotinin 1 μM; leupeptin 25 μM) (Sigma-Aldrich, St. Louis, EUA). After that, they were centrifuged (380 ×*g* at 4 °C for 30 min), and the supernatant was stored at −80 °C until immunoglobulin dosage. The assay was directly performed on microplates (Nunc-Immuno places, MaxiSorp) reversed with goat anti-mouse IgA antibody (Sigma, St. Louis, USA), and the levels of sIgA were determined based on IgA standard curve (Sigma-Aldrich, St. Louis, USA). *O*-phenylenediamine (OPD) (1 mg/mL) (Sigma-Aldrich, St. Louis, USA) was used as an enzymatic substrate for the reaction. The absorbance (492 nm) was measured using a microplate reader (Epoch, BioTek Instruments), and sIgA concentration was expressed in μg/mL of intestinal content.

2.11. Colonic gene expression by RT-qPCR

Total RNA isolation of the distal colon was performed with Pure Link™ RNA Mini Kit (Invitrogen, Carlsbad, USA), following the recommended instructions. RNA integrity was performed in 1.5 % agarose gel. 2 μg of RNA, previously determined by NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, USA), was converted to complementary DNA using a High-Capacity cDNA Reverse Transcription kit (ThermoFisher, Waltham, USA), following the manufacturer's instructions. The PowerUp™ SYBR® Green Master Mix (ThermoFisher) was used in quantitative PCR (qPCR) that was performed on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems™) (steps: 95 °C for 10 min; 40 cycles of 95 °C for 15 s, and 60 °C for 1 min). Relative target gene expression was normalized based on the transcript levels of *Gapdh* (glyceraldehyde 3-phosphate dehydrogenase) and *Actb* (actin beta) genes, following the $2^{-\Delta\Delta CT}$ method [34] (Table 1).

2.12. Gut microbiota profile

DNA extraction from mouse fecal samples was performed following the method of Yu and Morrison [42], with some modifications. The 16S rRNA sequencing and libraries were performed by Neoprospectra Microbiome Technologies (https://www.neoprospecta.com/) using the MiSeq Sequencing System (Illumina Inc., USA). The amplification was performed with primers 341F (CCTACGGGRSGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) for region V3-V4 of the 16S rRNA gene. Adapters were trimmed with Trimmomatic [43]. The sequences were clustered into Operational Taxonomic Units (OTU) (97 % similarity) and taxonomically assigned using UCHIME v. 4.2.40 [44] and VSEARCH v. 2.22.1 [45] pipelines. Alpha diversity (Shannon's and Simpson's index) data were obtained using PAST 4.13 software [46].

2.13. Statistical analysis

The data were analyzed using "one way" or "two way" ANOVA plus Tukey's test or Kruskal-Wallis plus Dunn's test. A Mann-Whitney or Student *t*-test was performed when two groups were compared. Pearson's correlation coefficient was used to explore the relationship between colonic microbiota and cytokines profile in the development of DSS-induced colitis. GraphPad Prism (8.0.2) (GraphPad Software, San

Table 1
Primer sequences used in qPCR.

Gene	Primer sequence (5′……. 3′)	Reference
Actb	F: GCTGAGAGGGAAATCGTGCGTG R: CCAGGGAGGAAGAGGATGCGG	[35]
Gapdh	F: TCACCACCATGGAGAAGGC R: GCTAAGCAGTTGGTGGTGCA	[36]
Tlr2	F: ACAATAGAGGGAGACGCCTTT R: AGTGTCTGGTAAGGATTTCCCAT	[37]
Nfkb1	F: GTGGAGGCATGTTCGGTAGTG R: TCTTGGCACAATCTTTAGGGC	[38]
Tnf	F: ACGTGGAACTGGCAGAAGAG R: CTCCTCCACTTGGTGGTTTG	[39]
Il6	F: GAGGATACCACTCCCAACAGACC R: AAGTGCATCATCGTTGTTCATACA	[36]
Nlrp3	F: AGAGCCTACAGTTGGGTGAAATG R: CCACGCCTACCAGGAAATCTC	[40]
Cxcl5	F: GTTCATCTCGCCATTCATGC R: GCGGCTATGACTGAGGAAGG	[41]
Ocln	F: ACTCCTCCAATGGACAAGTG R: CCCCACCTGTCGTGTAGTCT	[35]
Hp	F: CCACCTCTGTCCAGCTCTTC R: CACCGGAGTGATGGTTTTCT	[35]
F11r	F: CACCTTCTCATCCAGTGGCATC R: CTCCACAGCATCCATGTGTGC	[35]
Cldn2	F: GTCATCGCCCATCAGAAGAT R: ACTGTTGGACAGGGAACCAG	[35]
Muc2	F: GATGGCACCTACCTCGTTT R: GTCCTGGCACTTGTTGGAAT	[35]

Note: F (Forward); R (Reverse); *Actb* (actin beta); *Gapdh* (glyceraldehyde 3-phosphate dehydrogenase); *Tlr2* (Toll-like receptor 2); *Nfkb1* (nuclear factor kappa B subunit 1); *Tnf* (Tumor necrosis factor alpha); *Il6* (interleukin 6); *Nlrp3* (Nod-like receptor family pyrin domain containing 3); *Cxcl5* (C-X-C motif chemokine 5); *Ocln* (occludin); *Hp* (zonulin); *F11r* (junctional adhesion molecule A); *Cldn2* (claudin 2); *Muc2* (mucin 2).

Diego, USA) conducted all data analyses and created figures. The data with statistical significance were represented as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

3. Results

3.1. Proteome analysis of Lactobacillus delbrueckii CIDCA 133

SDS-PAGE analysis was performed on proteins extracted from *L. delbrueckii* CIDCA 133 using 5 M Guanidine Hydrochloride (GuHCl). The results showed multiple distinct bands ranging from approximately 25 to 100 kDa. Compared to proteins from the SDS extract, 25 proteins were detected in the guanidine extract by proteomic analysis (Table S1). Of these, 23 proteins presented coverage of >20 % (Table 2), including phosphopyruvate hydratase, D-2-hydroxy-acid dehydrogenase, elongation factor Tu, pyruvate kinase, ATP-dependent Clp protease, chaperone DnaK, and C40 family peptidase. SurfG+ analysis of these proteins revealed that all proteins are cytoplasmic, except for the C40 family peptidase, which was classified as an extracellular protein. Chaperonin GroEL (*groL*) (57 kDa), phosphoglycerate kinase (*pgk*) (42 kDa), and type I glyceraldehyde-3-phosphate dehydrogenase (*gap*) (36 kDa) were the most abundant proteins present in the guanidine extract (Fig. 1a). All proteins identified by mass spectrometry are shown in Supplementary Table S1.

3.2. Postbiotics proteins reduced the severity of DSS-induced colitis

The therapeutic efficacy of extractable postbiotic proteins derived from *Lactobacillus delbrueckii* CIDCA 133 (100 μg/day, orally administrated) was assessed in a DSS-induced acute colitis mouse model (Fig. 1b). Compared to the control group (NC), the severity of DSS-induced colitis in mice (DSS group) was mainly characterized by significant body weight loss (NC: 3.94 ± 1.74 %; DSS: −7.81 ± 2.48 %) ($p < 0.0001$) (Fig. 1c, d), an elevated DAI score (Fig. 1e, f), and colon shortening (NC: 8.00 ± 0.28 cm; DSS: 6.70 ± 0.53 cm) (Fig. 1g, h) ($p = 0.0007$), indicating successful establishment of the ulcerative colitis model. Subsequently, the CIDCA 133 postbiotic proteins (PP group) were evaluated concerning their ability to ameliorate these parameters. Although no significant protective effect was observed on mice's body weight loss ($p = 0.0540$) (Fig. 1c, d), treatment with postbiotic proteins reduced the DAI score ($p = 0.0079$) (Fig. 1e, f) and prevented colon length shortening (7.54 ± 0.33 cm) ($p = 0.0154$) (Fig. 1g, h), indicating partial alleviation of colitis symptoms.

3.3. Postbiotic proteins alleviated colonic epithelial damage

After seven days of DSS administration, drastic changes in the colonic mucosa architecture were observed in the DSS group compared to the control group (NC), as illustrated in Fig. 2a. These alterations led to an enhanced histopathological score ($p < 0.001$) (Fig. 2b), transmural inflammatory cell infiltration by neutrophils (MPO activity; NC: 0.11 ± 0.26 AU/mg; DSS: 1.20 ± 0.32 AU/mg; $p < 0.001$) (Fig. 2c), and

Table 2
Proteins present in the guanidine extract.

Accession	Gene	Description	Cell localization	Coverage	emPAI
WP_002879985.1	*gap*	type I glyceraldehyde-3-phosphate dehydrogenase	Cytoplasm	78.40 %	371.7594
WP_002879986.1	*pgk*	phosphoglycerate kinase	Cytoplasm	78.16 %	35.74662
WP_002879863.1	*eno*	phosphopyruvate hydratase	Cytoplasm	74.12 %	99
WP_013439976.1	*groL*	chaperonin GroEL	Cytoplasm	73.93 %	30.62278
WP_013438907.1	*ddh*	D-2-hydroxyacid dehydrogenase	Cytoplasm	72.37 %	120.1528
WP_003617518.1	*tuf*	elongation factor Tu	Cytoplasm	68.94 %	243.8437
WP_013439390.1	*pyk*	pyruvate kinase	Cytoplasm	68.08 %	108.2601
WP_013440298.1	*tag*	ATP-dependent Clp protease	Cytoplasm	58.76 %	26.06652
WP_003611409.1		FAD-dependent oxidoreductase	Cytoplasm	51.67 %	6.943282
WP_013440090.1		PTS sugar transporter subunit IIB	Cytoplasm	51.16 %	25.82696
WP_003615406.1	*dnaK*	chaperone DnaK	Cytoplasm	46.42 %	6.196857
WP_013439226.1	*ptsP*	phosphoenolpyruvate–protein phosphotransferase	Cytoplasm	46.26 %	53.11695
WP_003617641.1	*rpsA*	30S ribosomal protein S1	Cytoplasm	45.39 %	17.4785
WP_013439025.1	*tyrS*	tyrosine–tRNA ligase	Cytoplasm	44.66 %	7.912509
WP_003611798.1	*fabF*	beta-ketoacyl-ACP synthase II	Cytoplasm	44.09 %	5.309573
WP_013440068.1	*pfk*	6-phosphofructokinase	Cytoplasm	33.43 %	5.309573
WP_013438958.1		C40 family peptidase	Extracellular	33.33 %	22.71374
WP_013439895.1	*glnA*	type I glutamate–ammonia ligase	Cytoplasm	33.03 %	3.084239
WP_013439809.1	*proS*	proline–tRNA ligase	Cytoplasm	31.50 %	2.162278
WP_013440196.1	*dhaK*	dihydroxyacetone kinase subunit DhaK	Cytoplasm	30.82 %	4.623413
WP_002879226.1	*thrS*	threonine–tRNA ligase	Cytoplasm	24.88 %	2.775053
WP_002879146.1	*mreC*	rod shape-determining protein	Cytoplasm	23.35 %	2.511192
WP_013439310.1	*atpC*	F0F1 ATP synthase subunit gamma	Cytoplasm	21.25 %	1.253934

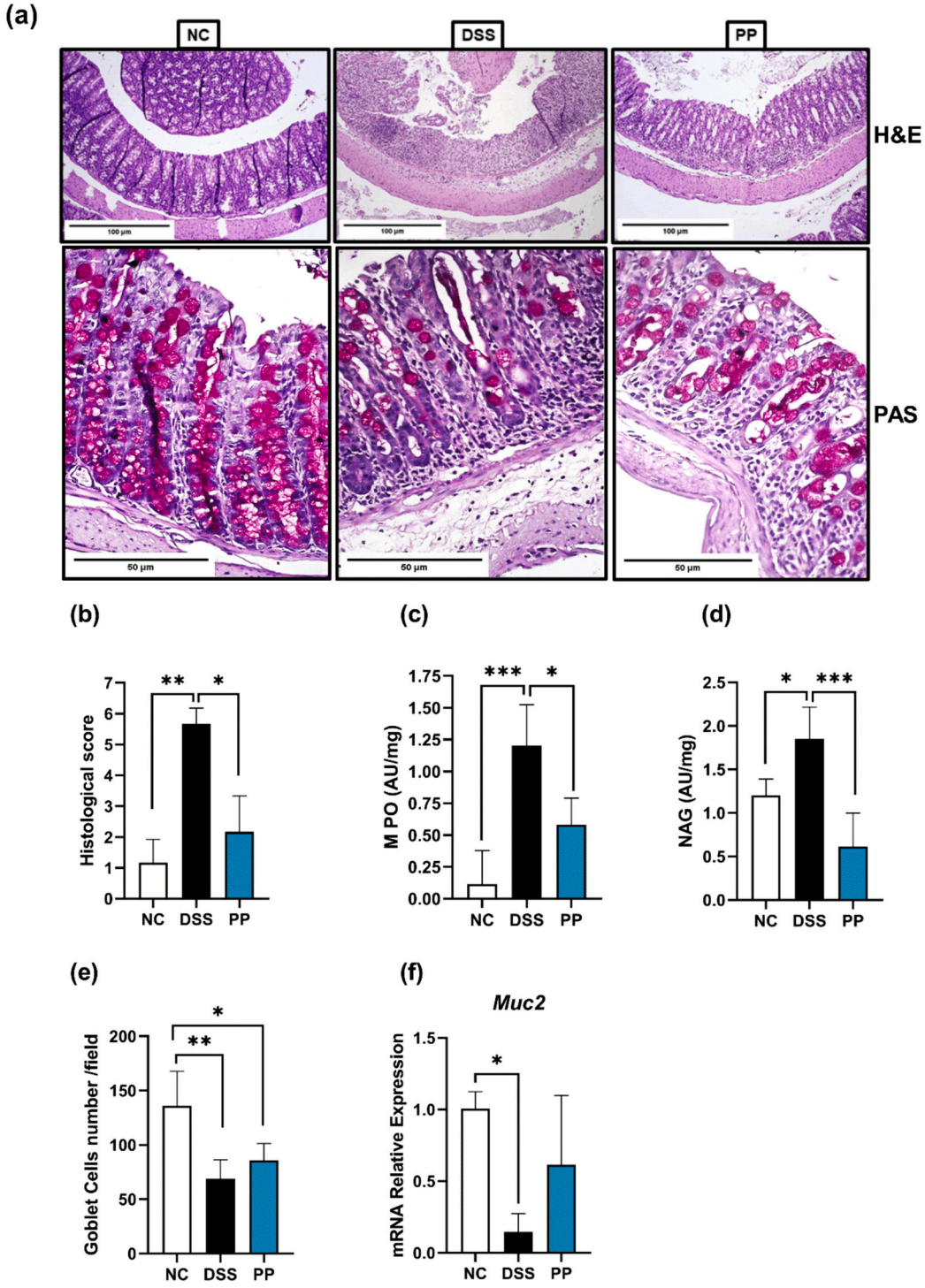

Fig. 2. Protective effect of postbiotic proteins from *Lactobacillus delbrueckii* CIDCA 133 on colonic mucosa architecture. (a) Representative images of H&E and PAS staining of colon tissue; (b) histopathological score; (c) MPO and (d) NAG activity; (e) goblet cell count; and (f) mucin 2 gene expression. Asterisks indicate statistically significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) according to ANOVA plus Tukey's post-test (b, c, f) or Kruskal-Wallis plus Dunn's post-test (d). NC: negative control (healthy mice); DSS: dextran sodium sulfate (colitic mice); PP: postbiotic proteins (colitic mice consuming postbiotic proteins).

macrophages (NAG activity; NC: 1.20 ± 0.18 AU/mg; DSS: 1.85 ± 0.36 AU/mg; $p = 0.0474$) into the lamina propria (Fig. 2d), and depletion of goblet cells (NC: 123.8 ± 41.01 cell/field; DSS: 77.62 ± 22.40 cell/field; $p = 0.0436$) (Fig. 2e) with a subsequent reduction in the transcript levels of mucin 2 (NC: 1.00 ± 0.01; DSS: 0.14 ± 0.12; $p = 0.0331$) (Fig. 2f). In contrast, the consumption of postbiotic proteins (PP group) ameliorated the damage inflicted on the colonic mucosal architecture by the DSS treatment. This was evidenced by the restoration of the inflammation-related histological score ($p < 0.05$) (Fig. 2b), and the reduction in DSS-associated recruitment of neutrophils (MPO: 0.58 ± 0.20 AU/mg; $p = 0.0121$) and macrophages (NAG: 0.61 ± 0.32 AU/mg; $p = 0.0006$) (Fig. 2c, d). No beneficial effect was observed on goblet cell count ($p = 0.4748$) (Fig. 2e) and the relative mucin 2 gene expression ($p = 0.2474$) (Fig. 2f) after postbiotic proteins administration.

3.4. Postbiotic proteins regulated colonic inflammatory responses

To elucidate the underlying mechanism by which postbiotic proteins from CIDCA 133 protect mice from the colonic mucosal damage caused by DSS, we analyzed the gene expression of some inflammatory markers (*Tlr2, Nfkb1, Nlrp3, Cxcl5, Tnf,* and *Il6*) by RT-qPCR.

Mice with colitis (DSS group) exhibited enhanced colonic gene expression of *Tlr2* (3.073 ± 1.31), *Nfkb1* (2.42 ± 0.53), *Nlrp3* (3.73 ± 0.79), *Tnf* (21.23 ± 11.37) and *Il6* (67.15 ± 28.92) compared to the expression levels in the control group (NC) ($p < 0.05$) (Fig. 3a). In contrast, treatment with postbiotic proteins (PP group) reduced the mRNA expression levels of these inflammatory markers: *Tlr2* (1.33 ± 0.52), *Nfkb1* (1.08 ± 0.76), *Nlpr3* (1.35 ± 1.08), *Tnf* (1.77 ± 1.75) and *Il6* (27.28 ± 3.67) ($p < 0.05$) (Fig. 3a). DSS-induced acute colitis did not affect the transcript levels of *Cxcl5* ($p = 0.1680$) (Fig. 3a).

Further immunoregulatory profile of postbiotic protein fraction was evaluated by measuring cytokine levels (IFNγ, IL17A, IL1β, TGFβ, and IL10) in colonic tissue homogenates.

Administration of DSS increased levels of the anti-inflammatory cytokines TGFβ (132.2 ± 18.14 pg/mL) and IL10 (269.0 ± 53.97 pg/mL) ($p < 0.05$) (Fig. 3b, c), as well as the pro-inflammatory cytokines IL1β (317.6 ± 105.5 pg/mL) and IFNγ (61.66 ± 6.81 ng/mL) ($p < 0.01$) (Fig. 3d, e) in the colonic mucosa of colitic mice. These cytokines levels were reduced in colitic mice following administration of postbiotic proteins (TGFβ: 94.73 ± 12.90 pg/mL; IL10: 159.3 ± 36.76 pg/mL; and IL1β: 75.26 ± 73.37 pg/mL; $p < 0.05$) (Fig. 3b-d). DSS-induced acute colitis did not affect IL17A concentration ($p = 0.2748$) (Fig. 3 f) compared to the control group. Furthermore, treatment with postbiotic proteins did not modulate the levels of IFNγ ($p = 0.7694$) (Fig. 3e) compared to the DSS group.

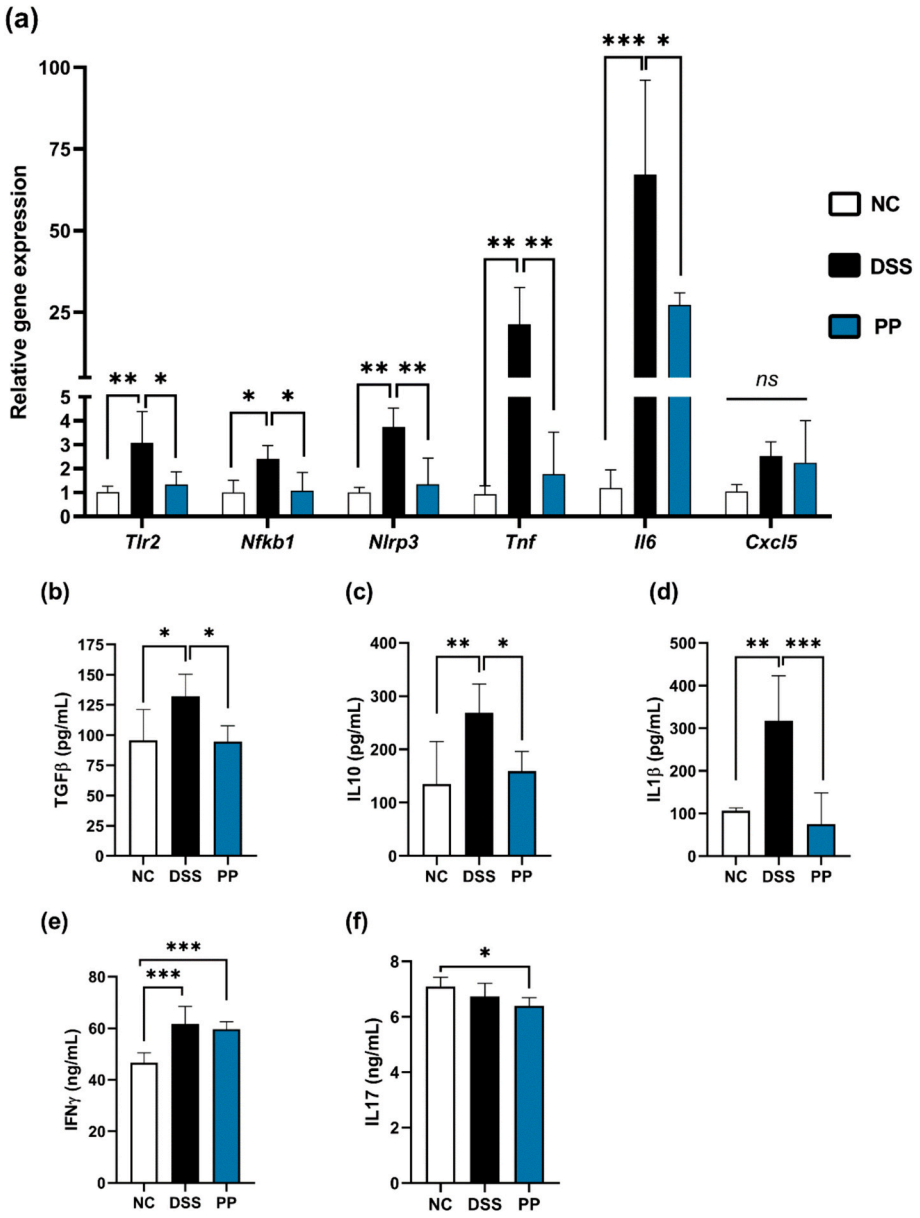

Fig. 3. Postbiotic proteins from *Lactobacillus delbrueckii* CIDCA 133 modulate colonic inflammation in colitic mice. (a) Gene expression of *Tlr2, Nfkb1*, *Nlpr3*, *Tnf*, *Il6* and *Cxcl5*. Levels of cytokines: (b) TGFβ (c) IL10 (d) IL1β, (e) IFNγ, and (f) IL17A. Asterisks indicate statistically significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) according to ANOVA plus Tukey's post-test. NC: negative control (healthy mice); DSS: dextran sodium sulfate (colitic mice); PP: postbiotic proteins (colitic mice consuming postbiotic proteins).

3.5. Postbiotic proteins protected the intestinal barrier function

DSS-induced acute colitis leads to several changes in mice, including elevated levels of secretory IgA, enhanced intestinal permeability, and concomitant downregulation of genetic markers associated with epithelial barrier function.

Administration of DSS increased sIgA levels (344 ± 22.84 μg/mL) compared to the control group (280.6 ± 21.60 μg/mL) ($p = 0.0284$). Mice treated with postbiotic proteins (PP group) exhibited higher secretory IgA levels (400.7 ± 48.92 μg/mL) compared to those in the DSS group ($p = 0.0496$) (Fig. 4a).

Furthermore, the DSS group exhibited significantly higher intestinal permeability (0.83 ± 0.07 % ID/g) than the control group (0.50 ± 0.11 % ID/g) ($p = 0.0004$) (Fig. 4b), indicating an impact of DSS 2.5 % on colonic barrier function. This group also displayed down-regulation of transcript levels of the tight junction proteins junctional adhesion molecule (*F11r*) (0.09 ± 0.09) and claudin-2 (*Cldn2*) (0.73 ± 0.12) compared to the control group (NC) ($p < 0.05$) (Fig. 4c, e). In contrast, treatment with postbiotic proteins (PP group) significantly reduced the intestinal permeability (0.56 ± 0.11 % ID/g) ($p = 0.0020$) (Fig. 4b) and upregulated the mRNA expression levels of the tight junction protein *Fr11* (0.46 ± 0.23) ($p = 0.0209$) (Fig. 4c) in colitic mice.

No effect on transcript levels of zonulin (*Hp*) and occludin (*Ocln*) was observed after DSS administration (DSS group) compared to the control group ($p > 0.05$) (Fig. 4d, f). In constrast, treatment with postbiotic proteins upregulated the gene expression of *Hp* (3.21 ± 1.71; $p = 0.0296$) while downregulating the transcript levels of *Cldn2* (0.40 ± 0.09; $p = 0.0085$) (Fig. 4d, e).

3.6. Postbiotic proteins regulate dysbiosis induced by DSS

We also explored the impact of postbiotic proteins from CIDCA 133 on gut microbiota using 16S rRNA gene sequencing analysis. The DSS group exhibited a significant loss in bacterial diversity compared to the control group ($p < 0.01$) (Fig. 5a, b). Nevertheless, oral administration of postbiotics proteins from CIDCA 133 (PP group) restored gut microbiota diversity ($p < 0.05$) (Fig. 5a, b). Further analysis at the phylum level revealed that administration of DSS (DSS group) decreased Firmicutes and Verrucomicrobia abundance and increased the relative abundance of Bacteroidetes ($p < 0.05$) (Fig. 5c, d). Additionally, a significant correlation ($r = 0.8713$; $p = 0.0204$) between Bacteroidetes and Firmicutes abundance was observed (Fig. 5e). The consumption of postbiotic proteins modulated the profile of gut microbiota, reducing the abundance of Bacteroidetes and increasing the abundance of Firmicutes. No impact of postbiotic proteins was observed on the Verrucomicrobia and Proteobacteria profile (Fig. 5d) ($p > 0.05$).

The impact of postbiotic proteins on the gut microbiota at the genus level was also explored. The administration of DSS (DSS group) increased the relative abundance of *Bacteroides* (Fig. 6a) and *Dorea* (Fig. 6d) genera and decreased the abundance of *Lactobacillus* (Fig. 6b) and *Arkkemansia* (Fig. 6c) compared to the control group ($p < 0.05$). This dysbiosis was partially countered by the consumption of postbiotic proteins from CIDCA 133, which reduced the relative abundance of *Bacteroides* (Fig. 6a) and *Dorea* (Fig. 6d) while increasing the abundance of *Arkkemansia* (Fig. 6c) ($p < 0.05$). A positive impact of postbiotic proteins from CIDCA 133 was also observed in the relative abundance of the *Oscillospira* genus (Fig. 6f) compared to the DSS group ($p < 0.05$). No impact of DSS was observed on *Helicobacter* (Fig. 6e), and no impact of postbiotic proteins was observed on the abundance of *Lactobacillus* ($p > 0.05$) (Fig. 6b).

Further analysis was performed to evaluate the correlation between the abundance of *Lactobacillus_Arkkemansia* (average abundance of both genera) and *Bacteroides* genera and the levels of cytokines IL1β and IL10, respectively, in the progression of DSS-induced colitis. A positive correlation was observed between the high levels of IL10 and the increased abundance of *Bacteroides* in the DSS group ($r = 0.9813$; $p = 0.0011$) (Fig. 6g). Furthermore, the reduction in the levels of the cytokine IL1β after the consumption of postbiotic proteins (PP group) was correlated

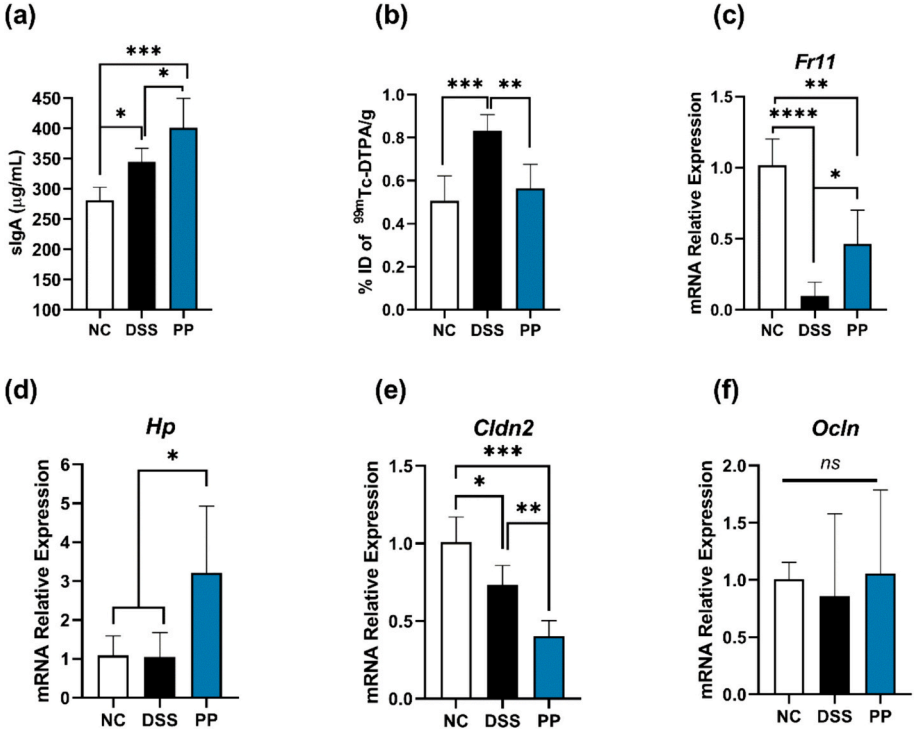

Fig. 4. Postbiotic proteins from *Lactobacillus delbrueckii* CIDCA 133 mitigate intestinal permeability in colitic mice. (a) Secretory IgA levels; (b) Intestinal permeability; Gene expression of tight junction proteins: (c) junctional adhesion molecule, (d) zonulin, (e) claudin 2, and (f) occludin. Asterisks indicate statistically significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) according to ANOVA plus Tukey's post-test. NC: negative control (healthy mice); DSS: dextran sodium sulfate (colitic mice); PP: postbiotic proteins (colitic mice consuming postbiotic proteins).

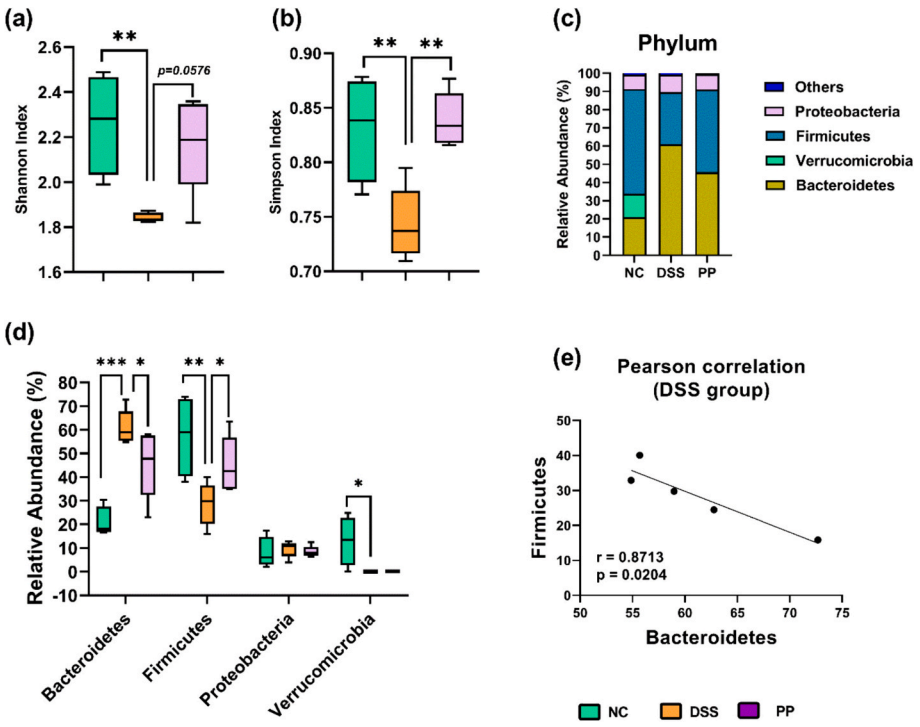

Fig. 5. Gut microbiota profile in colitic mice. (a) Shannon and (b) Simpson diversity indexes of gut microbiota in mice treated with postbiotic proteins from CIDCA 133; (c) Gut microbiota profile at the phylum level; (d) Relative abundance of gut microbiota at the phylum level; (e) Pearson's correlation between Bacteroidetes and Firmicutes abundance. Each dot on the scatter plot represents a pair of correlated data points from the two phyla. Asterisks indicate statistically significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) according to ANOVA plus Tukey's post-test (a, b) or Mann-Whitney or Student *t*-test (d). NC: negative control (healthy mice); DSS: dextran sodium sulfate (colitic mice); PP: postbiotic proteins (colitic mice consuming postbiotic proteins).

with increased abundance of health-promoting bacteria *Lactobacillus_Arkkemansia* ($r = -0.8442$; $p = 0.0360$) (Fig. 6h). No correlation was observed between other microbiota components and the parameters assessed in this study (data not shown).

4. Discussion

Understanding the specific components responsible for the anti-inflammatory effects of probiotic bacteria is crucial for optimizing their therapeutic efficacy. In this study, we demonstrated for the first time that extractable proteins (postbiotics) from *Lactobacillus delbrueckii* CIDCA 133 effectively mitigated inflammation in an acute colitis model in mice.

We initially demonstrated that the consumption of postbiotic proteins attenuated leukocyte invasion into the colonic mucosa, thereby reducing the tissue damage caused by excessive recruitment or activation of these cells following DSS administration. Improvement of inflammatory bowel disease (IBD) through the reduction of leukocytic cells has been reported for other postbiotic molecules, such as protein extract from *Limosilactobacillus fermentum* MTCC 5689 [47] and the soluble p40 protein derived from *Lacticaseibacillus rhamnosus* GG [48].

Activation or invasion of leukocytes into the colonic mucosa caused by DSS administration results in excessive secretion of pro-inflammatory cytokines, which have been directly implicated in the severity of colitis [49]. In our study, the protective effect of postbiotic proteins from CIDCA 133 on the colonic mucosa may be related to the immunological response driven by these proteins, including the reduction in transcript levels of pro-inflammatory markers *Nfkb1, Nlrp3*, *Tnf,* and *Il6*, as well as the local modulation in the levels of the cytokines IL1β, TGFβ, and IL10, which were altered by DSS administration.

The pro-inflammatory cytokines IL1β, IL6, and TNF exert various pro-inflammatory functions in the inflamed mucosa in IBD, such as activation of inflammatory pathways (e.g., NLRP3 inflammasome and NF-κB pathways), induction of epithelial cell death, alteration of barrier integrity, and production of other pro-inflammatory cytokines by macrophages and T cells [49]. Therefore, immunomodulation of these cytokines after consumption of postbiotic proteins evidences the anti-inflammatory properties of these molecules in regulating the balance between Th1, Th2, Th17, and Treg responses, confirming findings from previous studies [16,21,47].

Our data also revealed that colitic mice had increased levels of the anti-inflammatory cytokines IL10 and TGFβ, which is consistent with findings from previous preclinical [50,51] and clinical studies [52,53] reporting elevated levels of these cytokines in UC patients and DSS-induced colitis in mice. Both TGFβ and IL10 act as mediators to suppress mucosal inflammation [54,55], suggesting that the high secretion levels of these cytokines in our IBD model may represent a host mechanism to alleviate tissue damage and persistent inflammatory changes, thereby restoring colonic homeostasis. Moreover, considering that macrophages are one of the cell types responsible for producing high levels of the immunoregulatory cytokine IL10 in the colon [56], we hypothesize that the elevated levels of this cytokine following DSS administration, and its subsequent reduction after consumption of postbiotic protein, may be partly attributed to the observed decrease in macrophage activation observed in our study, as indicated by NAG enzyme activity. Another plausible explanation is that the postbiotic proteins of CIDCA 133 may exert their entero-protective effects by activating other anti-inflammatory molecules, such as IL4 and IL22. However, further studies are needed to validate these hypotheses, including those utilizing IL10 knockout mice.

Mechanistically, although postbiotic proteins from CIDCA 133 alleviated colitis, we observed that DSS increased the IFNγ cytokine level in the colonic mucosa, and its production was not altered after the administration of postbiotic protein from CIDCA 133. IFNγ is a pro-inflammatory cytokine strongly expressed in most lymphocytes. This cytokine has pleiotropic functions, including antiviral activity and the

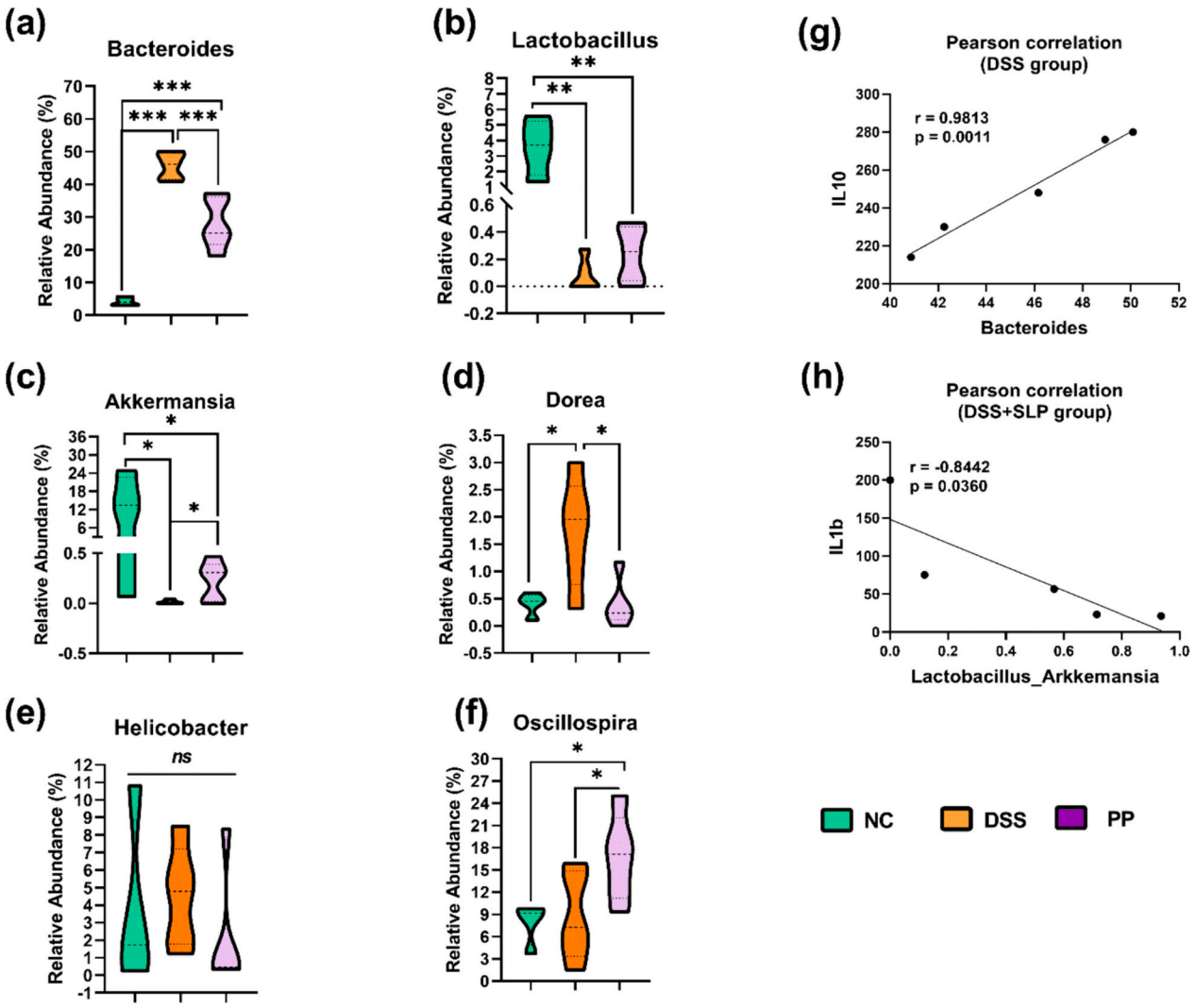

Fig. 6. Postbiotics proteins from CIDCA 133 modulate gut microbiota profile in colitic mice. Relative abundance of the genus (a) *Bacteroides;* (b) *Lactobacillus*; (c) *Arkkemansia*; (d) *Dorea*; (e) *Helicobacter*; (f) *Oscillospira*; Pearson correlation between (g) IL10 cytokine level and Bacteroides abundance; (h) *Lactobacillus_Arkkemansia* abundance and IL1β cytokine levels. Each dot on the scatter plot represents a pair of correlated data points from the two variables (genus versus cytokine). Asterisks indicate statistically significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) according to the Mann-Whitney or Student *t*-test. NC: negative control (healthy mice); DSS: dextran sodium sulfate (colitic mice); PP: postbiotic proteins (colitic mice consuming postbiotic proteins).

inhibition of intracellular microbes [57]. Additionally, IFNγ can contribute to mucosal inflammation by promoting the disruption of adherens junction protein VE-cadherin, leading to the breakdown of the vascular barrier [58]. It has also been shown that IFNγ induces the loss of mucus-containing goblet cells and the extrusion and death of anti-microbial peptides-secreting Paneth cells [59,60]. This corroborates our study's findings, in which 2.5 % DSS induced the loss of mucus-producing goblet cells and reduced the transcript levels of mucin 2. However, the administration of postbiotics proteins from CIDCA 133 did not improve these factors.

IFNγ can also collaborate with or antagonize other pro-inflammatory cytokines, such as TNF and IL17. For instance, IFNγ in synergy with TNF regulates epithelial cell proliferation, differentiation, and apoptosis through modulation of the β-catenin signaling pathway, an essential pathway for maintaining intestinal stem cells and epithelial regeneration [61]. Regarding the IL17 cytokine, it has been shown that this inflammatory marker plays a vital role in regulating the host immune response against pathogens [62]. For instance, IL17 can significantly enhance the immunogenicity of a mucosal herpes simplex virus vaccine by boosting the Th1 response and subsequent IFNγ production in the female genital tract [63]. Another study reported that the protective effect of IL17 against *Francisella tularensis* infection was mediated through IFNγ production in macrophages, improving bacterial killing [64]. The cross-regulation of T-cell subsets has also been evaluated in mucosal inflammation. It has been reported that IFNγ antagonizes IL17 responses in a CD4 T cell-induced colitis model. The absence of IFNγ results in a predominant IL17-driven immune response sufficient to induce colitis [65]. Thus, given that IFNγ-deficient mice exhibited increased IL17 levels and Th-17 responses and, similarly, IL17-deficient mice displayed exacerbated Th1 responses [66,67], we suggest that low levels of IL17 in our colitis model can be explained partially by the high levels of IFNγ.

It is important to recognize that Th1-type cytokine IFNγ is one of the key mediators in the IBD pathogenesis, but not the only contributing factor. Thus, we propose that in our report, the intervention with postbiotic proteins from CIDCA 133 against DSS may also operate through mechanisms independent of direct IFNγ modulation, thereby involving other cytokines, such as TNF, IL1β, and IL6, which also contribute to the pathogenesis of colitis [49,52]. This hypothesis is corroborated by Brasseit et al. [65], who reported that CD4 T cell-induced colitis develops without the participation of IFNγ. A similar pattern was also observed by Sandes et al. [68]. The authors showed that *Weissella*

paramesenteroides WpK4 treatment mitigated colonic damage and inflammation in 2.5 % DSS-induced colitis even though the immunobiotic does not affect IFNγ levels, but reduces TNF, IL1β and CXCL1 levels.

The observed anti-inflammatory effects in this study might be associated with the predominant proteins identified through proteomic analysis in the guanidine extract, such as chaperonin Hsp60 (*groL*), phosphoglycerate kinase (*pgk*), type I glyceraldehyde-3-phosphate dehydrogenase (*gap*), phosphopyruvate hydratase (*eno*), and elongation factor Tu (*tuf*), which are well-recognized as moonlighting (multifunctional) proteins inducing immune responses [69]. For instance, the *Limosilactobacillus reuteri* GroEL protein (a heat shock protein) decreased the pro-inflammatory cytokine IFNγ while increasing anti-inflammatory cytokines IL10 and IL13, and the expression of M2-like macrophage markers to promote mucosal healing in a mouse model of colitis [16]. Recombinant *Mycobacterium* Hsp65 also prevented DSS-induced colitis by decreasing pro-inflammatory cytokines (IL6, IFNγ, and TNFα) and expanding CD4 + Foxp3+ and CD4 + LAP+ regulatory T cells in an IL10- and TLR2-dependent manner in mice with DSS-induced colitis [70]. Proteins in the glycolytic metabolic pathway also play a crucial role in regulating or inducing immune responses. It has been shown that GAP and PGK were some of the most immunoreactive or immunogenic proteins isolated from *Lacticaseibacillus rhamnosus* or *Lactobacillus johnsonii* strains [71]. The protein ENO was predicted as a candidate possibly involved with the anti-inflammatory properties of *P. freudenreichii* [14]. Additionally, a proteinaceous extract from *Lactiplantibacillus plantarum* MTCC 5690 and *Limosilactobacillus fermentum* MTCC 5689, containing EF-Tu as one of the most abundant proteins, relieved acute colitis in mice by enhancing the levels of IL10 and mitigating leukocyte infiltrate and pro-inflammatory cytokines (TNFα and IFNγ) [47]. Altogether, these data show that these proteins may be essential for directing anti-inflammatory activity in probiotic bacteria.

The immunological changes promoted by DSS-induced colitis impaired the intestinal barrier function, consistent with the loss of the colonic epithelial cells during the inflammatory processes [72,73]. In our study, the administration of postbiotic proteins from CIDCA 133 strengthened the integrity of the intestinal epithelial barrier by reducing intestinal paracellular permeability and upregulating the transcript levels of the tight junction protein zonulin and junctional adhesion molecule. These outcomes indicate that these bacterial proteins may prevent the entry of harmful compounds and pathogenic microorganisms into the mucosa, thereby reducing inflammation. Surprisingly, our results revealed that DSS-induced colitis reduced the transcript levels of claudin-2, which contrasts with findings from other studies [74,75]. This reduction was further potentialized after postbiotic treatment. Other studies have also observed downregulation of claudin-2 following DSS exposure [76,77].

Leaky pore-forming claudin-2 is uniquely expressed by undifferentiated and proliferative colonocytes at the crypt base [78], leading to altered tight junction structure and pronounced barrier dysfunction, thereby increasing intestinal paracellular permeability by forming cation-selective and water channels on the intestinal epithelium [79,80]. Elevated expression of claudin-2 during gut inflammation is driven by immune signals, including IL13 and IL22 cytokines [81,82], and may be involved in the early stages of IBD-associated carcinogenesis [83]. Nevertheless, the precise role of claudin-2 in regulating colonic homeostasis remains unclear, with controversial reports. For instance, despite increased mucosal permeability, overexpression of claudin-2 has been shown to protect the intestinal epithelium against DSS-induced colitis in villin-claudin-2 transgenic mice [84]. Another study demonstrated that elevated claudin-2 expression exacerbated the T cell transfer-mediated colitis model, while claudin-2 knockout limited the progression of immune-mediated colitis severity [85]. Furthermore, other reports have shown that loss of claudin-2 exacerbated colitis [78,86]. According to Ahmad et al. [78], the regulation of claudin-2 expression during colitis is biphasic, with its expression downregulated during DSS-induced acute colitis, but upregulated during recovery from colitis and chronic colitis. Additionally, the authors showed that claudin-2 expression decreased in a dose-dependent manner in DSS-treated cells. In our study, we induced acute colitis with 2.5 % of DSS for seven days. Thus, our data aligns with the findings of Ahamad et al. [78], evidencing that the pronounced downregulation of claudin-2 is associated with colitis severity. Nevertheless, it remains unclear why this reduction was further potentialized after postbiotic treatment. Therefore, further studies are needed to evaluate the influence of DSS and postbiotic proteins from CIDCA 133 on the colonic tight junction profile.

Another important feature evaluated in our study was the levels of secretory IgA. High levels of this class of antibodies were observed after DSS administration, as its secretion was significantly higher after treatment with postbiotic proteins from CIDCA 133. Secretory IgA serves as the first line of defense in protecting mucosal surfaces from toxins and commensal bacteria [87]. Thus, increased secretion of IgA in the intestinal fluids after postbiotic treatment may serve as a mechanism to stimulate the host's innate immune system and control the dysbiosis that occurs in DSS-induced IBD [88,89]. The high antigenic potential of the proteins GAP, ENO, PGK, EF-TU, and GroEL has been previously reported [71,90,91]. Additionally, in our study, the first evidence of dysbiotic commensal microbiota may be associated with the upregulation of TLR2 and NLRP3 gene expression after DSS administration and its down-regulation after postbiotic protein treatment. These receptors are important for recognizing microbial-associated molecules and inducing immunological responses that may protect the host from infection [92,93].

Gut microbiota dysbiosis is strongly associated with IBD development [94]. In our study, 16S rRNA sequencing results revealed that postbiotic proteins from CIDCA 133 mitigate gut microbiota dysbiosis triggered by DSS administration. This postbiotic fraction increased both the bacterial diversity and abundance of *Oscillospira* and *Arkkemansia* genera while reducing the abundance of *Bacteroides* and *Dorea*, which were increased by DSS administration.

Bacteroides are commensal gram-negative exclusive anaerobic microorganisms with conflicting data about their effect on IBD progression [95,96]. *Bacteroides* is a producer of butyrate and acetate, both short-chain fatty acids (SCFAs) known to have immunomodulatory effects [97,98]. Additionally, this genus can activate IL10-producing FoxP3+ CD4+ regulatory T cells (Tregs) [99] and stimulate immunomodulatory M2 macrophages [98] to alleviate inflammation. In our study, *Bacteroides* was the most abundant genus after DSS administration, showing a positive correlation with IL10 cytokine levels. This suggests that in colitic mice, this bacterial genus could help restore colon mucosa homeostasis by inducing immunoregulatory IL10-producing cells. Furthermore, the reduction in the abundance of this genus after the administration of postbiotic proteins from CIDCA 133 was accompanied by a decrease in IL10 levels. Similar outcomes were reported by De Jesus et al. [100]. Therefore, this suggests that the anti-inflammatory effects of this protein fraction may involve other anti-inflammatory markers. However, further studies, including those utilizing IL10 knockout mice, are needed to validate these hypotheses.

Administration of postbiotic proteins from CIDCA 133 also reduced the abundance of the *Dorea* genus. Data about the relationship between *Dorea* (Firmicutes phylum) and the pathogenesis of ulcerative colitis are limited. However, a higher abundance of this genus has been reported in the colitis mice model induced by DSS [101], and its positive correlation with higher expression of pro-inflammatory cytokines IL1β in these mice [102] corroborates our study's results. In addition, it has been reported that some species of *Dorea* can induce inflammation by stimulating IFNγ production and by degrading mucin, thus potentially increasing gut permeability [103].

Oral administration of postbiotic proteins from CIDCA 133 also restored the beneficial *Arkkemansia* genus, which DSS suppressed, as well as increased the *Oscillospira* abundance. *Oscillospira* has been

pointed out as a candidate for next-generation probiotics [104], capable of producing health-beneficial butyrate, a metabolite well-known for its anti-inflammatory properties [105]. Therefore, the modulation of this bacterial genus by postbiotic proteins from CIDCA 133 could also be associated with their beneficial effects observed in colitic mice. Furthermore, a positive correlation was observed between the average abundance of beneficial bacterial *Lactobacillus_Arkkemansia* and the reduced inflammatory cytokine IL1β. These results prove that postbiotic proteins from CIDCA 133 modulate the gut microbiota to induce an anti-inflammatory effect in colitic mice. These findings corroborated other studies which demonstrated that both *Lactobacillus* and *Arkkemansia* play a vital role in restoring and maintaining intestinal homeostasis disrupted in colitis condition, either by stimulating the Treg cells [106,107], producing tolerogenic DCs [108] and reducing pro-inflammatory cytokines [8,20], or by reinforcing the epithelial barrier through mucin 2, expression of tight junction proteins [20,109], and increasing SCFA-producing bacteria [107,110].

It is crucial to emphasize that all the promising outcomes related to postbiotic protein administration culminated in improved clinical signs in mice, including better fecal consistency, reduction in rectal bleeding, and colon shortening, which are prominent changes observed in DSS-induced colitis [29,111].

Although our study provides exciting information about the anti-inflammatory properties of postbiotic proteins extracted from *L. delbrueckii* CIDCA 133, several limitations should be considered. One of the key limitations of our study is the absence of immunolocalization analysis of tight junction proteins, which would have offered a deeper understanding of how these proteins are distributed within cells and maintained under conditions following DSS exposure and postbiotic treatment. Moreover, our investigation was restricted to a narrow selection of tight junction proteins, potentially overlooking other important contributors to barrier integrity. Furthermore, employing IL10 knockout (KO) mice could have provided valuable insights into the role of IL10 in mediating the effects of DSS and the immunoregulatory functions of postbiotic proteins from CIDCA 133. Moreover, the lack of flow cytometry analyses to characterize immune cell populations, including macrophages, regulatory T cells, and neutrophils, represents a missed opportunity to comprehensively understand the dynamic shifts in immune cell composition following postbiotic protein administration. Thus, to address these gaps and gain a more thorough understanding of our findings, we reinforce that further studies should be conducted.

Notwithstanding these limitations, our research has shown that oral administration of *Lactobacillus delbrueckii* CIDCA 133-derived postbiotic proteins ameliorated the DSS-induced colonic inflammation and, therefore, can mediate host-probiotic interactions. This protective effect was observed at the histological level, evidencing that these bacterial components drive, at least partially, the anti-inflammatory effects of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133. This postbiotic fraction opens a new avenue as an innovative therapeutic tool to be explored for alleviating acute ulcerative colitis.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2024.134216.

Funding

This work was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES) (grant number 88887.745552/2022-00) and the Brazilian National Council for Scientific and Technological Development (CNPq) (grant number 152434/2022-4).

Ethics statements

All experimental procedures were approved by the Animal Ethics Committee of the Federal University of Minas Gerais (protocol number 116/2023) and followed the guidelines of the Brazilian National Council for the Control of Animal Experimentation (CONCEA).

CRediT authorship contribution statement

Andria dos Santos Freitas: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Fernanda Alvarenga Lima Barroso:** Writing – review & editing, Methodology. **Gabriela Munis Campos:** Writing – review & editing, Methodology. **Monique Ferrary Américo:** Writing – review & editing, Methodology. **Rhayane Cristina dos Santos Viegas:** Writing – review & editing, Methodology. **Gabriel Camargos Gomes:** Writing – review & editing, Methodology. **Kátia Duarte Vital:** Writing – review & editing, Methodology, Formal analysis. **Simone Odília Antunes Fernandes:** Writing – review & editing, Methodology. **Rodrigo Dias de Oliveira Carvalho:** Writing – review & editing, Methodology, Formal analysis. **Julien Jardin:** Writing – review & editing, Methodology, Formal analysis. **Ana Paula Gomes dos Santos Miranda:** Writing – review & editing, Methodology, Formal analysis. **Enio Ferreira:** Writing – review & editing, Methodology, Data curation. **Flaviano Santos Martins:** Writing – review & editing, Methodology, Data curation. **Juliana Guimarães Laguna:** Writing – review & editing, Methodology, Formal analysis. **Gwénaël Jan:** Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. **Vasco Azevedo:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition. **Luís Cláudio Lima de Jesus:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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.

Data availability

The data for this study will be made available upon request.

Acknowledgments

We thank the CIDCA Center and Pablo F. Pérez from the National University of La Plata-Argentine for providing the bacteria for the study. The authors would also like to acknowledge the Funding agencies for their financial support: the Brazilian National Council for Scientific and Technological Development (CNPq), the Foundation for Research Support of the State of Minas Gerais (FAPEMIG), the Coordination for the Improvement of Higher Education Personnel (CAPES), and Pró-Reitoria de Pesquisa - Universidade Federal de Minas Gerais.

References

[1] S.C. Ng, H.Y. Shi, N. Hamidi, F.E. Underwood, W. Tang, E.I. Benchimol, R. Panaccione, S. Ghosh, J.C.Y. Wu, F.K.L. Chan, J.J.Y. Sung, G.G. Kaplan, Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies, Lancet 390 (2017) 2769–2778, https://doi.org/10.1016/S0140-6736(17)32448-0.

[2] R. Ungaro, S. Mehandru, P.B. Allen, L. Peyrin-Biroulet, J.-F. Colombel, Ulcerative colitis, Lancet 389 (2017) 1756–1770, https://doi.org/10.1016/S0140-6736(16)32126-2.

[3] R.J. Porter, R. Kalla, G.-T. Ho, Ulcerative colitis: recent advances in the understanding of disease pathogenesis, F1000Res 9 (2020) 294, https://doi.org/10.12688/f1000research.20805.1.

[4] M.S. Nadeem, V. Kumar, F.A. Al-Abbasi, M.A. Kamal, F. Anwar, Risk of colorectal cancer in inflammatory bowel diseases, Semin. Cancer Biol. 64 (2020) 51–60, https://doi.org/10.1016/j.semcancer.2019.05.001.

[5] Z. Cai, S. Wang, J. Li, Treatment of inflammatory bowel disease: a comprehensive review, Front. Med. (Lausanne) 8 (2021), https://doi.org/10.3389/fmed.2021.765474.

[6] B. Yue, Z.-L. Yu, C. Lv, X.-L. Geng, Z.-T. Wang, W. Dou, Regulation of the intestinal microbiota: an emerging therapeutic strategy for inflammatory bowel disease, World J. Gastroenterol. 26 (2020) 4378–4393, https://doi.org/10.3748/wjg.v26.i30.4378.

[7] P.J. Basso, N.O.S. Câmara, H. Sales-Campos, Microbial-based therapies in the treatment of inflammatory bowel disease – an overview of human studies, Front. Pharmacol. 9 (2019), https://doi.org/10.3389/fphar.2018.01571.
[8] X. Bian, W. Wu, L. Yang, L. Lv, Q. Wang, Y. Li, J. Ye, D. Fang, J. Wu, X. Jiang, D. Shi, L. Li, Administration of *Akkermansia muciniphila* ameliorates dextran sulfate sodium-induced ulcerative colitis in mice, Front. Microbiol. 10 (2019), https://doi.org/10.3389/fmicb.2019.02259.
[9] S. Salminen, M.C. Collado, A. Endo, C. Hill, S. Lebeer, E.M.M. Quigley, M. E. Sanders, R. Shamir, J.R. Swann, H. Szajewska, G. Vinderola, The international scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics, Nat. Rev. Gastroenterol. Hepatol. 18 (2021) 649–667, https://doi.org/10.1038/s41575-021-00440-6.
[10] L. Ruiz, S. Delgado, P. Ruas-Madiedo, A. Margolles, B. Sánchez, Proteinaceous molecules mediating *Bifidobacterium*-host interactions, Front. Microbiol. 7 (2016), https://doi.org/10.3389/fmicb.2016.01193.
[11] S. Delgado, B. Sánchez, A. Margolles, P. Ruas-Madiedo, L. Ruiz, Molecules produced by probiotics and intestinal microorganisms with immunomodulatory activity, Nutrients 12 (2020) 391, https://doi.org/10.3390/nu12020391.
[12] L. Macia, J. Tan, A.T. Vieira, K. Leach, D. Stanley, S. Luong, M. Maruya, C. Ian McKenzie, A. Hijikata, C. Wong, L. Binge, A.N. Thorburn, N. Chevalier, C. Ang, E. Marino, R. Robert, S. Offermanns, M.M. Teixeira, R.J. Moore, R.A. Flavell, S. Fagarasan, C.R. Mackay, Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome, Nat. Commun. 6 (2015) 6734, https://doi.org/10.1038/ncomms7734.
[13] R. Jastrząb, D. Graczyk, P. Siedlecki, Molecular and cellular mechanisms influenced by postbiotics, Int. J. Mol. Sci. 22 (2021) 13475, https://doi.org/10.3390/ijms222413475.
[14] S.-M. Deutsch, M. Mariadassou, P. Nicolas, S. Parayre, R. Le Guellec, V. Chuat, V. Peton, C. Le Maréchal, J. Burati, V. Loux, V. Briard-Bion, J. Jardin, C. Plé, B. Foligné, G. Jan, H. Falentin, Identification of proteins involved in the anti-inflammatory properties of *Propionibacterium freudenreichii* by means of a multi-strain study, Sci. Rep. 7 (2017) 46409, https://doi.org/10.1038/srep46409.
[15] D. Pradhan, G. Gulati, R. Avadhani, R. H.M., K. Soumya, A. Kumari, A. Gupta, D. Dwivedi, J.K. Kaushik, S. Grover, Postbiotic Lipoteichoic acid of probiotic *Lactobacillus* origin ameliorates inflammation in HT-29 cells and colitis mice, Int. J. Biol. Macromol. 236 (2023) 123962, https://doi.org/10.1016/j.ijbiomac.2023.123962.
[16] A.M.M. Dias, R. Douhard, F. Hermetet, M. Regimbeau, T.E. Lopez, D. Gonzalez, S. Masson, G. Marcion, K. Chaumonnot, B. Uyanik, S.Z. Causse, A. Rieu, T. Hadi, C. Basset, J. Chluba, J. Grober, J. Guzzo, F. Neiers, P. Ortega-Deballon, O. N. Demidov, F. Lirussi, C. Garrido, *Lactobacillus* stress protein GroEL prevents colonic inflammation, J. Gastroenterol. 56 (2021) 442–455, https://doi.org/10.1007/s00535-021-01774-3.
[17] L. Zhou, M. Zhang, Y. Wang, R.G. Dorfman, H. Liu, T. Yu, X. Chen, D. Tang, L. Xu, Y. Yin, Y. Pan, Q. Zhou, Y. Zhou, C. Yu, *Faecalibacterium prausnitzii* produces butyrate to maintain Th17/Treg balance and to ameliorate colorectal colitis by inhibiting histone deacetylase 1, Inflamm. Bowel Dis. 24 (2018) 1926–1940, https://doi.org/10.1093/ibd/izy182.
[18] A.A. Hugo, I.S. Rolny, D. Romanin, P.F. Pérez, *Lactobacillus delbrueckii* subsp. *lactis* (strain CIDCA 133) stimulates murine macrophages infected with Citrobacter rodentium, World J. Microbiol. Biotechnol. 33 (2017) 48, https://doi.org/10.1007/s11274-017-2219-4.
[19] L.C.L. De Jesus, M.M. Drumond, A. de Carvalho, S.S. Santos, F.S. Martins, Ê. Ferreira, R.S. Fernandes, A.L.B. de Barros, F.L.R. do Carmo, P.F. Perez, V. Azevedo, P. Mancha-Agresti, Protective effect of *Lactobacillus delbrueckii* subsp. *Lactis* CIDCA 133 in a model of 5 fluorouracil-induced intestinal mucositis, J. Funct. Foods 53 (2019) 197–207, https://doi.org/10.1016/j.jff.2018.12.027.
[20] F.A.L. Barroso, L.C.L. de Jesus, T.F. da Silva, V.L. Batista, J. Laguna, N.D. Coelho-Rocha, K.D. Vital, S.O.A. Fernandes, V.N. Cardoso, E. Ferreira, F.S. Martins, M. M. Drumond, P. Mancha-Agresti, A. Birbrair, D. Barh, V. Azevedo, *Lactobacillus delbrueckii* CIDCA 133 ameliorates chemotherapy-induced mucositis by modulating epithelial barrier and TLR2/4/Myd88/NF-κB signaling pathway, Front. Microbiol. 13 (2022), https://doi.org/10.3389/fmicb.2022.858036.
[21] V.L. Batista, L.C.L. De Jesus, L.M. Tavares, F.L.A. Barroso, L.J. da S. Fernandes, A. dos S. Freitas, M.F. Americo, M.M. Drumond, P. Mancha-Agresti, E. Ferreira, J. G. Laguna, L.C.J. Alcantara, V. Azevedo, Paraprobiotics and Postbiotics of *Lactobacillus delbrueckii* CIDCA 133 mitigate 5-FU-induced intestinal inflammation, Microorganisms 10 (2022) 1418, https://doi.org/10.3390/microorganisms10071418.
[22] L.C.L. de Jesus, M.M. Drumond, F.F. Aburjaile, T. de J. Sousa, N.D. Coelho-Rocha, R. Profeta, B. Brenig, P. Mancha-Agresti, V. Azevedo, Probiogenomics of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133: In Silico, In Vitro, and In Vivo Approaches, Microorganisms 9 (2021) 829, https://doi.org/10.3390/microorganisms9040829.
[23] C. Le Maréchal, V. Peton, C. Plé, C. Vroland, J. Jardin, V. Briard-Bion, G. Durant, V. Chuat, V. Loux, B. Foligné, S.-M. Deutsch, H. Falentin, G. Jan, Surface proteins of *Propionibacterium freudenreichii* are involved in its anti-inflammatory properties, J. Proteomics 113 (2015) 447–461, https://doi.org/10.1016/j.jprot.2014.07.018.
[24] J. Sambrook, D.W. Russell, SDS-Polyacrylamide Gel Electrophoresis of Proteins, Cold Spring Harb Protoc 2006, 2006, https://doi.org/10.1101/pdb.prot4540 pdb.prot4540.
[25] T.F. da Silva, R. de A. Glória, T.J. de Sousa, M.F. Americo, A. dos S. Freitas, M.V. C. Viana, L.C.L. de Jesus, L.C. da Silva Prado, N. Daniel, O. Ménard, M.-F. Cochet, D. Dupont, J. Jardin, A.D. Borges, S.O.A. Fernandes, V.N. Cardoso, B. Brenig, E. Ferreira, R. Profeta, F.F. Aburjaile, R.D.O. de Carvalho, P. Langella, Y. Le Loir, C. Cherbuy, G. Jan, V. Azevedo, É. Guédon, Comprehensive probiogenomics analysis of the commensal *Escherichia coli* CEC15 as a potential probiotic strain, BMC Microbiol. 23 (2023) 364, https://doi.org/10.1186/s12866-023-03112-4.
[26] F. Tarnaud, F. Gaucher, F.L.R. do Carmo, N. Illikoud, J. Jardin, V. Briard-Bion, F. Guyomarc'h, V. Gagnaire, G. Jan, Differential adaptation of *Propionibacterium freudenreichii* CIRM-BIA129 to cow's milk versus soymilk environments modulates its stress tolerance and proteome, Front. Microbiol. 11 (2020), https://doi.org/10.3389/fmicb.2020.549027.
[27] O. Langella, B. Valot, T. Balliau, M. Blein-Nicolas, L. Bonhomme, M. Zivy, X! TandemPipeline: a tool to manage sequence redundancy for protein inference and Phosphosite identification, J. Proteome Res. 16 (2017) 494–503, https://doi.org/10.1021/acs.jproteome.6b00632.
[28] A. Barinov, V. Loux, A. Hammani, P. Nicolas, P. Langella, D. Ehrlich, E. Maguin, M. van de Guchte, Prediction of surface exposed proteins in *Streptococcus pyogenes*, with a potential application to other gram-positive bacteria, Proteomics 9 (2009) 61–73, https://doi.org/10.1002/pmic.200800195.
[29] S. Wirtz, V. Popp, M. Kindermann, K. Gerlach, B. Weigmann, S. Fichtner-Feigl, M. F. Neurath, Chemically induced mouse models of acute and chronic intestinal inflammation, Nat. Protoc. 12 (2017) 1295–1309, https://doi.org/10.1038/nprot.2017.044.
[30] H.S. Cooper, S.N. Murthy, R.S. Shah, D.J. Sedergran, Clinicopathologic study of dextran sulfate sodium experimental murine colitis, Lab. Invest. 69 (1993) 238–249.
[31] P.A.V. de Barros, M.E. Rabelo Andrade, S. de Vasconcelos Generoso, S.E. Mendes Miranda, D.C. dos Reis, P.C. Lacerda Leocádio, É.L. de Sales e Souza, F. dos Santos Martins, M.A.S. da Gama, G.D. Cassali, J.I. Alvarez Leite, S.O. Antunes Fernandes, V.N. Cardoso, Conjugated linoleic acid prevents damage caused by intestinal mucositis induced by 5-fluorouracil in an experimental model, Biomed. Pharmacother. 103 (2018) 1567–1576, https://doi.org/10.1016/j.biopha.2018.04.133.
[32] C.M. Queiroz-Junior, R.L.C. Bessoni, V.V. Costa, D.G. Souza, M.M. Teixeira, T. A. Silva, Preventive and therapeutic anti-TNF-α therapy with pentoxifylline decreases arthritis and the associated periodontal co-morbidity in mice, Life Sci. 93 (2013) 423–428, https://doi.org/10.1016/j.lfs.2013.07.022.
[33] F.A.L. Barroso, L.C.L. de Jesus, C.P. de Castro, V.L. Batista, Ê. Ferreira, R. S. Fernandes, A.L.B. de Barros, S.Y. Leclerq, V. Azevedo, P. Mancha-Agresti, M. M. Drumond, Intake of *Lactobacillus delbrueckii* (pExu:hsp65) prevents the inflammation and the disorganization of the intestinal mucosa in a mouse model of mucositis, Microorganisms 9 (2021) 107, https://doi.org/10.3390/microorganisms9010107.
[34] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method, Methods 25 (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.
[35] V. Volynets, A. Rings, G. Bárdos, M.J. Ostaff, J. Wehkamp, S.C. Bischoff, Intestinal barrier analysis by assessment of mucins, tight junctions, and α-defensins in healthy C57BL/6J and BALB/cJ mice, Tissue Barriers 4 (2016) e1208468, https://doi.org/10.1080/21688370.2016.1208468.
[36] A. Giulietti, L. Overbergh, D. Valckx, B. Decallonne, R. Bouillon, C. Mathieu, An overview of real-time quantitative PCR: applications to quantify cytokine gene expression, Methods 25 (2001) 386–401, https://doi.org/10.1006/meth.2001.1261.
[37] C.-W. Chang, H.-C. Lee, L.-H. Li, J.-S. Chiang Chiau, T.-E. Wang, W.-H. Chuang, M.-J. Chen, H.-Y. Wang, S.-C. Shih, C.-Y. Liu, T.-H. Tsai, Y.-J. Chen, Fecal microbiota transplantation prevents intestinal injury, upregulation of toll-like receptors, and 5-fluorouracil/oxaliplatin-induced toxicity in colorectal cancer, Int. J. Mol. Sci. 21 (2020) 386, https://doi.org/10.3390/ijms21020386.
[38] L. Zheng, Y.-L. Zhang, Y.-C. Dai, X. Chen, D.-L. Chen, Y.-T. Dai, Z.-P. Tang, Jianpi Qingchang decoction alleviates ulcerative colitis by inhibiting nuclear factor-κB activation, World J. Gastroenterol. 23 (2017) 1180, https://doi.org/10.3748/wjg.v23.i7.1180.
[39] M.-K. Song, M.-Y. Park, M.-K. Sung, 5-fluorouracil-induced changes of intestinal integrity biomarkers in BALB/C mice, J. Cancer Prev. 18 (2013) 322–329, https://doi.org/10.15430/JCP.2013.18.4.322.
[40] Y. Chen, Q. Qian, J. Yu, Carbenoxolone ameliorates insulin sensitivity in obese mice induced by high fat diet via regulating the IκB-α/NF-κB pathway and NLRP3 inflammasome, Biomed. Pharmacother. 115 (2019) 108868, https://doi.org/10.1016/j.biopha.2019.108868.
[41] H.-L. Li, L. Lu, X.-S. Wang, L.-Y. Qin, P. Wang, S.-P. Qiu, H. Wu, F. Huang, B.-B. Zhang, H.-L. Shi, X.-J. Wu, Alteration of gut microbiota and inflammatory cytokine/chemokine profiles in 5-fluorouracil induced intestinal mucositis, Front. Cell. Infect. Microbiol. 7 (2017), https://doi.org/10.3389/fcimb.2017.00455.
[42] Z. Yu, M. Morrison, Improved extraction of PCR-quality community DNA from digesta and fecal samples, Biotechniques 36 (2004) 808–812, https://doi.org/10.2144/04365ST04.
[43] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinformatics 30 (2014) 2114–2120, https://doi.org/10.1093/bioinformatics/btu170.
[44] R.C. Edgar, B.J. Haas, J.C. Clemente, C. Quince, R. Knight, UCHIME improves sensitivity and speed of chimera detection, Bioinformatics 27 (2011) 2194–2200, https://doi.org/10.1093/bioinformatics/btr381.
[45] T. Rognes, T. Flouri, B. Nichols, C. Quince, F. Mahé, VSEARCH: a versatile open source tool for metagenomics, PeerJ 2016 (2016) e2584, https://doi.org/10.7717/PEERJ.2584.
[46] O. Hammer, D.A.T. Harper, P.D. Ryan, PAST: paleontological statistics software package for education and data analysis, Palaeontol. Electron. 4 (2021) 1–9.

[47] P.R. Chandhni, D. Pradhan, K. Sowmya, S. Gupta, S. Kadyan, R. Choudhary, A. Gupta, G. Gulati, R.H. Mallappa, J.K. Kaushik, S. Grover, Ameliorative effect of surface proteins of probiotic lactobacilli in colitis mouse models, Front. Microbiol. 12 (2021), https://doi.org/10.3389/fmicb.2021.679773.
[48] X. Shen, L. Liu, R.M. Peek, S.A. Acra, D.J. Moore, K.T. Wilson, F. He, D.B. Polk, F. Yan, Supplementation of p40, a *Lactobacillus rhamnosus* GG-derived protein, in early life promotes epidermal growth factor receptor-dependent intestinal development and long-term health outcomes, Mucosal Immunol. 11 (2018) 1316–1328, https://doi.org/10.1038/s41385-018-0034-3.
[49] M. Friedrich, M. Pohin, F. Powrie, Cytokine networks in the pathophysiology of inflammatory bowel disease, Immunity 50 (2019) 992–1006, https://doi.org/10.1016/j.immuni.2019.03.017.
[50] Y.J. Jang, W.-K. Kim, D.H. Han, K. Lee, G. Ko, *Lactobacillus fermentum* species ameliorate dextran sulfate sodium-induced colitis by regulating the immune response and altering gut microbiota, Gut Microbes 10 (2019) 696–711, https://doi.org/10.1080/19490976.2019.1589281.
[51] Abdullah G. Alkushi, A. Abdelfattah-Hassan, H. Eldoumani, S.T. Elazab, S.A. M. Mohamed, A.Sh. Metwally, E.S. El-Shetry, A.A. Saleh, N.A. ElSawy, D. Ibrahim, Probiotics-loaded nanoparticles attenuated colon inflammation, oxidative stress, and apoptosis in colitis, Sci. Rep. 12 (2022) 5116, https://doi.org/10.1038/s41598-022-08915-5.
[52] G. Roda, M. Marocchi, A. Sartini, E. Roda, Cytokine networks in ulcerative colitis, Ulcers 2011 (2011) 1–5, https://doi.org/10.1155/2011/391787.
[53] S. Wang, J. Wang, R. Ma, S. Yang, T. Fan, J. Cao, Y. Wang, W. Ma, W. Yang, F. Wang, H. Zhang, IL-10 enhances T cell survival and is associated with faster relapse in patients with inactive ulcerative colitis, Mol. Immunol. 121 (2020) 92–98, https://doi.org/10.1016/j.molimm.2020.03.001.
[54] B. Li, R. Alli, P. Vogel, T.L. Geiger, IL-10 modulates DSS-induced colitis through a macrophage–ROS–NO axis, Mucosal Immunol. 7 (2014) 869–878, https://doi.org/10.1038/mi.2013.103.
[55] S. Ihara, Y. Hirata, K. Koike, TGF-β in inflammatory bowel disease: a key regulator of immune cells, epithelium, and the intestinal microbiota, J. Gastroenterol. 52 (2017) 777–787, https://doi.org/10.1007/s00535-017-1350-1.
[56] T.L. Morhardt, A. Hayashi, T. Ochi, M. Quirós, S. Kitamoto, H. Nagao-Kitamoto, P. Kuffa, K. Atarashi, K. Honda, J.Y. Kao, A. Nusrat, N. Kamada, IL-10 produced by macrophages regulates epithelial integrity in the small intestine, Sci. Rep. 9 (2019) 1223, https://doi.org/10.1038/s41598-018-38125-x.
[57] M.F. Neurath, Strategies for targeting cytokines in inflammatory bowel disease, Nat. Rev. Immunol. (2024), https://doi.org/10.1038/s41577-024-01008-6.
[58] V. Langer, E. Vivi, D. Regensburger, T.H. Winkler, M.J. Waldner, T. Rath, B. Schmid, L. Skottke, S. Lee, N.L. Jeon, T. Wohlfahrt, V. Kramer, P. Tripal, M. Schumann, S. Kersting, C. Handtrack, C.I. Geppert, K. Suchowski, R.H. Adams, C. Becker, A. Ramming, E. Naschberger, N. Britzen-Laurent, M. Stürzl, IFN-γ drives inflammatory bowel disease pathogenesis through VE-cadherin–directed vascular barrier disruption, J. Clin. Investig. 129 (2019) 4691–4707, https://doi.org/10.1172/JCI124884.
[59] H.F. Farin, W.R. Karthaus, P. Kujala, M. Rakhshandehroo, G. Schwank, R.G. J. Vries, E. Kalkhoven, E.E.S. Nieuwenhuis, H. Clevers, Paneth cell extrusion and release of antimicrobial products is directly controlled by immune cell–derived IFN-γ, J. Exp. Med. 211 (2014) 1393–1405, https://doi.org/10.1084/jem.20130753.
[60] Y. Eriguchi, K. Nakamura, Y. Yokoi, R. Sugimoto, S. Takahashi, D. Hashimoto, T. Teshima, T. Ayabe, M.E. Selsted, A.J. Ouellette, Essential role of IFN-γ in T cell–associated intestinal inflammation, JCI Insight 3 (2018), https://doi.org/10.1172/jci.insight.121886.
[61] P. Nava, S. Koch, M.G. Laukoetter, W.Y. Lee, K. Kolegraff, C.T. Capaldo, N. Beeman, C. Addis, K. Gerner-Smidt, I. Neumaier, A. Skerra, L. Li, C.A. Parkos, A. Nusrat, Interferon-γ regulates intestinal epithelial homeostasis through converging β-catenin signaling pathways, Immunity 32 (2010) 392–402, https://doi.org/10.1016/j.immuni.2010.03.001.
[62] U. Sahu, D. Biswas, V.K. Prajapati, A.K. Singh, M. Samant, P. Khare, Interleukin-17—a multifaceted cytokine in viral infections, J. Cell. Physiol. 236 (2021) 8000–8019, https://doi.org/10.1002/jcp.30471.
[63] P. Bagri, V.C. Anipindi, P.V. Nguyen, D. Vitali, M.R. Stämpfli, C. Kaushic, Novel role for Interleukin-17 in enhancing type 1 helper T cell immunity in the female genital tract following mucosal herpes simplex virus 2 vaccination, J. Virol. 91 (2017), https://doi.org/10.1128/JVI.01234-17.
[64] Y. Lin, S. Ritchea, A. Logar, S. Slight, M. Messmer, J. Rangel-Moreno, L. Guglani, J.F. Alcorn, H. Strawbridge, S.M. Park, R. Onishi, N. Nyugen, M.J. Walter, D. Pociask, T.D. Randall, S.L. Gaffen, Y. Iwakura, J.K. Kolls, S.A. Khader, Interleukin-17 is required for T helper 1 cell immunity and host resistance to the intracellular pathogen *Francisella tularensis*, Immunity 31 (2009) 799–810, https://doi.org/10.1016/j.immuni.2009.08.025.
[65] J. Brasseit, C.K.C. Kwong Chung, M. Noti, D. Zysset, N. Hoheisel-Dickgreber, V. Genitsch, N. Corazza, C. Mueller, Divergent roles of interferon-γ and innate lymphoid cells in innate and adaptive immune cell-mediated intestinal inflammation, Front. Immunol. 9 (2018), https://doi.org/10.3389/fimmu.2018.00023.
[66] A. Awasthi, V.K. Kuchroo, IL-17A directly inhibits TH1 cells and thereby suppresses development of intestinal inflammation, Nat. Immunol. 10 (2009) 568–570, https://doi.org/10.1038/ni0609-568.
[67] W. O'Connor Jr., M. Kamanaka, C.J. Booth, T. Town, S. Nakae, Y. Iwakura, J. K. Kolls, R.A. Flavell, A protective function for interleukin 17A in T cell–mediated intestinal inflammation, Nat. Immunol. 10 (2009) 603–609, https://doi.org/10.1038/ni.1736.
[68] S. Sandes, N. Figueiredo, S. Pedroso, F. Sant'Anna, L. Acurcio, M. Abatemarco Junior, P. Barros, F. Oliveira, V. Cardoso, S. Generoso, M. Caliari, J. Nicoli, E. Neumann, Á. Nunes, *Weissella paramesenteroides* WpK4 plays an immunobiotic role in gut-brain axis, reducing gut permeability, anxiety-like and depressive-like behaviors in murine models of colitis and chronic stress, Food Res. Int. 137 (2020) 109741, https://doi.org/10.1016/j.foodres.2020.109741.
[69] G. Wang, Y. Xia, J. Cui, Z. Gu, Y. Song, Y.Q. Chen, H. Chen, H. Zhang, W. Chen, The roles of moonlighting proteins in bacteria, Curr. Issues Mol. Biol. 16 (2014) 15–22.
[70] A.C. Gomes-Santos, R.P. de Oliveira, T.G. Moreira, A.B. Castro-Junior, B.C. Horta, L. Lemos, L.A. de Almeida, R.M. Rezende, D.C. Cara, S.C. Oliveira, V.A. C. Azevedo, A. Miyoshi, A.M.C. Faria, Hsp65-producing *Lactococcus lactis* prevents inflammatory intestinal disease in mice by IL-10- and TLR2-dependent pathways, Front. Immunol. 8 (2017), https://doi.org/10.3389/fimmu.2017.00030.
[71] S. Górska, B. Buda, E. Brzozowska, M. Schwarzer, D. Srutkova, H. Kozakova, A. Gamian, Identification of *Lactobacillus* proteins with different recognition patterns between immune rabbit sera and nonimmune mice or human sera, BMC Microbiol. 16 (2016) 17, https://doi.org/10.1186/s12866-016-0631-9.
[72] D.D. Eichele, K.K. Kharbanda, Dextran sodium sulfate colitis murine model: an indispensable tool for advancing our understanding of inflammatory bowel diseases pathogenesis, World J. Gastroenterol. 23 (2017) 6016–6029, https://doi.org/10.3748/wjg.v23.i33.6016.
[73] L.S. Poritz, K.I. Garver, C. Green, L. Fitzpatrick, F. Ruggiero, W.A. Koltun, Loss of the tight junction protein ZO-1 in dextran sulfate sodium induced colitis, J. Surg. Res. 140 (2007) 12–19, https://doi.org/10.1016/j.jss.2006.07.050.
[74] T. Oshima, H. Miwa, T. Joh, Changes in the expression of claudins in active ulcerative colitis, J. Gastroenterol. Hepatol. 23 (2008) S146–S150, https://doi.org/10.1111/j.1440-1746.2008.05405.x.
[75] Y. Tan, Y. Guan, Y. Sun, C. Zheng, Correlation of intestinal mucosal healing and tight junction protein expression in ulcerative colitis patients, Am. J. Med. Sci. 357 (2019) 195–204, https://doi.org/10.1016/j.amjms.2018.11.011.
[76] S. Tang, W. Guo, L. Kang, J. Liang, MiRNA-182-5p aggravates experimental ulcerative colitis via sponging Claudin-2, J. Mol. Histol. 52 (2021) 1215–1224, https://doi.org/10.1007/s10735-021-10021-1.
[77] L. Luiskari, J. Lindén, M. Lehto, H. Salmenkari, R. Korpela, Ketogenic diet protects from experimental colitis in a mouse model regardless of dietary fat source, Nutrients 16 (2024) 1348, https://doi.org/10.3390/nu16091348.
[78] R. Ahmad, B. Kumar, I. Thapa, R.L. Tamang, S.K. Yadav, M.K. Washington, G. A. Talmon, A.S. Yu, D.K. Bastola, P. Dhawan, A.B. Singh, Claudin-2 protects against colitis-associated cancer by promoting colitis-associated mucosal healing, J. Clin. Investig. 133 (2023), https://doi.org/10.1172/JCI170771.
[79] S. Zeissig, N. Burgel, D. Gunzel, J. Richter, J. Mankertz, U. Wahnschaffe, A. J. Kroesen, M. Zeitz, M. Fromm, J.-D. Schulzke, Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease, Gut 56 (2007) 61–72, https://doi.org/10.1136/gut.2006.094375.
[80] J. Luettig, R. Rosenthal, C. Barmeyer, J. Schulzke, Claudin-2 as a mediator of leaky gut barrier during intestinal inflammation, Tissue Barriers 3 (2015) e977176, https://doi.org/10.4161/21688370.2014.977176.
[81] F. Heller, P. Florian, C. Bojarski, J. Richter, M. Christ, B. Hillenbrand, J. Mankertz, A.H. Gitter, N. Bürgel, M. Fromm, M. Zeitz, I. Fuss, W. Strober, J. D. Schulzke, Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution, Gastroenterology 129 (2005) 550–564, https://doi.org/10.1053/j.gastro.2005.05.002.
[82] Y. Wang, J.B. Mumm, R. Herbst, R. Kolbeck, Y. Wang, IL-22 increases permeability of intestinal epithelial tight junctions by enhancing Claudin-2 expression, J. Immunol. 199 (2017) 3316–3325, https://doi.org/10.4049/jimmunol.1700152.
[83] C.R. Weber, S.C. Nalle, M. Tretiakova, D.T. Rubin, J.R. Turner, Claudin-1 and claudin-2 expression is elevated in inflammatory bowel disease and may contribute to early neoplastic transformation, Lab. Invest. 88 (2008) 1110–1120, https://doi.org/10.1038/labinvest.2008.78.
[84] R. Ahmad, R. Chaturvedi, D. Olivares-Villagómez, T. Habib, M. Asim, P. Shivesh, D.B. Polk, K.T. Wilson, M.K. Washington, L. Van Kaer, P. Dhawan, A.B. Singh, Targeted colonic claudin-2 expression renders resistance to epithelial injury, induces immune suppression, and protects from colitis, Mucosal Immunol. 7 (2014) 1340–1353, https://doi.org/10.1038/mi.2014.21.
[85] P. Raju, N. Shashikanth, P.-Y. Tsai, P. Pongkorpsakol, S. Chanez-Paredes, P. R. Steinhagen, W.-T. Kuo, G. Singh, S. Tsukita, J.R. Turner, Inactivation of paracellular cation-selective claudin-2 channels attenuates immune-mediated experimental colitis in mice, J. Clin. Investig. 130 (2020) 5197–5208, https://doi.org/10.1172/JCI138697.
[86] M. Nishida, M. Yoshida, S. Nishiumi, M. Furuse, T. Azuma, Claudin-2 regulates colorectal inflammation via myosin light chain kinase-dependent signaling, Dig. Dis. Sci. 58 (2013) 1546–1559, https://doi.org/10.1007/s10620-012-2535-3.
[87] N.J. Mantis, N. Rol, B. Corthésy, Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut, Mucosal Immunol. 4 (2011) 603–611, https://doi.org/10.1038/mi.2011.41.
[88] Z.-H. Shen, C.-X. Zhu, Y.-S. Quan, Z.-Y. Yang, S. Wu, W.-W. Luo, B. Tan, X.-Y. Wang, Relationship between intestinal microbiota and ulcerative colitis: mechanisms and clinical application of probiotics and fecal microbiota transplantation, World J. Gastroenterol. 24 (2018) 5–14, https://doi.org/10.3748/wjg.v24.i1.5.

[89] A. Pisani, P. Rausch, C. Bang, S. Ellul, T. Tabone, C. Marantidis Cordina, G. Zahra, A. Franke, P. Ellul, Dysbiosis in the gut microbiota in patients with inflammatory bowel disease during remission, Microbiol. Spectr. 10 (2022), https://doi.org/10.1128/spectrum.00616-22.

[90] A.-L. Prangli, M. Utt, I. Talja, E. Sepp, M. Mikelsaar, T. Rajasalu, O. Uibo, V. Tillmann, R. Uibo, Antigenic proteins of *Lactobacillus acidophilus* that are recognised by serum IgG antibodies in children with type 1 diabetes and coeliac disease, Pediatr. Allergy Immunol. 21 (2009) e772–e779, https://doi.org/10.1111/j.1399-3038.2009.00904.x.

[91] K.R. Fourie, H.L. Wilson, Understanding GroEL and DnaK stress response proteins as antigens for bacterial diseases, Vaccines (Basel) 8 (2020) 773, https://doi.org/10.3390/vaccines8040773.

[92] S.-U. Seo, N. Kamada, R. Muñoz-Planillo, Y.-G. Kim, D. Kim, Y. Koizumi, M. Hasegawa, S.D. Himpsl, H.P. Browne, T.D. Lawley, H.L.T. Mobley, N. Inohara, G. Núñez, Distinct commensals induce interleukin-1β via NLRP3 Inflammasome in inflammatory monocytes to promote intestinal inflammation in response to injury, Immunity 42 (2015) 744–755, https://doi.org/10.1016/j.immuni.2015.03.004.

[93] S.R. El-Zayat, H. Sibaii, F.A. Mannaa, Toll-like receptors activation, signaling, and targeting: an overview, Bull. Natl. Res. Cent. 43 (2019) 187, https://doi.org/10.1186/s42269-019-0227-2.

[94] T. Zuo, S.C. Ng, The gut microbiota in the pathogenesis and therapeutics of inflammatory bowel disease, Front. Microbiol. 9 (2018), https://doi.org/10.3389/fmicb.2018.02247.

[95] S. Zamani, S. Hesam Shariati, M.R. Zali, H. Asadzadeh Aghdaei, A. Sarabi Asiabar, S. Bokaie, B. Nomanpour, L.A. Sechi, M.M. Feizabadi, Detection of enterotoxigenic *Bacteroides fragilis* in patients with ulcerative colitis, Gut Pathog. 9 (2017) 53, https://doi.org/10.1186/s13099-017-0202-0.

[96] F.D. Ihekweazu, M.A. Engevik, W. Ruan, Z. Shi, R. Fultz, K.A. Engevik, A. L. Chang-Graham, J. Freeborn, E.S. Park, S. Venable, T.D. Horvath, S. J. Haidacher, A.M. Haag, A. Goodwin, D.A. Schady, J.M. Hyser, J.K. Spinler, Y. Liu, J. Versalovic, *Bacteroides ovatus* Promotes IL-22 Production and Reduces Trinitrobenzene Sulfonic Acid–Driven Colonic Inflammation, Am. J. Pathol. 191 (2021) 704–719, https://doi.org/10.1016/j.ajpath.2021.01.009.

[97] M.H. Sofi, Y. Wu, T. Ticer, S. Schutt, D. Bastian, H.-J. Choi, L. Tian, C. Mealer, C. Liu, C. Westwater, K.E. Armeson, A.V. Alekseyenko, X.-Z. Yu, A single strain of *Bacteroides fragilis* protects gut integrity and reduces GVHD, JCI Insight 6 (2021), https://doi.org/10.1172/jci.insight.136841.

[98] W. Zhou, W. Wu, Z. Si, H. Liu, H. Wang, H. Jiang, Y. Liu, R.N. Alolga, C. Chen, S. Liu, X. Bian, J. Shan, J. Li, N. Tan, Z. Zhang, The gut microbe *Bacteroides fragilis* ameliorates renal fibrosis in mice, Nat. Commun. 13 (2022) 6081, https://doi.org/10.1038/s41467-022-33824-6.

[99] J.L. Round, S.K. Mazmanian, Inducible Foxp3 $^{+}$ regulatory T-cell development by a commensal bacterium of the intestinal microbiota, Proc. Natl. Acad. Sci. 107 (2010) 12204–12209, https://doi.org/10.1073/pnas.0909122107.

[100] L.C.L. de Jesus, A. dos S. Freitas, J. da C.F. Dutra, G.M. Campos, M.F. Américo, J. G. Laguna, E.G. Dornelas, R.D. de O. Carvalho, K.D. Vital, S.O.A. Fernandes, V. N. Cardoso, J.S. de Oliveira, M.F.A. de Oliveira, A.M.C. Faria, E. Ferreira, R. De O. Souza, F.S. Martins, F.A.L. Barroso, V. Azevedo, *Lactobacillus delbrueckii* CIDCA 133 fermented milk modulates inflammation and gut microbiota to alleviate acute colitis, Food Res. Int. 186 (2024) 114322, https://doi.org/10.1016/j.foodres.2024.114322.

[101] L. Gong, L. Hu, H. Wang, R. Chen, J. Wang, Protective effect of feruloylated oligosaccharides on dextran sulfate sodium-induced ulcerative colitis in rats, Food Front. 3 (2022) 517–528, https://doi.org/10.1002/fft2.140.

[102] F. Li, Y. Han, X. Cai, M. Gu, J. Sun, C. Qi, T. Goulette, M. Song, Z. Li, H. Xiao, Dietary resveratrol attenuated colitis and modulated gut microbiota in dextran sulfate sodium-treated mice, Food Funct. 11 (2020) 1063–1073, https://doi.org/10.1039/C9FO01519A.

[103] M. Schirmer, S.P. Smeekens, H. Vlamakis, M. Jaeger, M. Oosting, E.A. Franzosa, R. ter Horst, T. Jansen, L. Jacobs, M.J. Bonder, A. Kurilshikov, J. Fu, L.A. B. Joosten, A. Zhernakova, C. Huttenhower, C. Wijmenga, M.G. Netea, R. J. Xavier, Linking the human gut microbiome to inflammatory cytokine production capacity, Cell 167 (2016) 1125–1136.e8, https://doi.org/10.1016/j.cell.2016.10.020.

[104] J. Yang, Y. Li, Z. Wen, W. Liu, L. Meng, H. Huang, *Oscillospira* - a candidate for the next-generation probiotics, Gut Microbes 13 (2021), https://doi.org/10.1080/19490976.2021.1987783.

[105] N. Recharla, R. Geesala, X.-Z. Shi, Gut microbial metabolite butyrate and its therapeutic role in inflammatory bowel disease: a literature review, Nutrients 15 (2023) 2275, https://doi.org/10.3390/nu15102275.

[106] C.S. Rocha, A.C. Gomes-Santos, T. Garcias Moreira, M. de Azevedo, T. Diniz Luerce, M. Mariadassou, A.P. Longaray Delamare, P. Langella, E. Maguin, V. Azevedo, A.M. Caetano de Faria, A. Miyoshi, M. van de Guchte, Local and systemic immune mechanisms underlying the anti-colitis effects of the dairy bacterium *Lactobacillus delbrueckii*, PloS One 9 (2014) e85923, https://doi.org/10.1371/journal.pone.0085923.

[107] R. Zhai, X. Xue, L. Zhang, X. Yang, L. Zhao, C. Zhang, Strain-specific anti-inflammatory properties of two *Akkermansia muciniphila* strains on chronic colitis in mice, Front. Cell. Infect. Microbiol. 9 (2019), https://doi.org/10.3389/fcimb.2019.00239.

[108] T.K. Hoang, B. He, T. Wang, D.Q. Tran, J.M. Rhoads, Y. Liu, Protective effect of *Lactobacillus reuteri* DSM 17938 against experimental necrotizing enterocolitis is mediated by toll-like receptor 2, American Journal of Physiology-Gastrointestinal and Liver Physiology 315 (2018) G231–G240, https://doi.org/10.1152/ajpgi.00084.2017.

[109] Y. Liu, Q. Liu, C. Zhang, J. Zhao, H. Zhang, W. Chen, Q. Zhai, Strain-specific effects of *Akkermansia muciniphila* on the regulation of intestinal barrier, Food Sci. Human Wellness 12 (2023) 1526–1537, https://doi.org/10.1016/j.fshw.2023.02.022.

[110] Q. Gu, C. Xia, N. Liu, Z. Chen, Q. Zhou, P. Li, *Lactobacillus plantarum* ZJ316 alleviates ulcerative colitis by inhibiting inflammation and regulating short-chain fatty acid levels and the gut microbiota in a mouse model, Food Funct. (2023), https://doi.org/10.1039/D2FO02567A.

[111] B. Chassaing, J.D. Aitken, M. Malleshappa, M. Vijay-Kumar, Dextran sulfate sodium (DSS)-induced colitis in mice, Curr. Protoc. Immunol. 104 (2014), https://doi.org/10.1002/0471142735.im1525s104.