Lactobacillus plantarum 17–5 attenuates Escherichia coli-induced inflammatory responses via inhibiting the activation of the NF-κB and MAPK signalling pathways in bovine mammary epithelial cells

Ke Li1, Ming Yang1, Mengyue Tian2, Li Jia1, Jinliang Du1,3, Yinghao Wu1, Lianmin Li1, Lining Yuan1 and Yuzhong Ma1*

Abstract

Background: Mastitis is one of the most prevalent diseases and causes considerable economic losses in the dairy farming sector and dairy industry. Presently, antibiotic treatment is still the main method to control this disease, but it also brings bacterial resistance and drug residue problems. Lactobacillus plantarum (L. plantarum) is a multifunctional probiotic that exists widely in nature. Due to its anti-inflammatory potential, L. plantarum has recently been widely researched in complementary therapies for various inflammatory diseases. In this study, the apoptotic ratio, the expression levels of various inflammatory mediators and key signalling pathway proteins in Escherichia coli-induced bovine mammary epithelial cells (BMECs) under different doses of L. plantarum 17–5 intervention were evaluated.

Results: The data showed that L. plantarum 17–5 reduced the apoptotic ratio, downregulated the mRNA expression levels of TLR2, TLR4, MyD88, IL1β, IL6, IL8, TNFa, COX2, iNOS, CXCL2 and CXCL10, and inhibited the activation of the NF-κB and MAPK signalling pathways by suppressing the phosphorylation levels of p65, IκBα, p38, ERK and JNK.

Conclusions: The results proved that L. plantarum 17–5 exerted alleviative effects in Escherichia coli-induced inflammatory responses of BMECs.

Keywords: Lactobacillus plantarum, Escherichia coli, Inflammation, NF-κB, MAPK, Bovine mammary epithelial cell

Background

Mastitis is one of the most prevalent diseases in dairy cows, causing severe economic losses and restricting the development of the dairy cow industry [1]. It is usually caused by infection of the mammary gland with pathogens, such as Escherichia coli, one of the major environmental pathogens responsible for dairy cow mastitis [2, 3]. Coliform mastitis is normally characterized by severe local inflammatory responses and systemic symptoms, and can even cause death under the most serious circumstances [4]. Therefore, it is essential to determine the primary pathogens and inhibit inflammatory reactions to control this disease.

Currently, there is no exact and effective treatment for dairy cow mastitis in the clinic, and antibiotics are often used to control the progression of disease. Despite the positive results of this treatment approach, the excessive
use of antibiotics also brings drug resistance and residue problems [5, 6]. Considering these potential issues, many countries, such as China and the European Union, have already restricted antibiotics in animal feed [7]. Hence, finding alternative safe and effective drugs is of great importance for human health and animal welfare.

*L. plantarum* is a versatile and abundant probiotic found in diverse environments ranging from food to animal and human gastrointestinal tracts [8]. *L. plantarum* is widely used for food processing and livestock feed due to its potential health benefits and biosafety [9]. In recent years, with the deepening of research, the potential anti-inflammatory properties of *L. plantarum* have come into the sight of researchers. Yue et al. found that *L. plantarum* could reduce the expression of TLR4, IL6, and TNFα as well as jejunal injury and had a protective effect on diarrhoea caused by enterotoxigenic *Escherichia coli* [10]. Tian et al.'s research showed that oral supplementation with *L. plantarum* TW1–1 decreased inflammation and modulated gut microbiota in DEHP-induced testicular damage mice [11]. Frola et al. pointed out that *L. plantarum* CRL 1716 provided good therapeutic effects on dairy cow mastitis after intramammary inoculation in lactating cows [12]. Thus, we hypothesized that *L. plantarum* might have a protective effect against inflammatory injury in mastitis cows. However, our analysis of the present literature reveals that related basic research is still limited.

In this study, the probiotic *L. plantarum* 17–5 with possible anti-inflammatory activity was selected. This study aimed to investigate the potential protective effects of this probiotic on *Escherichia coli*-induced inflammatory responses of BMECs and lay an excellent foundation for developing relevant microecological preparations.

### Results

#### Dose effect of *L. plantarum* 17–5 on cell viability

To evaluate the toxicity of *L. plantarum* 17–5 on BMECs, the CCK-8 assay was performed to detect cell viability. As shown in Fig. 1, none of the tested concentrations showed cytotoxicity on BMECs, except the 10^7 CFU/mL dose. Therefore, three concentrations (10^4, 10^5 and 10^6 CFU/mL) were selected for subsequent experiments.

#### Effect of *L. plantarum* 17–5 on apoptosis of BMECs

The effects of varying *L. plantarum* 17–5 doses on the apoptosis of BMECs were analysed (Fig. 2A). The results showed that the apoptotic ratio in the ECOL group increased significantly (*P* < 0.05) compared with those in the CON group and decreased significantly (*P* < 0.05) in the *L. plantarum* 17–5 preconditioning group compared with those in the ECOL group (Fig. 2B). Similar results were seen in the necrotic ratio. The necrotic ratio in each dose preconditioning group decreased significantly (*P* < 0.05) compared with those in the ECOL group (Fig. 2C).

#### Effect of *L. plantarum* 17–5 on the mRNA expressions of TLRs and MyD88

The expression levels of TLR2, TLR4 and MyD88 were measured by real-time PCR. As shown in Fig. 3, after *E. coli* induction, the expression levels of TLR2, TLR4 and MyD88 mRNA increased compared with those in the CON group (*P* < 0.05). Pretreatment with three different doses of *L. plantarum* 17–5 significantly reduced the expression of TLR2, TLR4 and MyD88 mRNA after *E. coli* infection (*P* < 0.05) (Fig. 3A-C).

#### Effect of *L. plantarum* 17–5 on the mRNA expression of inflammatory mediators and chemokines

The expression levels of IL1β, IL6, IL8, TNFα, COX2, iNOS, CXCL2 and CXCL10 were examined using the same method as above. The results showed that *E. coli* increased the mRNA levels of all the indicators (*P* < 0.05). However, these increases were significantly mitigated by pretreatment of *L. plantarum* 17–5 (*P* < 0.05) (Fig. 4A-H).

#### Effect of *L. plantarum* 17–5 on the protein expression of the NF-κB and MAPK pathways

Western blot analysis demonstrated that *E. coli* upregulated the phosphorylation levels of p65, IκBα, p38, ERK and JNK (*P* < 0.05). However, these upregulations were inhibited by pretreatment of *L. plantarum* 17–5 (Fig. 5A, B).
Fig. 2  
(A) Apoptosis was determined by Annexin V-FITC/PI staining. The apoptotic cells showed green fluorescence (FITC-positive and PI-negative), necrotic cells showed both green and red fluorescence (FITC-positive and PI-positive), and normal cells had no fluorescence signal (FITC-negative and PI-negative). Scale bars: 100 μm. 
(B, C) The apoptotic ratio and necrotic ratio in each group. Values from five visual fields were shown as mean ± SEM. The same letter on top of the bars indicated no significant difference, however, different letters indicated significant difference (P < 0.05). The same as the following figures.

Fig. 3  
Effects of L. plantarum 17–5 on TLRs and MyD88 mRNA expression in E. coli-treated BMECs. The mRNA expression levels of TLR2, TLR4 and MyD88 (A-C) were evaluated with qRT-PCR in BEECs. The values were presented as the means ± SEM of three independent experiments.
Discussion

Due to the multiple problems caused by the overuse of antibiotics, several alternative biologics, including beneficial microbes, are being considered to treat and prevent dairy cow mastitis [13]. Several studies suggested that some lactic acid bacteria showed promising effects in treating bovine mastitis [14, 15], among which *L. plantarum* is a typical *Lactobacillus* species with various beneficial effects on host metabolic health. Martín et al. successfully isolated *Lactobacillus fermentum*, *Lactobacillus aerogenes* and *Lactobacillus salivarius* from the milk of healthy females, thereby confirming the existence of probiotics in normal healthy mammary gland tissue [16], this study provides a basis for probiotic treatment in humans. However, there are still some potential problems with this biological therapy, especially when it acts directly on the mammary gland tissue. A recent study reported that intramammary injections of *Lactococcus lactis* (approximately 10⁷ CFU) might elicit a suppurative inflammatory response in the murine model [17]. In light of these challenges, we used a lower dose and treatment time than the above report. Meanwhile, the cytotoxicity assay showed that test doses of *L. plantarum* 17–5 were not toxic to BMECs. Given this result and subsequent indicators, we believe that the test conditions were safe and effective for the cell.

Identifying pathogens is the first step in defense against invading pathogens in the immune system [18]. Mammary epithelial cells have many pattern recognition receptors (PRRs) that are distributed on the cell surface or in the cytoplasm, such as toll-like receptors (TLRs) [19]. Some TLRs, which span the cell membrane, can recognize pathogen-associated molecular patterns (PAMPs). For example, TLR2 recognizes bacterial lipoproteins, and TLR4 recognizes exogenous ligands such as LPS [20]. *Escherichia coli* is an important causative agent of dairy cow mastitis due to its higher incidence rate than other pathogenic microbes [21]. After *E. coli* invades cow mammary tissue, the TLR2 and TLR4 receptors are activated, which mostly leads to MyD88 downstream signalling and consequently activates a series of downstream pathways, kinases and transcription factors [18, 22]. In this study, we found that the mRNA expression levels of *TLR2*, *TLR4* and *MyD88* were upregulated after incubation with *E. coli* at 8h, whereas preincubation with *L. plantarum* 17–5 significantly suppressed these changes. Based on these results, we suspect that *L. plantarum* 17–5 has regulatory effects on downstream inflammatory genes and pathways.

Bacterial infections are usually accompanied by severe inflammatory responses [23]. Excessive inflammatory mediator production can promote inflammatory injury and induce cell apoptosis [24, 25]. TNFα is an inflammatory mediator that plays a proinflammatory role in early inflammation [26]. IL1β and IL6 participate in the occurrence and development of inflammation by activating the expression of other proinflammatory cytokines and modulating chemokine expression [27]. PGE2 and NO were proven to induce intense inflammation with trace amounts in previous research, whereas COX2 and iNOS are key enzymes in their biosynthesis, respectively [28].

![Fig. 4 Effects of *L. plantarum* 17–5 on the inflammatory mediator and chemokine mRNA expression in *E. coli* treated BMECs. The mRNA expression levels of *IL1β*, *IL6*, *TNFα*, *COX2*, *iNOS*, *CXCL2* and *CXCL10* (A–H) in BEECs were detected with the same methods as above. Values were presented as the means ± SEM from three independent experiments.](image-url)
Furthermore, some chemokines, such as IL8, CXCL2 and CXCL10, have also been implicated in inflammatory injury [29]. Previous studies have shown that *E. coli* can stimulate host cells to release various proinflammatory mediators, including IL1β, IL6, TNFα and NO, while inducing cell apoptosis [30, 31]. Our results showed that pretreatment with different doses of *L. plantarum* 17–5 reduced the expression of IL1β, IL6, IL8, TNFα, COX2, iNOS, CXCL2 and CXCL10 during *E. coli* infection. We also evaluated the effect of *L. plantarum* 17–5 on *E. coli*-induced apoptosis. As expected, *L. plantarum* 17–5 inhibited the apoptosis of these cells. This result is consistent with a previous report that probiotics can inhibit induced apoptosis [31].

The MAPK and NF-κB signalling pathways, which are downstream of TLRs, play a key role in regulating cellular proliferation, apoptosis, and inflammation [32]. NF-κB is a pleiotropic transcription factor involved in the control of proinflammatory gene expression, such as TNFα, IL6, COX2 and iNOS [33]. In the quiescent state, NF-κB, as an inactive complex, binds to IκB, an NF-κB inhibitor. Under the action of upstream factors, IκB is phosphorylated and dissociates from NF-κB, allowing NF-κB p65 to translocate to the nucleus and thus activate related gene transcription [34]. The MAPK pathways include p38 MAPK, ERK1/2, and JNK, and this pathway controls the synthesis and release of cytokines during the inflammatory response [35]. The activation of each MAPK depends on multiple upstream kinases with unique cascade reactions [36]. A previous report indicated that *Lactobacillus plantarum* could inhibit the inflammatory response by modulating the NF-κB and MAPK pathways [37, 38]. Similarly, our data suggest that *L. plantarum* 17–5 markedly decreased the phosphorylation of key proteins in the NF-κB and MAPK signalling pathways.

![Fig. 5 Inhibitory effects of *L. plantarum* 17–5 on NF-κB (A) and MAPK (B) phosphorylation in BMECs. Data were represented as the mean ± SEM of three independent experiments](image-url)
Conclusions
In conclusion, L. plantarum 17–5 can attenuate E. coli-induced inflammatory responses by inhibiting the mRNA expression of inflammatory mediators and the activation of the NF-κB and MAPK signalling pathways in BMECs and may be a potential therapeutic agent for dairy cow mastitis. However, due to the limitations of the in vitro model, the biological significance of these findings needs further investigation in vivo.

Materials and methods

Chemicals and reagents
 Dulbecco's modified Eagle's medium/Ham's F-12 nutrient mixture (DMEM/F12) and foetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY), hydrocortisone from Sigma–Aldrich (MO, USA), de Man Rogosa Sharpe (MRS) broth and Luria-Bertani (LB) broth from Aobox (Beijing, China), cell counting kit-8 (CCK-8), RIPA lysis buffer, BCA protein assay kit and BCIP/NBT colour development kit from Solarbio (Beijing, China), and ultrapure RNA extraction kit from CWBIO (Beijing, China). Uelris Il RT–PCR System for First-Strand cDNA Synthesis and AugeGreen™ qPCR Master Mix from US Everbright Inc. (CA, USA), and an Annexin V-FITC Apoptosis Detection Kit from Beyotime (Shanghai, China). Primary antibodies against p38 (Catalog #8690T), phospho-p38 (Catalog #4511T), ERK (Catalog #4695T), phospho-ERK (Catalog #4370T), JNK (Catalog #9252T), phospho-JNK (Catalog #4668T) and IκBα (Catalog #4812S) were acquired from Cell Signaling Technology (Danvers, MA, USA), and antibodies against NF-κB p65 (Catalog #bs-0465R), NF-κB phospho-p65 (Catalog #bs-0982R), phospho-IκBα (Catalog #bsm-52169R) and β-actin (Catalog #bs-0061R) were purchased from Bioss (Woburn, MA, USA).

Culture of bacterial strains and cells
Lactobacillus plantarum 17–5 (ATCC 8014, American Type Culture Collection, Manassas, VA, USA) was cultured by static culture with MRS broth at 37 °C for 24 h. Escherichia coli O111:K58 (B4) (ATCC 43887) was grown in LB broth at 37 °C for 12 h with shaking. After three generations, bacterial strains at the logarithmic growth phase were used for subsequent experiments. Primary cultures of bovine mammary epithelial cells (BMECs) from the mammary glands of Holstein dairy cows by modification of previously reported protocol [39], these changes were made to make cells grow better. The acquired mammary tissues were washed using PBS with constant stirring to remove residual milk, finely sliced into small pieces (0.5 cm³) and cultured in humidified air containing 5% CO₂ at 37 °C. When the cells were 60–80% confluent, the tissue was removed. The fibroblasts were removed and subsequent epithelial cells were enriched according to their different sensitivity to 0.25% trypsin-EDTA. Next, the cells were inoculated into new flasks and cultured in DMEM/F12 supplemented with 15% FBS, 0.1% hydrocortisone, 0.025 M HEPES, 100 U/mL penicillin–streptomycin, and maintained in the same culture conditions as described above.

Cell viability assay
The L. plantarum 17–5 cytotoxicity to BMECs was evaluated using the CCK-8 assay according to the manufacturer's instructions. BMECs were seeded into 96-well microplates at a concentration of 1 × 10⁴ cells/well and cultured to 80–90% confluence. Cells were treated with varying concentrations of L. plantarum 17–5 (10³ to 10⁷ CFU/mL) for 3 h. Then, 10 μL CCK-8 solution was added to each well and further incubated for 2 h at 37°C. Subsequently, the absorbance at 450 nm was measured using a microplate reader.

Cell immunofluorescence assay
BMECs were seeded in 96-well plates and cultured to 70–80% confluence as described above. The confluent cells were then treated as follows: CON group, DMEM/F12 alone; ECOL group, E. coli (10⁷ CFU/mL) infection alone; LP group, L. plantarum 17–5 (10⁷ CFU/mL) incubation alone for 3 h; (L, M, H) LP + ECOL group, L. plantarum 17–5 (10⁴, 10⁵ and 10⁶ CFU/mL) preincubation for 3 h before the addition of E. coli (10⁷ CFU/mL). After E. coli infection for 8 h, the medium was discarded, and the cells were washed with PBS. Subsequently, the cells were stained with an Annexin V-FITC / Propidium Iodide (PI) Apoptosis Detection Kit and observed under a fluorescence microscope. Five visual fields were randomly selected for microscopic observation and the positive cells were counted by ImageJ software (NIH, Bethesda, USA). The apoptotic ratio was calculated as the ratio between the number of apoptotic cells in the treatment group and the untreated control group. The same was done for the necrotic ratio.

qRT–PCR analysis
Total RNA from BMECs was extracted using the Ultrapure RNA extraction kit according to the manufacturer's instructions. The RNA integrity was assessed by agarose gel electrophoresis and the concentration and purity (the ratio of the OD₂₆₀/OD₂₈₀ and OD₂₆₀/OD₂₃₀) were measured on the Nanodrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA). Then the above RNA was reversely transcribed into cDNA by a reverse transcription kit for quantitative real-time PCR (qRT–PCR) analyses. The reaction procedures were as follows: 300s at
95 °C followed by 40 cycles of 5 s at 95 °C, 30 s at 60 °C and
15 s at 72 °C. The PCR amplification efficiency was evalu-
ated using standard curve analysis. The level of target
gene expression was normalized to the GAPDH
reference
gene (For stability of the reference genes, please refer to
supplementary material) and calculated using the $2^{-\Delta\Delta C_T}$
method, and the primer sequences are listed in Table 1.

### Western blot analysis
Proteins from cells were extracted using RIPA lysis buffer
and quantified using a BCA protein assay kit. Protein
samples (20 μg) were separated on 12% polyacrylamide-
SDS gels, and resolved proteins were then transferred
onto nitrocellulose membranes. The membrane was
blocked with 5% skimmed milk for 1 h at room tem-
perature. After incubation with primary and secondary
antibodies, immunoblot signals were visualized with an
NBT/BCIP colour development kit. The densities of the
protein bands from three separate experiments were
quantified by ImageJ software.

### Statistical analysis
All data are presented as the mean ± SEM from at least
three independent experiments. Comparisons between
the groups were evaluated by one-way ANOVA test
with Tukey’s multiple comparisons test. P values < 0.05
were considered significantly different.

### Abbreviations
BMECs: Bovine mammary epithelial cells; DEHP: Diethylhexylphthalate; TLRs:
Toll-like receptors; PAMPs: Pathogen-associated molecular patterns; MyD88:
Myeloid differentiation Factor 88; FITC: Fluorescein isothiocyanate.

### Supplementary Information
The online version contains supplementary material available at https://doi.
org/10.1186/s12917-022-03355-9.

**Additional file 1.** The original, full length blots of western blot.

**Additional file 2.** Analysis of reference genes expression stability.

### Acknowledgements
We thank the Animal Clinical Laboratory of Hebei Agricultural University for
providing technical support.

---

**Table 1** Sequences of primer used for qRT-PCR

| Gene   | Primer sequence (5′-3′)                                      | Product sizes (bp) | GenBank accession no. |
|--------|---------------------------------------------------------------|--------------------|-----------------------|
| TLR2   | CATTCCTGGCAAGTGGATTATC                                       | 201                | AY634629              |
|        | GGAATGGCCCTTCTGATGCTAAGTATGG                                  |                    |                       |
| TLR4   | AGCTTCAACGGTATGCGGCTTCT                                       | 166                | NM_174198.6           |
|        | ACTAAGCACTGGCATGCTCCTCAT                                      |                    |                       |
| MyD88  | AAGTACAAGCAATGAAGAAGAGAG                                      | 102                | NM_001014382.2        |
|        | GAGGGCAAGAAGGAACAGCAG                                         |                    |                       |
| IL-1β  | CCTCGGTTCATGCGGAGATG                                          | 119                | NM_174093.1           |
|        | AGGCACTGTTCCTCAGGACAG                                         |                    |                       |
| IL-6   | TGAAGACACAGACAGACAGACACT                                       | 90                 | NM_173923.2           |
|        | TGATTGAACCAGATTGAAGAAGC                                       |                    |                       |
| TNF-α  | GGGCTTTACCTCATCTCAGCAG                                         | 132                | NM_173966.3           |
|        | GATGGCGACAGAGATGGGACC                                         |                    |                       |
| IL-8   | AACACATCCACACCTTTCCAC                                         | 149                | AF232704              |
|        | ACCCTCTGCACCACCTTTTC                                          |                    |                       |
| COX-2  | GGTCGTGCCTGTCTGATGAT                                          | 124                | NM_174445.2           |
|        | GATGACTGCCTGCTGTGCTGGA                                        |                    |                       |
| iNOS   | TGTCAGCGCAGACAGACAGCATT                                       | 289                | NM_001076799.1        |
|        | CGGCTGTTGCAATGAGAAACCT                                       |                    |                       |
| CXCL-2 | ACCGAAGTCAACGCACTCCTC                                         | 218                | NM_174299.3           |
|        | TCCAGATGCCCTAGAGGT                                            |                    |                       |
| CXCL-10| CTCAGACCGAAGGAGGAGGCA                                        | 117                | NM_001046551          |
|        | TCCAGCGACATATTAGGCGCTT                                         |                    |                       |
| GAPDH  | CACCTCATAGTGTCAGCA                                             | 103                | NM_001034034.2        |
|        | GGTGCTAATTTCCCAGCAA                                           |                    |                       |
Authors' contributions
KL and MYT designed the study. YHW, LML and LNY prepared materials. All experiments were performed by KL and MY. LJ and JLD analysed the data. KL drafted the manuscript, which was revised by YZM. All authors read and approved the final manuscript.

Funding
This research was supported by the Hebei Key Research and Development Program (19226611D).

Availability of data and materials
The original sequences we used for primer sequence design can be found in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under the accession numbers AY634629, NM_174198.6, NM_00104382.2, NM_174093.3, NM_173922.3. The original, full-length western blot blots and analysis of reference genes expression in the supplementary information (Additional files 1 and 2). Data generated during the presented study are available from the corresponding author (YZM) upon reasonable request.

Declarations

Ethics approval and consent to participate
The collection of mammary glands tissue was approved by a local abattoir (Lianchi, Hebei, China) from healthy adult dairy cows. All dairy cows in this abattoir were slaughtered for meat production, and none of the cows were used for tissue collection.

Consent for publication
Not applicable.

Competing interests
The authors declare no potential conflicts of interest with respect to the research, authorship, or publication of this article.

Author details
1College of Veterinary Medicine, Hebei Agricultural University, Baoding 071001, Hebei, China. 2College of Life Science and Food Engineering, Hebei University of Engineering, Handan 056038, Hebei, China. 3Key Laboratory of Freshwater Fisheries and Germplasm Resources Utilization, Ministry of Agriculture, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, WuXi 214081, China.

Received: 27 January 2022 Accepted: 21 June 2022
Published online: 28 June 2022

References
1. Bonsaglia ECR, Gomes MS, Canisso IF, Zhou Z, Lima SF, Rall VLM, et al. Milk microbiome and bacterial load following dry cow therapy without antibiotics in dairy cows with healthy mammary gland. Sci Rep. 2017;7(1):8067.
2. Xu T, Shen X, Seyfert HM. Stearoyl-CoA desaturase 1 expression is down-regulated in liver and udder during cow mastitis through inflammatory activation of C/EBP factors and reduced expression of the inducer SREBP1A. BMC Mol Biol. 2016;17(1):16.
3. Chang G, Petzl W, Vanselow J, Günter J, Shen X, Seyfert HM. Epigenetic mechanisms contribute to enhanced expression of immune response genes in the liver of cows after experimentally induced Escherichia coli mastitis. Vet J. 2015;203(3):339–41.
4. Liu M, Song S, Li H, Jiang X, Yin P, Wan C, et al. The protective effect of caffeic acid against inflammation injury of primary bovine mammary epithelial cells induced by lipopolysaccharide. J Dairy Sci. 2014;97(5):2865–66.
5. Boehler JL. Proteomic analyses of host and pathogen responses during bovine mastitis. J Mammary Gland Biol Neoplasia. 2011;16(4):323–38.
6. Kero Dego O, Oliver SP, Almeda RA. Host-pathogen gene expression profiles during infection of primary bovine mammary epithelial cells with Escherichia coli strains associated with acute or persistent bovine mastitis. Vet Microbiol. 2012;155(2-4):291–7.
7. Inatomi T, Otomaru K. Effect of dietary probiotics on the semen traits and antioxidative activity of male broiler breeders. Sci Rep. 2018;8(1):5874.
8. Vries, Vaughan EE, Kleerebezem M, de Vos WM. Lactobacillus plantarum-survival, functional and potential probiotic properties in the human intestinal tract, 2006.
9. Kaprasob R, Kerkoechochuen O, Laohakunjit N, Thumphanaran B, Shetty K. Changes in phycho-chemical, astrignency, volatile compounds and anti-oxidant activity of fresh and concentrated cashew juice fermented with Lactobacillus plantarum. J Food Sci Technol. 2018;55(10):3979–90.
10. Yue Y, He Z, Zhou Y, Ross RP, Stanton C, Zhao J, et al. Lactobacillus plantarum relieves diarrhea caused by enterotoxin-producing Escherichia coli through inflammation modulation and gut microbiota regulation. Food Funct. 2020;11(12):10362–74.
11. Tian X, Yu Z, Peng P, Ye Z, Li R, Liu J, et al. Lactobacillus plantarum TWC-1 alleviates Diethyihexylphthalate-induced testicular damage in mice by modulating gut microbiota and decreasing inflammation. Front Cell Infect Microbiol. 2019;9:221.
12. Fraia ID, Pellegrino MS, Espeche MC, Giraudo JA, Nader-Macias ME, Bogni CI. Effects of intramammary inoculation of Lactobacillus perolens CRL724 in lactating cows’ udders. J Dairy Res. 2012;79(1):84–92.
13. Fukuyama K, Islam MA, Takagi M, Ikeda-Ohsubo W, Kurata S, Aso H, et al. Evaluation of the Immunomodulatory ability of lactic acid Bacteria isolated from feedlot cattle against mastitis using a bovine mammary epithelial cells in vitro assay. Pathogens. 2020(5):410.
14. Klostermann K, Crispie F, Flynn J, Ross RP, Hill C, Meaney W. Intramammary infusion of a live culture of Lactococcus lactis for treatment of bovine mastitis: comparison with antibiotic treatment in field trials. J Dairy Res. 2008;75(3):365–73.
15. Armas F, Camperio C, Marianelli C. In vitro assessment of the probiotic potential of Lactococcus lactis LMG 7930 against ruminant mastitis-causing pathogens. PLoS One. 2017;12(1):e0169543.
16. Martin R, Olivares M, Marín ML, Fernández L, Xaus J, Rodríguez JM. Probiotic potential of 3 Lactobacilli strains isolated from milk breast. J Hum Lact. 2005;21(1):8–17 quiz 18–21, 41.
17. Camperio C, Armas F, Biasietti E, Frassanito P, Giovannelli C, Spuria L, et al. A mouse mastitis model to study the effects of the intramammary infusion of a food-grade Lactococcus lactis strain. PLoS One. 2017;12(9):e0184218.
18. Wang Y, Zerbe H, Petzl W, Brunner RM, Günter J, Draining C, et al. Bovine TLR2 and TLR4 properly transduce signals from Staphylococcus aureus and E. coli, but S. aureus fails to both activate NF-kappaB in mammary epithelial cells and to quickly induce TNFalpha and interleukin-8 (CXC8L) expression in the udder. Mol Immunol. 2008;45(5):1385–97.
19. Turner ML, Cronin JG, Healey GD, Sheldon RM. Epithelial and stromal cells of bovine endometrium have roles in innate immunity and initiate inflammatory responses to bacterial lipopeptides in vitro via toll-like receptors TLR2, TLR1, and TLR6. Endocrinology. 2014;155(4):1453–65.
20. Wang W, Huang F, Jiang W, Wang W, Xiang J. Brilliant blue G attenuates neuro-inflammation via regulating MAPK and NF-κB signaling pathways in lipopolysaccharide-induced BV2 microglia cells. Exp Ther Med. 2020;20(5):116.
21. Lamney ES, Ammar AM, Zaki E, Khairy N, Refai MK. Virulence Factors of Escherichia coli isolated from Recurrent Cases of Clinical and Subclinical Mastitis in Buffaloes, 2013.
22. Takeuchi O, Akira S. Toll-like receptors, their physiological role and signal transduction system. Int Immunopharmacol. 2001;1(6):625–35.
23. Herath S, Fischer DP, Werling D, Williams EJ, Lilly ST, Dobson H, et al. Expression and function of toll-like receptor 4 in the endometrial cells of the uterus. Endocrinology. 2006;147(1):562–70.
24. Chen J, Wang W, Shi C, Fang J. A comparative study of sodium houttuyfanate and 2-undecanone for their in vitro and in vivo anti-inflammatory activities and stabilities. Int J Mol Sci. 2014;15(12):22978–94.
25. Tang J, Luo K, Li Y, Chen Q, Tang D, Wang D, et al. Capsaicin attenuates LPS-induced inflammatory cytokine production by upregulation of LXRα. Int Immunopharmacol. 2015;28(1):264–9.
26. Wang S, Li R, He S, He L, Zhao H, Deng X, et al. Tripterygium wilfordii glycosides Uprgulate the new anti-inflammatory cytokine IL-37 through ERK1/2 and p38 MAPK signal pathways. Evid Based Complement Alternat Med. 2017;2017:9148523.
27. Huang WC, Liou CJ, Shen SC, Hu S, Hsiao CY, Wu SJ. Luteolin attenuates IL-1β-induced THP-1 adhesion to ARPE-19 cells via suppression of NF-kB and MAPK pathways. Mediat Inflamm. 2020;2020:9421340.

28. Jiang SH, Ping LF, Sun FY, Wang XL, Sun ZJ. Protective effect of taxasterol against rheumatoid arthritis by the modulation of inflammatory responses in mice. Exp Ther Med. 2016;12(6):4035–40.

29. Hughes CE, Nibbs RJB. A guide to chemokines and their receptors. FEBS J. 2018;285(16):2944–71.

30. Long E, Capuco AV, Wood DL, Sonstegard T, Tomita G, Paape MJ, et al. Escherichia coli induces apoptosis and proliferation of mammary cells. Cell Death Differ. 2001;8(6):808–16.

31. Liu M, Wu Q, Wang M, Fu Y, Wang J. Lactobacillus rhamnosus GR-1 limits Escherichia coli-induced inflammatory responses via attenuating MyD88-dependent and MyD88-independent pathway activation in bovine endometrial epithelial cells. Inflammation. 2016;39(4):1483–94.

32. Yao L, Sun T. Glycyrrhizin administration ameliorates Streptococcus aureus-induced acute lung injury. Int Immunopharmacol. 2019;70:504–11.

33. Zhou HY, Shin EM, Guo LY, Youn UJ, Bae K, Kang SS, et al. Anti-inflammatory activity of 4-methoxyhonokiol is a function of the inhibition of iNOS and COX-2 expression in RAW 264.7 macrophages via NF-kappaB, JNK and p38 MAPK inactivation. Eur J Pharmacol. 2008;586(1–3):340–9.

34. Ettari R, Pallio G, Pizzino G, Irrera N, Zappalà M, Maiorana S, et al. Non-covalent immunoproteasome inhibitors induce cell cycle arrest in multiple myeloma MM.1R cells. J Enzyme Inhib Med Chem. 2019;34(1):1307–13.

35. Kaminska B. MAPK signalling pathways as molecular targets for anti-inflammatory therapy—from molecular mechanisms to therapeutic benefits. Biochim Biophys Acta. 2005;1754(1–2):253–62.

36. Kyriakis JM, Avruch J. Mammalian MAPK signal transduction pathways activated by stress and inflammation: a 10-year update. Physiol Rev. 2012;92(2):689–737.

37. Yang J, Qiu Y, Hu S, Zhu C, Wang L, Wen X, et al. Lactobacillus plantarum inhibited the inflammatory response induced by enterotoxigenic Escherichia coli K88 via modulating MAPK and NF-κB signalling in intestinal porcine epithelial cells. J Appl Microbiol. 2021;130(5):1684–94.

38. Wu Y, Zhu C, Chen Z, Chen Z, Zhang W, Ma X, et al. Protective effects of Lactobacillus plantarum on epithelial barrier disruption caused by enterotoxigenic Escherichia coli in intestinal porcine epithelial cells. Vet Immunol Immunopathol. 2016;172:55–63.

39. Zhao K, Liu HY, Zhou MM, Liu JX. Establishment and characterization of a lactating bovine mammary epithelial cell model for the study of milk synthesis. Cell Biol Int. 2010;34(7):717–21.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.