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Lactobacillus crispatus attenuates *Escherichia coli*-induced inflammation and migration of cervical cancer cells

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Abstract

Comprehensive research has indicated that *Lactobacillus*, serving as the predominant bacterial genus in the female reproductive tract, effectively restrains pathogenic microorganisms and plays a vital role in upholding urogenital health. In the present study, *Lactobacillus crispatus*, a pivotal species within the *Lactobacillus* genus, was chosen to explore its antibacterial mechanisms against *Escherichia coli* and the molecular pathways implicated in alleviating the progression of cervical cancer complicated by *Escherichia coli* infection. *Lactobacillus crispatus* manifested notable inhibitory effects on the growth of *Escherichia coli*, with an average inhibition zone diameter of 23.86 ± 0.41 mm. The principal active agents accountable for this antimicrobial activity were ascertained to be viable *Lactobacillus crispatus* cells. Moreover, *Lactobacillus crispatus* significantly curbed the biofilm formation of *Escherichia coli*. Remarkably, *Escherichia coli* infection induced ferroptosis, exacerbated inflammatory responses, and promoted migratory capacity in human cervical cancer cell lines HeLa and C-33A. In contrast, *Lactobacillus crispatus* treatment effectively attenuated *Escherichia coli*-induced ferroptosis, inflammation, and cell migration in human cervical cancer cell lines HeLa and C-33A. As a microbial homeostasis regulator, *Lactobacillus crispatus* not only suppresses the growth of *Escherichia coli* but also improves the migratory potential of cervical cancer cells in the context of *Escherichia coli* infection, thus contributing to the preservation of female reproductive system health.

Keywords *Lactobacillus crispatus*, *Escherichia coli*, Ferroptosis, Cervical cancer, Inflammatory injury, Migration

Introduction

There exists a diverse array of microbial communities within the female vagina, collectively maintaining the balance and stability of vaginal microecology (De et al., 2022; Shen et al. 2022). However, any disruption to this equilibrium may lead to gynecological diseases, including inflammation and tumors. *Escherichia coli* exhibits specific distribution patterns in the female reproductive system, frequently serving as a common pathogen in gynecological infections. *Escherichia coli* infection can elicit an inflammatory response, releasing various cytokines and inflammatory mediators, such as IL-6、IL-8、TNF- α . These substances may promote cell proliferation, inhibit apoptosis, and create favorable conditions

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for the growth and survival of tumor cells. As a critical anatomical structure connecting the vagina and uterus, the cervix is also a region prone to colonization by *Escherichia coli*, potentially leading to cervical epithelial damage, inflammation, and even carcinogenesis (Lopez et al. 2021).

Ferroptosis is a newly identified form of programmed cell death that distinctly differs from apoptosis, necrosis, and autophagy. It is characterized by the iron-dependent accumulation of lipid peroxidation to cytotoxic levels. Recent studies have demonstrated a close association between microbial infection, localization and metastasis, ferroptosis, and the onset and progression of tumors. Ferroptosis plays a crucial regulatory role in the host immune response to infection and tumorigenesis. Pathogen invasion can trigger ferroptosis in host cells, leading to the release of damage-associated molecular patterns (DAMPs) and subsequent activation of innate immune signaling pathways (Saavedra-Torres et al., 2025). However, certain pathogenic microorganisms manipulate host iron homeostasis and suppress reactive oxygen species (ROS) production to facilitate their survival (Wang et al. 2025). For instance, *Salmonella* exploits the host transferrin receptor-mediated iron uptake pathway to enhance intracellular persistence and bolster its antioxidant defense mechanisms (Hos et al. 2017). Meanwhile, malignant tumor cells frequently exhibit dysregulated iron metabolism and impaired antioxidant systems, including altered glutathione peroxidase 4 (GPX4) activity. Within the tumor microenvironment, immune cells can influence tumor progression by modulating ferroptotic processes (Tong et al. 2022).

Lactobacillus crispatus serves as the dominant strain in the female vagina, playing a pivotal role in preserving the balance of vaginal microecology (Garza et al. 2021). Bacterial vaginosis is a condition characterized by the overgrowth of anaerobic bacteria, particularly *Gardnerella* species, and a concurrent reduction or depletion of *Lactobacillus crispatus* (Mändar et al. 2023). The administration of *Lactobacillus crispatus* lowers the pH of the vaginal milieu through lactic acid production, thereby inhibiting the proliferation of anaerobic pathogens (Marschalkó et al. 2023). Additionally, *Lactobacillus crispatus* can produce multiple antimicrobial agents, including hydrogen peroxide and bacteriocins, which contribute to its protective effect against microbial infections (Balkus et al. 2012; Nilsen et al. 2020). *Lactobacillus cristae* reduces the risk of inflammation by maintaining an acidic vaginal environment and generating hydrogen peroxide, which inhibits the proliferation of pathogenic and opportunistic microorganisms (Dricot et al. 2024). Currently, the mechanism by which *Lactobacillus crispatus* mitigates cervical cancer through the inhibition of *Escherichia coli* remains unclear. The objective of this

study is to further investigate the role of *Escherichia coli* in the onset of cervical cancer, which holds significant implications for enhancing early prevention, improving treatment efficacy, and reducing mortality rates. Additionally, the application of *Lactobacillus crispatus*-based microecological therapy to control the occurrence and progression of cervical cancer carries substantial practical significance.

Materials and methods

Strains and cell lines and their cultures

The freeze-dried powder of *Lactobacillus crispatus* was obtained from the Chinese General Microbiological Culture Collection Center (CGMCC, accession number CGMCC 1.2743) and is a wild-type strain. An appropriate volume of sterile water was added to reconstitute the lyophilized powder, which was subsequently inoculated into MRS liquid medium (Solarbio, Beijing, China). The culture was maintained under controlled conditions at 37 °C with a shaking speed of 180 × g for 18 h. Following successful growth of the strain, an inoculation loop was used to transfer the culture onto MRS solid medium, which was then incubated at 37 °C for 18 to 24 h. *Escherichia coli* ATCC25922 originates from the Laboratory of Shenyang Medical College Affiliated Central Hospital and is cultured in broth medium (Solarbio, Beijing, China) under conditions at 37 °C, 180 × g, for 8 h.

Human cervical cancer cell lines HeLa and C-33A were obtained from the Cell Bank of the Typical Culture Preservation Committee, Chinese Academy of Sciences. These cell lines were originally sourced from the American Type Culture Collection (ATCC), with the accession numbers CCL-2 for HeLa and HTB-31 for C-33A (Macville et al. 1999; Hendricks et al. 1997). Both HeLa and C-33A cells were cultured in DMEM (Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA), along with 100 units/mL of penicillin (Genview, Australia) and 100 units/mL of streptomycin solution (Genview, Australia). The cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂.

Antibodies

The primary antibodies include mouse monoclonal anti-NF-κB p65 (1:3000, Cat No. ab288751), rabbit polyclonal anti-LOX (1:5000, Cat No. ab174316), rabbit monoclonal anti-COX2 (1:3000, Cat No. ab179800), rabbit monoclonal anti-N-cadherin (1:6000, Cat No. ab76011), rabbit monoclonal anti-Vimentin (1:3000, Cat No. ab92547), rabbit monoclonal anti-Snail (1:1000, Cat No. ab216347), rabbit monoclonal anti-Slug (1:1000, Cat No. ab314086), rabbit polyclonal anti-groEL antibody (1:1000, Cat No. ab90522) were obtained from Abcam Biomedical Company (Cambridge, UK). The primary antibodies include

mouse monoclonal anti-GPX4 (1:3000, Cat No. 67763–1-Ig), rabbit polyclonal anti-SLC7A11 (1:1000, Cat No. 26864–1-AP), rabbit polyclonal anti-SLC3A2 (1:20,000, Cat No. 15193–1-AP), rabbit polyclonal anti-Lipoprotein Lpp (1:5000, Cat No. 25045–1-AP) were obtained from Proteintech Company (Wuhan, Hubei, China). The primary antibodies include rabbit polyclonal anti-ompA (1:2000, Cat No. PA5-117,575), rabbit polyclonal anti-ompC (1:2000, Cat No. PA5-117,701), were obtained from Thermo Fisher Scientific Company (Massachusetts, USA). HRP-Goat anti-rabbit recombinant secondary antibody (H+L) and HRP-Goat anti-mouse recombinant secondary antibody (H+L) were obtained from Protein-tech Company (Wuhan, Hubei, China).

Bacteriostatic active ingredient detection

The Oxford cup assay was employed to determine active ingredient. Briefly, single colonies of *Escherichia coli* were inoculated into 5 mL of LB broth and incubated aerobically at 37 °C with shaking (180 rpm) for 12 h. The resulting culture was then diluted with fresh LB broth to achieve a standardized inoculum density of 1×10^6 CFU/mL. Subsequently, 100 μ L of this suspension was uniformly spread onto the surface of MRS agar plates using a sterile glass spreader. Single colonies of *Lactobacillus crispatus* were isolated and inoculated into MRS medium (supplemented with 5 mL) under aseptic conditions. The cultures were incubated at 37 °C for 18 h with shaking at $180 \times g$ to prepare the seed solution. Subsequently, the fermentation broth was prepared by inoculating the seed culture into MRS medium (50 mL, containing 1% inoculum) and incubating it at 37 °C for 24 h with shaking at $180 \times g$. The fermentation broth of *Lactobacillus crispatus* was divided into five experimental groups: the first group consisted of untreated fermentation broth; the second group involved filtration of the supernatant through a 0.22 μ m filter membrane to remove bacterial cells; the third group involved centrifugation of the fermentation broth at $18,000 \times g$ for 10 min at 4 °C, followed by collection of the precipitated *Lactobacillus crispatus* cells and their resuspension in saline; the fourth group was similar to the third, except that the resuspended cells were subjected to heat treatment at 62 °C for 30 min to achieve bacterial inactivation; the fifth group served as a control and contained only saline. Sterile Oxford cups were placed equidistantly on the inoculated agar surface, and 150 μ L of the sample to be tested was added into each cup. Plates were incubated at 37 °C for 24 h under aerobic conditions, after which zones of inhibition were measured to assess antibacterial activity.

Determination of inhibition threshold of strain co-culture

The concentration of *Lactobacillus crispatus* was adjusted to 0×10^4 CFU/mL, 0.125×10^4 CFU/mL, 0.25×10^4 CFU/

mL, 0.5×10^4 CFU/mL and 1×10^4 CFU/mL using MRS medium. Subsequently, 100 μ L of *Lactobacillus crispatus* bacterial suspensions at varying concentrations were added to a 96-well plate, followed by the addition of 100 μ L of *Escherichia coli* suspension (1×10^6 CFU/mL) into each well. The 96-well plates were incubated aerobically at 37 °C for 24 h. Bacterial viability was quantified via colony-forming unit (CFU) enumeration, and colony PCR was performed to determine the inhibitory concentration threshold in the co-culture system.

Fermentation broth conditions determination

Single colonies of *Lactobacillus crispatus* were aseptically picked and inoculated into 5 mL of MRS medium. To investigate pH and temperature dependent growth responses, cultures were incubated across a pH range of 1–12 (adjusted with sterile HCl or NaOH) and at temperatures ranging from 28 to 43 °C for durations of 12–42 h. At the conclusion of each incubation period, bacterial cells were harvested by centrifugation at $18,000 \times g$ for 10 min at 4 °C, washed once with sterile PBS, and resuspended in fresh MRS medium (pH 6.5–7.0) to standardize the optical density. Antimicrobial efficacy was evaluated using the Oxford cup assay according to the standardized protocol described earlier.

Viable bacteria quantity detection

The viable bacterial count was quantified using the standard plate count method. Briefly, test samples were serially diluted tenfold in sterile physiological saline. Aliquots of 100 μ L from dilutions ranging from 10^4 to 10^7 were evenly spread onto LB or MRS agar plates. Plates were incubated aerobically at 37 °C for 24 h. Following incubation, visible colonies were enumerated, and the viable bacterial concentration was calculated based on the dilution factor and inoculum volume, and reported as colony-forming units per milliliter (CFU/mL).

MOI assay

HeLa and C-33A cells were seeded in DMEM medium (contains 10% fetal bovine serum, and devoid of penicillin and streptomycin) at density of 2×10^4 cells/mL (500 μ L per well) into 24-well tissue culture plates and incubated at 37 °C under 5% CO₂ for 18 h. Following incubation, the culture medium was aspirated, and cells were washed three times with PBS. Subsequently, the MOI was determined as follows: for *Escherichia coli*, 500 μ L of 1×10^6 CFU/mL was used at 4 h post-infection in both HeLa and C-33A cells; for *Lactobacillus crispatus*, 500 μ L of $1/16 \times 10^4$ CFU/mL, $1/8 \times 10^4$ CFU/mL and $1/4 \times 10^4$ CFU/mL were applied at 24 h post-infection in HeLa and C-33A cells, respectively. Cell-free DMEM medium for bacterial culture alone (contains 10% fetal bovine serum, and devoid of penicillin and streptomycin)

only group served as control. The MOI was determined using the viable plate count method. Calculation $\text{MOI} = \text{number of bacteria} / \text{number of cells}$.

Cell viability assay

Hela and C-33A cells were seeded in DMEM medium (contains 10% fetal bovine serum, and devoid of penicillin and streptomycin) at a density of 2×10^4 cells/mL (500 μL per well) into 24-well tissue culture plates and incubated at 37°C under 5% CO_2 for 18 h. Following incubation, the culture medium was aspirated, and cells were washed three times with PBS. Hela and C-33A cells were treated with either *Escherichia coli* or *Lactobacillus crispatus*. Following completion of the incubation period, 40 μL of cell counting kit-8 solution was added (Apexbio, USA) to each plate, and the plate was incubated for 4 h. The absorbance of each individual experiment at 460 nm was detected. Cell viability (%) = $[(\text{OD}_{\text{experimental}} - \text{OD}_{\text{blank}}) / (\text{OD}_{\text{control}} - \text{OD}_{\text{blank}})] \times 100\%$

Bacterial identification

The bacteria were identified by colony PCR. The specific method was as follows: single colony was selected, added to the pre-mixed PCR reaction tube, and treated at 95°C for 10 min to decompress and release DNA. The universal primers of 16SrRNA gene 27F (5'-AGAGTTTGATC CTGGCTCAG-3') / 1541R (5'-AAGGAGGTGATCCA GCC-3') were used for PCR amplification. The reaction tube was placed in the PCR instrument, and the reaction program was set as follows: the predenaturation stage was 95°C for 5 min; The denaturation stage was 95°C for 30 s. The annealing stage was 58°C for 35 s. The extension phase was 72°C for 80 s with 30 cycles to complete the amplification. The PCR products were detected by agarose gel electrophoresis, and the target bands were obtained. The PCR products were sent to Shanghai Sangon Company for sequencing. The sequencing results were compared in Ezbiocloud (<https://EzBioCloud.net>).

Ferroptosis determination

The effects of *Escherichia coli* and *Lactobacillus crispatus* on ferroptosis in Hela and C-33A cells were evaluated using a green fluorescent probe assay. The experiments were divided into DMEM medium (contains 10% fetal bovine serum, and devoid of penicillin and streptomycin) group containing *Escherichia coli* (1×10^6 CFU/mL, MOI 20), DMEM medium (contains 10% fetal bovine serum, and devoid of penicillin and streptomycin) group containing *Lactobacillus crispatus* at different concentrations ($1/16 \times 10^4$ CFU/mL MOI 35, $1/8 \times 10^4$ CFU/mL MOI 35, and $1/4 \times 10^4$ CFU/mL MOI 35) and *Escherichia coli* (1×10^6 CFU/mL, MOI 20). DMEM medium (contains 10% fetal bovine serum, and devoid of penicillin and streptomycin) only group served as the blank

control. The procedure was as follows: 500 μL of Hela or C-33A cell suspension (2×10^4 cells/mL) was seeded into each well of a 24-well plate and incubated at 37°C under 5% CO_2 for 18 h. After incubation, the culture medium was removed, and the cells were washed three times with PBS. Subsequently, 500 μL of the respective test solution for each experimental group was added and incubated for 4 h. The culture medium was subsequently replaced with DMEM supplemented with 10% fetal bovine serum (FBS) and devoid of penicillin and streptomycin, to which ferric ammonium citrate (Sigma-Aldrich, Germany) was added to a final concentration of 100 $\mu\text{mol/L}$. Cells were then incubated for 30 min under standard culture conditions. Cells were washed again with PBS, fixed with 4% paraformaldehyde at room temperature for 30 min, and permeabilized with 1% Triton X-100 for 20 min. Following an additional PBS wash, 500 μL of FerroBrite Green solution (final concentration: 5 $\mu\text{mol/L}$; AAT Bioquest, USA) was added to each well and incubated for 30 min under light-protected conditions. Fluorescence signals were observed and recorded using a fluorescence inverted microscope (Zeiss, Germany) with excitation at 450 nm and emission at 550 nm.

Glutathione (GSH) content detection

The level of GSH in cells was quantified using an GSH assay kit (mlbio, Shanghai, China), which primarily contained 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), glutathione reductase, and nicotinamide adenine dinucleotide phosphate (NADPH). In this assay, GSH in the samples reacted with DTNB to generate yellow-colored 5-thio-2-nitrobenzoic acid (TNB), and the absorbance was measured at 450 nm using a microplate reader (BioTek, USA) to determine GSH concentration. The experimental groups consisted of DMEM medium (contains 10% fetal bovine serum, and devoid of penicillin and streptomycin) supplemented with *Escherichia coli* (1×10^6 CFU/mL, MOI 20) and DMEM medium (contains 10% fetal bovine serum, and devoid of penicillin and streptomycin) alone as the blank control. The experimental procedure was as follows: Hela and C-33A cells were exposed to each treatment group for 4 h. After washing the cells with PBS, adherent cells were separated using trypsin and centrifuged at $1000 \times g$ for 10 min at 4°C to collect the resulting cell pellet. Harvested cells were resuspended in PBS to a final concentration of 1×10^7 cells/mL. Subsequently, the cell suspension was transferred to a grinding tube containing ceramic lysate beads and homogenized using a multifunctional homogenizer (Bertin, France) for 30 s at 6500 rpm under continuous cooling at 4°C . After lysis, samples were centrifuged at $12,000 \times g$ for 10 min at 4°C , and supernatants were collected for subsequent analysis. Subsequently, 150 μL of total glutathione assay working solution was added to each sample, thoroughly

mixed, and incubated for 5 min at room temperature. Then, 50 μ L of NADPH solution (0.5 mg/mL) was added, mixed again, and incubated for 30 min at room temperature before assay.

Reactive oxygen species (ROS) content detection

The level of ROS in cells was quantified using an ROS assay kit (mlbio, Shanghai, China). The assay kit primarily contained the 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) probe. Upon intracellular reactive oxygen species (ROS) generation, DCFH-DA is deacetylated and subsequently oxidized to the fluorescent compound 2',7'-dichlorofluorescein (DCF). The ROS levels were quantified by measuring the fluorescence intensity using a fluorescence microplate reader. The experimental groups consisted of DMEM medium (contains 10% fetal bovine serum, and devoid of penicillin and streptomycin) supplemented with *Escherichia coli* (1×10^6 CFU/mL, MOI 20), while DMEM medium (contains 10% fetal bovine serum, and devoid of penicillin and streptomycin) alone served as the blank control. The procedures were as follows: Hela and C-33A cells were exposed to each treatment groups for 4 h. Following washing of the cells with PBS, adherent cells were detached using trypsin, centrifuged at $1000 \times g$ for 10 min at 4 °C, and the resulting cell pellets were collected. For the preparation of the DCFH-DA working solution, a 10 mM DCFH-DA stock solution in DMSO was diluted in serum-free DMEM medium at a ratio of 1:1000 to achieve a final concentration of 10 μ M. The cell concentration was adjusted to 1×10^7 cells/mL in DCFH-DA working solution. Cells were then incubated at 37 °C for 20 min, followed by three washes with serum-free culture medium to ensure complete removal of extracellular DCFH-DA. The harvested cells were resuspended in PBS to a final concentration of 1×10^7 cells/mL. Subsequently, the cell suspension was transferred to a grinding tube containing ceramic lysis beads and homogenized using a multifunctional homogenizer (Bertin, France) at 6500 rpm for 30 s under continuous cooling at 4 °C. Following lysis, the sample was centrifuged at $12,000 \times g$ for 10 min at 4 °C, and the resulting supernatant was collected for downstream analysis. Fluorescence intensity was measured using a fluorescent microplate reader (Biotek, USA) with excitation set at 502 nm and emission at 530 nm.

Biofilm formation determination

To accurately assess the inhibitory effect of *Lactobacillus crispatus* on *Escherichia coli* biofilm formation, potential auto-biofilm formation by *Lactobacillus crispatus* itself must be rigorously excluded. Accordingly, the biofilm-forming capacity of *Lactobacillus crispatus* was first evaluated in both LB and MRS media under identical incubation conditions (37 °C, 24 h) and in the absence

of *Escherichia coli*. Three serial dilutions of *Lactobacillus crispatus* $1/16 \times 10^4$ CFU/mL, $1/8 \times 10^4$ CFU/mL, and $1/4 \times 10^4$ CFU/mL were independently cultured in each medium. Only upon confirmation that *Lactobacillus crispatus* did not form detectable biofilms under these conditions was co-culture with *Escherichia coli* initiated. For co-culture experiments, a standardized suspension of *Escherichia coli* (1×10^6 CFU/mL, MOI 20) was seeded into 48-well microtiter plates, followed by addition of the aforementioned *Lactobacillus crispatus* dilutions prepared in either LB or MRS medium. Uninoculated LB and MRS media served as negative controls. All experimental groups were incubated at 37 °C for 24 h prior to biofilm quantification. After incubation, the medium and non-adherent bacteria were removed, and the wells were washed with PBS buffer. The adherent bacteria were fixed with methanol for 20 min, stained with a 2% crystal violet solution for 15 min, and decolorized with 33% glacial acetic acid. Finally, the absorbance value was measured at 630 nm using a microplate reader and the optical microscope was used for observation using a $10 \times$ eyepiece and a $40 \times$ objective lens (total magnification: $400 \times$).

Cell scratch assay

Briefly, 2×10^5 cells/well Hela and C-33A cells were seeded in 6-well plates and incubated at 37 °C under 5% CO₂ for 24 h to achieve approximately 90% confluence. The experiments were divided into DMEM medium (contains 10% fetal bovine serum, and devoid of penicillin and streptomycin) group containing *Escherichia coli* (1×10^6 CFU/mL, MOI 20), DMEM medium (contains 10% fetal bovine serum, and devoid of penicillin and streptomycin) group containing *Lactobacillus crispatus* at different concentrations ($1/16 \times 10^4$ MOI 35, $1/8 \times 10^4$ MOI 35, and 1×10^4 CFU/mL MOI 35) and *Escherichia coli* (1×10^6 CFU/mL MOI 20). DMEM medium (contains 10% fetal bovine serum, and devoid of penicillin and streptomycin) only group served as the blank control. Following treatment, a uniform scratch was created in each well using a 200 μ L pipette tip, after which the monolayers were washed once with PBS. Subsequently, 2 mL of the respective treatment DMEM medium (contains 10% fetal bovine serum, and devoid of penicillin and streptomycin) was added to each well and maintained at 37 °C under 5% CO₂ for 24 h. The experiment was performed in triplicate, and wound healing was assessed by measuring the remaining scratch area using ImageJ software 1.8.0.

Adhesion ability determination

500 μ L of Hela and C-33A cells, suspended at a concentration of 2×10^5 cells/mL, were seeded into 6-well plates and incubated overnight under standard cell culture conditions (37 °C, 5% CO₂). Subsequently, the cells were cultured in DMEM medium (contains 10% fetal

bovine serum, and devoid of penicillin and streptomycin) supplemented with serum for 3 days, followed by serum-free DMEM medium (contains no penicillin and streptomycin) for 12 h. After removing the culture medium and washing the wells twice with PBS, the following treatments were added to each well: 1 mL of *Escherichia coli* suspension (MOI 20), 1 mL of *Escherichia coli* suspension (MOI 20) combined with $1/16 \times 10^4$ CFU/mL (MOI 35) *Lactobacillus crispatus* mixture, 1 mL of *Escherichia coli* suspension (MOI 20) combined with $1/8 \times 10^4$ CFU/mL (MOI 35) *Lactobacillus crispatus* mixture, 1 mL of *Escherichia coli* suspension (MOI 20) combined with 1×10^4 CFU/mL (MOI 35) *Lactobacillus crispatus* mixture, and 1 mL of DMEM medium (contains no fetal bovine serum, penicillin, and streptomycin) as a control. The contents were mixed thoroughly and co-incubated for 4 h. Following incubation, the cells were washed with PBS and fixed with 4% paraformaldehyde for 1 h. Cells were stained with crystal violet for 1 min, followed by gentle rinsing with phosphate-buffered saline (PBS). Subsequently, excess dye was removed by decolorization with 95% ethanol for 30 s, and the monolayer was washed again with PBS. Thereafter, cells were counterstained with diluted basic fuchsin for 1 min and thoroughly rinsed with PBS. Bacterial adherence was quantified by counting the number of bacteria associated with individual host cells at five standardized positions—upper, lower, left, right, and central—within each microscopic field. Counts from all five positions were averaged to yield a representative value per field. Imaging and enumeration were performed under bright-field microscopy using a $10\times$ eyepiece and a $40\times$ objective lens (total magnification: $400\times$).

Inflammatory cytokines detection

The levels of inflammatory factors in cells were quantified using the Enzyme-Linked Immunosorbent Assay (ELISA). Hela and C-33A cells, treated with *Escherichia coli* or a combination of *Escherichia coli* and *Lactobacillus crispatus*, were centrifuged at 1000 g for 20 min at 4 °C to collect the supernatant. According to the manufacturer's instructions (Mibio, Shanghai, China), standard wells and sample wells were prepared for TNF- α , IL-6, and IL-8 detection. Fifty microliters (50 μ L) of standards at varying concentrations were added to the standard wells, while 50 μ L of samples were added to the sample wells. Each well was subsequently incubated with 100 μ L of horseradish peroxidase (HRP)-conjugated detection antibody for 60 min at 37 °C. Following this, each well was washed five times with 300 μ L of washing buffer. Substrate A and substrate B (50 μ L each) were then added to each well and incubated for 15 min at 37 °C in the dark. Finally, 50 μ L of stop solution was added to

each well, and the absorbance was measured at a wavelength of 450 nm.

Western blot

Hela and C-33A cells were treated with *Escherichia coli* or a combination of *Escherichia coli* and *Lactobacillus crispatus*. The cells were subsequently lysed using RIPA lysate (Beyotime Biotechnology, Shanghai, China) containing 1 mM PMSF (Beyotime Biotechnology, Shanghai, China), and total protein extracts were prepared. Equal quantities of protein samples from each experimental group were resolved by SDS-PAGE at 80 V for 25 min and then at 120 V for an additional 50 min. The separated proteins were transferred onto PVDF membranes (Millipore, Boston, MA, USA) under constant conditions of 15 V and 1.0 A for 18 min. The membranes were extensively washed with TBST, blocked overnight at 4 °C with 5% skim milk (Sigma, Louis, MO, USA) in TBST, and incubated with the primary antibody for 2 h at room temperature. This was followed by incubation with the secondary antibody for 1 h under the same conditions. Protein bands were visualized using ECL reagent (Vazyme, Shanghai, China), and the gray values of the protein bands were calculated by Image J software.

Statistical analysis

All experiments were performed with 3–5 independent biological replicates. Data are presented as the mean $\pm$ standard deviation. Prior to conducting statistical analyses, the Shapiro–Wilk test was employed to assess the assumption of normality, and Levene's test was used to evaluate the homogeneity of variances. To compare the cell ferroptosis level, inflammatory factors, migration level with or without *Escherichia coli* intervention, using unpaired Student's t-test (for normal data) or the Mann–Whitney U test (for non-normal data). The impacts of *Escherichia coli* in combination with *Lactobacillus crispatus* at diverse concentrations on ferroptosis, inflammatory factors, and migration of human cervical cancer cells were evaluated via one-way analysis of variance (ANOVA). Subsequently, if the ANOVA results were significant, post-hoc analysis was carried out using Tukey's test. If assumptions were violated, the nonparametric Kruskal–Wallis test and Dunn's post hoc test were used. Significance levels were set as follows: * <0.05 , ** <0.01 and *** <0.001 . Statistical analysis of all data was performed using SPSS 22.0 software.

Results

Escherichia coli induces expression of inflammatory factors in cervical cancer cells through ferroptosis

Human cervical cancer cells Hela and C-33A were infected with *Escherichia coli*. As shown in Fig. 1-A, using fluorescence microscopy, the intracellular ferroptosis

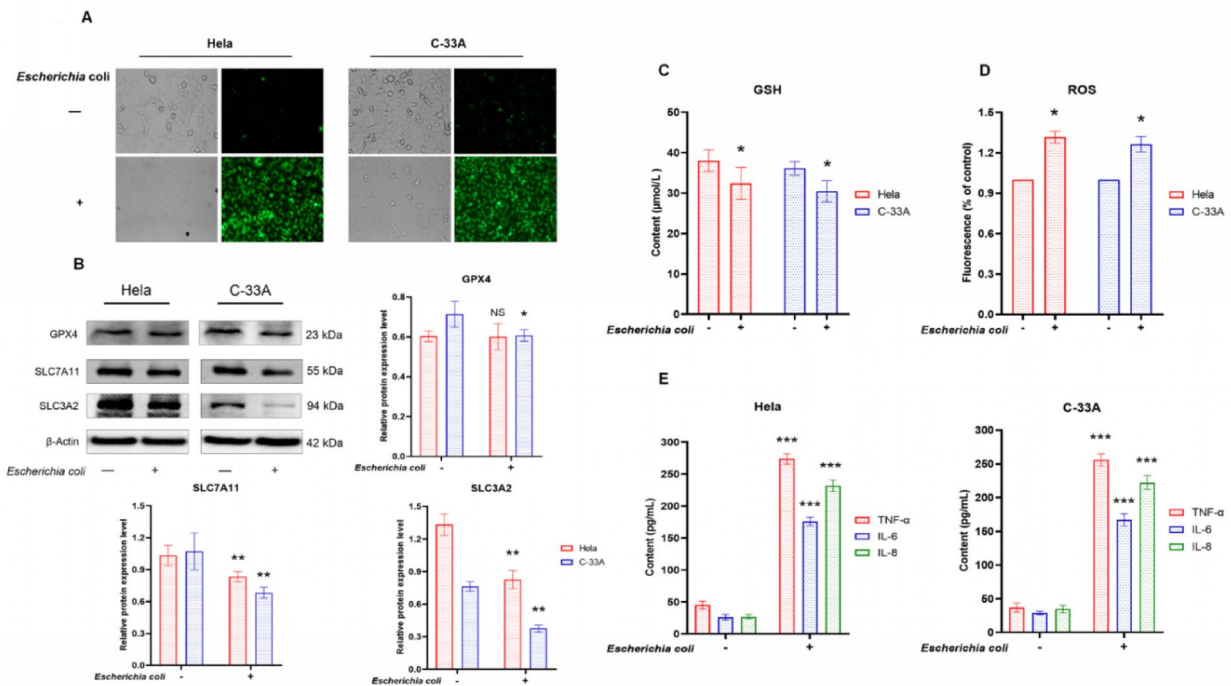


Fig. 1 *Escherichia coli* induces inflammatory damage through ferroptosis pathway in cervical cancer cells. (A) Detection of ferroptosis in HeLa and C-33A cells using *Escherichia coli* through fluorescence assays. (B) The expression of ferroptosis related protein in HeLa and C-33A cells was detected by Western blot. (C) The GSH content of HeLa and C-33A cells was detected by Elisa. (D) The ROS content of HeLa and C-33A cells was detected by fluorescence luminescence method. (E) The effects of *Escherichia coli* on inflammatory factors of HeLa and C-33A cells of cervical cancer were detected by Elisa. NS, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Full-length gels are presented in supplementary figure S1

fluorescence signal was significantly increased in HeLa and C-33A cells infected with *Escherichia coli* compared with those not infected with *Escherichia coli*. As shown in Fig. 1-B, the expression of SLC7A11 and SLC3A2 in HeLa cells was extremely significantly decreased after *Escherichia coli* infection ($p < 0.01$). The expression of GPX4 in C-33A cells was significantly decreased, and the expression of SLC7A11 and SLC3A2 was extremely significantly decreased ($p < 0.01$). Glutathione (GSH) functions as a scavenger of reactive oxygen species (ROS) through the catalytic action of GPX4. The enzymatic activity of ferroptosis-regulatory proteins was assessed by quantifying GSH and ROS levels. Following infection with *Escherichia coli*, a significant decrease in GSH content was observed in both HeLa and C-33A cells ($p < 0.05$). Additionally, *Escherichia coli* infection led to a marked significant increase in ROS levels in both HeLa and C-33A cells ($p < 0.05$) (Fig. 1-C and D). Studies have confirmed that ferroptosis is closely related to inflammatory injury. The results showed that the expression of TNF- α , IL-6 and IL-8 in HeLa and C-33A cells after *Escherichia coli* infection was extremely significantly increased ($p < 0.001$) (Fig. 1-E).

Escherichia coli promotes the migration of cervical cancer cells

We investigated the impact of *Escherichia coli* on the migratory capacity of cervical cancer HeLa and C-33A cells. As shown in Fig. 2-A, the migratory ability of cervical cancer HeLa and C-33A cells infected with *Escherichia coli* was extremely significantly enhanced compared to that of uninfected cells ($p < 0.001$). Western blot analysis revealed that the expression levels of adhesion-related proteins N-cadherin, Vimentin, Snail, and Slug were significantly upregulated in *Escherichia coli*-infected cervical cancer HeLa and C-33A cells relative to uninfected cells ($p < 0.05$) (Fig. 2-B).

Lactobacillus crispatus inhibited the growth of *Escherichia coli*

As shown in Fig. 3-A, we divided the cultured *Lactobacillus crispatus* bacterial solution into five groups: whole bacterial solution (a), sterile supernatant (b), active bacterial (c), inactivated bacterial (d), and saline (e). The whole solution of *Lactobacillus crispatus* and the active strain could inhibit the growth of *Escherichia coli*, but neither the supernatant nor the inactivated strain could inhibit the growth of *Escherichia coli*. The inhibitory diameters of the whole fermentation solution and the active

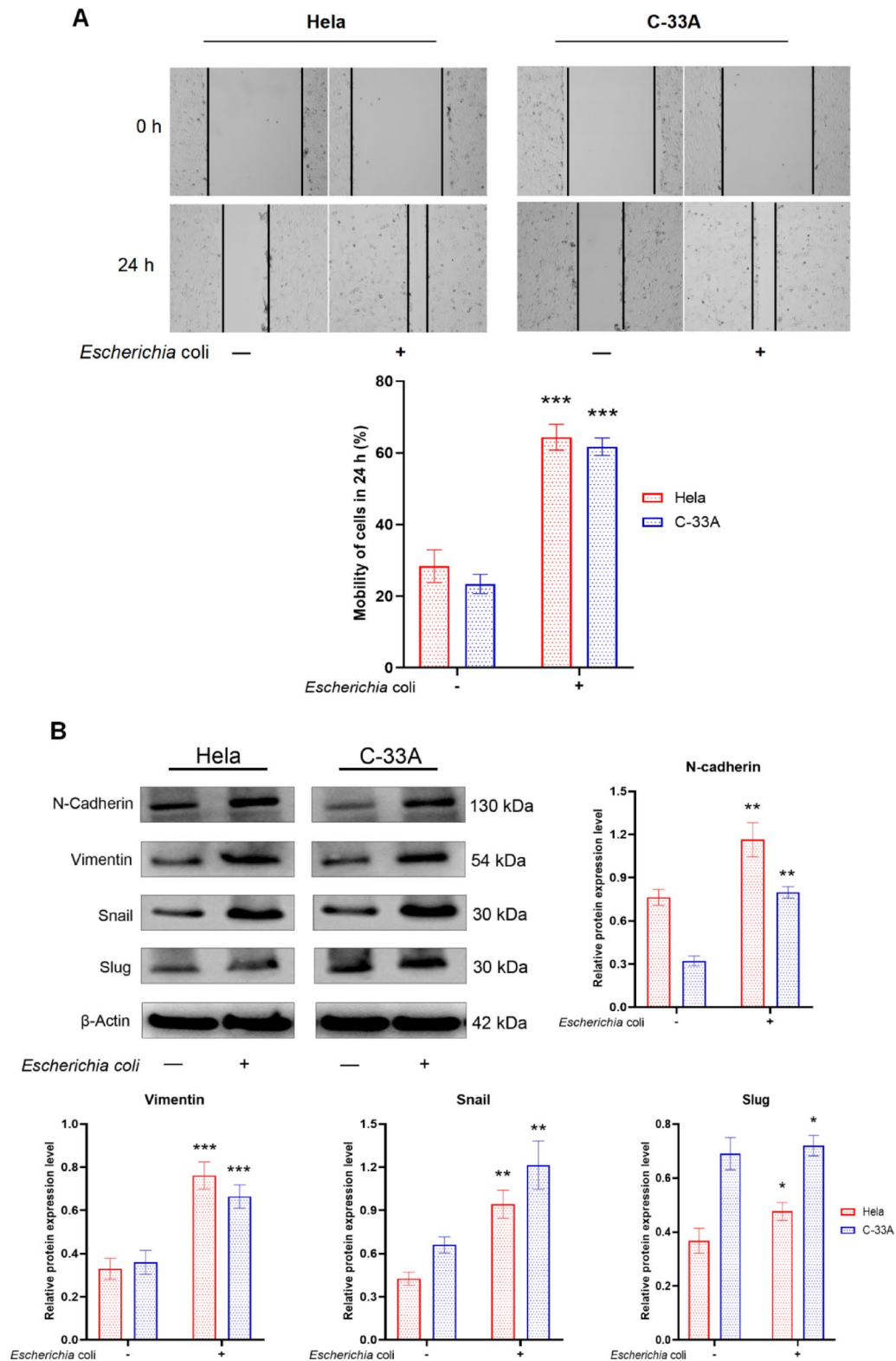


Fig. 2 *Escherichia coli* promotes the migration of cervical cancer Hela cells and C-33A cells. **(A)** The effect of *Escherichia coli* on the migration of cervical cancer Hela cells and C-33A cells was detected by scratch assay. **(B)** The effect of *Escherichia coli* on the expression of migration-related proteins in Hela cells and C-33A cells of cervical cancer was detected by Western blot. NS, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Full-length gels are presented in supplementary figure S1

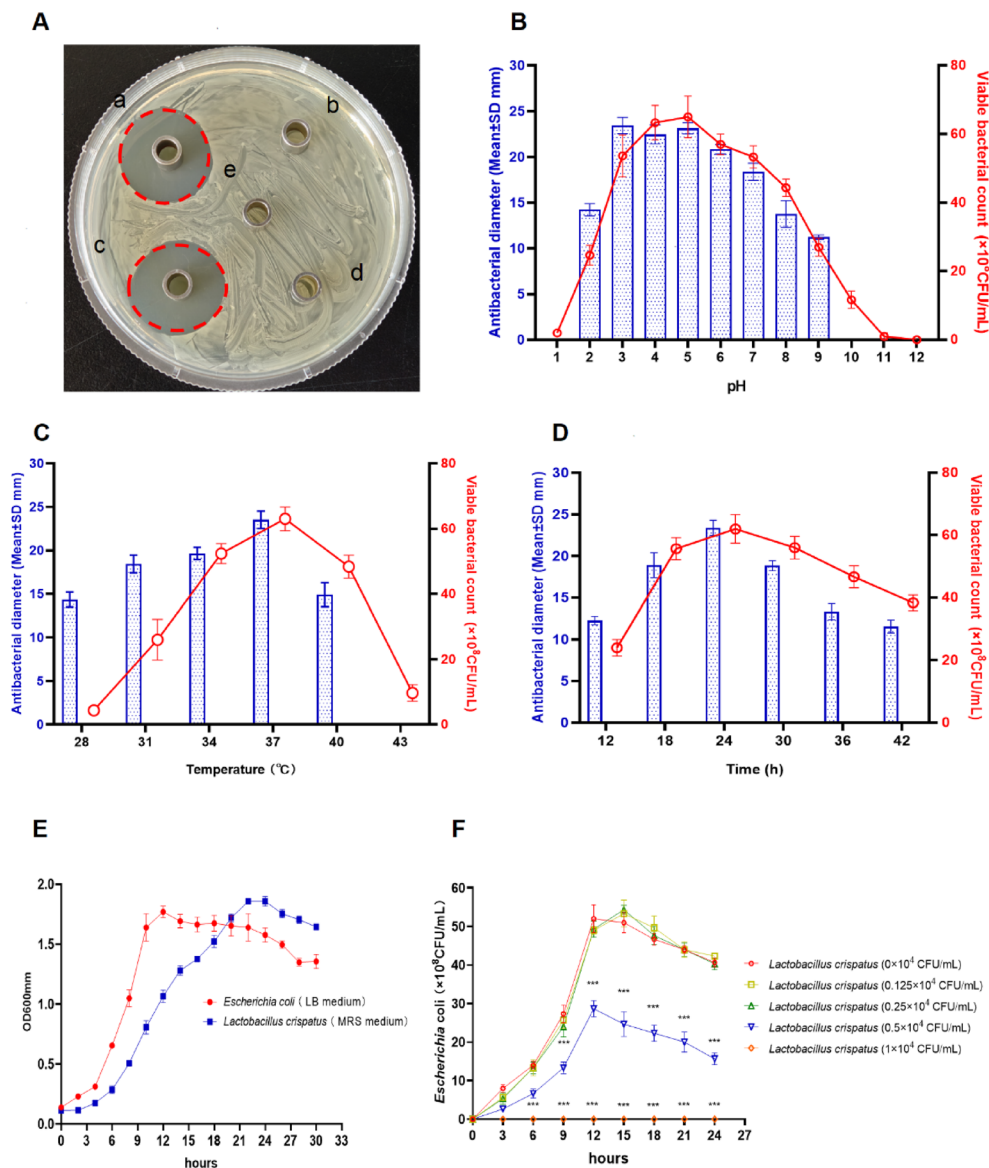


Fig. 3 Viable *Lactobacillus crispatus* could inhibit the growth of *Escherichia coli*. **(A)** The active components of *Lactobacillus crispatus* inhibiting the growth of *Escherichia coli* were detected by Oxford cup assay. **(B)** Effect of culture medium pH on inhibition of *Escherichia coli* growth by *Lactobacillus crispatus*. **(C)** Effect of incubation temperature on the inhibition of *Escherichia coli* growth by *Lactobacillus crispatus*. **(D)** Effect of incubation time on the inhibition of *Escherichia coli* growth by *Lactobacillus crispatus*. **(E)** Growth curves of *Escherichia coli* and *Lactobacillus crispatus* in LB broth or MRS broth. **(F)** The effect of *Lactobacillus crispatus* on the growth of *Escherichia coli* was detected in MRS broth. NS, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. a, whole culture medium; b, culture medium supernatant; c, viable bacteria; d, inviable bacteria; e, saline

strain against *Escherichia coli* were 18.45 ± 0.24 mm and 19.65 ± 0.32 mm, respectively. The culture medium's initial pH, incubation temperature, and incubation duration were systematically optimized. As illustrated in Fig. 3-B to D, the viable cell count of *Lactobacillus crispatus* correlated closely with the magnitude of its antibacterial activity. Under the optimized conditions, *Lactobacillus crispatus* exhibited an inhibition zone diameter of 23.86 ± 0.41 mm against *Escherichia coli*. As shown in Fig. 3-E, the growth curves of *Escherichia coli* and *Lactobacillus crispatus* are presented. When co-cultured in

MRS medium, the inhibition threshold of *Lactobacillus crispatus* against *Escherichia coli* was 1×10^4 CFU/mL (Fig. 3-F).

Inhibitory mechanism analysis of *Lactobacillus crispatus* against *Escherichia coli*

We selected 4 concentrations of *Lactobacillus crispatus* viable bacteria to analyze the bacteriostatic mechanism, and the 4 concentrations were divided into 0×10^4 CFU/mL, $1/16 \times 10^4$ CFU/mL, $1/8 \times 10^4$ CFU/mL, and $1/4 \times 10^4$ CFU/mL. As shown in Figs. 4-D, *Lactobacillus*

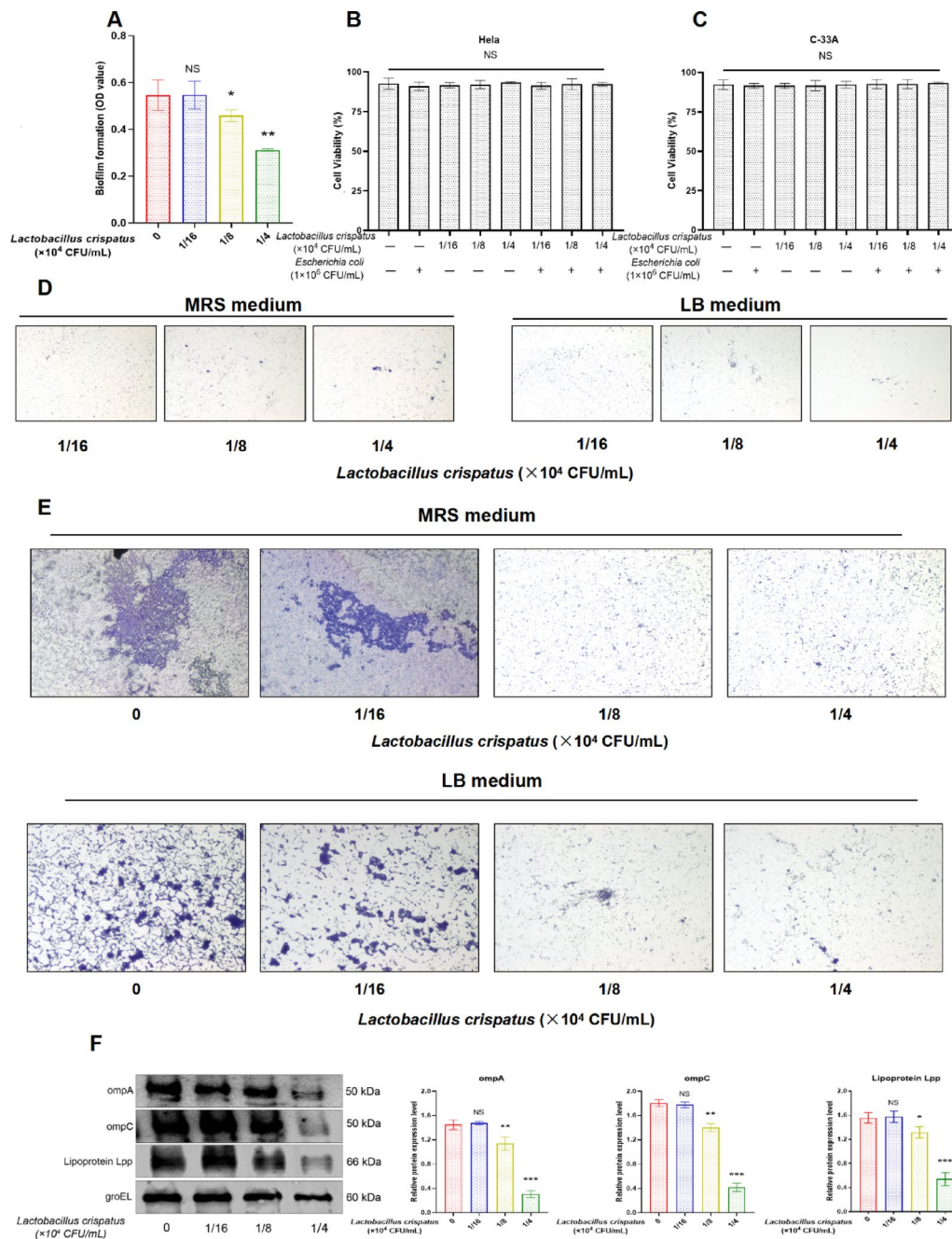


Fig. 4 Inhibitory mechanism analysis of *Lactobacillus crispatus* against *Escherichia coli*. **(A)** Effects of different concentrations of *Lactobacillus crispatus* on *Escherichia coli* biofilms. **(B)** Effect of *Escherichia coli* and *Lactobacillus crispatus* on the viability of HeLa cells. **(C)** Effect of *Escherichia coli* and *Lactobacillus crispatus* on the viability of C-33A cells. **(D)** Biofilm formation by *Lactobacillus crispatus* was assessed in both MRS and LB broth. **(E)** The effect of *Lactobacillus crispatus* on *Escherichia coli* biofilm formation was detected by direct observation in both MRS and LB broth. **(F)** The effect of *Lactobacillus crispatus* on the expression of key regulatory proteins in *Escherichia coli* biofilm was detected by western blot. NS, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Full-length gels are presented in supplementary figure S2

crispatus at different concentrations in MRS medium and LB medium almost did not form biofilm. LB medium was not suitable for the growth of *Lactobacillus crispatus*, while MRS medium showed rapid proliferation and almost no biofilm formation within 24 h. As shown in Figs. 4-A to C, *Lactobacillus crispatus* and

Escherichia coli exhibited no cytotoxic effects on HeLa and C-33A cells under the experimental conditions. Furthermore, at concentrations of *Lactobacillus crispatus* $\geq 1/8 \times 10^4$ CFU/mL, biofilm formation by *Escherichia coli* was significantly inhibited ($p < 0.05$). The protein gel imaging system showed that when the concentration

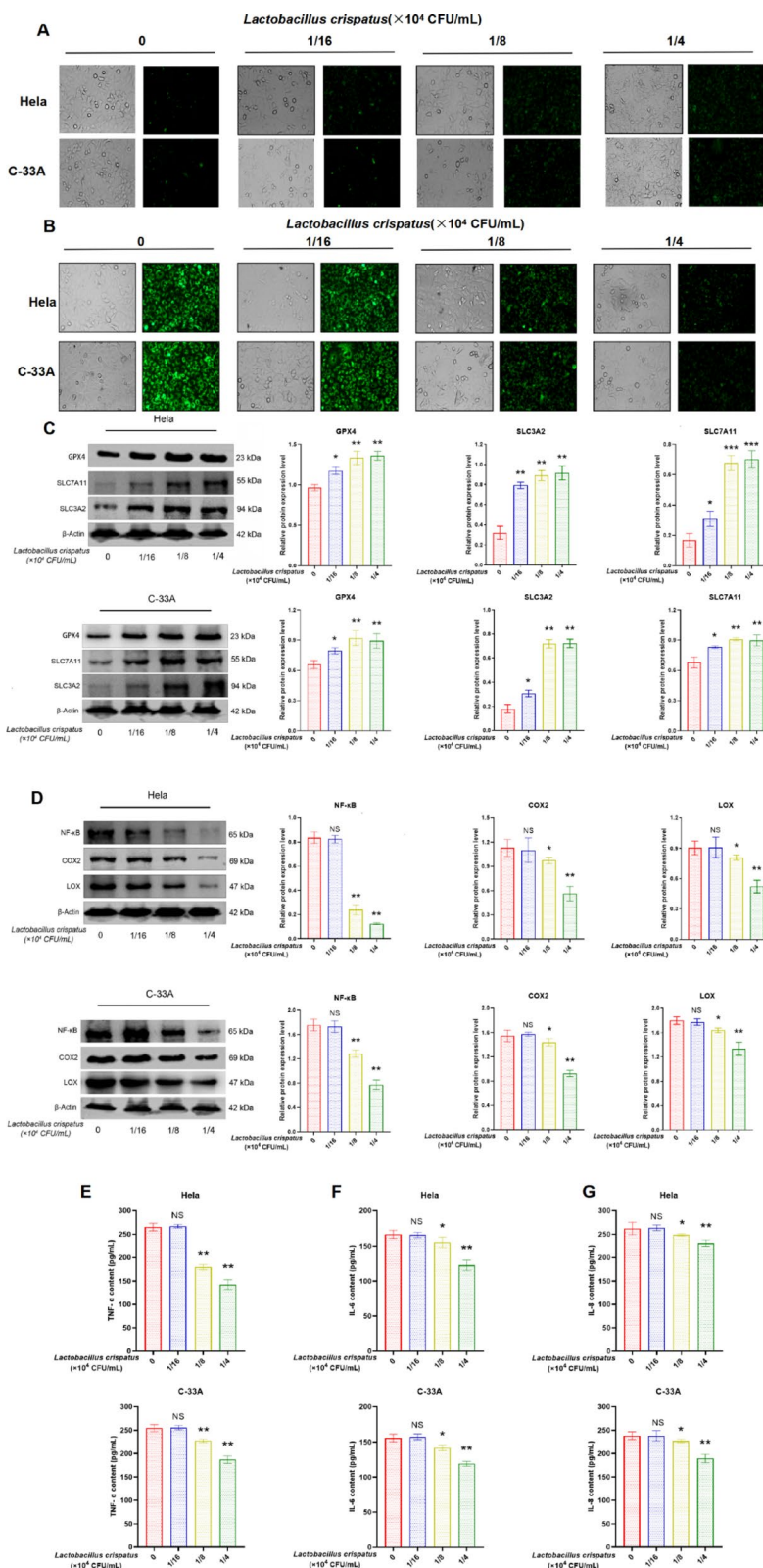


Fig. 5 (See legend on next page.)

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Fig. 5 *Lactobacillus* reverses the inflammatory injury of cervical cancer cells induced by *Escherichia coli* through ferroptosis pathway. **(A)** The effect of *Lactobacillus crispatus* monotherapy on ferroptosis in cervical cancer cells was detected by fluorescence method. **(B)** The effect of *Lactobacillus crispatus* at different concentrations on the ferroptosis of cervical cancer cells induced by *Escherichia coli* was detected by fluorescence method. **(C)** The effect of *Lactobacillus crispatus* at different concentrations on the expression of ferroptosis related proteins in cervical cancer cells induced by *Escherichia coli* were detected by Western blot. **(D)** The effect of *Lactobacillus crispatus* at different concentrations on the expression of proteins related to the regulation of inflammation in cervical cancer cells induced by *Escherichia coli* was detected by Western blot. The effect of *Lactobacillus crispatus* at different concentrations on the expression of inflammatory factors TNF- α (E), IL-6 (F), IL-8 (G) in cervical cancer cells induced by *Escherichia coli* was detected by Elisa. NS, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Full-length gels are presented in supplementary figure S3

of *Lactobacillus crispatus* was $\geq 1/8 \times 10^4$ CFU/mL, the expression level of soluble protein in *Escherichia coli* cells decreased (Fig. 4-C). Subsequently, we conducted a more in-depth analysis of the expression of biofilm-related proteins and key regulatory proteins involved in adhesion. The results demonstrated that the expression levels of *Escherichia coli* Outer membrane protein A (OmpA), Outer membrane protein C (OmpC), and Lipoprotein Lpp could be significantly suppressed when the *Lactobacillus crispatus* concentration was $\geq 1/8 \times 10^4$ CFU/mL ($p < 0.05$) (Fig. 4-F).

***Lactobacillus crispatus* reversed the inflammatory injury induced by *Escherichia coli* in cervical cancer cells**

We investigated the impact of *Lactobacillus crispatus* on the inflammatory response triggered by ferroptosis induced by *Escherichia coli* in HeLa and C-33A cells through the addition of *Lactobacillus crispatus*. As shown in Fig. 5-B, *Lactobacillus crispatus* (without *Escherichia coli*) at different concentrations did not activate ferroptosis in HeLa and C-33A cells. As shown in Fig. 5-B, fluorescence microscopy results demonstrated that when the concentration of *Lactobacillus crispatus* was $\geq 1/8 \times 10^4$ CFU/mL, the intensity of the ferroptosis-related fluorescence signal in *Escherichia coli*-infected HeLa and C-33A cells was significantly reduced. Additionally, at a concentration of *Lactobacillus crispatus* $\geq 1/16 \times 10^4$ CFU/mL, the expression levels of ferroptosis-associated regulatory proteins GPX4, SLC7A11, and SLC3A2 were significantly increased ($p < 0.05$) (Fig. 5-C). Moreover, when the concentration of *Lactobacillus crispatus* was $\geq 1/8 \times 10^4$ CFU/mL, the expression levels of key proteins involved in the inflammation-migration signaling pathway including NF- κ B (a master regulator of inflammatory activation), COX2 (a pro-inflammatory enzyme), and LOX (a critical mediator of cell migration) were significantly downregulated in both HeLa and C-33A cervical cancer cell lines ($p < 0.05$) (Fig. 5-D). The expression levels of TNF- α , IL-6, and IL-8 were significantly inhibited under the same conditions ($p < 0.05$) (Fig. 5-E to G).

***Lactobacillus crispatus* inhibits *Escherichia coli*-induced migration of cervical cancer cells**

As shown in Fig. 6-A, *Lactobacillus crispatus* (without *Escherichia coli*) at different concentrations had no effect

on the migration of HeLa and C-33A cells. As shown in Fig. 6-B, *Escherichia coli* infected HeLa and C-33A cells were able to significantly inhibit the migration of HeLa and C-33A cells when *Lactobacillus crispatus* was added at a concentration $\geq 1/8 \times 10^4$ CFU/mL ($p < 0.05$). In addition, Western blot results showed that the expression levels of migration-related proteins N-cadherin, vimentin, Snail, Slug were significantly decreased at the concentration of *Lactobacillus crispatus* $\geq 1/8 \times 10^4$ CFU/mL ($p < 0.05$) (Fig. 6-C).

Discussion

Escherichia coli, a prevalent bacterium in the intestinal and vaginal microbial communities, typically maintains a balanced population and species diversity within the vaginal microbiota of healthy women. However, an abnormal increase in bacterial load or alterations in the composition and structure of the vaginal microecosystem may disrupt this balance, potentially leading to gynecological inflammation and even tumor development (Farid et al., 2023). Recent evidence suggests that *Escherichia coli* may serve as a potential risk factor for the onset and progression of various cancers. colibactin, a genotoxin produced by *Escherichia coli* harboring the polyketide synthase (PKS) island, has been shown to induce DNA double-strand breaks, contributing to colon cancer progression (Oliero et al. 2021). Studies have demonstrated that cathepsin K (CTSK), a protein secreted by colon cancer cells stimulated by *Escherichia coli*-derived lipopolysaccharide (LPS), can bind to Toll-like receptor 4 (TLR4) via the mTOR signaling pathway, thereby promoting M2 polarization of tumor-associated macrophages (TAMs) and enhancing the migration and motility of colon cancer cells (Li et al. 2019). Additionally, reports indicate a significantly higher detection rate of *Escherichia coli* in patients with HPV16-positive cervical cancer. *Escherichia coli* induces upregulation of Foxp3 and promotes IL-10 expression in cervical cancer, exacerbating cervical intraepithelial neoplasia and enhancing the adhesion and invasion capabilities of cervical cancer cells (Zou et al. 2024). As a novel form of programmed cell death, ferroptosis plays a crucial role in the onset and progression of various diseases. Notably, the mechanism underlying ferroptosis offers a promising target for the development of novel anti-tumor drugs. Inducing ferroptosis in tumor cells can effectively achieve tumor suppression. However,

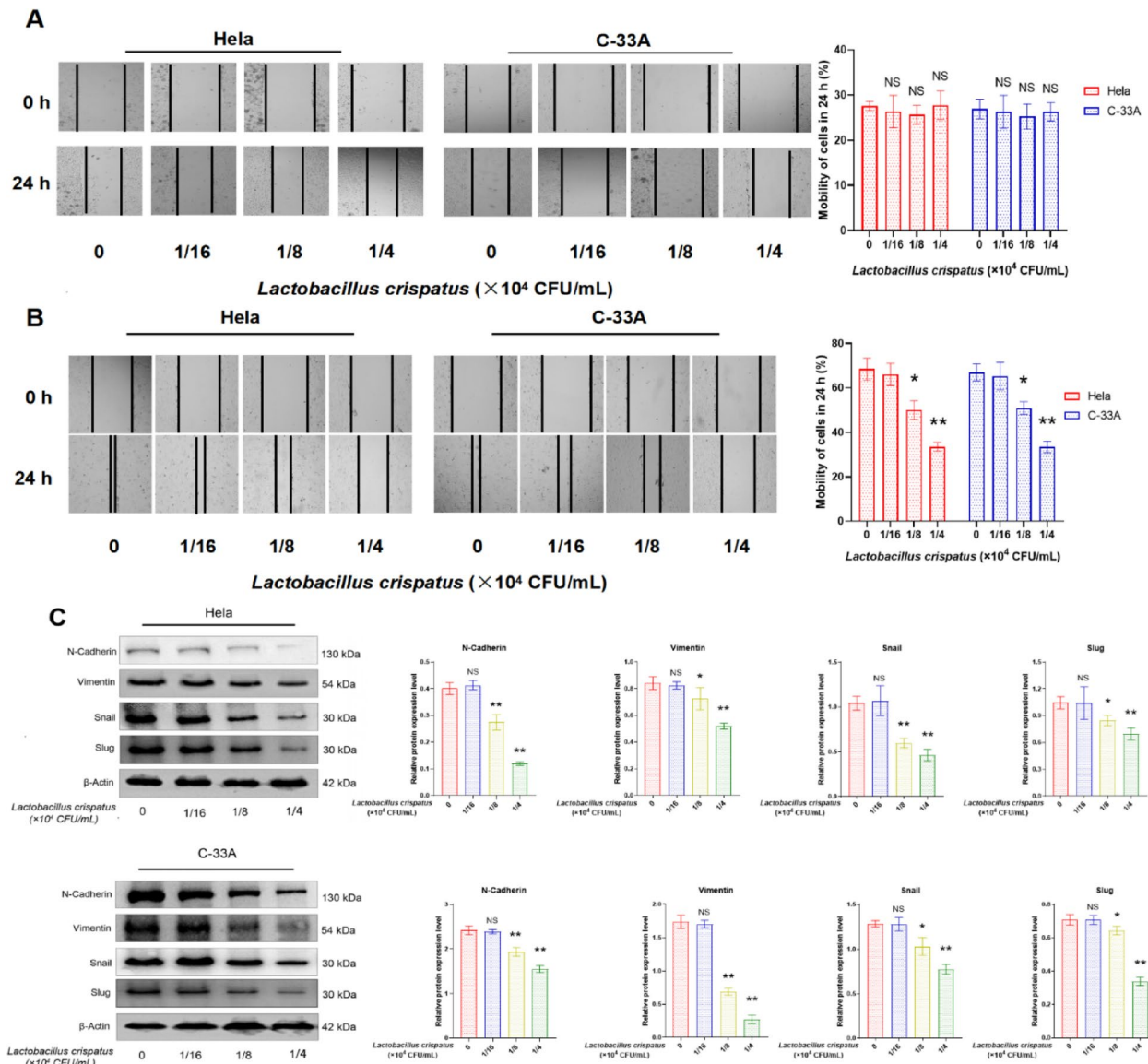


Fig. 6 *Lactobacillus crispatus* inhibits the migration of cervical cancer cells induced by *Escherichia coli*. **(A)** The effect of *Lactobacillus crispatus* mono-therapy on the migration of cervical cancer cells was detected by scratch method. **(B)** The effect of *Lactobacillus crispatus* at different concentrations on the migration of cervical cancer cells induced by *Escherichia coli* was detected by scratch method. **(C)** The effect of *Lactobacillus crispatus* at different concentrations on the expression of migration-related proteins in cervical cancer cells induced by *Escherichia coli* was detected by Western blot. NS, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Full-length gels are presented in supplementary figure S3

certain studies have demonstrated that the regulatory influence of ferroptosis on tumor development might be bidirectional (Ebrahimi et al. 2022; Vucetic et al. 2020). For instance, ferroptosis not only promotes EMT but also inhibits the MET of cancer cells during the terminal colonization phase (Guan et al. 2022). In this study, we investigated the effects of *Escherichia coli* on cervical cancer cells in vitro. Our results demonstrated that the expression levels of ferroptosis regulatory proteins in the cervical cancer cell model were reduced following *Escherichia coli* treatment, accompanied by an increased intracellular ferroptosis fluorescence signal. Zhuang et al. reported

that the protein expression levels of GPX4, SLC7A11, and SLC3A2 were down-regulated in bovine mammary epithelial cells infected with *Escherichia coli*, which is consistent with our experimental results (Zhuang et al. 2024). We subsequently refined the relevant assays and conducted a detailed analysis of GSH and ROS content. Extensive literature has demonstrated that GSH effectively scavenges ROS through GPX4 catalysis, and that GSH and ROS levels are closely associated with ferroptosis. Our results revealed a decrease in GSH content and an increase in ROS levels following *Escherichia coli* intervention in HeLa and C-33A cells, suggesting reduced

GPX4 activity. We also assessed the expression of TNF- α , IL-6, and IL-8 in HeLa and C-33A cervical cancer cells post-treatment with *Escherichia coli*. Our results are similar to those of other research results. Yang et al. demonstrated that *Escherichia coli* could increase the iron content in teleost erythrocytes, induce the Fenton reaction to release reactive oxygen species (ROS), increase the expression of inflammatory factors, and activate the ferroptosis signaling pathway (Yang et al. 2021). Extensive research has established that inflammatory injury can facilitate tumor cell migration. The expression profile of cell adhesion molecules changes significantly during tumor migration. The expression level of N-cadherin is significantly up-regulated. The switch of E-cadherin to N-cadherin expression (an important molecular event in the process of epithelial-mesenchymal transition) leads to the loss of intercellular adhesion and cell polarity, and enhances cell motility and invasiveness, making it easier for tumor cells to escape from the primary tumor, penetrate the basement membrane, and migrate and colonize the surrounding tissues or distant organs (Moori et al. 2024; Lee et al. 2024). It is a key link in the cascade of tumor metastasis. Following *Escherichia coli* treatment, we observed increased expression of N-cadherin, Vimentin, Snail, and Slug, which promoted the migration of HeLa and C-33A cells. Based on a comprehensive review of the current literature and our team's experimental findings, we propose that *Escherichia coli* may induce ferroptosis in cervical cancer cells, thereby triggering inflammatory responses and enhancing cellular migration. These results contribute novel insights into the intersection of microbial modulation, ferroptosis, and cancer progression, underscoring the need for rigorous mechanistic investigation of this pathway. Nevertheless, the present study lacks validation via ferroptosis-specific inhibitors or functional rescue experiments constituting a key methodological limitation. Future work will focus on elucidating the precise molecular mechanisms underlying *Escherichia coli* mediated inflammation and migration in cervical cancer, including identification of upstream regulators and downstream effectors.

Lactobacillus crispatus is the predominant microflora in the genital tract of healthy women and serves as a key indicator reflecting the vaginal microenvironment's health status (Vitale et al. 2021). Research has demonstrated that *Lactobacillus crispatus* maintains the vagina's mildly acidic environment through lactic acid secretion and produces antimicrobial substances such as hydrogen peroxide and bacteriocins, effectively inhibiting pathogenic bacterial growth and preventing genital infections (Breshears et al. 2015; Ding et al. 2022). In recent years, it has been increasingly reported that *Lactobacillus crispatus* inhibits *Escherichia coli* infection through the secretion of antibacterial substances and

modulation of immune responses (Santarelli et al. 2025; Song et al. 2022). In this study, we provide the first evidence that *Lactobacillus crispatus* can inhibit *Escherichia coli* growth, with its active component being viable bacteria. At temperatures of 4 °C and 25 °C, the bacteriostatic activity of the viable strain persists for up to 42 days and 7 days, respectively. Our results indicate that concentrations of *Lactobacillus crispatus* $\geq 1/8 \times 10^4$ CFU/mL inhibit *Escherichia coli* biofilm formation. We further analyzed the bacteriostasis mechanism and found that *Lactobacillus crispatus* could inhibit the expression levels of *Escherichia coli* Outer membrane protein A (OmpA), Outer membrane protein C (OmpC) and Lipoprotein Lpp. In earlier phases of this study, Our findings demonstrate that *Escherichia coli* infection induces ferroptosis in cervical cancer cells and concurrently enhances pro-inflammatory cytokine production and cellular migration. Consequently, we investigated *Lactobacillus crispatus*'s impact on *Escherichia coli*'s tumorigenic function. We found that *Lactobacillus crispatus* reverses *Escherichia coli*-induced ferroptosis in HeLa and C-33A cells, significantly increased GPX4, SLC7A11, and SLC3A2 expression levels while markedly diminishing intracellular ferroptosis fluorescence signals. NF- κ B is involved in many biological processes such as immune response, cell proliferation, differentiation and survival (Mao et al. 2025). Under normal conditions, NF- κ B exists in the cytoplasm in an inactive state and can activate the expression of inflammatory factors when stimulated. COX2 is an important indicator of inflammation (Li et al. 2025). Lysyl oxidase (LOX) promotes tumor cell migration by promoting Epithelial-Mesenchymal Transition (EMT) and participating in the formation of premetastatic microenvironment (Tzeng et al. 2021). Therefore, the exploration of NF- κ B /COX2/ LOX signaling pathway in this study plays a key role in elucidating the ferroptosis-inflammation-migration mechanism of *Lactobacillus crispatus*. Furthermore, *Lactobacillus crispatus* significantly suppressed the expression of including NF- κ B, COX2, LOX, TNF- α , IL-6, and IL-8 as well as inhibited the migratory capacity of HeLa and C-33A cells following *Escherichia coli* infection. Studies have shown that ferroptosis-related genes in cervical cancer can be used as biomarkers to predict the prognosis of gynecological tumors (Han et al. 2023). This suggests that ferroptosis based prognostic models are associated with enhanced immune checkpoint expression in low-risk populations, providing further evidence for the interplay between ferroptosis and the immune microenvironment. Recent studies have demonstrated the inhibitory effect of *Lactobacillus crispatus* on cervical cancer through metabolomic and proteomic analyses (Zhong et al. 2025). The current findings indicate that *Lactobacillus crispatus* activates ferroptosis-related pathways in cervical cancer

cells, a mechanism that aligns with the hypothesis proposed in our research. These results highlight the potential of *Lactobacillus crispatus* as a therapeutic agent targeting the ferroptosis pathway in cervical cancer, thereby providing new insights for future research and therapeutic development.

Despite the scientific findings, this study has several limitations that merit attention. First, the human gut contains multiple *Escherichia coli* strains, and genetic variation and phenotypic changes due to factors such as route of infection or antibiotic use complicate the selection of a single strain in studies. *Escherichia coli* ATCC25922 was selected because it is a reference strain for clinical microbiological quality control. It has clear genetic background, stable drug susceptibility phenotype and biochemical characteristics, no pathogenicity, and belongs to the normal flora of human gut. In this study, we focused on the role of non-pathogenic *Escherichia coli* in the pathogenesis of cervical cancer through ectopic growth in women. The experimental design of *Escherichia coli* ATCC25922 is suitable for our design. In the future, we will continue to select clinical *Escherichia coli* isolates and even drug-resistant strains to fully elaborate the scientific questions raised by this study. Second, all results were obtained from in vitro cell models, specifically focusing on the anti-inflammatory and anti-tumor effects of *Lactobacillus crispatus* mediated through the ferroptosis pathway at the cellular level. Future studies should further investigate the influence of diverse pathogenic bacteria on the gynecological tumor microenvironment and validate the role of *Lactobacillus crispatus* in modulating ferroptosis in cervical cancer using animal models or clinical specimens, thereby strengthening the translational potential of these findings.

Conclusions

Escherichia coli is a predominant microorganism in intestinal microecology; however, its impact on the health of the female reproductive system remains under-investigated. In this study, we demonstrate that *Escherichia coli* infection induces ferroptosis in cervical cancer cells, concomitant with elevated inflammatory injury and enhanced migratory capacity. Additionally, *Lactobacillus crispatus*, as a key probiotic strain, plays an essential role in maintaining microecological balance. Our findings revealed that *Lactobacillus crispatus* significantly inhibits the growth of *Escherichia coli*, thus alleviating *Escherichia coli*-induced inflammatory damage and suppressing the migration of cervical cancer cells. These results provide compelling evidence for the theoretical basis of employing *Lactobacillus crispatus* as a potential anti-infective and anti-tumor microecological agent in clinical applications, underscoring its promising prospects in gynecological health.

Abbreviations

GPX4	Glutathione peroxidase 4
SLC7A11	Solute carrier family 7, member 11
SLC3A2	Solute carrier family 3, member 2
TNF- α	Tumor necrosis factor- α
IL	Interleukin
pH	Potential of hydrogen
OmpA	Outer membrane protein A
OmpC	Outer membrane protein C
MOI	Multiplicity of infection
NF- κ B	Nuclear factor kappa-B
COX2	Cyclooxygenase-2
LOX	Lysyl oxidase
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
PBS	Phosphate-buffered saline
GSH	Glutathione
ROS	Reactive oxygen species

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13568-026-02055-z>.

Supplementary Material 1

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Author contributions

YZ: Experimental design (lead); funding support (lead).YS: Experimental operation (lead); data analysis (lead); article writing (lead).LD: Experimental operation; data analysis.DT: Experimental design.EL: Experimental operation. HL: Experimental design.FM and XC: Data analysis.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was conducted at the School of Life Sciences and Health at Northeastern University in accordance with the Declaration of Helsinki and approved by the local Council of Governance (no. NEU-EC-2023A079S).

Consent for publication

Not applicable.

Informed consent

Written informed consent was obtained from individual or guardian participants.

Clinical trial number

Not applicable.

Competing interests

The authors declare no competing interests.

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