Effects of Lactobacillus plantarum on morphology, survival, and immune response of Escherichia coli-infected porcine intestinal epithelial cells (Postprint)

Authors: Zeng Yanxia, Wang Sixin, Zhang Wei, Liu Hui, Zhang Dongyan, Wang Yamin, Wang Jing, Ji Haifeng

Date: 2018-12-24T00:00:00+00:00

Abstract

This study aimed to investigate the effects of Lactobacillus plantarum on the morphology, viability, and immune response of porcine intestinal epithelial cells (IPEC-J2 cells) infected with Escherichia coli. Under in vitro conditions, Giemsa and trypan blue staining methods were used to detect the effects of Lactobacillus plantarum on the morphology and viability of E. coli-infected IPEC-J2 cells, and real-time quantitative fluorescence PCR was employed to study its effects on the immune response of E. coli-infected IPEC-J2 cells. The results showed that Lactobacillus plantarum could alleviate the morphological damage of IPEC-J2 cells caused by E. coli. Compared with the E. coli treatment group, co-treatment with Lactobacillus plantarum and E. coli significantly or highly significantly reduced the cell death rate (1, 2 h, $P<0.01$; 3 h, $P<0.05$). Lactobacillus plantarum significantly inhibited the overexpression of Toll-like pattern recognition receptor 2 (TLR2) (2, 3 h, $P<0.01$), Toll-like pattern recognition receptor 6 (TLR6) (2 h, $P<0.05$; 3 h, $P<0.01$), NOD-like pattern recognition receptor 2 (NOD2) (1 h, $P<0.05$; 2 h, $P<0.01$), and inflammatory cytokines interleukin-6 (IL-6) (2, 3 h, $P<0.01$), interleukin-8 (IL-8) (2, 3 h, $P<0.01$) mRNA induced by E. coli in IPEC-J2 cells ($P<0.05$), and also promoted the expression of NOD-like pattern recognition receptor 1 (NOD1) mRNA in IPEC-J2 cells (2 h, $P<0.05$; 3 h, $P<0.01$). The results suggest that Lactobacillus plantarum can alleviate the morphological damage and death of IPEC-J2 cells caused by E. coli, and can alleviate the overexpression of cellular inflammatory cytokines IL-6 and IL-8 mRNA induced by E. coli by regulating the expression of pattern recognition receptors TLR2, TLR6, and NOD2 mRNA.

Full Text

Effects of *Lactobacillus plantarum* on Morphology, Survival, and Immune Response of *Escherichia coli*-Infected Porcine Intestinal Epithelial Cells

ZENG Yanxia, WANG Sixin, ZHANG Wei, LIU Hui, ZHANG Dongyan, WANG Yamin, WANG Jing*, JI Haifeng* *Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China*

Abstract

This study investigated the effects of *Lactobacillus plantarum* on the morphology, survival, and immune response of porcine intestinal epithelial cells (IPEC-J2) infected with *Escherichia coli*. In vitro experiments using Giemsa and trypan blue staining revealed that *L. plantarum* alleviated *E. coli*-induced morphological damage to IPEC-J2 cells. Compared with the *E. coli* treatment group, co-treatment with *L. plantarum* and *E. coli* significantly or extremely significantly reduced cell mortality at 1 and 2 h ($P<0.01$) and at 3 h ($P<0.05$). Real-time quantitative PCR analysis demonstrated that *L. plantarum* significantly inhibited the mRNA overexpression of Toll-like receptor 2 (TLR2) (2, 3 h, $P<0.01$), Toll-like receptor 6 (TLR6) (2 h, $P<0.05$; 3 h, $P<0.01$), NOD-like receptor 2 (NOD2) (1 h, $P<0.05$; 2 h, $P<0.01$), and inflammatory cytokines interleukin-6 (IL-6) (2, 3 h, $P<0.01$) and interleukin-8 (IL-8) (2, 3 h, $P<0.01$) induced by *E. coli* in IPEC-J2 cells. Additionally, *L. plantarum* promoted NOD-like receptor 1 (NOD1) mRNA expression (2 h, $P<0.05$; 3 h, $P<0.01$). These results indicate that *L. plantarum* can mitigate *E. coli*-induced morphological damage and cell death in IPEC-J2 cells, and alleviate *E. coli*-induced overexpression of inflammatory cytokines IL-6 and IL-8 by modulating the mRNA expression of pattern recognition receptors TLR2, TLR6, and NOD2.

Keywords: *Lactobacillus plantarum*; IPEC-J2 cells; *Escherichia coli*; damage; immune response

---

Post-weaning diarrhea in piglets severely affects growth performance and survival rates, directly impacting the economic benefits of swine producers. Therefore, investigating strategies to prevent and alleviate piglet diarrhea is crucial. Lactic acid bacteria, as a novel feed additive, represent a class of probiotics that have been extensively studied and demonstrate beneficial effects in animal production. These bacteria regulate intestinal flora structure, enhance immunity, and reduce pathogenic infections, thereby alleviating diarrhea caused by pathogenic bacteria[1-2]. Research indicates that the probiotic functions of lactic acid bacteria are primarily achieved through modulation of intestinal barrier function[3-6]. Porcine intestinal epithelial cells (IPEC-J2) are the main executors of intestinal barrier function, and their integrity and immune regulatory ca-

pacity are essential for barrier effectiveness[7]. Previous studies have shown that different strains of lactic acid bacteria protect intestinal cell integrity, alleviate damage induced by lipopolysaccharide, and suppress inflammatory responses triggered by harmful bacteria[8-9]. However, few reports have examined the effects of porcine-derived *Lactobacillus plantarum* on IPEC-J2 cells. Therefore, this study employed IPEC-J2 cells as an in vitro model to investigate the effects of *L. plantarum* on the morphology, survival, and immune response of *E. coli*-infected cells, providing a theoretical basis for elucidating its mechanism of action and guiding further development and application.

1.1.1 Strains and Cells

The porcine-derived *Lactobacillus plantarum* used in this study was isolated from the intestine of healthy piglets by the Animal Nutrition Laboratory at the Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences. This strain has been deposited in the China General Microbiological Culture Collection Center (CGMCC No. 7370) and designated *Lactobacillus plantarum* ZLP001. The *Escherichia coli* strain was enterotoxigenic *E. coli* (ETEC) serotype O149:K91:K88ac. IPEC-J2 cells (porcine intestinal epithelial cells) were purchased from Guangzhou Jennio Biological Technology Co., Ltd.

1.1.2 Reagents and Instruments

Reagents: DMEM/F12 basal medium, heat-inactivated fetal bovine serum (FBS), penicillin/streptomycin, and 0.25% trypsin-EDTA were purchased from Gibco (USA). Giemsa staining solution and 4% trypan blue solution were obtained from Beijing Coolaber Technology Co., Ltd. Trizol reagent was purchased from Invitrogen (USA). Chloroform and isopropanol were from Beijing Chemical Plant. Quant cDNA first-strand synthesis kit, conventional PCR kit, and SuperReal fluorescence quantitative premix were purchased from Tiangen Biotech Co., Ltd.

Instruments: HF151 UV CO cell incubator (Shanghai Lishen Scientific Instrument Co., Ltd.), HZQ-F160 full-temperature oscillation incubator (Harbin Donglian Electronic Technology Development Co., Ltd.), IX71 inverted microscope (Olympus, Japan), TC20 cell counter (Bio-Rad, USA), and QuantStudio 3 real-time PCR system (Thermo Fisher Scientific, USA).

1.2 Experimental Methods

1.2.1 IPEC-J2 Cell Culture IPEC-J2 cells were cultured in 75T flasks using DMEM/F12 basal medium supplemented with 10% FBS and 1% penicillin/streptomycin at 37°C in a 5% CO incubator with 95% relative humidity, with medium replacement every 24 h. Upon reaching 80-90% confluence, cells were digested with 0.25% trypsin-EDTA and centrifuged at 309×g for 5 min for passaging. After three passages, cells were seeded into 6-well plates at 5×10

cells/well in medium without penicillin/streptomycin. Experiments were conducted when cells formed a monolayer[10].

1.2.2 Strain Culture and Bacterial Suspension Preparation Strain Culture: *Lactobacillus plantarum* stock culture was inoculated at 1% into fresh MRS broth and incubated at 37°C for 20 h, with two subcultures prepared. *E. coli* stock culture was inoculated at 1% into fresh LB broth and cultured at 37°C on a shaker for 8 h, with two subcultures prepared.

Bacterial Suspension Preparation: Log-phase *L. plantarum* and *E. coli* were centrifuged at 3,300×g for 15 min to collect bacterial cells. After washing twice with sterile phosphate-buffered saline (PBS, pH 7.2-7.4), pellets were resuspended in DMEM/F12 basal medium. Bacterial concentrations were adjusted to 10 CFU/mL for *L. plantarum* and 10 CFU/mL for *E. coli* using microscopic counting and plate counting methods.

1.2.3 Effects of *L. plantarum* on Morphology of *E. coli*-Infected IPEC-J2 Cells IPEC-J2 monolayers were rinsed twice with sterile PBS and divided into four treatment groups: (1) untreated control, (2) *E. coli* treatment, (3) *L. plantarum* treatment, and (4) *L. plantarum* + *E. coli* co-treatment. After 1, 2, and 3 h of treatment, cells were washed three times with PBS to remove non-adherent bacteria, fixed with methanol for 15 min, and stained with Giemsa solution for 30 min. Following two washes with distilled water, cell morphology was observed under an inverted microscope at 200× magnification. Each experiment was repeated three times[11].

1.2.4 Effects of *L. plantarum* on Survival of *E. coli*-Infected IPEC-J2 Cells IPEC-J2 monolayers were rinsed twice with sterile PBS and treated as described in section 1.2.3. After 1, 2, and 3 h of treatment, the supernatant was collected in sterile 10 mL centrifuge tubes. Cells were washed three times with sterile PBS, digested with 300 L of 0.05% trypsin-EDTA, and centrifuged at 309×g for 5 min after termination of digestion. The pellet was resuspended in 500 L DMEM/F12 basal medium, and viable cells were counted using trypan blue staining on a TC20 cell counter. Each experiment was repeated three times[11].

1.2.5 Effects of *L. plantarum* on Immune Response of *E. coli*-Infected IPEC-J2 Cells IPEC-J2 monolayers were rinsed twice with sterile PBS and treated as described in section 1.2.3. After treatment, cells were incubated at 37°C for 1, 2, and 3 h. The supernatant was discarded, and cells were washed three times with sterile PBS. Total RNA was extracted using Trizol reagent (1 mL per well), lysed for 5 min at room temperature, transferred to 1.5 mL RNase-free tubes, and stored at -80°C. Each experiment was repeated three times.

Total RNA was extracted using the Trizol method and reverse-transcribed into cDNA using the Quant cDNA first-strand synthesis kit. Using cDNA as template, conventional PCR was performed according to the kit instructions to optimize reaction conditions and programs for target genes, with products verified by agarose gel electrophoresis. Primer sequences are listed in Table 1 . Optimized PCR conditions were then used for real-time quantitative PCR with SuperReal fluorescence quantitative premix to detect relative mRNA expression of Toll-like receptors (TLRs) including TLR2 and TLR6, NOD-like receptors (NLRs) including NOD1 and NOD2, and cytokines [interleukin-8 (IL-8), interleukin-6 (IL-6), tumor necrosis factor- (TNF-), and transforming growth factor- 1 (TGF- 1)].

Table 1 Primer sequences used for PCR

Genes	Sequence (5 -3)	Product size/bp	Reference
-actin	F: AAGGACCTC-TACGCCAACACGR: TGGAAGGTG-GACAGCGAGGC	200	Arce et al.[12]
TLR2	F: TCACTTGTCTAACT-TATCATCCTCR: TCAGCGAAGGTGT-CATTATTGC	150	This study
TLR6	F: GCCTGGATGTG-GTTCCTTTAR: ATGGATTGTTCCCT-GCTTTG	180	Mariani et al.[13]
NOD1	F: GGTGACCCTGAC-CGATGTR: TAGGCTGCGTCCT-GTTCA	120	Zhang et al.[14]
NOD2	F: GAGCGCATC-CTCTTAACTTTCGR: ACGCTCGTGATC-CGTGAAC	140	This study
IL-6	F: TTCGATGCCAGT-GCATAAATAR: CTGTACACCTTCTG-CACCCA	160	Xia[15]
IL-8	F: AAATGCTCTTCAC-CTCTCR: TCACACTTCTCAT-ACTTCTC	180	Zhou et al.[16]

Genes	Sequence (5 -3)	Product size/bp	Reference
TNF-	F: CCCCTCTGAAAAA-GACACCAR: TCGAAGT-GCAGTAGGCAGAA	200	Mariani et al.[13]
TGF- 1	F: TGTCTGTCCAC-CATTCATTTGR: CACCAGGAGTACCT-GCTCAAG	220	Mariani et al.[13]

F: forward; R: reverse.

PCR program: 95°C for 5 min; 95°C for 30 s; annealing at 60°C (61°C for NOD1 and NOD2, 62.5°C for TNF-) for 30 s; 72°C for 20 s; 32 cycles; 72°C for 5 min.

1.3 Statistical Analysis

Data were initially processed using Excel 2016 and analyzed by one-way ANOVA using SPSS 19.0 software. Tukey' s test was used for multiple comparisons when variances were homogeneous. $P<0.05$ was considered statistically significant and $P<0.01$ extremely significant. Data are expressed as mean ± standard error. Graphs were generated using GraphPad Prism 5 software.

2.1 Effects of *L. plantarum* on Morphology of *E. coli*-Infected IPEC-J2 Cells

Normal IPEC-J2 cells (control group) exhibited uniform epithelial morphology with irregular polygonal shapes arranged in a non-overlapping, cobblestone-like pattern. Cells were interconnected with oval nuclei, representing typical epithelial monolayers [Figure 1: see original paper]. Treatment with *L. plantarum* alone for 1, 2, and 3 h did not cause obvious morphological changes compared with control cells. In contrast, *E. coli* treatment for 1 h induced minor changes including cell shrinkage and marginalization. After 2 h of *E. coli* treatment, cells showed pronounced morphological alterations including shrinkage, rounding, chromatin condensation, large cytoplasmic vacuoles, marginalization, and incomplete cell morphology. By 3 h, *E. coli* caused severe morphological damage and cell death with detachment. Co-treatment with *L. plantarum* and *E. coli* effectively alleviated *E. coli*-induced morphological damage at 1 and 2 h. At 3 h, *L. plantarum* still mitigated morphological damage, though the protective effect was less pronounced than at earlier time points [Figure 2: see original paper].

2.2 Effects of *L. plantarum* on Survival of *E. coli*-Infected IPEC-J2 Cells

Treatment with *L. plantarum* alone for 1, 2, and 3 h did not significantly affect cell mortality compared with the control group (P>0.05). *E. coli* treatment alone for 1, 2, and 3 h significantly increased cell mortality compared with the control (P<0.01), indicating severe impairment of cell survival. Co-treatment with *L. plantarum* and *E. coli* extremely significantly reduced cell mortality at 1 and 2 h (P<0.01) and significantly reduced mortality at 3 h (P<0.05) compared with *E. coli* treatment alone [Figure 3: see original paper].

2.3.1 Effects of *L. plantarum* on TLRs and NLRs mRNA Expression in *E. coli*-Infected IPEC-J2 Cells

E. coli treatment stimulated mRNA expression of extracellular pattern recognition receptors TLR2 and TLR6, and intracellular receptor NOD2. Co-treatment with *L. plantarum* and *E. coli* significantly or extremely significantly inhibited *E. coli*-induced overexpression of TLR2 (2, 3 h), TLR6 (2, 3 h), and NOD2 (2 h) mRNA (P<0.05 or P<0.01). Interestingly, *L. plantarum* significantly or extremely significantly stimulated NOD1 mRNA expression (P<0.05 or P<0.01). Treatment with *L. plantarum* alone also extremely significantly stimulated NOD1 (2 h) and NOD2 (1 h) mRNA expression (P<0.01) [Figure 4: see original paper].

2.3.2 Effects of *L. plantarum* on Cytokine mRNA Expression in *E. coli*-Infected IPEC-J2 Cells

Compared with the control group, *E. coli* treatment extremely significantly increased mRNA expression of inflammatory cytokines IL-6 and TNF- at 2 h (P<0.01), and IL-6, IL-8, and TNF- at 3 h (P<0.01). Co-treatment with *L. plantarum* and *E. coli* for 2 and 3 h extremely significantly inhibited *E. coli*-induced overexpression of IL-6 and IL-8 mRNA (P<0.01). No significant differences in TGF- 1 mRNA expression were observed among any groups (P>0.05) [Figure 5: see original paper].

3.1 Effects of *L. plantarum* on Morphology and Survival of *E. coli*-Infected IPEC-J2 Cells

Intestinal epithelial cells are the primary executors of porcine intestinal barrier function, covering approximately 100 m^2 of intestinal surface area. Epithelial cell integrity is essential for maintaining selective permeability and resisting pathogen invasion[17]. Wine et al.[18] demonstrated that pathogenic bacteria adhere to and invade intestinal epithelial cells, proliferate extensively, destroy intestinal epithelial structure, and cause cell death, thereby damaging intestinal barrier function. Therefore, this study investigated *L. plantarum*' s protective effects against *E. coli*-induced damage using both morphological and viability

assessments. Results showed that *E. coli* treatment alone caused severe morphological disruption and extensive cell death, while *L. plantarum* alone had no significant effect on cell morphology or survival. Co-treatment with *L. plantarum* and *E. coli* markedly alleviated *E. coli*-induced morphological damage and significantly or extremely significantly inhibited cell death. These findings align with Yang et al.[19], who reported that *E. coli* adheres to intestinal cells, damages the cytoskeleton, and increases intercellular spaces, while co-culture with lactic acid bacteria significantly mitigates this damage. This demonstrates that probiotics inhibit intestinal *E. coli* infection. Additionally, Zhao[20] found that *Lactobacillus amylovorus* metabolites inhibit *E. coli*-induced morphological disruption, suggesting that *L. plantarum* metabolites may also contribute to protecting intestinal cell morphology, warranting further investigation.

3.2 Effects of *L. plantarum* on Immune Response of *E. coli*-Infected IPEC-J2 Cells

The porcine intestinal innate immune system plays a critical role in maintaining intestinal barrier function and preventing pathogen-induced damage. The intestinal immune system recognizes pathogenic and commensal microorganisms through a complex receptor system, primarily including extracellular TLRs and intracellular NLRs. Under normal conditions, moderate immune signaling through TLRs and NLRs produces pro-inflammatory cytokines, chemokines, and anti-inflammatory factors that regulate host immune responses and maintain health[21-22]. However, pathogen stimulation can hyperactivate immune responses, leading to excessive secretion of inflammatory cytokines such as TNF- , IL-6, and IL-8, thereby causing immune stress. Studies have shown that probiotics alleviate *E. coli*-induced inflammatory damage by suppressing inflammatory cytokine overexpression in intestinal epithelial cells[23]. This study demonstrated that *E. coli* alone stimulated IL-6, IL-8, and TNF- mRNA expression in IPEC-J2 cells at 2 and 3 h, while co-treatment with *L. plantarum* and *E. coli* inhibited *E. coli*-induced overexpression of IL-6, IL-8, and TNF- mRNA. These results are consistent with Wang et al.[24], possibly because *E. coli* activates cytokine expression pathways through specific receptors while *L. plantarum* mitigates this excessive immune activation. Furthermore, this study examined the effects on pattern recognition receptors. *E. coli* treatment stimulated TLR2, TLR6, and NOD2 mRNA expression, while co-treatment with *L. plantarum* inhibited this overexpression. This suggests that *L. plantarum* maintains immune homeostasis by modulating these pattern recognition receptor signals, thereby preventing inflammatory damage to intestinal epithelium. Notably, *E. coli* did not stimulate NOD1 mRNA expression, whereas *L. plantarum* promoted it, possibly due to the specificity of pattern recognition receptors as described by Trinchieri et al.[25]. This indicates that NOD2 may be the primary intracellular pattern recognition receptor mediating *L. plantarum*' s effects, while TLR2 and TLR6 may be the main extracellular receptors. Regarding TGF- 1, an epidermal growth factor-related gene important for cell differentiation, growth, and immunity, no significant differences were observed among groups. This contrasts

with Hou[26], who reported that *Lactobacillus reuteri* significantly stimulated TGF- 3 mRNA expression after 6 h, possibly due to strain differences.

4 Conclusion

Lactobacillus plantarum alleviates *Escherichia coli*-induced morphological damage and death in porcine intestinal epithelial cells. It suppresses *E. coli*-induced overexpression of inflammatory cytokines IL-6 and IL-8 by modulating mRNA expression of pattern recognition receptors TLR2, TLR6, and NOD2.

References

[1] MURALIDHARA K S, SHEGGEBY G G, ELLIKER P R, et al. Effect of feeding *Lactobacillus* on the coliform and *Lactobacillus* flora of intestinal tissue and feces from piglets[J]. *Journal of Food Protection*, 1977, 40(5): 288-295.

[2] GIANG H H, VIET T Q, OGLE B, et al. Growth performance, digestibility, gut environment and health status in weaned piglets fed a diet supplemented with potentially probiotic complexes of lactic acid bacteria[J]. *Livestock Science*, 2010, 129(1/2/3): 95-103.

[3] MENNIGEN R, BRUEWER M. Effect of probiotics on intestinal barrier function[J]. *Annals of the New York Academy of Sciences*, 2009, 1165(1): 183–189.

[4] KUMAR N S N, BALAMURUGAN R, JAYAKANTHAN K, et al. Probiotic administration alters the gut flora and attenuates colitis in mice administered dextran sodium sulfate[J]. *Journal of Gastroenterology and Hepatology*, 2008, 23(12): 1834–1839.

[5] OHLAND C L, MACNAUGHTON W K. Probiotic bacteria and intestinal epithelial barrier function[J]. *American Journal of Physiology: Gastrointestinal and Liver Physiology*, 2010, 298(6): G807–G819.

[6] MENNIGEN R, BRUEWER M. Effect of probiotics on intestinal barrier function[J]. *Annals of the New York Academy of Sciences*, 2009, 1165(1): 183-189.

[7] COSTA L F, PAIXÃO T A, TSOLIS R M, et al. Salmonellosis in cattle: advantages of being an experimental model[J]. *Research in Veterinary Science*, 2012, 93(1): 1–6.

[8] YANG F J, WANG A N, ZENG X F, et al. *Lactobacillus reuteri* I5007 modulates tight junction protein expression in IPEC-J2 cells with LPS stimulation and in newborn piglets under normal conditions[J]. *BMC Microbiology*, 2015, 15: 32.

[9] PALÓCZ O, PÁSZTI-GERE E, GÁLFI P, et al. Chlorogenic acid combined with *Lactobacillus plantarum* 2142 reduced LPS-induced intestinal inflammation and oxidative stress in IPEC-J2 cells[J]. *PLoS One*, 11(11): e0166642.

[10] LIU Y Y, FATHEREE N Y, MANGALAT N, et al. Human-derived probiotic *Lactobacillus reuteri* strains differentially reduce intestinal inflammation[J]. *American Journal of Physiology: Gastrointestinal and Liver Physiology*, 2010, 299(5): G1087–G1096.

[11] ZHU J. Study on the interaction among enterotoxigenic *Escherichia coli*, intestinal epithelial cells and lactic acid bacteria[D]. PhD thesis. Shanghai: Shanghai Jiao Tong University, 2011.

[12] ARCE C, RAMÍREZ-BOO M, LUCENA C, et al. Innate immune activation of swine intestinal epithelial cell lines (IPEC-J2 and IPI-2I) in response to LPS from *Salmonella typhimurium*[J]. *Comparative Immunology, Microbiology and Infectious Diseases*, 2010, 33(2): 161–174.

[13] MARIANI V S, PALERMO S, FIORENTINI S, et al. Gene expression study of two widely used pig intestinal epithelial cell lines: IPEC-J2 and IPI-2I[J]. *Veterinary Immunology and Immunopathology*, 2009, 131(3/4): 278–284.

[14] ZHANG W, ZHU Y H, YANG J C, et al. A selected *Lactobacillus rhamnosus* strain promotes EGFR-independent Akt activation in an enterotoxigenic *Escherichia coli* K88-infected IPEC-J2 cell model[J]. *PLoS One*, 2015, 10(4): e0125717.

[15] XIA L. Effects of *Bacillus subtilis* on TLRs and cytokine mRNA expression in RV-infected IPEC-J2 cells[D]. Master' s thesis. Harbin: Northeast Agricultural University, 2014.

[16] ZHOU C L, LIU Z Z, YU Y, et al. Immune stimulation of F4ac enterotoxigenic *Escherichia coli* culture supernatant on porcine small intestinal epithelial cells[J]. *Scientia Agricultura Sinica*, 2014, 47(4): 779–785.

[17] ZHANG Y G, DAVIN R. New discoveries in porcine small intestinal epithelial cell function[J]. *Chinese Journal of Animal Science*, 2015, 51(8): 57–60.

[18] WINE E, GAREAU M G, JOHNSON-HENRY K, et al. Strain-specific probiotic (*Lactobacillus helveticus*) inhibition of *Campylobacter jejuni* invasion of human intestinal epithelial cells[J]. *FEMS Microbiology Letters*, 2009, 300(1): 146–152.

[19] YANG J, ZHANG Z W, QIN H L. Protective effect of lactic acid bacteria on invasive *Escherichia coli*-induced injury in intestinal epithelial cells[J]. *World Chinese Journal of Digestology*, 2008, 16(30): 3394–3399.

[20] ZHAO Y B. Antiviral effects of *Lactobacillus amylovorus* metabolites on regulating cell integrity and barrier function[D]. Master's thesis. Harbin: Northeast Agricultural University, 2014.

[21] MEDZHITOV R. Recognition of microorganisms and activation of the immune response[J]. *Nature*, 2007, 449(7164): 819–826.

[22] SANSONETTI P J. War and peace at mucosal surfaces[J]. *Nature Reviews Immunology*, 2007, 4(1): 953–964.

[23] SKJOLAAS K A, BURKEY T E, DRITZ S S, et al. Effects of *Salmonella enterica* serovar Typhimurium, or serovar Choleraesuis, *Lactobacillus reuteri* and *Lactobacillus licheniformis* on chemokine and cytokine expression in the swine jejunal epithelial cell line, IPEC-J2[J]. *Veterinary Immunology and Immunopathology*, 2007, 115(3/4): 299–308.

[24] WANG Z L, WANG L, ZHUANG C, et al. In vitro evaluation of swine-derived *Lactobacillus reuteri*: probiotic properties and effects on intestinal porcine epithelial cells challenged with enterotoxigenic *Escherichia coli* K88[J]. *Journal of Microbiology and Biotechnology*, 2016, 26(6): 1018–1025.

[25] TRINCHIERI G, SHER A. Cooperation of Toll-like receptor signals in innate immune defence[J]. *Nature*, 2007, 7(3): 179–190.

[26] HOU C L. Whole genome sequence analysis of *Lactobacillus reuteri* and its regulation of intestinal mucosal immune function in piglets[D]. PhD thesis. Beijing: China Agricultural University, 2015.

Note: Figure translations are in progress. See original paper for figures.