Article

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Lactobacillus Shielding Against *E. coli O157:H7* in Mouse Enterocytes: A Prophylactic Approach

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Background: *Escherichia coli* (*E. coli O157:H7*), a Shiga-like toxin-producing *Escherichia coli* (STEC) serotype, is harmful, especially for children, elderly people, and immunocompromised persons. This strain can induce serious enterocyte problems, requiring careful mitigation. We intend to study *Lactobacillus*' prophylactic ability against *E. coli O157:H7*-induced cellular damage in mouse enterocytes.

Methods: The binding affinity of bacterial intimin and its human target, integrin, was assessed using a docking study. Next, *Lactobacillus* supplementation was tested in *E. coli O157:H7*-infected mice enterocytes using transmission electron microscopy (TEM) and light microscope (LM). The rats, weighing between 100 and 200 g, were randomly categorized into three groups (n = 6): uninfected (control), STEC-infected, and probiotics + STEC-infected. The randomization process was conducted using a computer-generated method that considered factors such as age, sex, and initial body weight. This approach was employed to minimize potential biases during the grouping process. Subsequently, specimens from the ileum were meticulously examined utilizing both transmission electron microscopy (TEM) and light microscopy (LM).

Results: ClusPro showed *Lactobacillus* bound more efficiently than *E. coli* (–632.5). In STEC-infected mice, LM revealed intestinal epithelial cells (IECs) compromises that probiotic improved. Moreover, TEM showcased structural disruptions in infected mice, rectified by probiotics. Statistical scrutiny, using parameters such as the number of intact microvilli per enterocyte and the extent of membrane damage, underscored significant improvements in STEC-infected mice treated with probiotics, emphasizing their pivotal role in preserving cellular integrity.

Conclusions: Certain probiotics may protect enterocytes from pathogens. These results imply that probiotics may reduce pathogenicity, offering new avenues for enteric infection research.

Keywords: probiotics; *Escherichia coli*; *O157:H7*; TEM; ileal epithelial cells

Introduction

Escherichia coli (*E. coli*) is a bacterium found in both human and animal intestines. Although most of these bacteria are innocuous, some strains, including Shiga-like toxin-producing *Escherichia coli* (STEC), have been reported for their harmful effects on human health. The STEC has been implicated in worldwide epidemics of severe diarrheal diseases in older people and children. The extreme complications include bloody diarrhea, hemorrhagic colitis of toxin generation and release that further involves neurological disorders, hemolytic uremic syndrome, and kidney failure [1]. Moreover, STEC is the causative agent of extreme gastrointestinal diseases in developed nations and a serious public health concern, with serotype *O157:H7* infection being frequently documented [2]. Consuming contaminated food, including raw meat, sprouts, seeds, beef products, and raw vegetables, poses a significant risk for STEC transmission by cross-contamination [3]. Enterohemorrhagic *E. coli* (EHEC) is the STEC subclass that causes human disease including hemorrhagic colitis and hemolytic uremia syn-

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drome [4]. Because cattle are a main source of EHEC, intervention strategies aim at reducing its excretion in cattle to reduce the risk of human infection [5].

The intestinal epithelial cells (IECs) coat the surface of the intestinal epithelium, assisting in food digestion and nutritional absorption, representing a body-protection defense against microbial pathogens. Furthermore, IECs establish a metabolic and physical barrier between the host tissue and the pathogen to maintain epithelial integrity. Discharging antimicrobial peptides and mucins by secretory IECs aids this function [6]. The IEC lining is a very flexible interface that can accommodate various interactions with others in the environment. The gut epithelial barrier is continually subjected to commensal bacteria, food-borne antigens, and invading entero-pathogens. In addition, IECs and immune cells are major participants in preserving the dynamic equilibrium between intestinal tolerability and inflammation [7]. Diseases lead to a substantial decline in the functionality of IECs [8].

Probiotics are living, nonpathogenic bacteria that may enhance the health of the host and have been extensively studied for their ability to regulate the gut microbiota and host inflammatory responses [9]. These probiotics have been used to treat various inflammatory and pathogenic intestinal disorders, including inflammatory bowel disease [10] and children's infectious diarrhea [11]. Probiotics have also been employed in animal models of infectious gastroenteritis [12]. Multiple studies have demonstrated the anti-inflammatory and anti-adhesive properties of probiotic therapy. However, more research is required regarding the specific protective mechanisms by which probiotics can fortify the cellular integrity of the intestinal barrier against invading pathogens. In this context, exploring the therapeutic potential of probiotics has gained traction. Therefore, our study delves into the prophylactic efficacy of *Lactobacillus* against *E. coli O157:H7* infection in mouse enterocytes [13]. The rationale for this investigation stems from the urgent need to identify preventive measures against the damaging effects of this pathogenic strain.

Our hypotheses were twofold: docking study and *in vivo* evaluation. Regarding the docking study, we hypothesized a discernible binding affinity between bacterial intimin and integrin, potentially shedding light on the mechanistic aspects of *E. coli O157:H7* pathogenesis. Concerning *in vivo* evaluation, we anticipated that the group supplemented with *Lactobacillus* will exhibit preserved enterocyte morphology akin to the uninfected (control) group. Selecting the mouse model for our study was primarily driven by its physiological relevance to human enterocyte responses and its established usage in gastrointestinal infection studies. The anatomical and physiological similarities between murine and human intestinal epithelia make mice an appropriate model for studying enteric infections and probiotic interventions. Ethical considerations and feasibility also played roles in choosing an appropriate model for the *in vivo* experimentation. These hypotheses suggest the potential of *Lactobacillus* to shield enterocytes from the deleterious effects of *E. coli O157:H7* infection [14]. The choice of *Lactobacillus* strains was based on several factors, including their documented safety profiles, known probiotic properties such as adhesion to intestinal epithelial cells, and prior studies demonstrating their efficacy against pathogenic bacteria [15]. Accordingly, we aimed to probe the interaction between bacterial intimin and its human target, integrin, through a docking study to elucidate potential mechanisms underlying the pathogenicity of *E. coli O157:H7*. Moreover, we aim to examine the impact of *Lactobacillus* supplementation on *E. coli O157:H7*-infected mouse enterocytes *in vivo* utilizing transmission electron microscopy (TEM).

Materials and Methods

Molecular Docking

Initially, we interrogated whether the differential affinity between *Lactobacillus* and *E. coli* could contribute to the protective effects of *Lactobacillus*. The crystal structure of pilus adhesin SpaC from *Lactobacillus rhamnosus* GG and intimin from *E. coli* were retrieved by accessing the protein data bank (https://www.rcsb.org/) with protein IDs of 6M7C and 2ZQK, respectively. From the same source, we obtained the crystal structure of the human integrin with protein ID: 1QCY. A computational docking analysis was performed on the ClusPro web server (https://cluspro.bu.edu/home.php).

Animal

Herein, 18 specific pathogen-free (SPF) adult male white mice (*Mus Musculus*) were obtained from the animal house in King Khalid University's Faculty of Medicine. The mice were supplied with a special diet and sterilized water (126 °C for 30 min) *ad libitum*. The water contained Cl_2 at a final concentration of 1.5 ppm (µg/mL) and streptomycin sulfate (10 mg/mL; batch number S6501, Sigma Chemical, St. Louis, MO, USA), with water bottles being provided with freshly prepared water every three days. The animal experiments followed the Laboratory Animal Care and Use Guide guidelines, ensuring the ethical and humane treatment of the animals throughout the study. The Research Ethics Committee at King Khalid University approved the experimental procedures on animals (protocol number ECM#2022-115; dated March 11, 2022). All mice were equally and randomly allocated into three groups (n = 6): uninfected (control), STEC infected, and probiotics + STEC infected, per the Institutional Animal Ethics Committee guidelines. All animal groups were housed in a particulate with high efficiency, arresting-filtered, easily accessible, separately ventilated cages to sterilize water and food throughout most of the experiment trials.

Probiotics Strains and Growth Conditions

Murine Model and Infection Protocol

A small inoculum of 5×10^3 colony forming unit (CFU) of STEC, *O157:H7* in tap water (5 mg/mL) was used to infect mice. After 18 h, a significant increase of 50% was observed in the amount of bacteria excreted, reaching 10^9 CFU/g of feces [16]. Control mice were only given distilled water. A soft polyethylene catheter was used to orally provide the probiotics suspension (100 µL/mouse) to the infection + probiotics group. After 24 h inoculation of the probiotic combination, each mouse received 100 µL of STEC suspension (1.0×10^7 CFU/mL) in the same manner. Seven days post-STEC infection, mice were anesthetized with isoflurane administered at 3% for induction and maintained at 1.5–2% in oxygen via a nose cone, until the reflexes were absent, just before euthanasia via cervical dislocation. Subsequently, the ileum from all groups was collected for further analysis.

Tissue Preparation for TEM

Toluidine Blue Staining

The extracted ileum was processed in 2.5% glutaraldehyde for 24 h, washed in phosphate buffer (0.1 M, pH 7.4; Glutaraldehyde, Cat. No. 76847, Sigma-Aldrich, St. Louis, MO, USA), and post-fixed at 4 °C for 1–2 h in 1% osmium tetroxide (Osmium Tetroxide, Cat. No. 251755, Sigma-Aldrich, St. Louis, MO, USA) buffered to pH 7.4 with 0.1 M phosphate buffer. After washing in phosphate buffer to remove any remaining fixative, the samples were dehydrated using ethanol grades in ascending order and cleared in propylene oxide. Semi-thin sections of 1 mm thickness were stained with toluidine blue for orientation and observation. Toluidine blue-stained sections were examined using a Nikon Eclipse Ti2 Inverted Microscope (Nikon Corporation, Tokyo, Japan) light microscope (LM) to ensure proper staining and tissue quality.

TEM Sectioning following Toluidine Blue Staining

Tissue samples from the ileum of mice were initially fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h at room temperature. After fixation, the samples were rinsed in 0.1 M phosphate buffer three times for 10 min each and post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 1–2 h. Subsequently, the samples underwent dehydration through a graded series of ethanol concentrations and were infiltrated with epoxy resin before embedding. Semi-thin sections (0.5–1 µm) were cut from the embedded tissue blocks using an ultramicrotome equipped with a glass or diamond knife. These sections were mounted on glass slides, stained with 1% toluidine blue solution for 1–2 min, and rinsed with distilled water. Following toluidine blue staining, the blocks were further trimmed, and ultrathin sections (approximately 100 nm) were cut using a JEOL ultramicrotome. The ultrathin sections were collected onto uncoated copper grids for TEM analysis. TEM imaging was conducted using appropriate voltage and magnification settings to capture high-resolution images of cellular structures. Analysis of TEM images included assessing cellular morphology, ultrastructural changes, and quantification of parameters such as microvilli integrity and membrane damage. The findings were compared between experimental groups to evaluate the effects of treatments or interventions.

Histopathological and Ultrastructural Assessments

Histopathological and ultrastructural evaluations were conducted to assess the impact of *E. coli O157:H7* infection and probiotics intervention on the intestines of mice. Specifically, TEM was employed for a detailed examination of cellular structures.

Histopathological Assessments

For histopathological evaluations, intestinal specimens were collected from each experimental group, including the control, infected mice, probiotics, and STEC-infected mice. These specimens were processed and stained using standard histological techniques. Histopathological parameters, such as the status of intestinal glands, epithelial cells, cytoplasm, nuclei, mitochondria, rough endoplasmic reticulum, Golgi apparatus, intraepithelial lymphocytes, vacuoles, the lumen of the intestinal gland, brush border, *E. coli*, and goblet cells, were assessed and scored based on predefined criteria [17].

Ultrastructural Assessments Using TEM

The TEM was utilized to explore ultrastructural changes in the enterocytes. The prepared ultrathin sections (100 nm) were double stained with lead citrate (Lead Citrate, Cat. No. 6107-83-1, Sigma-Aldrich, St. Louis, MO, USA) and uranyl acetate (Uranyl Acetate, Cat. No. 715645-66-2, Sigma-Aldrich, St. Louis, MO, USA). Subsequently, the sections were inspected and photographed using a TEM (JEM-1011, JEOL Ltd., Tokyo, Japan).

Data Analysis

The data were extracted, reviewed, categorized, and inputted into the statistical software IBM SPSS version 22 (SPSS Inc., Chicago, IL, USA). Descriptive analysis relied on the percentage of reported change per area among different study groups, visually represented in bar charts.

Results

Differential Affinity between Lactobacillus and E. coli Binding to Human Integrin

The output from the ClusPro web server showed that the affinity of the binding protein to human integrin *Lactobacillus* was higher than that of *E. coli*, with binding

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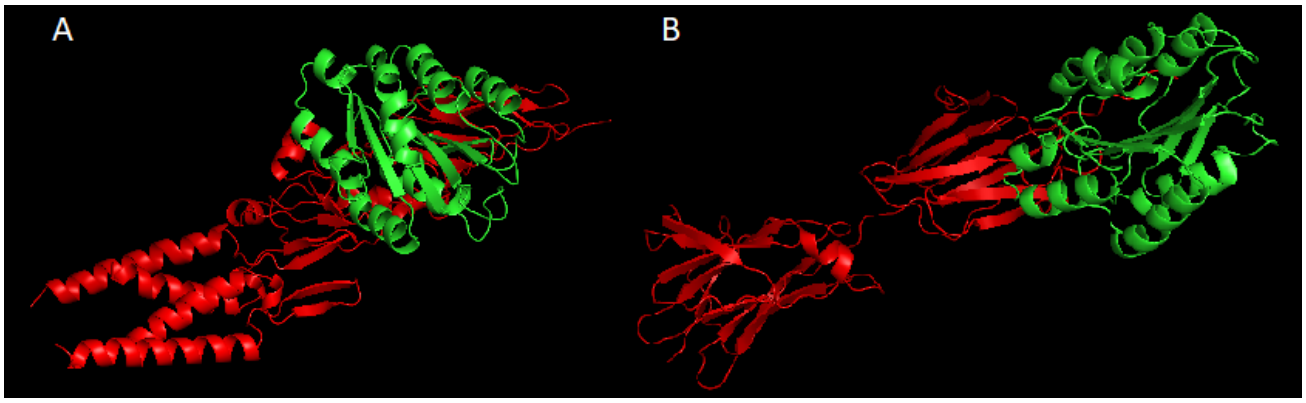

Fig. 1. The generated complexes between the binding domains of *E. coli O157:H7*, *Escherichia coli* (A), and *Lactobacillus* (B) with the human integrin. *E. coli*, *Escherichia coli*.

scores of –632.5 and –549.5, respectively. Fig. 1 depicts the formed complex between *E. coli* and *Lactobacillus* binding domains to human integrin.

LM Observations

Uninfected (Control) Group

The ileal specimens of the control mice revealed several IECs with brush borders and evident intercellular boundaries closely related to mucous-secreting goblet cells after staining with toluidine blue. Paneth cells were visible at the base of the intestine gland (Fig. 2A,B).

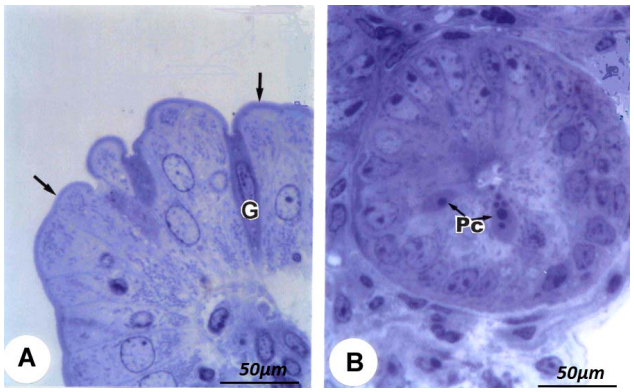

Fig. 2. Micrographs of semi-thin sections from the control (uninfected) group stained with toluidine blue stain (scale bar = 50 µm). (A) Uninfected mice epithelium covering the ileum showing numerous columnar cells with their brush border (arrows) interspersed with mucous-secreting goblet cells (G). (B) Uninfected mice epithelium covering the ileum showing the basal portion of the gland with Paneth cells (Pc) (arrows).

STEC-Infected Group

In certain areas of the ileal specimens of infected mice, the IECs showed diminished brush border, displaying cytoplasmic pallor. Moreover, a damaged mucous-secreting goblet cell with a dense nucleus as well as intraepithelial lymphocytes, were observed. Paneth cells were observed within the lumen of the intestinal glands, along with the presence of cells undergoing mitosis (Fig. 3A,B).

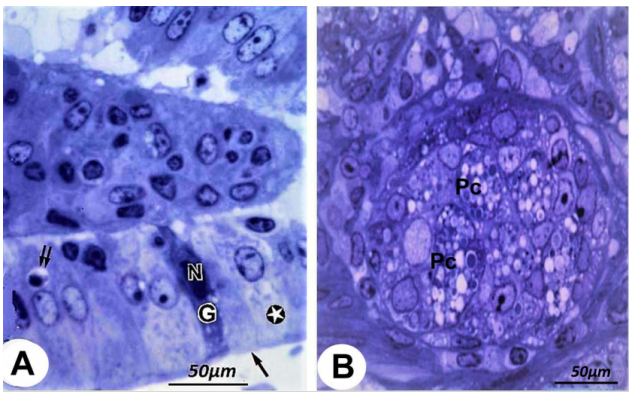

Fig. 3. Micrographs of semi-thin sections from mice of the infected group stained with toluidine blue stain (scale bar = 50 µm). (A) Mice intestinal epithelial cells (IECs) of the ileum after infection show the disappearance of the brush border (arrow) with cytoplasm pallor (star) in some areas. The micrographs depict disrupted mucous-secreting goblet cells (G) with dense nuclei (N) as well as intraepithelial lymphocytes (double arrows). (B) After infection, IECs of the ileum show two intestinal glands with numerous Paneth cells (Pc) accumulating towards its lumen and cells in mitosis.

Probiotics + STEC-Infected Group

Regarding the mice infected with STEC and administered probiotics, the IECs were completely covered by epithelial columnar cells. Additionally, the goblet cells were normal, but the brush border of the microvilli was damaged. The ileal sections showed healthy Paneth cells with visible granules in several crypts, and several pyknotic nuclei were observed (Fig. 4A,B).

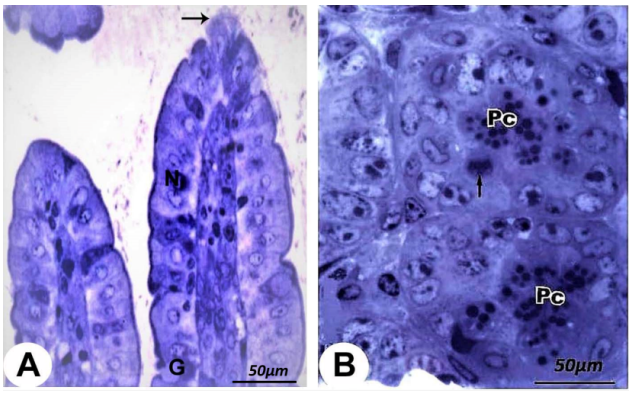

Fig. 4. Micrographs of semi-thin sections from the probiotics + infection group stained with toluidine blue stain (scale bar = 50 µm). (A) The probiotics + infected group exhibits intact IECs of the ileum, completely covered with epithelial columnar cells with nuclei (N) and goblet cells (G). Some disrupted brush border (arrow) of the microvilli is observed. (B) The probiotics + infected group exhibiting intact Paneth cells (Pc) with prominent granules (arrow) in most of the crypts. Some pyknotic nuclei (arrow) are observed.

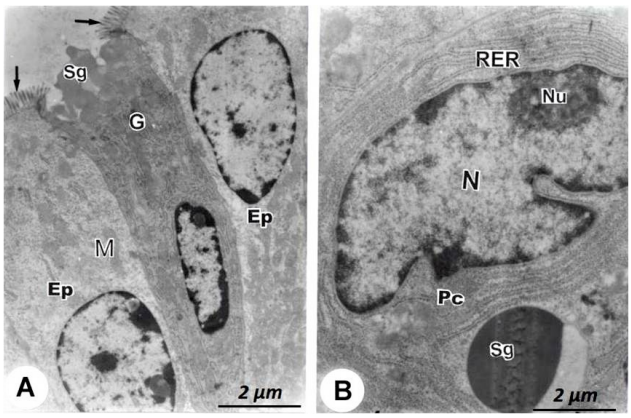

Fig. 5. Transmission electron microscopy (TEM) micrographs from the control (uninfected) group (scale bar = 2 µm). (A) Uninfected mice intestinal epithelial cells (IECs; Ep) of the ileum show scattered mitochondria (M) and luminal surfaces covered with a brush border of densely packed microvilli (arrows). A goblet cell with basal rough endoplasmic reticulum and apical secretory granules (Sg) are observed. (B) Uninfected mice Paneth cells (Pc) of the ileum showing basal nucleus (N) with prominent nucleolus (Nu), abundant rough endoplasmic reticulum (RER), and large secretory granules (SGs) with a protein core surrounded by a halo of polysaccharide-rich material. G, goblet cells.

TEM Observations

Uninfected (Control) Group

The IEC lumen was covered with a brush border of heavily populated microvilli and scattered mitochondria throughout the cytoplasm. The rough endoplasmic reticulum comprised most of the basal part of goblet cells, whereas the cell apex was densely packed with secretory granules. Paneth cells had a large secretory granule holding a protein surrounded by a polysaccharide-rich halo, a massive rough endoplasmic reticulum, and a basal nucleus containing a prominent nucleolus (Fig. 5A,B).

STEC-Infected Group

The ileal brush border was intact after infection, but vacuolation and damaged mitochondria were clearly visible besides vacuolation in the goblet cell (Fig. 6A,B). Paneth secretory granules, with various sizes and forms (including a core of protein covered with a large halo of polysaccharide-rich material), migrated to the lumen of the intestinal gland. The IECs had a significant amount of Golgi apparatus, rough endoplasmic reticulum, and many disrupted mitochondria. Moreover, bacteria were observed in the intestine gland lumen (Fig. 7A,B). After infection, many secretory granules and membrane breakdown were identified in the goblet cell. Several bacteria were identified in the IEC lumen (Fig. 7C). Microvilli in IECs were increasingly disordered, and the underlying membrane became disturbed (Fig. 7D). Regarding bacterial presence and correlations with gut entrecote, the secretory granules of Paneth cells were observed to trap a portion of bacteria. In addition, intercellular bridges between IECs with disrupted mitochondria and microvilli were noted (Fig. 8A). Eventually, the bacteria was found to be swelled and degraded (Fig. 8B).

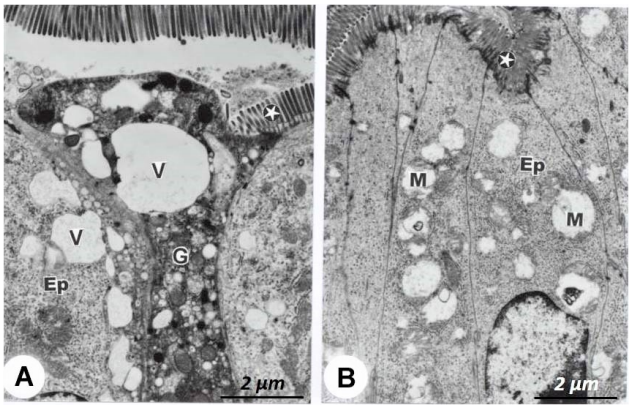

Fig. 6. Transmission electron microscopy (TEM) micrographs from mice of the infected group (scale bar = 2 µm). (A) Mice intestinal epithelial cells (IECs; Ep) of the ileum after infection show an intact brush border (star) with vacuolations (V) in its cytoplasm and the neighboring goblet cells (G). (B) Mice intestinal epithelial cells (IECs; Ep) of the ileum after infection show an intact brush border (star) with vacuolated and disrupted mitochondria (M).

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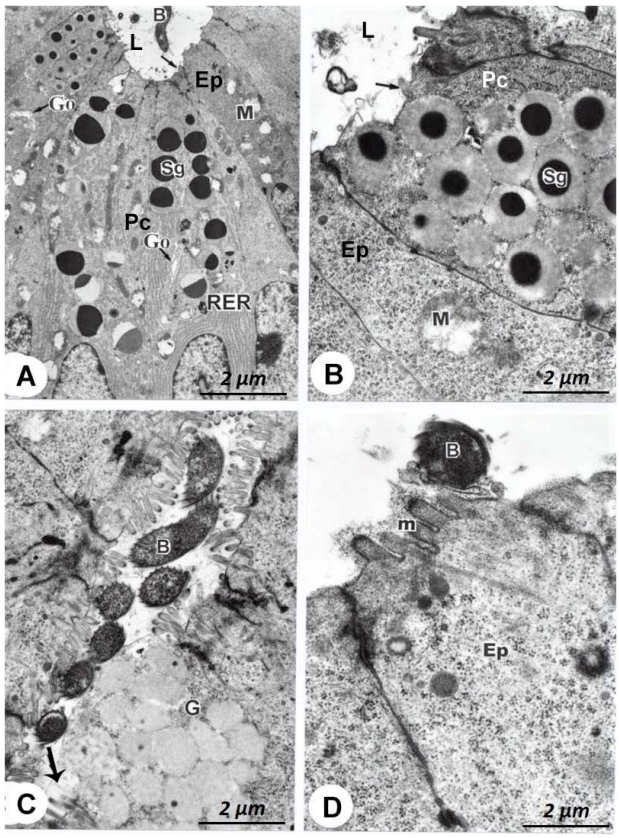

Fig. 7. Transmission electron microscopy (TEM) micrographs from mice of the infected group (scale bar = 2 µm). (A) Mice intestinal epithelial cells (IECs; Ep) with microvilli (arrow) of the ileum after infection show the migration of Paneth cells (Pc) into the lumen (L) of the intestinal gland that contains secretory granules (Sg) of different sizes and shapes and abundant rough endoplasmic reticulum (RER) and Golgi apparatus (Go). Disrupted mitochondria (M) in IECs and the presence of bacteria (B) in the lumen (L) of the intestinal gland are noticed. (B) Mice IECs with microvilli (arrow) of the ileum after infection show large secretory granules (Sg) of Paneth cells (Pc) with a protein core surrounded with a large halo of polysaccharide-rich- material. Destruction of microvilli (arrows) on the apical surface of IECs and damaged mitochondria (M) are observed. (C) The intestinal gland, after infection, shows goblet cells (G) with numerous secretory granules with a destructed membrane (arrow). Numerous bacteria (B) in the lumen of the intestinal gland are observed. (D) Intestinal epithelial cells (IECs; Ep) of the ileum after infection show absorptive columnar cells with abnormal microvilli (m) and dense bacteria (B) with damaged surrounding membranes that rest on the surface of the intestinal cell.

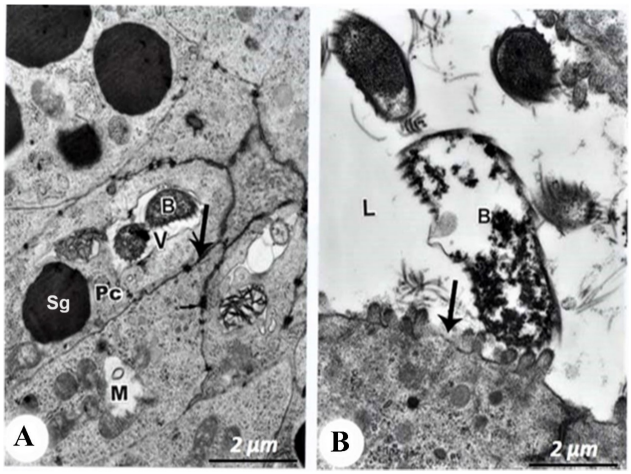

Fig. 8. Transmission electron microscopy (TEM) micrographs from mice of the infected group (scale bar = 2 µm). (A) Shows intercellular bridges (arrow) between IECs with disrupted mitochondria (M). Autophagic vacuole (V) engulfed two bacteria (B) is observed within the cytoplasm of Paneth cells (Pc). Large secretory granules (Sg) of Paneth cells (Pc) are also seen. (B) Reveals swelled and degraded bacteria (B) in the lumen (L) of the intestinal gland. Damaged microvilli (arrow) of epithelial cells are also seen.

Probiotics + STEC-Infected Group

The appearance of IECs in all ultrathin sections of the probiotics + infected mice group was significantly similar to that of the control. A brush border of tightly packed microvilli was incorporated on the luminal surface, and scattered mitochondria were detected. There were intact goblet cells with nuclei along the bottom, secretory granules at the tops, and multiple vacuoles in the cytoplasm. Large secretory granules covered with a large halo of polysaccharide material, undamaged nuclei, and some vacuoles were observed in the Paneth cells of the ileum in mice from the same group (Fig. 9A,B).

Statistical Observations

In STEC-infected mice, all components of intestinal glands, particularly intraepithelial lymphocytes, and the Golgi apparatus displayed significant changes. The control group exhibited the smallest change, followed by the probiotics + STEC group (Fig. 10A; Table 1). The STEC-infected mice showed the most significant changes in the intestinal gland, particularly the lumen region, whereas the control group showed the least significant changes, followed by the probiotics + STEC group (Fig. 10B; Table 1). Finally, the STEC-infected group revealed the highest changes, particularly in secretory granules, followed by Paneth cells of the intestinal gland, whereas the control group demonstrated the least change, followed by the probiotics + STEC group (Fig. 10C; Table 1).

Discussion

The intestinal epithelial barrier is a crucial physical barrier that communicates with the host gut microbiota and many immune system components to maintain gut home-

Table 1. Histopathological and ultrastructural parameters of intestine structures of control, infected, and probiotics + Shiga-like toxin-producing *Escherichia coli* (STEC)-infected mice.

Aspect	Control	Infected mice	Probiotics + STEC-infected mice
Intestinal gland	Normal	Destroyed	Intact
Epithelial cells	Intact	Abnormalities	Partial damaged
Cytoplasm	Intact	Interspersed with mucous secretions	Partial damaged
Nuclei	Healthy	Pyknotic	Normal
Mitochondria	Normal	Destructed &lost	Partial damaged
Rough endoplasmic reticulum	Intact	Reconstruction	Disrupted
Golgi apparatus	Healthy	Lost	Damaged
Intraepithelial lymphocytes	Non	Numerous	Few
Vacuoles	Non	Numerous	Few
The lumen of the intestinal gland	Healthy	Abnormalities	Normal
Brush border	Intact	Damaged	Partial damaged
Escherichia coli	Non	Numerous and damaged	Non
Goblet cells of the intestinal gland	Healthy	Disrupted	Intact

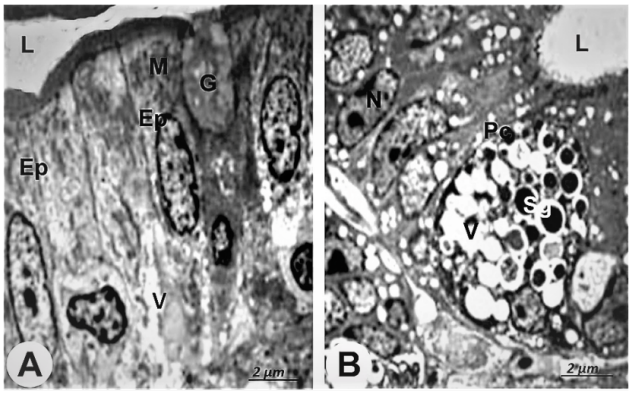

Fig. 9. Transmission electron microscopy (TEM) micrographs from mice of the infected + probiotics group (scale bar = 2 µm). (A) Mice intact intestinal epithelial cells (IECs; Ep) of the ileum after probiotics + infection exhibit scattered mitochondria (M) and luminal surface (L) covered with a brush border of densely packed microvilli. A goblet cell with apical secretory granules and a basal nucleus is observed. Some vacuolations (V) are observed in the cytoplasm. (B) Mice Paneth cells (Pc) of the ileum after probiotics + infection shows large secretory granules (Sg) with a protein core that are surrounded by a large halo of polysaccharide-rich- material, intact nucleus (N), and some vacuoles (V) are observed as well as the lumen (L). G, goblet cells.

ostasis and ensure proper functioning while providing immunoprotective effects [18]. Herein, we expand on the relevance of these backgrounds in relation to the influence of gut microbiota on epithelial cell modulation and immune system regulation. The influence of the gut microbiota on epithelial cell modulation and immune system regulation emerges as a critical aspect when interpreting the observed alterations. Barbara *et al*. [19] and Markowiak *et al*. [20] have highlighted the intricate interplay between gut microbiota and the intestinal epithelial barrier. The disruptions observed in intercellular bridges and epithelial morphology might indicate microbial-induced changes within the gut environment. Understanding these changes in the context of microbial modulation of epithelial cells and immune system interactions provides a crucial framework for elucidating how probiotics might intervene in maintaining or restoring intestinal homeostasis in the face of bacterial challenges. Reducing microflora composition diversity in the gut microbiome caused by invading pathogens has been related to various immune system complications. These issues can negatively impact the barrier functions of the gut, resulting in inflammatory and metabolic disorders [21]. Numerous probiotics have been reported to enhance the intestinal microbial balance and keep the epithelial barrier integrity. Accordingly, they have been employed as immune system-modulating factors, anti-obesity, and protective agents against invading intestinal pathogens [22]. Both intestinal pathogens and probiotics follow the same way of adhering to the surface of epithelial cells to perform their respective harmful and protective functions. Various *in vitro* and *in vivo* methods have demonstrated the ability of administered probiotics to inhibit the adherence of multiple intestinal pathogens to the surface of host intestinal cells [23–25]. Our study investigated this previously reported protective effect on the cellular level by employing microscopic approaches to compare three induced conditions in a mouse model. The first condition represented the natural or normal state of the intestinal cells, and the second condition showed the damaging effects of *E. coli O157:H7* on several cellular components. Meanwhile, the third condition demonstrated the protective roles of the employed probiotics *Lactobacillus rhamnosus* and *Lactobacillus helveticus*.

Here, the TEM images revealed significant alterations in the intestinal epithelium following exposure to *E. coli O157:H7* and subsequent treatment with *Lactobacillus* probiotics. Precise descriptions of these changes mani-

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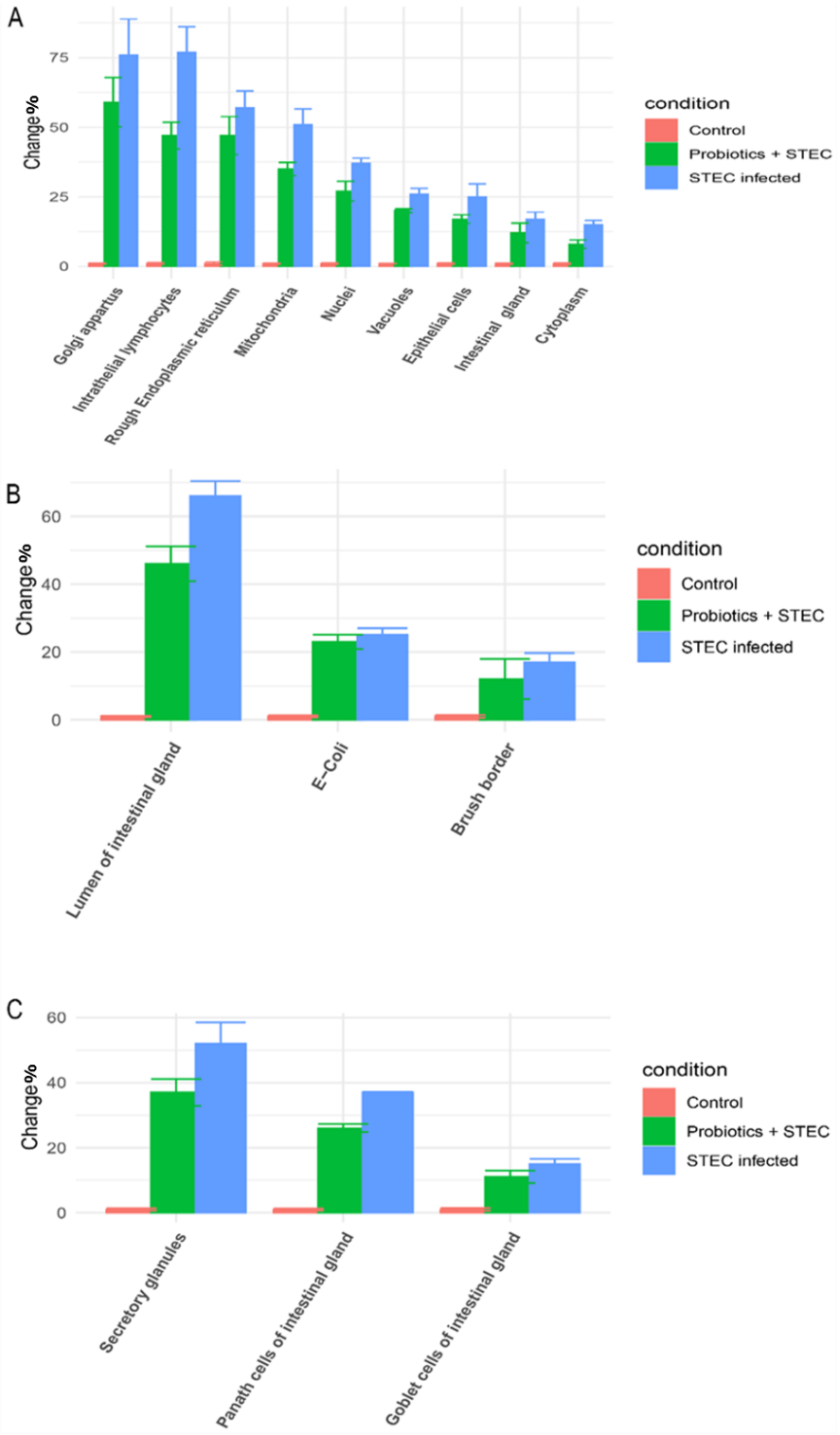

Fig. 10. Histopathological and ultrastructural parameters diagram of intestine structures of the control and infected mice. (A) Intestinal gland change %: infected mice demonstrated significant change for all intestinal gland tissues, particularly intraepithelial lymphocytes and the Golgi apparatus. The uninfected mice (control group) show the lowest change, followed by the probiotics + STEC group. (B) Lumen of intestinal gland change %: infected mice showed the most significant change for the lumen of intestinal gland issue, especially for the lumen part and *E. coli*, with the lowest change for the uninfected mice (control group), then for probiotics + STEC group. (C) Infected mice showed the most significant change, especially for secretory granules, followed by Paneth cells of the intestinal gland with the lowest change for the uninfected mice (control group), then for probiotics + STEC group. STEC, Shiga-like toxin-producing *Escherichia coli*.

fested disruptions in intercellular bridges between IECs, potentially compromising the intestinal epithelial barrier integrity. Such disruptions are of paramount significance, given the pivotal role of the barrier in regulating the passage of harmful antigens and microorganisms from the gut lumen into the systemic circulation. These observations underscore the vulnerability of the intestinal epithelium to pathogenic insults and hint at potential mechanisms. These probiotics, like *Lactobacillus*, might exert protective effects, warranting further investigation into their role in preserving epithelial integrity. The brush border is the microvilli-covered surface of simple columnar epithelium in various body parts. The brush border morphology enhances the surface area of the cell, which is a crucial characteristic of absorptive cells [26]. Several pathogenic serotypes of *E. coli* have been found to adhere to and affect the brush border integrity. Injecting enteropathogenic *E. coli* (EPEC) into enterocytes has resulted in actin rearrangement and stimulated the generation of a pedestal that lifts the bacterium high, away from the host defense mechanisms [27]. Shiga toxin produced by EHEC has been analyzed for its role in pedestal formation, where it used actin modifiers, such as vinculin, cortactin, and α-actinin [28,29]. These modifiers consequently affect actin polymerization, resulting in a force that pushes the apical plasma membrane and generates the pedestal [30]. On the other hand, probiotics can upregulate the host mucosal alkaline sphingomyelinase, which in turn hydrolyzes sphingomyelin into ceramide [31], modifying the apical cortical F-actin network that applies resistance against invading pathogens [32]. Consistent with these findings, our study showed numerous columnar cells with intact brush borders that experienced a disappearance and cytoplasm pallor formation in some areas due to *E. coli O157:H7* infection where the protective effect of probiotics was clear in the probiotics + infection group that manifested only a few disrupted brush borders. Furthermore, identifying damaged mitochondria with disrupted cristae raises intriguing implications in the context of bacterial-induced mitochondrial dysfunction. Maurice *et al*. [33] and Reitsema *et al*. [34] have emphasized the significance of bacterial manipulation of mitochondrial function during infections. The ability of bacterial pathogens to target mitochondria and induce dysfunction poses substantial challenges to cellular bioenergetics, dynamics, and immune responses. These findings underscore the broader context of bacterial infections and the potential therapeutic interventions targeting mitochondrial dysfunction to mitigate the downstream effects of infections and bolster host resilience.

Goblet cells are important components of the intestine, where they represent single-cell glands that can generate mucin. Mucin constitutes a mucus layer that isolates the materials in cavities from the intestinal epithelium and fights against the invasion of pathogenic microorganisms. Furthermore, goblet cells regulate the immune response through nonspecific endocytosis and function in antigen passages that deliver the antigens to the underlying antigen-presenting cells. The immune system reacts back by promoting the differentiation and maturation of goblet cells and inducing mucin secretion through a series of immuno-regulatory factors [35]. The immunostaining and PCR have been deployed to determine the deleterious impact of pathogens on the integrity and functions of goblet cells. This has revealed that the count of goblet cells and *Muc2* gene expression (responsible for mucus formation) was significantly reduced by day ten post-*C. rodentium* infection [36]. Meanwhile, the beneficial effect of probiotics on goblet cells has also been reported, where probiotics positively influenced intestinal *Mucin* gene expression [37]. In addition, *Lactobacillus reuteri* administration induced intestinal epithelial proliferation and stimulated its differentiation into goblet cells [38]. Our microscopy observations consistently demonstrated a disruption in mice goblet cells due to *E. coli O157:H7* infection. This effect was modulated by probiotics, as observed in the probiotics + infection group.

Paneth cells play major roles in fighting against invading intestinal pathogens as they secrete antimicrobial peptides and act as a key mediator of host-microbe interactions [39]. Small intestinal crypts contain stem cells that act as a source to compensate for dead epithelial cells. The location of Paneth cells next to these stem cells suggests that they function in keeping epithelial cell renewal [40]. Moreover, Paneth cells employ cell-autonomous MyD88-dependent toll-like receptor (TLR) activation to overexpress several antimicrobials and control the bacterial invasion of the host tissues. This elucidates its functions in keeping intestinal homeostasis [41]. For these protective roles, Paneth cells disruption results in intestinal complications such as necrotizing enterocolitis [42]. Probiotics have a supporting effect on Paneth cells where the administration of *Lactobacillus casei* and *L. paracasei* elevated the count of Paneth cells in the small intestine and stimulated the antimicrobial activity against *Staphylococcus aureus* and *Salmonella typhimurium* in the intestinal fluids [43,44]. This study demonstrated that administrating probiotics, in conjunction with *E. coli O157:H7* infection, preserved the integrity of Paneth cells. In contrast, the mice group infected with *E. coli O157:H7* without probiotic protection experienced disruption of Paneth cells function.

However, our study is limited to the absence of quantitative measures, including counts of disrupted microvilli, assessment of mitochondrial damage, and quantification of bacterial colonization. These measures might offer a deeper understanding of the cellular changes induced by *E. coli O157:H7* and the potential protective effects of probiotics. Accordingly, future investigations delving deeper into these specific quantitative measures will complement and strengthen the qualitative TEM observations presented in this study.

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Although mouse models offer valuable insights into initial biological responses and interactions, extrapolating these findings to human health requires careful consideration and further exploration. The observed effects of *Lactobacillus* supplementation in mitigating the detrimental impact of *E. coli O157:H7* on mouse enterocytes provide a foundational understanding of potential probiotic interventions. Translating these insights to human health necessitates additional studies, including clinical trials or *ex vivo* models closely mimicking human intestinal physiology and responses. Understanding the nuances and differences in the gut microbiota composition, immune system intricacies, and epithelial cell dynamics between mice and humans is critical. Future research endeavors should bridge this gap by incorporating diverse model systems or employing advanced *in vitro* models that better simulate human intestinal conditions. Moreover, exploring the relevance of our findings in clinical settings or human trials would be invaluable. Investigating the efficacy and safety of specific probiotic strains, like *Lactobacillus*, in human populations susceptible to enteric infections could shed light on their potential therapeutic applications. By delineating our limitations and highlighting the need for further studies, we aim to encourage and guide future research efforts that can validate and extend the implications of our findings to human health.

Conclusions

The *E. coli O157:H7* infection had a detrimental impact on the crucial cellular component integrity, effectively counteracted by probiotics. Administration of *Lactobacillus* species to *E. coli O157:H7*-infected mice improves mucosal resistance to the invading bacteria by limiting its adhesion to epithelial cell surfaces and inhibiting bacterial translocation. Additionally, probiotics maintained the intestinal cells in a state more closely resembling their normal status. Incorporating a microscopy-based approach proved the protecting effects of the administered probiotics against the invading intestinal pathogen. Our study offers insights into the prophylactic role of specific probiotics, particularly *Lactobacillus*, against *E. coli O157:H7*-induced damage in enterocytes. Understanding the protective mechanisms of probiotics could pave the way for innovative strategies to prevent or mitigate the adverse effects of this pathogenic strain, presenting novel avenues for therapeutic interventions.

Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author.

Author Contributions

Conceptualization, MSZ, RAE, MAS, MEM and MAE; methodology, MSZ, RAE, MAS, WKA, SSA, and MAE; data curation, MAA, MA, AMA, TGK, EF, and RAE; writing—original draft preparation, MSZ, RAE, MAS, and MAE; writing—review and editing, MAA, and TGK; supervision, MSZ, and TGK; project administration, MSZ, MAS, and RAE; funding acquisition, MAA, MSZ. All authors contributed to important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The Research Ethics Committee approved this study at King Khalid University (protocol number ECM#2022-115; dated March 11, 2022).

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Conflict of Interest

The authors declare no conflict of interest.

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