Kyereh *et al.*
Food Production, Processing and Nutrition (2022) 4:34
https://doi.org/10.1186/s43014-022-00111-2

Food Production, Processing and Nutrition

RESEARCH

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Immobilization of *Lactobacillus plantarum* NCIMB 8826 ameliorates *Citrobacter rodentium* induced lesions and enhances the gut inflammatory response in C57BL/6 weanling mice

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Abstract

Infectious diarrhea is a major cause of infant mortality in most developing countries. In this research, we evaluated the potential of immobilized *Lactobacillus plantarum* NCIMB 8826 on *weanimix* infant cereal and its effectiveness in reducing the severity of *Citrobacter rodentium*-induced diarrhea in weanling mice. Thirty-six C57BL/6 weanling mice were placed into four groups ($n = 9$ each; negative, positive, prevention and cure). Mice received either *L. plantarum* (10^9 CFU/g) immobilized on *weanimix* infant cereal 3 days before *C. rodentium* (10^9 CFU/ ml) infection (Prevention) or 3 days after *C. rodentium* infection (Cure). A positive control group was infected with *C. rodentium* only, while a negative control group received neither *L. plantarum* nor *C. rodentium*. Positive control mice showed colonic mucosal and submucosal inflammation, erosion, and mucosal epithelia hyperplasia with the *C. rodentium* infection. Mice in the prevention and cure groups had less severe histologic alterations in the colon. Some beneficial effect of *L. plantarum* was observed in cecal short-chain fatty acid concentrations, which stimulates water and electrolytes absorption to reduce diarrhea. Our findings demonstrated that *L. plantarum* NCIMB 8826 could be immobilized on *weanimix* infant cereal to help reduce diarrhea during weaning.

Keywords: Probiotics, *Citrobacter rodentium*, *Lactobacillus plantarum*, Inflammation, Weanimix

Approved for publication by the director of the Louisiana Agricultural Experimental Station as manuscript number 2021.

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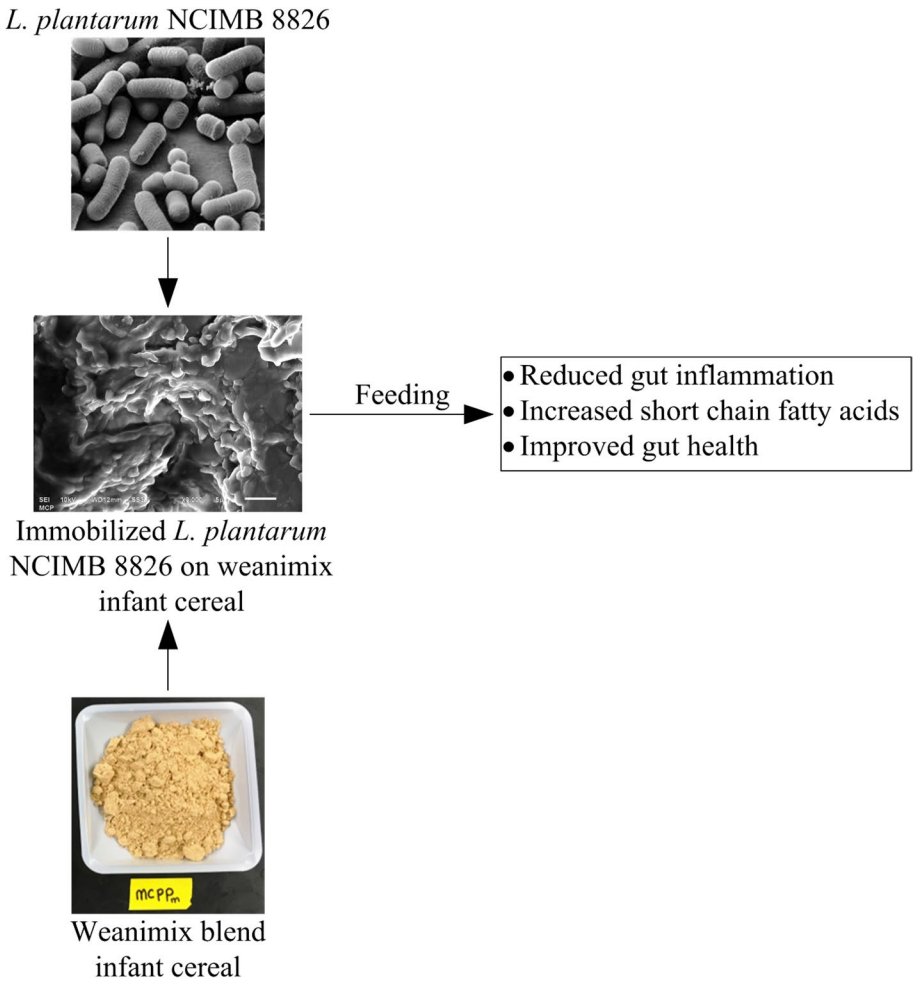
Graphical Abstract



Introduction

Lactic acid bacteria (LAB) are safe and beneficial microorganisms that improve disrupted gut microbiota and reduce infectious inflammatory diseases (Chung et al. 2008; Dahiya & Nigam 2022; Jang et al. 2014; Seme et al. 2017; Seo et al. 2017). LAB, such as *Lactobacillus plantarum*, have been employed in the probiotic industry in many diverse ways, such as competing with pathogenic bacteria on the skin and mucosal surfaces to prevent and treat infections (Brachkova et al. 2012; Corbo et al. 2013; Martín & Langella 2019).

According to Singh et al. (2018), the prevalence of chronic diarrhea was 6.6% in United States adults, and they found that lifestyle and dietary factors contributed to chronic diarrhea. Irritable Bowel Syndrome (IBS) is a common chronic functional bowel disorder, and probiotics are effective for overall IBS symptoms (Sun et al. 2020).

Lactobacillus plantarum protects against rotavirus-induced diarrhea, regulates inflammatory response (Kim et al. 2018), and significantly alleviates bowel frequency (Yang et al. 2021). *Lactobacillus plantarum* can relieve diarrhea caused by *Escherichia coli* by regulating the inflammatory cytokines, rebalancing the gut microbiota, and modulating short-chain fatty acids (SCFAs) generation (Yue et al. 2020). Irritable Bowel Syndrome (IBS) is a common chronic functional bowel disorder, and probiotics are effective for overall IBS symptoms (Sun et al. 2020). Yan et al. (2019) showed that probiotics could alleviate colitis by modulating the inflammation-related cytokines, maintaining the normal mucosal barrier in the gut, and restoring microbiota changes.

Probiotics can modulate microbiome composition, anti-inflammatory activity, and intestinal barrier function in patients with diarrhea-predominant IBS (Caviglia et al. 2020).

To alleviate diarrhea due to pathogenic microorganisms and reduce IBS, the cell viability of probiotics is an essential factor. It is vital to provide a physical barrier to probiotics to protect them from the processing and gastrointestinal environments. Immobilization of probiotic bacteria has significantly enhanced probiotics' viability (Calumba et al. 2021; Jayani et al. 2020; Terpou et al. 2017). The best application of immobilization technology is the controlled and constant release of live bacteria in the gut after surviving the acid environment in the stomach and the bile conditions in the small intestine (Mitropoulou et al. 2013). Cell immobilization involves retaining microorganisms in a discrete location within a fermentation system to maintain high cell concentrations. Immobilization is advantageous compared to free cells because it fosters high diversity, improves resistance to contamination and bacteriophage attack, prevents washing-out during continuous cultures, and provides physical and chemical protection of cells (Doleyres & Lacroix 2005). Immobilization results in the entrapment of live probiotic cells in a porous food matrix that assists the high density of the probiotic cells to pass through the gastrointestinal tract and deliver them to the colon. However, some prerequisites are needed for a food product to be considered an immobilization material. For example, the food matrix must have immobilization support, support the biological and metabolic activities of the immobilized cells, and preserve the food product's quality characteristics (Mitropoulou et al. 2013).

Citrobacter rodentium, a mucosal pathogen of mice, is an ideal model for investigating pathogen-host immune interaction in the gut because it colonizes the intestinal mucosa through the formation of attaching and effacing (A/E) lesions (Collins et al. 2014; Kimizuka et al. 2021; Mundy et al. 2005). *C. rodentium* is the causative agent of Transmissible Murine Colonic Hyperplasia characterized by epithelial cell proliferation in the mucosa of the descending colon (Berger et al. 2018; Johnson-Henry et al. 2005; Schauer & Falkow 1993). This mouse-restricted pathogen harbors a locus of enterocyte effacement pathogenicity island like enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 and enteropathogenic *Escherichia coli* (EPEC). EHEC and EPEC are two clinically significant human gastrointestinal pathogens that are common causes of infantile diarrhea and lead to high morbidity and mortality rates in developing countries (Collins et al. 2014; Mullineaux-Sanders et al. 2019). Hence it serves as an appropriate small-animal model to mimic infectious diarrhea infection in humans. This study evaluates the effect of *Lactobacillus plantarum* NCIMB 8826 immobilized on *weanimix* infant cereal to attenuate colonic infection by *C. rodentium* in C57BL/6 weanling mice. *Weanimix* is a cereal-legume-fortified infant food prepared into a slurry for most children from developing countries, primarily in sub-Saharan Africa.

Materials and methods

Bacterial strains

Citrobacter rodentium strain DBS 100 (ATCC 51459) was purchased from the American Type Culture Collection (ATCC), Manassas, VA, USA. The stock culture was prepared according to the vendor's instructions and stored at −80 °C in 10% glycerol. The stock was reactivated in Luria Bertani broth (LB) according to the method described by Wine et al. (2010) and Bhinder et al. (2013) with some modifications. Inoculated LB was prepared to a final concentration of 10^9 CFU/ ml. Bacteria were grown from frozen stocks on LB agar plates at 37 °C and then regrown in LB broth in a falcon culture tube overnight on a benchtop incubation shaker (Lab-line 3532 incubator-shaker, Melrose Park, IL) at 200 rpm. Mice were infected with the cloudy LB inoculum. Weanling mice were orally gavaged 100 µL of the bacterial inoculum on the 20th day after birth. The negative control group was gavaged with equal amounts of distilled water.

The probiotic bacteria, *L. plantarum* NCIMB 8826 (ATCC BAA-793), were purchased from the American Type Culture Collection. Freeze-dried *L. plantarum* (LP), and were rehydrated to a concentration of 10^9 CFU/L, as described by Chotiko and Sathivel (2014). The cell culture (*L. plantarum* NCIMB 8826, ATCC BAA-793) was grown in MRS broth for 24 h at 37 °C and then for 16 h to reach the stationary phase. The cells were harvested as pellets by centrifugation at 6000×g for 10 min at 4 °C (Beckman J2-HC, Beckman Coulter, Inc., Brea, CA, USA). The harvested pellets were then suspended in distilled water and immobilized on cereal-legume infant cereal (*weanimix*) as described by Kyereh and Sathivel (2021). The *L. plantarum* immobilized on the infant cereal was fed to the prevention group (feeding started on the 17th day after birth) and cure group (feeding started on the 23rd day after birth). In contrast, the positive and negative control groups were fed the *weanimix* infant cereal without *L. plantarum.*

Animals

Fifteen germ-free C57BL/6 adult mice (10 females and 5 males, Charles River) were paired in a 2:1 female to male ratio and bred for 36 weanling pups for this study. All procedures were approved by the Louisiana State University Institutional Animal Care and Use Committee

(LSU-IACUC protocol number 16-074) and the Inter-Institutional Biological and Recombinant DNA Safety Committee (IBRDSC project reference number 16-018).

Experimental diet

Dried corn kernels (*Zea mays*), shelled cowpea (Cowpea, *Vigna unguiculata*), shelled peanut (*Arachis hypogaea*), and powdered milk were the components in the fortified *weanimix* cereal diet (Maize: Cowpea: Peanut: Powdered milk, $MCPP_m$ = 70:15:10:5% ratio) and was prepared for this study as described by Kyereh and Sathivel (2021). Animals were fed with $MCPP_m$ (*weanimix* infant cereal) prior to the study to ensure diet acclimatization. After the acclimatization period, the positive and negative control groups continued to receive the $MCPP_m$ diet. The prevention treatment group was fed $MCPP_m$ with *L. plantarum* 3 days before *C. rodentium* infection. In comparison, the cure treatment group was fed $MCPP_m$ with *L. plantarum* 3 days after *C. rodentium* infection.

Experimental design

Thirty-six pups were divided into 4 groups (n = 9), and treatments were administered as summarized in Fig. 1. Mice were housed in two or three per cage, considering the sexes. Animals were housed in a 12 h light/dark cycle with free access to water and feed. The *L. plantarum* was administered in immobilized form (on $MCPP_m$ *weanimix* cereal). Mice were treated as follows: (a) Prevention group (n = 9) received immobilized *L. plantarum* (10^9 log CFU/g) for 3 days before (i.e., at day 17 after birth) they were orally gavaged with 100 µL of *C. rodentium* (10^9 CFU/ml) and continued to receive the immobilized *L. plantarum* with *weanimix* cereal throughout the study; (b) Cure group (n = 9) was treated with immobilized *L. plantarum* 3 days after the mice were orally gavaged with 100 µL of *C. rodentium* (i.e., at day 23 after birth) and continued to receive immobilized *L. plantarum* diet throughout the study; (c) Positive control group (n = 9) was gavaged with *C. rodentium* only; and (d) Negative control (n = 9) received neither *L. plantarum* nor *C. rodentium*. Mice in the positive and negative control groups were fed with *weanimix* cereal formulation without *L. plantarum* during the study, while mice in the prevention and cure groups received *weanimix* cereal with immobilized *L. plantarum*. Mice were fed 10 g of the prepared diet daily, and diets were changed every other day. Mice were euthanized at 10 days post-infection, and intestinal tissue samples and cecal contents were collected for histologic evaluation and short-chain fatty acid (SCFA) analysis, respectively.

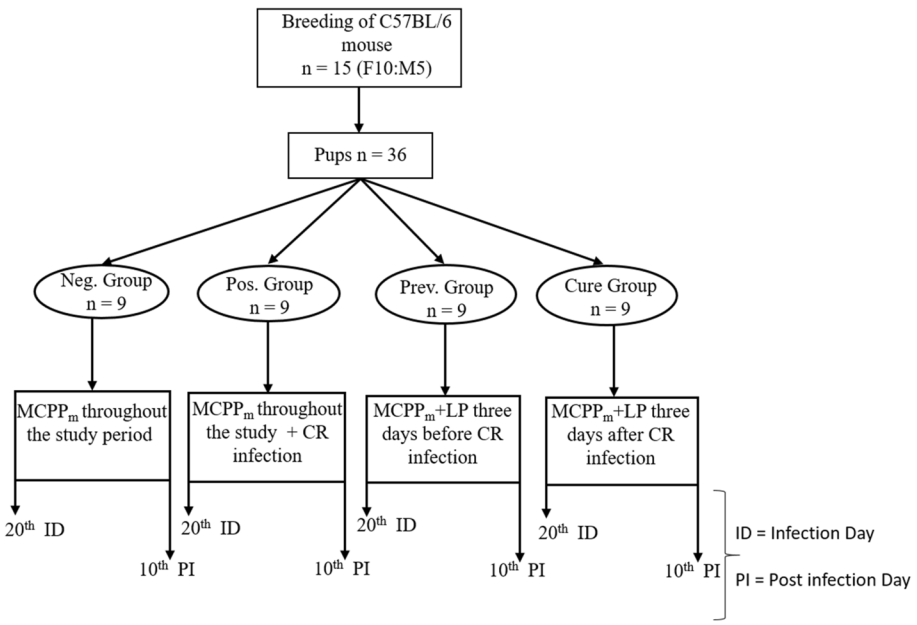


Fig. 1 Experimental design of the animal study. Upper rectangular boxes indicate breeding of C57BL/6 mice from Charles River (Charles River Laboratories, Wilmington, MA) bred for 36 weanling pups. The round circles indicate the different treatments with the number of weanling mice used in each group. The lower rectangular boxes indicate the diet treatment each group received, and the arrows are the days when mice were infected with *Citrobacter rodentium* and euthanized, respectively. *C. rodentium* (100 µL of 10^9 Log CFU/ml) was administered through oral gavage (on day 20 after birth) with the exception of the negative control. All mice groups were fed with the *weanimix* infant cereal diet 5 days prior to their treatment diet to ensure acclimatization. LP = *Lactobacillus plantarum* NCIMB 8826 (ATCC BAA-793); CR = *Citrobacter rodentium* strain DBS 100 (ATCC 51459); ID = Infection Day; PI = Post-infection Day

Tissue collection

Sections of the colon were collected and fixed in 10% neutral-buffered formalin to be routinely processed for histopathological analysis. Sections were then stained with hematoxylin-eosin (H&E) to assess the presence of bacterial colonization and the degree of inflammation, erosion/ulceration, and epithelial cell hyperplasia. Additional serial sections were stained with Alcian Blue and Periodic Acid Schiff (AB/PAS) to highlight the goblet cells in the mucosa. Histological evaluation was performed by a blinded board-certified pathologist using a previously developed scoring system (Table 1).

Preparation of serum for cytokine measurements

Blood samples obtained from euthanasia through cardiac puncture were allowed to sit at room temperature for 2 h. Serum was collected by centrifuging at 2000 x *g* for 20 min (Thermo Electron Corporation, Forma 1 L GP centrifuge, PA, USA) and stored at − 80 °C until analysis. Levels of interleukin − 10 (IL10) and interferon-gamma (IFN-γ) in the serum were measured with enzyme-linked immunosorbent assay (ELISA) kits. The preparations of all standards, reagents, and protocol were according to the manufacturer's instructions (R&D System Inc. Minneapolis, MN; Fisher Scientific, Waltham, MA, USA).

Quantification of short-chain fatty acids in cecal content

A stock solution of internal standard 25% MPA/EBA mix was prepared by mixing 0.2 g ethyl butyric acid (EBA) and 25 g meta-phosphoric acid (MPA) in a 100 ml volumetric flask. A volatile fatty acid mixture (Matreya, LLC, State College, PA, USA) was used as a standard. Samples were stored at − 80 °C before analyzing for acetate, butyrate, and propionate levels. Cecal contents samples (0.5 g) were weighed into a falcon tube, and 5 ml of distilled water was added. The content was vortexed for 1 min, and 1 ml of the internal standard 25% MPA/EBA was added. Samples were vortexed for 1 min and centrifuged at 4 °C at 3000 x *g* for 10 min (Allegra 6R Centrifuge, Beckman Coulter, Brea, CA, USA). The supernatant was filtered through a 5-cc syringe attached to a Millipore filter (MILX HA 33 mm, 0.45 µm) into a 2 ml autosampler vial and cap. Samples were analyzed by GC/MS (Agilent 6890 Gas Chromatography, Santa Clara, CA, USA) using a Grace Alltech EC-1000 (15 m × 0.53 mm ID × 1.2 µm (100% polyethylene glycol acid modified) capillary column (Fisher Scientific). The program for temperature control was: 115 °C for 0.1 min, increase rate of temperature 10 °C per minute up to 150 °C and held for 0.1 min, then increased the temperature at 11 °C per minute up to 170 °C and held for 2 min. The injector temperature was 250 °C.

Table 1 Histopathological scoring system to quantify the degree of intestinal lesions

Criterion	Score			
	0	1	2	3
Mucosal Hyperplasia	–	↑	↑↑	↑↑↑
Mucosal Inflammation	–	↑	↑↑	↑↑↑
Submucosal Inflammation	–	–	↑	↑↑
Goblet Cell Number	–	↓	↓↓	↓↓↓
Erosion/Ulceration	–	↑	↑↑	↑↑↑

The scoring system adapted from Van der Sluis et al. (2006) was also used by Wine et al. (2010)

↑Indicates an increase in the degree of intestinal lesions levels

↓Indicates a decrease in the degree of intestinal lesions levels

—Indicates that the scoring system did not quantify the degree of intestinal lesions. The number of arrows reflects the degree of the lesion

Statistical analysis

The statistical significance of differences observed among treatment groups means was evaluated by Analysis of Variance (ANOVA) (SAS Version 9.4, SAS Institute Inc., Cary, NC, USA) with Tukey's post hoc analysis, and differences at $p < 0.05$ were considered statistically significant. Results were presented as the mean plus standard error of the mean.

Results and discussion

Behavioral and activity assessment of the mice

During the study period, one mortality was observed in the prevention group 2 days after administration of 100 µL of 10^9 CFU/ ml of *C. rodentium*. Necropsy findings suggested it to be a consequence of the gavage procedure. Feed intake and fluid consumption did not differ significantly between the two treatment groups and the two control groups. There was no weight loss over the 10 days post-infection period, irrespective of the treatment (data not shown). Nonetheless, some mice in the prevention ($n = 4$), cure ($n = 6$), and positive control groups ($n = 6$) showed coat ruffling and hunched posture during the early days of post-infection. These mice also had some degree of dehydration and were treated with a constant supply of Plasma-Lyte 148 (Sigma-Aldrich, St Louis, MO, USA).

Effect of pre-and post-treatment of *C. rodentium*-infected mice with immobilized *L. plantarum* on colonic damage and inflammation responses

At 10 days post-infection, *C. rodentium* infected mice had a colon partially devoid of content with feces that were softer than normal (Fig. 2B). The distal colon was thickened and rigid (Fig. 2B) compared to the negative control mice (Fig. 2A), which had normal content. Pretreatment of immobilized *L. plantarum* 3 days prior to *C. rodentium* infection (prevention) of the weanling mice

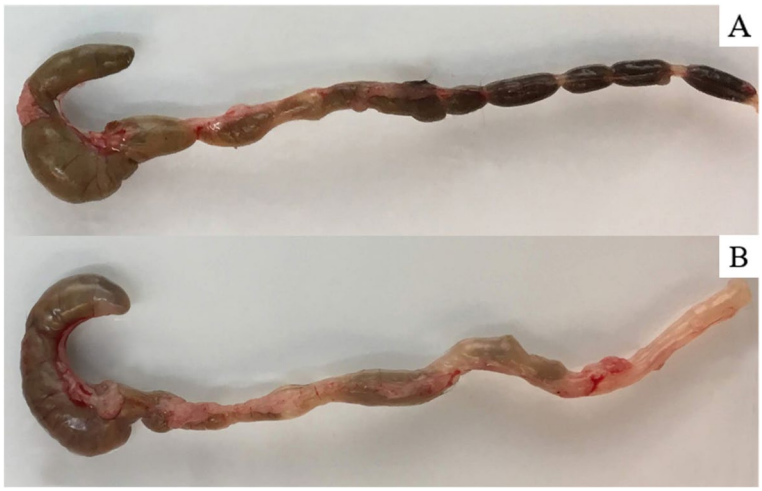


Fig. 2 Cecum and colon from (**A**) an uninfected mouse and (**B**) a mouse orally gavaged with *Citrobacter rodentium*, resulting in a thick and rigid distal colon partially devoid of content. The fecal material that remains in the colon of this mouse (**B**) is not as well formed as that in the colon shown in (**A**)

was beneficial in reducing colonic damage and inflammation (Fig. 3C). Likewise, mice in the cure group exhibited only mild inflammation (Fig. 3D) compared to the moderate inflammation in the mice of the positive control group (Fig. 3B). The mice in the positive control group also had superficial sloughing of the mucosal epithelial cells as is typical with A/E bacterial infection (Fig. 3B). In addition, the mice in this group had crypt cell hyperplasia and goblet cell depletion (Fig. 3B). Comparatively, mice that received either immobilized *L. plantarum* 3 days before or after *C. rodentium* infection did not show a difference in epithelial cell damage (Fig. 3C and D). Histologically, the positive control group recorded higher scores in all the indicators of inflammation and tissue damage (mucosal inflammation, submucosal inflammation, mucosal thickening, goblet cell number reduction, and erosion/ulceration; Fig. 3E). Moreover, there was not much difference between the scores of the two probiotics groups (prevention and cure). Ryu et al. (2016) have shown similar ameliorated effects of probiotics on *C. rodentium* infection in mice. However, the current study is the first to use the immobilization process with a commonly used weaning mix for children in underdeveloped nations to deliver probiotics. Restoring the integrity and architectural structure of damaged colonic epithelial cells is an essential consideration in a healthy gastrointestinal tract. Infection with *C. rodentium* is characterized by colonic inflammation and hyperplasia associated with intimate bacterial attachment and effacement of the epithelial brush border microvilli with localized actin polymerization, usually in the distal colon (Collins et al. 2014; Papapietro et al. 2013). The formation of these attaching and effacing (A/E) lesions, also seen with EPEC and EHEC, distinguishes *C. rodentium* from other pathogenic bacteria (Collins et al. 2014; Luperchio & Schauer 2001). Probiotics attenuate these A/E bacteria by competitively excluding the binding of the pathogenic organisms to the host epithelium and acidifying the luminal environment (Kim et al. 2010; Rodrigues et al. 2012; Ryu et al. 2016; Sellin et al. 2009).

Effect of *Lactobacillus plantarum* on immune modulation

Levels of the important immunoregulatory cytokine (IFN)-γ were increased in the serum of *C. rodentium* infected mice (positive, 101.2 ± 42.3 pg/ml) compared to that of mice that received *L. plantarum* either 3 days before (prevention, 45.7 ± 5.1 pg/ml) or after (cure, 48.2 ± 18.1 pg/ml) *C. rodentium* infection (Fig. 4A). Mao et al. (2021) have also reported the detection of increased production of IFN-γ in mice with *C. rodentium.* Levels of the anti-inflammatory cytokine IL-10 levels were higher in mice that received *L. plantarum* 3 days prior to *C. rodentium* infection (prevention, 6.6 ± 3.7 pg/ml) and in mice within the positive group (6.0 ± 4.4 pg/ml) compared to mice in the cure group (4.3 ± 2.6 pg/ml) and the uninfected mice (negative, 4.2 ± 3.4 pg/ml) (Fig. 4B).

Most model studies involving different probiotic strains, such as *Lactobacillus rhamnosus* or *Lactobacillus acidophilus,* reported the bacteria can have the colonic hyperplasia and clinical signs of intestine disease by modulating the mucosal immune function in adult mice (Johnson-Henry et al. 2005). Mohamad et al. (2021) have reported that probiotics can stabilize the mucosal immune function in fatty liver disease patients preventing increased intestinal permeability. The immune response to pathogens involves the rapid activation of

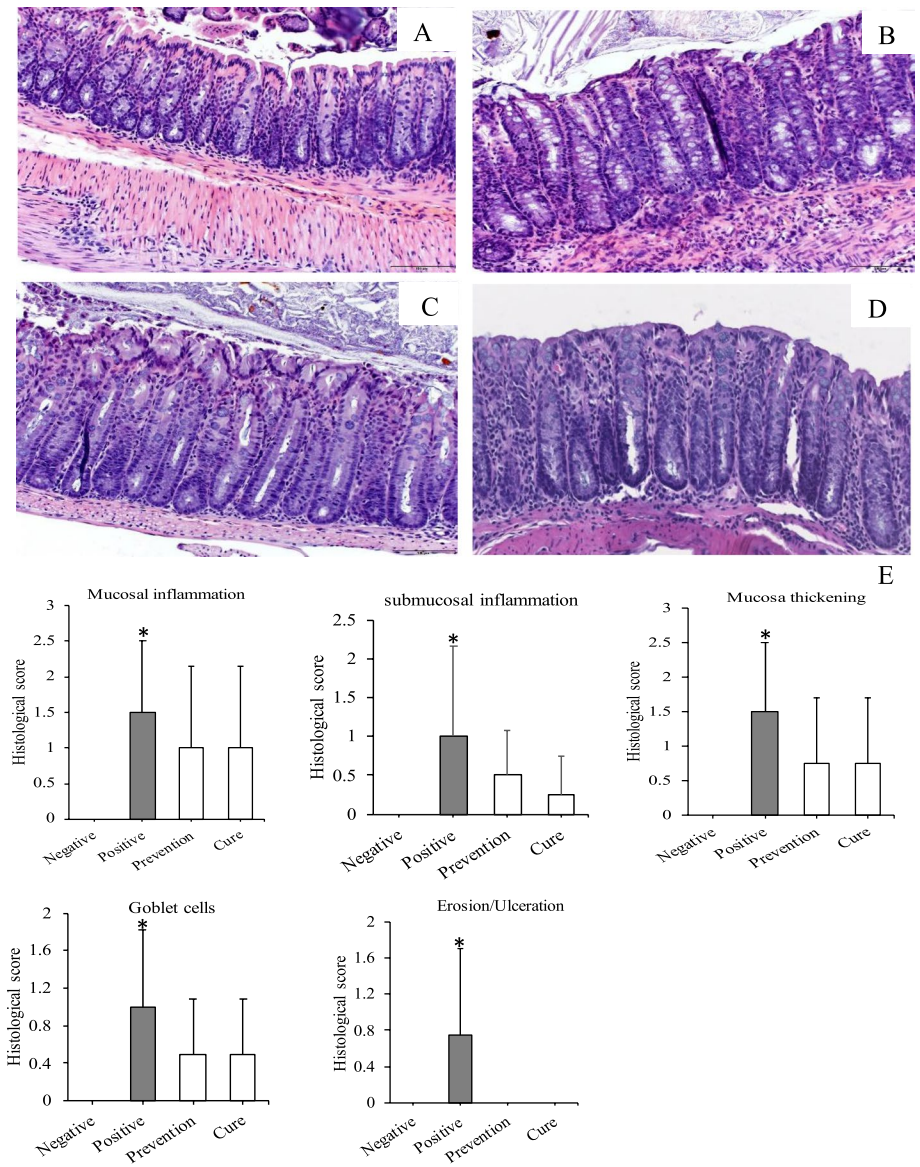


Fig. 3 Hematoxylin & Eosin (H&E) stained colon sections of C57/BL 6 weanling mice. **A** Non-infected mouse in the negative control group. Normal morphological characteristics of the mucosal epithelium. **B** *Citrobacter rodentium* infected mouse in the positive control group. Moderately hyperplastic mucosal epithelium with superficial sloughing of cells accompanied by inflammation in the mucosal and submucosa. **C** *Citrobacter rodentium*-infected mouse in the prevention group, with immobilized *Lactobacillus plantarum* initiated 3-days before infection. Mucosal hyperplasia and inflammation are mild. **D** *Citrobacter rodentium*-infected mouse in the cure group, with immobilized *L. plantarum* initiated 3-days after infection. Mucosal hyperplasia and inflammation are mild. (Scale bars = 100 µm). **E** Blinded histological scores showing significant differences between *C. rodentium* and *L. plantarum* treated mice (*$p < 0.05$)

pro-inflammatory cytokines that initiate the host defense against microbial invasion. However, systemic metabolic and hemodynamic disturbances harmful to the host can occur due to excess pro-inflammation (Lyer & Cheng 2012). The immune system develops a concurrent anti-inflammatory response to reduce the tissue damage and maintain or restore tissue homeostasis (Moore et al. 2002; Mosser 2009). Probiotics have been identified as key players in different immune cell modulatory pathways by stimulating some cytokines production. In many cases, probiotics are directly taken up through transcytosis by the microfold epithelial cells of the intestinal epithelium and are engulfed by macrophages or dendritic cells, a process that eventually triggers an immune response (Dicks & Botes 2010; Komiyama et al. 2011). A study with 8 different probiotics in a mixture demonstrated that levels of the anti-inflammatory cytokine IL-10 in the human intestine increased after the

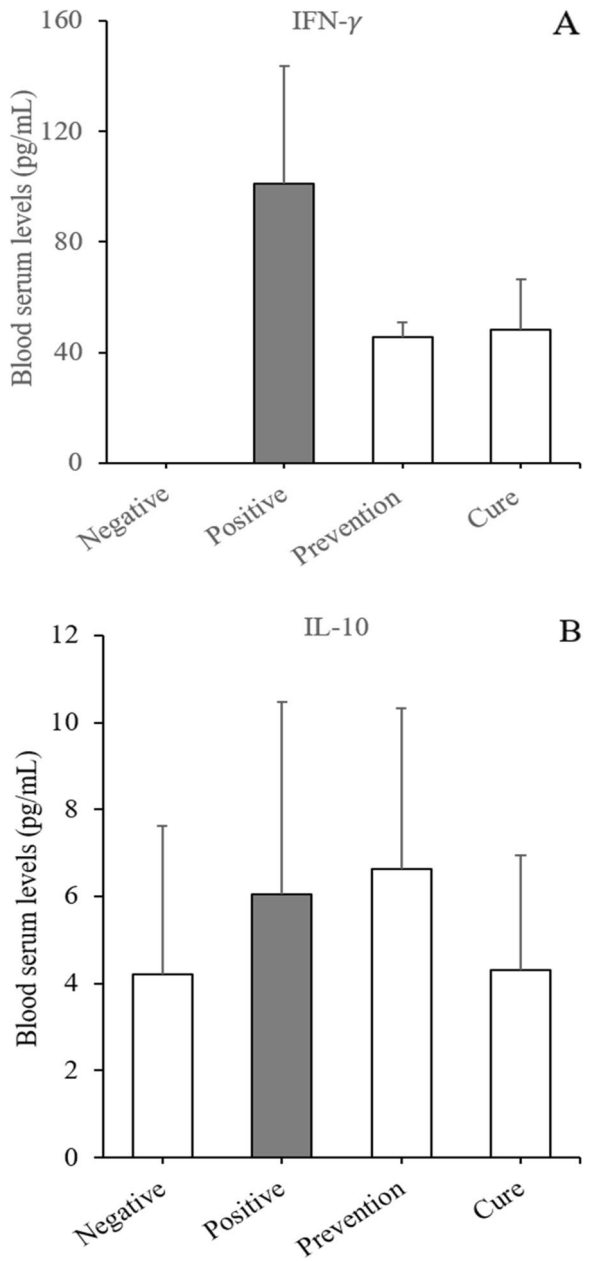


Fig. 4 Cytokine levels in cecal contents of mice with and without *Citrobacter rodentium* infection. **A** Interferon (IFN) gamma levels increased in all mice infected with this bacterium, with the highest levels seen in the positive control group and the medium levels in the two groups that received immobilized *L. plantarum* (**B**) Increases in the levels of interleukin (IL)-10 were observed in the positive control and prevention groups. The results are shown as the mean ± standard deviation

probiotic administration, with no effect on IFN-γ secretion (Mimura et al. 2004). In the present study, administration of probiotics prior to *C. rodentium* infection resulted in higher secretion of a potent anti-inflammatory cytokine IL-10, with its role being essential in preventing excessive pro-inflammation and autoimmune pathogenesis. Nevertheless, we observed elevated IL-10 and IFN-γ levels for the positive group. This finding indicates that IL-10 played a key role in controlling and resolving inflammation in this situation, which contrasts with observations made by Dann et al. (2014) in IL-10 deficient mice infected with *C. rodentium*. However, this finding shows some limitations in predicting inflammation and other autoimmune pathogenesis solely on cytokine production.

Effect of *Lactobacillus plantarum* on cecal short-chain fatty acids production

Although we observed no significant differences in SCFAs between the various treatment groups, the mice in the probiotics group (prevention and cure groups) had numerically higher levels of cecal acetate, propionate, and butyrate concentrations compared to the non-probiotic groups (negative and positive control groups) (Fig. 5A-C). Mice treated with *L. plantarum* either before or after *C. rodentium* infection had a higher acetic acid concentration (6.09 ± 0.65 mmol/L and 6.51 ± 0.21 mmol/L, respectively) compared to both the negative and positive control groups (5.51 ± 0.80 mmol/L and 5.22 ± 0.41 mmol/L, respectively; Fig. 5A). Butyrate, the preferred energy source for colonic epithelial cells, was found in slightly reduced level in the positive control group (0.99 ± 0.30 mmol/L) and negative (1.02 ± 0.12 mmol/L) groups, while the levels were slightly higher in the prevention (1.24 ± 0.02 mmol/L) and cure (1.11 ± 0.10 mmol/L) treatment groups (Fig. 5B). Furthermore, immobilized *L. plantarum*-treated mice showed equivalently higher propionic acid level per cecum after 10 days post-infection in both the prevention and cure group compared to the positive and negative control groups (Fig. 5C). For example, cure (1.27 ± 0.18) and prevention (1.10 ± 0.10) groups had numerically higher propionate levels (mmol/L) than negative (0.94 ± 0.30) and positive (0.85 ± 0.12) groups. Increased amounts of SCFAs have been associated with reduced inflammatory bowel disease and irritable bowel syndrome (Bugaut 1987; Hijova & Chmelarova, 2007). SCFAs also serve as energy for intestinal epithelial cells by modulating colonic and intracellular pH and regulating ion transport, cell proliferation, and cellular differentiation (Hijova & Chmelarova 2007). Their production may be attributed to undigested or unabsorbed dietary carbohydrates in the colon, which could stimulate the absorption of water and electrolytes. Probiotics play an important role in the production of SCFAs. Wang et al. (2014) reported a significant increase in fecal acetate and propionate in three adults after consuming

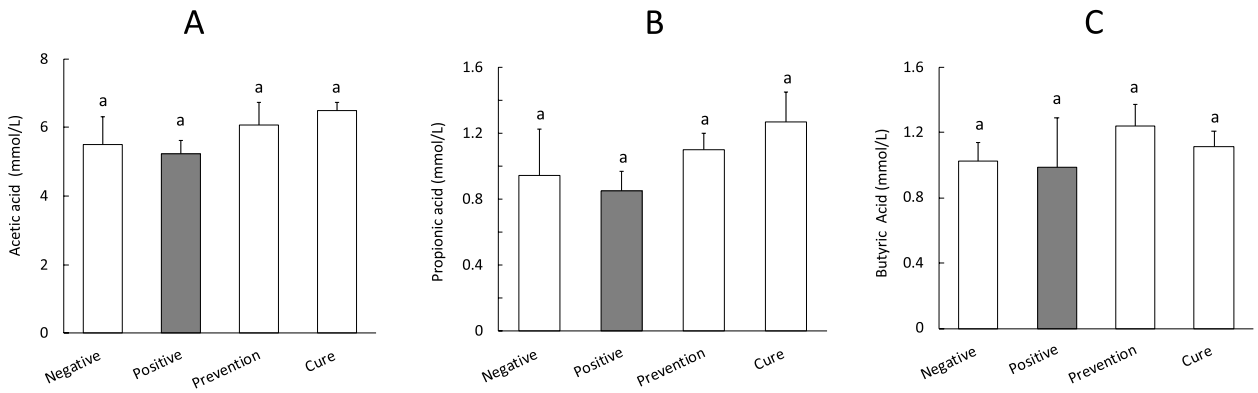


Fig. 5 Short chain fatty acid profiles in the cecal contents of *Citrobacter rodentium*-infected and non-infected mice, some of which were subjected to immobilized *L. plantarum* intervention. Levels of (**A**) Acetic acid; (**B**) Butyric Acid and (**C**) Propionic Acid slightly increased with *Lactobacillus plantarum* intervention. [a]means with the same letters within a particular SCFA are not significantly different ($p < 0.05$)

Lactobacillus plantarum P-8. The quantity of SCFAs generated depends on the diet, especially the type of fibers (de Vos et al. 2022); Liu et al. 2022; Kleigrewe et al. 2022). In our study, the milk constituents in the diet could also play a key role in SCFAs production levels. For example, Li et al. (2022) have documented that milk contains non-esterified or free fatty acids (FA), including SCFA (C2-C5), which are all products of ruminal bacterial activities. The results with SCFAs also demonstrate that despite infection, there was still fermentation of the fermentable fiber components in the *weanimix*. Using the mouse model allowed for very good control of environmental factors, including fermentable dietary fiber, and demonstrated that the protective effects of immobilized probiotics likely had a beneficial effect on gut health beyond the production of SCFAs. All four groups in the study had an equal amount of fiber contained in the *weanimix* (corn kernels, cowpea, and peanuts) and that is likely the reason we did not observe significant differences in the SCFAs. Thus, fiber in the diets and not the *L. plantarum* had the greater effect on production of SCFAs.

Similar results were observed in a study by Wang et al. (2014), during which *L. plantarum* P-8 was consumed by adults of different ages for 4-weeks. Generally, the negative correlation between the probiotic group and small production of SCFAs was not expected, as the probiotic could enhance the levels through intestinal modulation. Nevertheless, the limited production could be strain specific. When comparing the cecal SCFAs levels of the mice in the prevention and cure groups, the cure group produced higher levels of propionate and acetate. In contrast, the prevention group produced higher levels of butyrate. This was also reported by Hemalatha et al. (2017), who recorded higher levels of fecal acetate and propionate in post-intervention children who had experienced diarrhea. Acetate enhances the effects of propionate and butyrate in stimulating the absorption of magnesium and other cations in the colon, which is believed to assist in more efficient fluid absorption and prevention of diarrhea (Scharrer & Lutz 1990). El-Salhy et al. (2020) have reported that fecal SCFAs levels vary between patients with IBS and healthy individuals. SCFAs are produced by gut bacteria using undigested and unabsorbed carbohydrates. Among the SCFAs, butyric acid has anti-inflammatory and intestinal regenerative effects, and it could be beneficial in managing IBS (El-Salhy et al. 2020; Facchin et al. 2020).

Conclusion

In this study, we investigated the ability of *L. plantarum* immobilized on *weanimix* infant cereal to ameliorate *C. rodentium* induced lesions in C57BL/6 weanling mice. The present study demonstrated that *L. plantarum* immobilized on *weanimix* infant cereal reduced colonic damage caused by *C. rodentium* infection in C57BL/6 weanling mice regardless of the time of *L. plantarum* administration (pre- or post- *C. rodentium* infection). Feeding immobilized *L. plantarum* reduced colonic damage and inflammation and numerically increased the concentration of SCFAs (acetate, propionate, and butyrate) in the cecal contents. Therefore, *L. plantarum* immobilized on infant weaning mix reduced diarrhea during weaning. Based on cytokine expression analyses and histopathological data, *L. plantarum* protected mice against *C. rodentium* intestinal inflammation. A better understanding of *L. plantarum* and immobilized *L. plantarum's* role in regulating gut inflammations will facilitate the development of novel therapeutics for maintaining gut health that positively affects public health, especially in underdeveloped nations. The current study is also the first to use the immobilization process with a commonly used weaning mix for children for the delivery of probiotics.

Abbreviations
CR: *Citrobacter rodentium* strain DBS 100 (ATCC 51459); EBA: Ethyl butryic acid; ID: Infection Day; IFN-γ: Interferon-gamma; IL-10: Interleukin 10; LAB: Lactic acid bacteria; LB: Luria Bertani broth; LP: *Lactobacillus plantarum* NCIMB 8826 (ATCC BAA-793); $MCPP_m$: *Weanimix* infant cereal (mixture of maize, cowpea, peanut, and powdered milk); MPA: Meta-phosphoric acid; PI: Post-infection Day; SCFA: Short-chain fatty acid.

Acknowledgements
Emmanuel Kyereh is grateful to the Borlaug Higher Education for Agriculture and Higher Education (BHEARD) program for allowing him to pursue graduate studies at Louisiana State University.

Authors' contributions
SS, EK, and MK designed the study. EK, IL, DC, and AR performed the experiments and obtained the data. EK and IL analyzed and interpreted the results. EK wrote the article and SS and MK made constructive edits. All authors read and approved the final manuscript.

Funding
This work was supported by the Borlaug Leadership Enhancement in Agriculture Program (Borlaug LEAP) and the Borlaug Higher Education for Agriculture and Higher Education (BHEARD) under the CGIAR fund [grant number BFS-G-11-00002] and the predecessor fund, the Food Security and Crisis Mitigation II [grant number EEM-G-00-04-00013].

Availability of data and materials
All data generated during this study are included in this published article. Further details are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
All procedures involved with the animal study were approved by the Louisiana State University Institutional Animal Care and Use Committee (LSU-IACUC protocol number 16-074) and the Inter-Institutional Biological and Recombinant DNA Safety Committee (IBRDSC project reference number 16-018).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 18 April 2022 Accepted: 22 October 2022
Published online: 03 December 2022

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