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Application of Lactobacillus-Derived Bacteriocins to Control Escherichia coli O157:H7 in Food Systems

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ABSTRACT

Escherichia coli O157:H7 is an important foodborne pathogen that can cause serious gastrointestinal illness, and represents a significant public health threat because it has the potential to contaminate many different foods. There is rising interest in the use of bacteriocins produced by lactic acid bacteria as natural bio-preservatives for food, due to a growing demand for safe and natural food preservation methods. The present study was designed to assess and characterize the antimicrobial activity of bacteriocins originating from Lactobacillus acidophilus towards E. coli O157:H7 in different food systems. A controlled, randomized design laboratory-based experimental study was performed between winter 2024 and the beginning of summer 2025. Food matrices included meat (minced beef) and dairy products (pasteurized milk). Samples were inoculated with E. coli O157:H7, which were divided into four groups: negative control; positive control; treatment with crude bacteriocin and treatment with partially purified bacteriocin. The antimicrobial efficacy was determined by quantifying the count of bacteria (log CFU/g or mL) at various time intervals. Bacteriocin treatment resulted in a statistically significant reduction of E. coli O157:H7 counts in both food matrices ($p < 0.001$). In minced meat, the bacterial count decreased from 6.25 log CFU/g in the positive control group to 3.42 log CFU/g following treatment with crude bacteriocin, and further to 2.15 log CFU/g with purified bacteriocin treatment. Dairy products showed a more pronounced reduction, reaching counts of 5.80 log CFU/mL to 2.95 and 1.72 log CFU/mL, respectively. In both food systems, the partially purified form of bacteriocin was more antimicrobial than its crude counterpart. Finally, Lactobacillus acidophilus bacteriocins showed strong inhibition against E. coli O157:H7 in meat and dairy products and purification increased its effect. This discovery emphasizes the potential of bacteriocins as a natural and efficient bio-preservative in food systems which could support better food quality and safety.

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INTRODUCTION

One of the major global public health problem is food safety especially due to the rise in the number of foodborne diseases associated with pathogenic microorganisms. Of these, Escherichia coli O157:H7 is one of the most important foodborne pathogens, leading to critical clinical outcomes such as hemorrhagic colitis and hemolytic uremic syndrome. The pathogen with low infectious dose and high virulence makes it one of the principal threats both in developed and developing countries (Puligundla & Lim, 2022).

Due to frequent association of E. coli O157:H7 outbreaks with contaminated food products (particularly meat, dairy and fresh produce), the disease urgently needs effective control methods (Singha et al., 2023).

Physical and chemical techniques, such as thermal approaches, radiation, and chemical sanitizers, have been routinely used to regulate foodborne pathogens. While these strategies can be efficient, they are also associated with certain limitations, including negative effects on food quality, possible toxicity, and growing consumer pressure for reduced chemical residue (Xu et al., 2025). A growing need for alternatives that are natural and sustainable, further fostering the balance of food safety with nutritional and sensory properties has arisen in recent years. Therefore, the biological control of pathogenic bacteria in food has received much interest as an effective measure (Gaur et al., 2025).

In biological approaches, application of beneficial microorganisms particularly Lactobacillus has become an effective and environmental friendly approach. The lactic acid bacteria (LAB) represent a group of GRAS organisms known to produce an array of antimicrobial products (e.g., organic acids, hydrogen peroxide, and bacteriocins) (Baillo et al., 2023). Bacteriocins are ribosomally synthesized antimicrobial peptides, which can inhibit the growth of wide spectrum of bacteria including foodborne pathogens. In general, they act by disrupting the bacterial cell membrane, causing cell death (Puligundla & Lim, 2022).

Food preservation by bacteriocins has attracted considerable interest because they are quite specific, safe, and non-destructive to food property. Bacteriocins behave unlike the conventional preservatives as they are biodegradable in nature and further do not change the physicochemical properties of the food products. Additionally, their specificity towards antimicrobial activity helps them to act against pathogenic bacteria leaving the beneficial microbiota (Yu et al., 2023). Nisin is one of the most thoroughly examined and frequently used bacteriocins with significant antimicrobial action against a broad spectrum of pathogenic microorganisms, including E. coli O157:H7, specifically when introduced in novel forms such as nano-delivery systems (Elsherif et al., 2024).

Lactobacillus species bacteriocins displayed antimicrobial activity against E. coli O157:H7 in various food matrices in recent studies. The strains that produced bacteriocin exhibited a very effective antimicrobial activity against those in dairy products with vital effects such as fat levels, temperature and the initial levels of bacteria (Öncül & Yıldırım, 2019). Also, the synergistic approach of bacteriocins along with other preservation techniques, including hurdle technology, has been suggested to improve their antimicrobial activity and circumvent problems with resistant Gram-negative bacteria (Puligundla & Lim, 2022).

However, these promising results are faced with significant obstacles to the use of bacteriocins in the food system. This variability is attributed to the specificity of antimicrobial activity for specific food compositions, the problem of binding to some food components, and the requirement to deliver effective antimicrobials and maintain their activities during storage (Darbandi et al., 2021). In addition, E. coli O157:H7 and other Gram-negative bacteria have an outer membrane that limits the penetration of bacteriocins and therefore strategies for improving bacteriocin activity need to be developed (Xu et al., 2025).

Explore Lactobacillus Bacteriocin as bio preservatives for food safety. It assess the efficacy of sedi5ing the E. coli O157:H7 in bacteriocins produced by Lactobacillus species in food systems. It establishes conditions for their applications so that this work could provide insight into the development of safe, sustainable, and efficient strategies to control foodborne pathogens and improve food quality and safety by examining conditions for their antimicrobial activity.

PATIENTS AND METHODS

Study Design and Setting

We conducted this study as a controlled, randomized, laboratory-based experimental study to determine the inhibitory effects of bacteriocins produced by Lactobacillus acidophilus against Escherichia coli O157:H7 in selected food matrices. All experimental work was performed in the microbiology laboratory during the summer 2024 to the early winter 2025.

Bacterial Strains and Culture Conditions

Escherichia coli O157:H7 (ATCC® 43895™) was used as the indicator organism in this study. Bacteriocin producing strain Lactobacillus acidophilus from authenticated microbial culture collection. L. acidophilus isolated from patients was cultured in De Man, Rogosa, and Sharpe (MRS) broth and incubated anaerobically at 37 °C for 24–48 hours. E. coli O157:H7 was cultured in nutrient broth and incubated aerobically at 37 °C for 18–24h before use.

Preparation of Bacteriocin

Bacteriocin Extraction Bacteriocin was extracted from culture of L. acidophilus as per standard procedure. In short, 8000 rpm for 15 minutes followed by collection of cell-free supernatant of the bacterial culture. To remove the antimicrobial effect of organic acids, the pH was neutralized ($6.5 \leq pH \leq 7.0$) with sterile NaOH. The resulting supernatant was filtered with a 0.22 µm membrane filter to yield crude bacteriocin. A 70% saturation of ammonium sulfate precipitation was used for partial purification and resuspended in phosphate-buffered saline (PBS). The baceriocin activity, expressed in arbitrary units per milliliter (AU/mL).

Food Sample Preparation

For this study, two kinds of food matrices were chosen: Meat products (fresh minced beef) and Dairy products (pasteurized milk). Fresh food samples were collected from local markets and transported under aseptic conditions. Each of the samples obtained was split into equal parts (25 g or mL), exposed to UV or treated as necessary to reduce background microbiota, and kept at 4 °C until needed.

Experimental Design and Inoculation

The food samples were randomly allocated to the following groups:

1. Control (−) : Food without bacterial inoculation
2. Control (+) : Food contaminated with E. coli O157:H7 alone.
3. Treatment 1: Pathogen pre-exposed to the crude bacteriocin in a pasteurizer.
4. Treatment 2: Partial formulation of bacteriocin for food + pathogen

E. coli O157:H7 (10^5–10^6 CFU/g or mL, suspended in sterile phosphate-buffered saline (PBS; pH 7.4)) was added to each food sample. Bacteriocin was subsequently used at specific concentrations. Individual samples were stored at two different storing conditions, namely, 4 °C to represent refrigeration and room 25 °C temperature, respectively.

Outcome Measurement (Dependent Variable)

The dependent variable for this study was the count of E. coli O157:H7, and was expressed as colony-forming units per gram or milliliter (CFU/g or CFU/mL).

Microbiological Analysis

Samples were microbiologically assessed at the scheduled points (0, 24, 48, and 72 h). Serial dilutions were made in sterile saline, and were plated in aliquots onto selective media (Sorbitol MacConkey agar supplemented for selective isolation of E. coli O157:H7). Colonies were counted to assess bacterial load after incubating plates aerobically for 24 hours at 37 °C.

Quality Control

To confirm precision and reproducibility, all sha—standard microbiological methodologies were strictly adhered to. For validation, all experiments were done in triplicate using reference strains. That left room for the experiments and also for sterility control, to test for contamination.

Statistical Analysis

Data were analysed using a proper statistical package. For bacterial counts, results are expressed as mean ± standard deviation (SD). Between control and treatment groups, one-way ANOVA with post hoc Tukey test was used. P-value less than 0.05 was considered statistically significant.

Biosafety Considerations

Experimental procedures involving E. coli O157:H7 were conducted at biosafety level 2. All waste was collected according to strict aseptic techniques and disposal in this study.

RESULTS

Substantial differences in the E. coli O157:H7 count occurred among the groups evaluated test samples of ground flesh ($F = 152.6$, $p < 0.001$). It was shown that it the microbiological safety of the experiment for individual population, bacterial grow like as not detected in negative control group and test group. Meanwhile, the control group manifested high numbers of colonies, indicating that minced meat is a good culture for growth and replication of Escherichia coli O157:H7. Bacteriocins obtained from Lactobacillus acidophilus decreased the bacterial count significantly. Although the crude bacteriocin (Treatment I) showed a significant inhibitory effect, it was observed that the partially purified bacteriocin (Treatment II) exhibited a higher reduction in bacterial load. This increased potency is likely due to an increased concentration and activity of antimicrobial peptides post-purification (47.5%) (Table 1, figure 1).

Table 1. Comparison of *E. coli* O157:H7 Count in minced meat among study groups

Groups	E. coli O157:H7 Count	F Test (P value)
Control (-)	0.00 ± 0.00	F = 152.6 (p < 0.001)
Control (+)	6.25 ± 0.18	
Treatment I	3.42 ± 0.25	
Treatment II	2.15 ± 0.20	

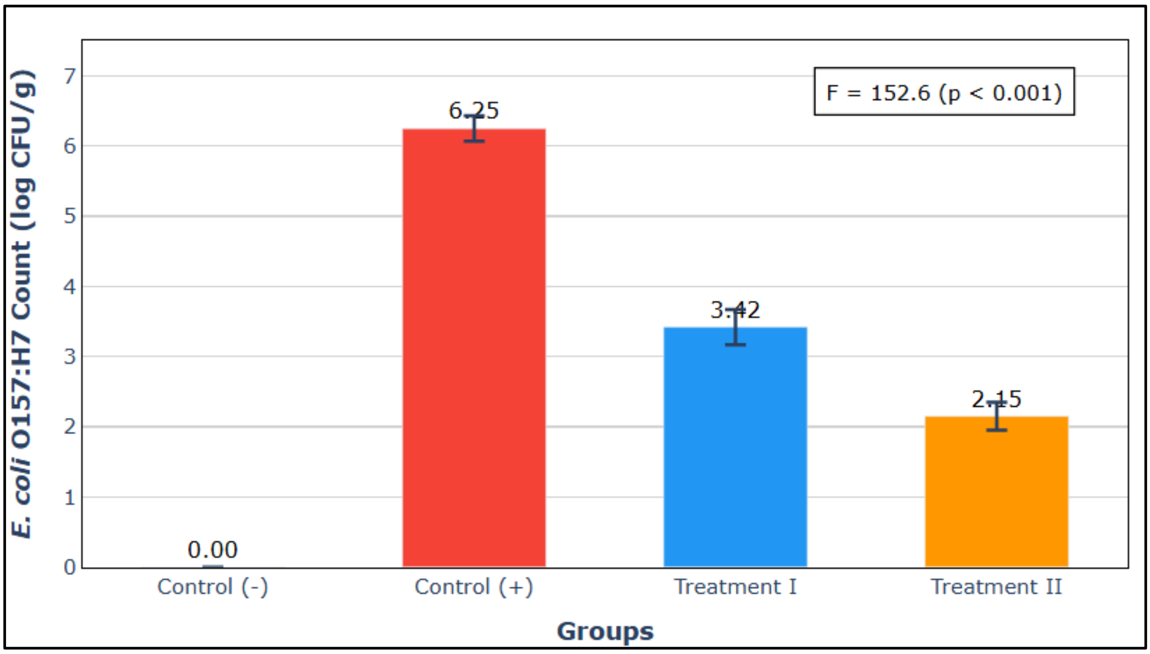

Figure 1. Comparison of *E. coli* O157:H7 Count in minced meat among study groups

Statistically significant difference was observed between the study groups regarding E. coli O157:H7 counts in dairy products (F = 138.4, p < 0.001). No bacterial growth was detected in the negative control group, verifying both no contamination and experiment conditions are trustworthy. On the contrary, even though all of the positive control groups showed a high count of bacteria growing in their dairy product plate which reaffirm that dairy products can enhance the survival and multiplication of the pathogen. Bacteriocins obtained from Lactobacillus acidophilus drastically reduced the bacterial load. Both I: crude bacteriocin and II: partially purified bacteriocin treatment markedly reduced the E. coli O157:H7 at levels, with a more pronounced effect for second variant (Table 2, figure 2).

Table 2. Differences in *E. coli* O157:H7 Count in dairy products among study groups

Groups	E. coli O157:H7 Count	F Test (P value)
Control (-)	0.00 ± 0.00	F = 138.4 (p < 0.001)
Control (+)	5.80 ± 0.22	
Treatment I	2.95 ± 0.27	
Treatment II	1.72 ± 0.19	

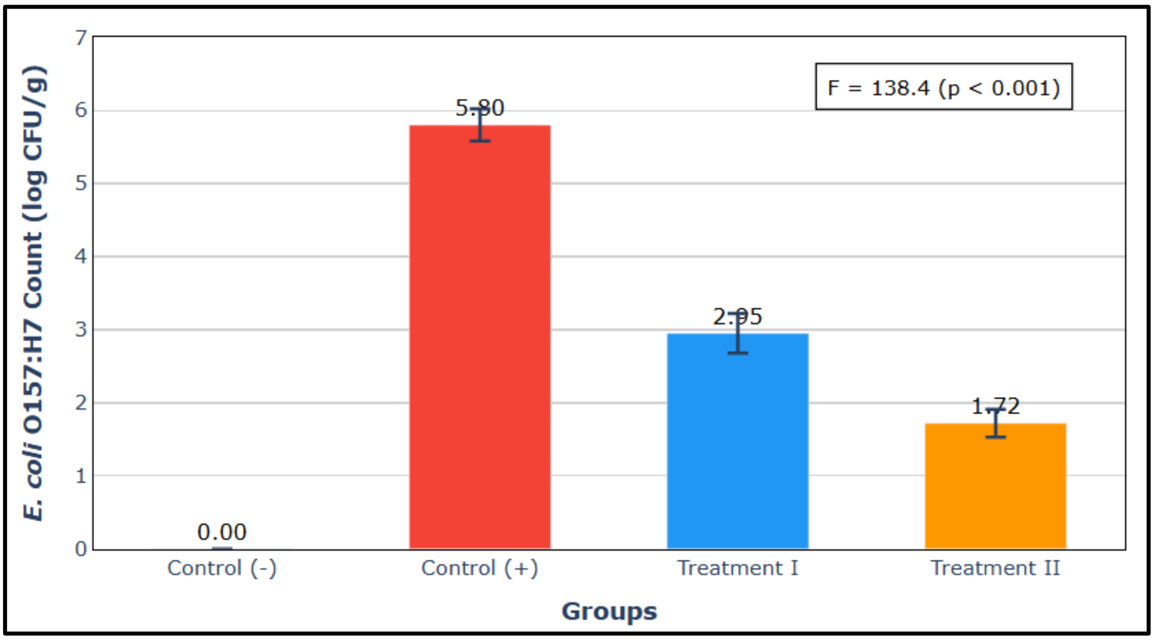

Figure 2. Comparison of *E. coli* O157:H7 Count in dairy products among study groups

Minced meat as well as dairy products were capable of increasing E. coli O157:H7 growth (6.25, 5.80 log CFU/g) Significant bacterial reductions were observed in both media at purified bacteriocin (Lactobacillus acidophilus) levels accounting for a final load of 2.15 log CFU/g in meat and 1.72 log CFU/g in dairy food matrixes respectively. In dairy Products, however, the degree of reduction was much greater reflecting the matrix effect by a slight enhancement in potency. Significance of all treatment effects were confirmed by statistical analysis ($F = 145.5$, $p < 0.001$). Thus purified bacteriocins can be regarded as natural bio-preservatives in food systems with a slight preference towards dairy products.

Table 3. Comparative antibacterial activity of bacteriocins from Lactobacillus acidophilus against E. coli O157:H7 in minced meat versus dairy products

Food Matrix	Positive Control (log CFU/g)	Crude Bacteriocin (Treatment I) (log CFU/g)	Purified Bacteriocin (Treatment II) (log CFU/g)	Total Reduction (log CFU/g)	F Test (P value)
Minced Meat	6.25	3.42	2.15	4.1	145.5 (<0.001)
Dairy Products	5.8	2.95	1.72	4.08	

DISCUSSION

This study approved the antimicrobial activity of Lactobacillus acidophilus bacteriocins produced against Escherichia coli O157:H7 in two importants food matrices (minced meat and dairy products). Results: A significant reduction of the bacterial counts was observed in all treated groups in comparison to positive control (P<0.05), being that for samples treated with partially purified bacteriocines the highest. Findings like these highlight the potential of bacteriocins as powerful natural food antimicrobials. O157:H7. These positive control groups (minced meat and dairy) showed appreciable levels of bacterial counts which implies that these food products were suitable habitats for the proliferation of these bacteria. In agreement with the previous reports that this pathogen is able to grow in protein-rich foods namely beef and milk, constituting an important public health hazard (Puligundla & Lim, 2022). Finally, the lack of growth in the negative control groups provides further evidence that the experimental methods and conditions are reliable and demonstrates that contamination is only due to deliberate inoculation.

The strong decrease in bacterial loads after treatment with bacteriocins further supports the hypothesis that antimicrobial peptides derived from Lactobacillus have potent activity against Gram-negative pathogens. Historically, bacteriocins are expected to be more active against Gram-positive bacteria, but accumulating evidence indicates that they can also suppress the growth of Gram-negative organisms such as E. coli O157:H7 when environmental conditions allow them interaction with the bacterial membrane (Xu et al., 2025). The major part of the mechanism is due to disruption, pore formation and depletion of proton motive force resulting in cell death (Puligundla & Lim, 2022).

In the current study, partially purified bacteriocins were more potent antimicrobials than crude preparations. This observation may be due to the increased concentration and removal of similar compounds in purification, which stabilize and increase the bioactivity of bacteriocin. Additionally, bacteriocins purified from doctor was found to exhibit more potent inhibition of E. coli O157:H7 than crude extract [61]. Furthermore, the purification minimizes interferences of organic acids and others metabolites which positively affect on the specificity of the bacteriocin in relation to crude extract (Öncül & Yıldırım, 2019).

Diverging activity of bacteriocins also was observed between matrices from various foods. For minced meat, there was still a lower but significant reduction in bacterial numbers over dairy products. This might be attributed to with the complicated nature of meat composition (high protein and fat thereof), which has the potential to protect bacterial cells, thereby decreasing the diffusion radius as well as the action of antimicrobial compounds (Lahiri et al., 2022). Unlike other-ripening substrata, dairy products are compositions (eg milk) that have a more homogeneous environment that allows proper distribution of bacteriocins for an effective action. This finding agrees with earlier studies that the composition of food, mainly fat and interactions of proteins affect bacteriocin activity (Öncül & Yıldırım, 2019).

Furthermore, emerging studies indicate the successful application of nisin and other bacteriocins in milk and yogurt systems which confirm this enhanced antimicrobial effect in dairy products. For instance, Elsherif et al. (2024) reported reduction of E.coli O157:H7 in yogurt by nisin and nanoform treatments, confirming the potential berry matrix as carriers for bacteriocin-based preservation strategies. Such results were in agreement with the present study, confirming that the high bacteriocin efficacy may be obtained from liquid or semi-solid food matrices.

Among the most relevant results in this paper is the impressive log decrease of bacteria achieved via bacteriocin treatments that has practical implications for food safety. For foodborne pathogens, a reduction in the range of 2–4 log CFU is generally regarded as meaningful with respect to risk of infection. Results summary from this study describes that bacteriocin action could be introduced as an additional hurdle to shelf life extension in food preservation systems circumferences data up through May 2005, including with the assumption of a possible modes of action thta may permit distinction multiple precedence (Mills et al., 2017). The combination of bacteriocins with other preservation processes can enhance their antimicrobial efficacy and hinder resistance mechanisms in Gram-negative bacteria (Puligundla & Lim, 2022).

Though results are promising, a few caveats remain. Gram-negative bacteria such as E. coli O157:H7 may also be resistant to bacteriocin penetration through their outer membrane barrier. Environmental factors such as pH, temperature and the composition of food may also influence bacteriocin activity. Therefore, further investigation is recommended for the ideal utilization of bacteriocin by using combined approaches, encapsulation processes or other natural antimicrobials (Muthuvelu et al., 2023).

These results contribute to the ongoing research progress in food biopreservation based on the use of natural antimicrobials as a potential safe and sustainable replacement for chemical preservatives. Since bacteriocins, probiotics, and bacteriophages are naturally present in food itself; biological control strategies are gaining more attention as an effective way to control foodborne pathogens without sacrificing the quality of the product (Breijyeh et al., 2020).

CONCLUSION

It has been found that *Lactobacillus acidophilus* bacteriocins had potent antibacterial activity against E. coli O157:H7 in both meat food and dairy products of which the activity was more pronounced when purified aliquots were used and dairy matrices were tested. Our findings provide scientific justification for the proposed application of bacteriocin-producing LAB as natural bio-preservatives in food systems, resulting in improved safety and a reduction of public health hazards.

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