

Zamorano University
Food Science and Technology Department
B.S. in Food Science and Technology



*“Control of *Listeria monocytogenes* on Contact Surfaces Through the Application of *Enterococcus faecium* J19 and *Lactobacillus sakei* L15”*
(Title of the SGP)

Student

Rosa Cristina Pagoada Romero

Advisors

Mindy Brashears Ph.D.

Ligia Luna M.Sc.

Honduras, november 2025

Authorities

KEITH L. ANDREWS

President i.a.

ANA M. MAIER ACOSTA

Vice President and Academic Dean

ADELA ACOSTA MARCHETTI

Director of Food Science and Technology Department

JULIO NAVARRO

Secretary General

Contents

List of Tables	4
Abstract.....	5
Resumen	6
Introduction	7
Methodology.....	11
Experiment location.....	11
Bacterial Strains and Growth Conditions	11
Surface Preparation and Inoculation	12
Lactic Acid Bacteria (LAB) Treatment Application	12
Sampling Procedure	13
Statistical Analysis.....	13
Results and Discussion	14
Reduction of <i>Listeria monocytogenes</i> on Stainless Steel Surfaces.....	14
Reduction of <i>Listeria monocytogenes</i> on Drain Surfaces	15
Conclusions	19
Recommendations	20
References	21

List of Tables

Table 1 Description of treatments applied with <i>Enterococcus faecium</i> J19 and <i>Lactobacillus sakei</i> L15, including dilutions, estimated concentrations, and surface types.	12
Table 2 Mean log CFU/cm ² of <i>Listeria monocytogenes</i> on stainless steel surfaces by treatment and contact time.	15
Table 3 Mean log CFU/cm ² of <i>Listeria monocytogenes</i> on drain surfaces by treatment and contact time.	17

Abstract

This study conducted at Texas Tech University evaluates the antimicrobial effectiveness of probiotic lactic acid bacteria, *Enterococcus Faecium J19* and *Lactobacillus sakei L15*, in controlling *Listeria monocytogenes* on food contact surfaces. Stainless steel and drain-type surfaces were inoculated with a cocktail containing approximately 10^5 CFU/mL of *L. monocytogenes* and subsequently treated with three dilutions (1:10, 1:100, and 1:1000) of each probiotic strain. Surfaces were sampled at 0, 6, and 24 hours post-treatment, and bacterial populations were quantified and expressed as \log_{10} CFU/cm². Both strains significantly reduced *L. monocytogenes* counts, with the greatest reductions observed at higher concentrations. *L. sakei* L15 exhibited sustained antimicrobial activity, particularly on porous drain surfaces, while *E. faecium* J19 achieved rapid reductions but showed slight rebounds over time. Statistical analysis revealed significant interactions among treatments, surfaces, and sampling times ($p < 0.05$). These results demonstrate the potential of LAB-based interventions as biocontrol agents, providing an environmentally friendly alternative or complement to conventional chemical sanitizers in food processing environments.

Keywords: Food-contact safety, LAB-derived bacteriocins, planktonic cell reduction, probiotic antimicrobial strategies, sustainable surface sanitation.

Resumen

Este estudio, realizado en la Universidad Texas Tech , evaluó la efectividad antimicrobiana de las bacterias lácticas probióticas *Enterococcus faecium* J19 y *Lactobacillus sakei* L15 en el control de *Listeria monocytogenes* sobre superficies de contacto alimentario. Las superficies de acero inoxidable y tipo drenaje fueron inoculadas con un cóctel que contenía aproximadamente 10^5 UFC/mL de *L. monocytogenes*, y posteriormente tratadas con tres diluciones (1:10, 1:100 y 1:1000) de cada cepa probiótica. Se tomaron muestras de las superficies a las 0, 6 y 24 horas posteriores al tratamiento, y las poblaciones bacterianas se cuantificaron y expresaron como \log_{10} UFC/cm². Ambas cepas redujeron significativamente los recuentos de *L. monocytogenes*, observándose mayores reducciones en las concentraciones más altas. *L. sakei* L15 mostró una actividad antimicrobiana sostenida, particularmente en superficies porosas tipo drenaje, mientras que *E. faecium* J19 logró reducciones rápidas pero presentó ligeros repuntes con el tiempo. El análisis estadístico reveló interacciones significativas entre tratamientos, superficies y tiempos de muestreo ($p < 0.05$). Estos resultados demuestran el potencial de las intervenciones basadas en BAL (bacterias ácido lácticas) como agentes de biocontrol, proporcionando una alternativa o un complemento ambientalmente amigable a los desinfectantes químicos convencionales en entornos de procesamiento de alimentos.

Palabras clave: Bacteriocinas derivadas de LAB, estrategias antimicrobianas probióticas, reducción de células planctónicas, sanitización sostenible de superficies, seguridad en superficies de contacto alimentario.

Introduction

Listeria monocytogenes, a gram-positive bacterium, is an ubiquitous, intracellular pathogen that has been implicated in the past decade as the causative organism in several outbreaks of foodborne disease (Campos et al., 2025). *L. monocytogenes* is one of the most significant foodborne illnesses in the U.S. and globally. In most cases, even though the disease is expressed as a light fever, it may also appear as a systemic (invasive) illness with severe symptoms and hospitalization, and even death. (Buchanan et al., 2017).

“Ninety-five percent of the total illnesses, hospitalizations, and deaths were estimated to be caused by only 15 pathogens, including *Listeria monocytogenes*, non-typhoidal *Salmonella*, and *Escherichia coli* O157:H (Hoffmann et al., 2015). *L. monocytogenes* can grow from 0 to 45°C with an optimum range of 30-35°C. Nonetheless, it is also known to be able to grow at refrigeration temperatures, which is of concern to food safety. The pH range for the growth of *L. monocytogenes* is 4.1 to 9.6 (Gourama, 2020).

Listeria monocytogenes is considered one of the most impactful foodborne pathogens due to its strong capacity to form biofilms and establish ecological niches within processing environments (Mazaheri et al., 2021). These biofilm structures enhance the microorganism’s ability to adapt and persist in food production settings. Processing facilities are highly vulnerable, as *L. monocytogenes* can be introduced through contaminated raw materials, such as livestock, and subsequently spread throughout the processing environment (Lakicevic et al., 2015). Once established, the pathogen can persist for extended periods by colonizing a wide range of surfaces within factories, including walls, floors, drains, carts, tool cabinets, and door handles, among others (Bolocan et al., 2016).

Over time, inadequate food handling or cooking procedures, breakage of the cold chain, and cross-contamination have been identified as the main drivers of foodborne illnesses (Augustin et al., 2020). Among these factors, cross-contamination has been highly involved in recent years, accounting for up to 91.7% of cases (Londero et al., 2019). Microbial cross-contamination refers to the direct or

indirect transfer of microorganisms (bacteria, viruses, parasites, or fungi) from a contaminated item to a non-contaminated one (Patrignani et al., 2016).

This contamination can happen during any stage of food processing, making the application of control and prevention systems with a global perspective crucial in the food industry (Mazaheri et al., 2021). While different raw and processed foods, such as inadequately pasteurized milk and ready-to-eat (RTE) products containing meat, eggs, and fish, have been identified as major sources of *L. monocytogenes* contamination (“The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010,” 2012), other food products have also been linked with the pathogen due to cross-contamination from industrial surfaces.

The application of lactic acid bacteria (LAB) as a bio-preservation strategy represents a promising alternative for ensuring food safety while minimizing the health risks linked to chemical preservatives and preventing the loss of quality often caused by heat-based processing (Silva et al., 2023). Lactic Acid Bacteria (LAB) are ubiquitously found in nature, a non-spore-forming genus of Gram-positive bacterium, which are mesophilic, psychotropic, and thermophilic. LAB are a large group of lactic acid-producing bacteria, such as *Lactobacilli*, *Lactococci*, *Enterococci*, *Streptococci*, *Leuconostoc*, and *Pediococci*. Lactic acid bacteria (LAB) are excellent candidates for preventing the growth of pathogenic bacteria in food products because they show bacteriostatic effects towards many bacterial species through various mechanisms, without causing unacceptable sensory changes in foodstuffs (Ghanbari et al., 2013).

LAB and LAB metabolites have been reported to prevent the growth of *L. monocytogenes*. The potential anti-listerial mechanisms are the production of bacteriocin and lactic acid, as well as nutrient competition (Pessione, 2012). LAB can produce antimicrobial compounds such as organic acids, hydrogen peroxide, carbon dioxide, oxidation potential, antifungal, and bacterial peptides (Mishra & Lambert, 1996).

The antimicrobial activity of LAB is strongly associated with the production of bacteriocins, ribosomally synthesized peptides with potent inhibitory effects against gram-positive bacteria, including *Listeria monocytogenes* (Saranraj et al., 2013). Among these, enterocins produced by *Enterococcus faecium* and sakacins produced by *Lactobacillus sakei* are well characterized for their stability and effectiveness in food environments (Gao et al., 2019; Saranraj et al., 2013). Their mode of action typically involves pore formation in the cell membrane of target pathogens, nutrient competition, and acidification of the surrounding microenvironment, resulting in bactericidal or bacteriostatic effects (Gao et al., 2019).

In food processing settings, *L. monocytogenes* poses a particular challenge due to its ability to form resilient biofilms on contact surfaces such as stainless steel and floor drains, which are difficult to eradicate with conventional sanitizers (Schöbitz et al., 2014). Recent studies have demonstrated that applying LAB or their bacteriocins directly to contaminated surfaces can significantly reduce *Listeria* populations. For example, biocontrol strategies utilizing *Enterococcus* and *Carnobacterium* bacteriocins embedded in alginate matrices achieved reductions greater than 3 log CFU/cm² within 24 hours, maintaining suppression for several days under refrigeration (Concha-Meyer et al., 2011; Schöbitz et al., 2014).

This study highlights the importance of exploring biological alternatives for controlling *Listeria monocytogenes* on food processing surfaces. Specifically, it evaluates the antimicrobial effect of *Enterococcus faecium* J19 and *Lactobacillus Sakei* L15 as potential protective agents during post-processing stages. By assessing their capacity to reduce *Listeria* contamination under conditions relevant to the food industry, this research provides valuable insights for developing safer and more sustainable control strategies, reducing reliance on conventional chemical disinfectants.

This study aimed to evaluate the antimicrobial potential of lactic acid bacteria strains *Enterococcus faecium* J19 and *Lactobacillus sakei* L15 in inhibiting *Listeria monocytogenes* on stainless steel and drain-type surfaces under simulated food processing conditions, to quantify and compare

the effects on *L.monocytogenes* populations when applying both strains at different concentrations and exposure times, and to assess the potential of implementing *E.faecium* J19 and *L.sakei* L15 as biocontrol agents and viable alternatives to conventional chemical sanitizers for strengthening hygienic practices and enhancing food safety within processing facilities.

Methodology

Experiment Location

The study was developed in the laboratory of the Experimental Science Building 1, Department of Animal and Food Science, at Texas Tech University, Lubbock, Texas, USA.

Bacterial Strains and Growth Conditions

All cultures of this study were stored in an ultra-low -80°C freezer after they were grown for 24 hours, and 10% glycerol was added prior to freezing. Strains were removed from the freezer stock collections at Texas Tech University to begin this experiment. Three strains of *L. monocytogenes* were used in this study: *L. monocytogenes* TTU N1-002, *L. monocytogenes* TTU N1-022. *L. monocytogenes* TTU N1-014 was provided by the International Center for Food Industry Excellence (ICFIE) at Texas Tech University. These *L. monocytogenes* strains were selected based on their association with foodborne outbreaks and relevance to surface contamination in food processing environments. Each *L. monocytogenes* strains were removed from the -80°C freezer and aseptically opened. Each strain was individually streaked onto Brain Heart Infusion (BHI) agar to obtain a well-isolated colony. From the isolated colony, individual strains were grown in 9 mL of Brain Heart Infusion (BHI) broth (Criterion brand) + Yeast Extract and incubated at 37 °C for 24 hours. A cocktail for incubation was prepared by mixing equal volumes (9ml) of each strain of the 24-hour cultures. All 3 strains were vortex mixed, and the concentration achieved was approximately 10⁹ CFU/ml.

Lactic acid bacteria (LAB) strains were prepared similarly. Two strains of Lactic Acid Bacteria (LAB) were removed from the freezer and aseptically opened. The strains J19 (*Enterococcus faecium*) and L15 (*Lactobacillus sakei*) were individually streaked onto De Man, Rogosa, and Sharp (MRS) agar (Criterion Brand) to obtain an isolated colony. From the isolated colony, individual LAB strains were selected and grown in MRS broth (Criterion™ by Hardy Diagnostics), anaerobically, and incubated at 37 °C for 24 hours. After 24-hour incubation, the LAB strains in MRS broth reached a concentration of approximately 10⁸ CFU/mL.

Surface Preparation and Inoculation

Stainless steel slates (100 cm²) and drains (91.52 cm²) were used as representative food contact and drain surfaces. All surfaces were initially cleaned with 70% ethanol, rinsed with sterile distilled water, and sterilized in an autoclave at 121°C for 15 minutes. After cooling, each surface was placed in the biosafety cabinet to maintain aseptic conditions and facilitate bacterial attachment.

A three-strain cocktail of *Listeria monocytogenes* (TTU N1-002, TTU N1-022, TTU N1-014), previously prepared, was used to inoculate surfaces. The inoculation was carried out using a sterile spray bottle to ensure homogenous distribution of the bacterial suspension across the surface area. Each surface was sprayed on one side until visibly wet and left for one hour at room temperature inside the biosafety cabinet to allow bacterial attachment.

Lactic Acid Bacteria (LAB) Treatment Application

After attachment, the treatments were applied using the two LAB strains *Enterococcus faecium* J19 and *Lactobacillus sakei* L15. Both LAB were diluted to achieve a final concentration of 10⁵ CFU/ml. From these base solutions, three initial dilutions were prepared using sterile buffered peptone water (BPW) as diluent. Each dilution was applied to the corresponding coupon for its treatment group using a sterile spray bottle. The control groups for (J19 and L15) were inoculated with *L. monocytogenes* and sprayed only with sterile MRS broth, without LAB. Each surface was inoculated with 1ml of the LAB treatment using the previously made dilutions (Table 1).

Table 1

Description of treatments applied with Enterococcus faecium J19 and Lactobacillus sakei L15, including dilutions, estimated concentrations, and surface types.

Treatment Code	LAB Strain	Dilution	Approx. CFU/mL	Surface Type
Trt1	<i>E. faecium</i> J19	1:10	10 ⁷ CFU/mL	Stainless steel / Drain
Trt2	<i>E. faecium</i> J19	1:100	10 ⁶ CFU/mL	Stainless steel / Drain
Trt3	<i>E. faecium</i> J19	1:1000	10 ⁵ CFU/mL	Stainless steel / Drain
Trt4	<i>L. sakei</i> L15	1:10	10 ⁷ CFU/mL	Stainless steel / Drain
Trt5	<i>L. sakei</i> L15	1:100	10 ⁶ CFU/mL	Stainless steel / Drain
Trt6	<i>L. sakei</i> L15	1:1000	10 ⁵ CFU/mL	Stainless steel / Drain
Control	No LAB (MRS only)	—	—	Stainless steel / Drain

Sampling Procedure

Samples were taken at 0, 6, and 24 hours after LAB application of LAB treatments. At each time point, the entire surface area was swabbed using a sterile cotton swab moistened with 0.1% buffered peptone water (BPW). Swabbing was performed in three directions- horizontal, vertical, and circular- to ensure full surface coverage and maximum recovery. Swabs were then placed into Whirl-pack filter bags containing 9ml of BPW and processed in a Stomacher at 230 rpm for 30 seconds to promote homogeneous bacterial suspension. After the agitation, 1ml of the homogenized solution was transferred into a test tube containing 9ml of BPW, vortexed for 30 seconds, and used to prepare 10^{-1} and 10^{-2} serial dilutions. From each dilution tube, 100 μ L was plated in duplicate onto Modified Oxford Agar (MOX) overlaid with Tryptic Soy Agar (TSA). This MOX+TSA overlay system was used to enhance recovery and visualization of *Listeria monocytogenes* colonies. Plates were incubated at 37°C for 24 hours, after which typical *Listeria* colonies -characterized by black colonies with a black halo due to esculin hydrolysis- were counted. Results were expressed as \log_{10} colony-forming units per square centimeter (\log_{10} CFU/cm²).

Statistical Analysis

The experiment followed a completely randomized design (CRD) with a factorial treatment arrangement of 7 treatments included *E. faecium* J19 (1:10, 1:100, 1:1000), *L.sakei* L15 (1:10, 1:100, 1:1000), and a control (no LAB). Each treatment was applied to stainless steel plates (100 cm²) and - drain-type sections (91.52 cm²). Samples were collected at 0, 6, and 24 hours post-treatment. All conditions were tested in three repetitions for a total of 126 samples. An ANOVA was applied to evaluate the effects of treatment, surface type, sampling time, and their interactions on *Listeria monocytogenes* counts. Least square means (LSMEANS) comparisons were performed for pairwise differences between treatments and timepoints. Duncan's multiple range test was used to separate statistically significant means at $p < 0.05$. All analyses were conducted using SAS software (version 9.4).

Results and Discussion

Reduction of *Listeria monocytogenes* on Stainless Steel Surfaces

The antimicrobial efficacy of probiotic strains *Enterococcus faecium* J19 and *Lactobacillus sakei* L15 was evaluated on stainless steel surfaces inoculated with a 10^5 CFU/mL cocktail of *Listeria monocytogenes*. Each treatment was applied at three dilution levels (1:10, 1:100, 1:1000) and was compared against a control group treated only with sterile MRS broth.

In Table 2, the control showed a moderated reduction from 4.35 ± 0.30 log CFU/cm² at 0 hours to 3.39 ± 0.18 at 24 hours, likely due to passive desiccation or nutrient depletion under static conditions, a phenomenon also reported by (Gao et al., 2019) in similar abiotic surface studies.

The control group showed a gradual reduction in *L. monocytogenes* counts from 4.35 ± 0.3 log CFU/cm² at 0 hours to 3.39 ± 0.18 at 24 hours. This reduction can be attributed to passive desiccation and nutrient depletion under static conditions, as previously reported in similar studies involving abiotic surfaces (Gao et al., 2019).

Among the treatments with *E. faecium* J19, Trt 1 (1:10) achieved the lowest bacterial count at 24 hours (2.42 ± 0.11 log CFU/cm²), followed by Trt3 (1:1000) with 2.86 ± 0.04 log CFU/cm² and Trt 2 (1:100) (2.77 ± 0.16). The antimicrobial effect of J19 was both concentration-dependent and sustained, with no evidence of bacterial rebounds. These findings confirm the short-term and persistent inhibitory action of *E. faecium*, consistent with its known enterocin production and rapid bactericidal effect on gram-positive bacterial pathogens (Dinçer, 2024).

Statistical analysis revealed that Trt1, Trt2, and Trt3 differed significantly from the control and from each other at multiple time points ($p < 0.05$), as indicated by different superscript letters in Table 2. The presence of distinct letters within rows and columns confirms significant differences between treatments and between sampling times.

In contrast, *L. sakei* L15 treatments (Trt4-Trt6) demonstrated even more stable and prolonged reduction. Trt4 (1:10) achieved the lowest final count at 24 hours (2.40 ± 0.07 log CFU/cm²), while Trt5

(1:100) and Trt6 (1:1000) also maintained reductions below the control throughout the experiment, with minimal variation (CV% between 3.51 and 4.52). This sustained effect supports prior evidence that *L. sakei* strains provide strong and persistent anti-*Listeria* activity through sakacin production and organic acid release (Lira et al., 2023; Webb et al., 2022). Moreover, the differences between J19 and L15 treatments highlight strain-specific bacteriocin profiles, as class II a enterocins from *E. faecium* and sakacins from *L. sakei* differ in spectrum and stability (Piras et al., 2025).

Table 2

Mean log CFU/cm² of Listeria monocytogenes on stainless steel surfaces by treatment and contact time.

Treatment	O HR	6 HR	24 HR	CV%	P value
	Mean ± SD	Mean ± SD	Mean ± SD		
Control	4.35 ± 0.3 ^{ABx}	3.84 ± 0.15 ^{Ay}	3.39 ± 0.18 ^{Ay}	6.03	0.0069
Trt 1	4.40 ± 0.29 ^{Ax}	3.47 ± 0.22 ^{By}	2.42 ± 0.11 ^{Cz}	6.62	0.0001
Trt 2	3.99 ± 0.14 ^{BCx}	3.57 ± 0.20 ^{ABx}	2.77 ± 0.16 ^{BCy}	7.79	0.0004
Trt 3	3.64 ± 0.12 ^{CDx}	3.41 ± 0.19 ^{Bx}	2.86 ± 0.04 ^{By}	5.36	0.0011
Trt 4	3.66 ± 0.20 ^{CDx}	3.55 ± 0.10 ^{ABx}	2.40 ± 0.07 ^{Cy}	4.52	<.0001
Trt 5	3.78 ± 0.02 ^{Cx}	2.76 ± 0.15 ^{Cy}	2.56 ± 0.49 ^{BCy}	9.79	0.0050
Trt 6	3.38 ± 0.12 ^{Dx}	2.84 ± 0.14 ^{Cy}	2.75 ± 0.005 ^{BCy}	3.51	0.0006
CV%	5.21	5.43	7.77		
P value	0.0008	0.0002	0.0032		

Note Trt: Treatment, HR: Hour, P value: Probability, CV: coefficient of variation, Different superscript letters (A-D) within the same column indicate statistically significant differences (Duncan, $p < 0.05$) between treatments, Different superscript letters (x-z) within the same row indicate statistically significant difference among times.

Reduction of *Listeria monocytogenes* on Drain Surfaces

In Table 3, the antimicrobial performance of *Enterococcus faecium* J19 and *Lactobacillus sakei* L15 was further evaluated on drain-type surfaces inoculated with a 10^5 CFU/mL cocktail of *Listeria monocytogenes*. Again, each treatment was applied in three dilutions (1:10, 1:100, 1:1000) and compared against a control group receiving sterile MRS broth.

The control group displayed a moderate reduction in counts from 5.52 ± 0.23 log CFU/cm² at 0 hours to 4.64 ± 0.36 CFU/cm² at 24 hours. This is consistent with desiccation and nutrient depletion effects described in static surface studies (Gao et al., 2019).

Among the *E.faecium* J19 Trt1 (1:10), reaching 3.62 ± 0.16 log CFU/cm², followed closely by Trt2 (1:100) with 3.65 ± 0.01 , while Trt3 (1:1000) was less effective, ending at 4.05 ± 0.06 . Contrary to earlier assumptions, no rebound was observed; all treatments resulted in steady reductions over time. Statistical analysis using Duncan's multiple range test confirmed that Trt1 and Trt2 were significantly different ($p < 0.05$) from the control at 24 hours and showed time-dependent variation across sampling points.

Lactobacillus sakei L15 treatments produced more consistent and sustained reductions. Trt4 (1:10) achieved the most pronounced decrease, from 5.20 ± 0.29 at 0 hours to 3.34 ± 0.14 at 24 hours. Trt5 (1:100) and Trt6 (1:1000) also performed well, ending at 3.65 ± 0.11 and 3.82 ± 0.06 , respectively. These reductions were statistically significant and remained stable over the 24-hour period, suggesting superior adaptability of *L. sakei* to porous or rough surfaces.

These findings are in line with previous research indicating that *L.sakei* strains exhibit persistent anti-listerial effects on complex surfaces through bacteriocin production and acidification mechanisms (Lira et al., 2023; Webb et al., 2022). Furthermore, their ability to maintain activity under environmental stress makes them ideal candidates for use in challenging environments such as drains.

Overall, reductions on drain surfaces were lower than those observed on the stainless steel contact surface (Table 3). The reduced efficacy may be explained by the higher roughness around the holes and grooves in the drain materials, which can promote biofilm formation and shield *Listeria* cells from direct exposure to bacteriocins and acids (Buchanan et al., 2017; Smoot & Pierson, 1998). Compared to the smooth surface of the stainless steel, the added pores and grooves in the drains might have harbored bacteria that were not easily accessible to the lactic acid bacteria. During sampling, the malleable nature of the sponge swabs likely recover from the remaining colonies. This

difference in surface design probably contributed to the lower reduction observed in the drains compared to the stainless-steel surfaces.

This observation highlights the importance of targeting high-risk surfaces like drains when implementing LAB-based biocontrol strategies in processing environments.

Table 3

Mean log CFU/cm² of Listeria monocytogenes on drain surfaces by treatment and contact time.

Treatment	O HR	6 HR	24 HR	CV%	P value
	Mean ± SD	Mean ± SD	Mean ± SD		
Control	5.52 ± 0.23 ^{Ax}	4.84 ± 0.09 ^{Ay}	4.64 ± 0.36 ^{Ay}	5.1	0.0134
Trt 1	5.14 ± 0.15 ^{Bx}	4.45 ± 0.31 ^{ABy}	3.62 ± 0.16 ^{CDz}	5.02	0.0005
Trt 2	5.06 ± 0.33 ^{Bx}	4.58 ± 0.27 ^{ABx}	3.65 ± 0.01 ^{CDy}	5.63	0.0013
Trt 3	5.11 ± 0.04 ^{Bx}	4.59 ± 0.09 ^{ABy}	4.05 ± 0.06 ^{Bz}	1.57	0.0001
Trt 4	5.20 ± 0.29 ^{ABx}	4.24 ± 0.15 ^{By}	3.34 ± 0.14 ^{Dz}	7.6	0.0012
Trt 5	4.84 ± 0.13 ^{Bx}	4.18 ± 0.20 ^{By}	3.65 ± 0.11 ^{CDz}	3.78	0.0003
Trt 6	4.89 ± 0.36 ^{Bx}	4.28 ± 0.22 ^{By}	3.82 ± 0.06 ^{BCy}	5.77	0.0058
CV%	3.65	5.92	4.63		
P value	0.0081	0.1215	<0.0001		

Note Trt: Treatment, HR: Hour, P value: Probability, CV: coefficient of variation Different superscript letters (A-D) within the same column indicates statistically significant differences (Duncan, $p < 0.05$) between treatments. Different superscript letters (x-z) within the same row indicate statistically significant differences among times.

The project aimed to evaluate the efficacy of *Enterococcus faecium* J19 and *Lactobacillus sakei* L15 in controlling *Listeria monocytogenes* on stainless steel and drain surfaces under conditions simulating a food processing environment. Overall, both probiotic strains significantly reduced *Listeria* counts, especially at higher concentrations (1:10) and after 24 hours of exposure. These results are relevant for food safety management since *Listeria* persistence on processing surfaces represents a major hazard for ready-to-eat foods (Buchanan et al., 2017).

When comparing both surface types, reductions were consistently more pronounced on stainless steel than on drain-type surfaces (Tables 2 and 3). This difference highlights the impact of

surface topography, with smoother surfaces facilitating more effective LAB-mediated biocontrol, likely due to reduced microbial adhesion and biofilm protection (Smoot & Pierson, 1998).

E. faecium J19 treatments demonstrated significant reductions on both surfaces. On stainless steel, the 1:10 dilution (Trt1) reduced counts to 2.42 ± 0.11 log CFU/cm² at 24 hours, while on drains it reached 3.62 ± 0.16 . *L. sakei* L15, on the other hand, showed greater and more stable suppression, achieving the lowest final count of 2.40 ± 0.07 on stainless steel (Trt4) and 3.34 ± 0.14 on drains (also Trt4). Unlike previous assumptions, no rebound effects were observed in either strain across the 24-hour period, confirming the sustained antimicrobial effect of LAB under the tested conditions.

These differences are likely attributed to the characteristics of their bacteriocins and acidification profiles: class IIa enterocins produced by *E. faecium* act rapidly but may be less stable under environmental stress, while sakacins from *L. sakei* are more resistant to pH and temperature variations, ensuring longer-lasting effects (Piras et al., 2025; Webb et al., 2022).

The stability of LAB-derived bacteriocins under acid and thermal conditions is a valuable property for industrial application, as it ensures continued antimicrobial activity even after cleaning cycles (Lira et al., 2023). Moreover, lactic acid bacteria isolated from food products are generally considered safe, with no virulence genes detected and minimal concern regarding antibiotic resistance (Keter et al., 2022). Incorporating these probiotic strains into routine sanitation protocols could offer an additional biological barrier against *Listeria monocytogenes*, especially when used alongside conventional chemical or physical cleaning agents.

Conclusions

Enterococcus faecium J19 and *Lactobacillus sakei* L15 demonstrated effective antimicrobial activity in reducing *Listeria monocytogenes* populations on stainless steel and drain-type surfaces under simulated food processing conditions, confirming their potential as biocontrol agents.

Treatments with higher concentrations (1:10) showed the most immediate and substantial antimicrobial effect, while lower dilutions maintained moderate effectiveness over 24 hours.

L. sakei L15 treatments were particularly effective in maintaining sustained reductions of *L. monocytogenes* on porous drain-type surfaces, suggesting their potential utility in complex industrial environments.

Overall, probiotic lactic acid bacteria present a promising, environmentally friendly alternative or complementary strategy to traditional chemical sanitizers for controlling pathogenic microorganisms on food-contact surfaces.

Recommendations

Additional studies are recommended to evaluate the effects on varying environmental conditions, such as humidity, organic matter presence, and temperature, on the efficacy of probiotic treatments.

Future research should explore potential synergies between *Enterococcus faecium* J19, *Lactobacillus sakei* L15, and conventional chemical disinfectants to develop integrated sanitation protocols.

Pilot studies should be conducted in commercial processing plants to validate laboratory findings under real-world conditions, evaluating both the practicality and cost-effectiveness of probiotic application methods.

References

- Augustin, J.-C., Kooh, P., Bayeux, T., Guillier, L., Meyer, T., Jourdan-Da Silva, N., Villena, I., Sanaa, M., Cerf, O., & On, B. (2020). Contribution of Foods and Poor Food-Handling Practices to the Burden of Foodborne Infectious Diseases in France. *Foods (Basel, Switzerland)*, *9*(11). <https://doi.org/10.3390/foods9111644>
- Bolocan, A. S., Nicolau, A. I., Alvarez-Ordóñez, A., Borda, D., Oniciuc, E. A., Stessl, B., Gurgu, L., Wagner, M., & Jordan, K. (2016). Dynamics of *Listeria monocytogenes* colonisation in a newly-opened meat processing facility. *Meat Science*, *113*, 26–34. <https://doi.org/10.1016/j.meatsci.2015.10.016>
- Buchanan, R. L., Gorris, L. G., Hayman, M. M., Jackson, T. C., & Whiting, R. C. (2017). A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control*, *75*, 1–13. <https://doi.org/10.1016/j.foodcont.2016.12.016>
- Campos, D. L., Perdomo, A., Nightingale, K., Franco, J., Jimenez, L., & Brashears, M. M. (2025). Lactic Acid Bacteria Synergy: Electrostatic Spraying, Dipping, and Formulation Applications of *Lactobacillus salivarius* L28 and *Enterococcus faecium* J19 to Enhance Cheese Safety Against *Listeria monocytogenes*. *Journal of Food Protection*, *88*(6), 100507. <https://doi.org/10.1016/j.jfp.2025.100507>
- Concha-Meyer, A., Schöbitz, R., Brito, C., & Fuentes, R. (2011). Lactic acid bacteria in an alginate film inhibit *Listeria monocytogenes* growth on smoked salmon. *Food Control*, *22*(3-4), 485–489. <https://doi.org/10.1016/j.foodcont.2010.09.032>
- Dinçer, E. (2024). Impact of lactic acid bacteria strains against *Listeria monocytogenes* biofilms on various food-contact surfaces. *Archives of Microbiology*, *206*(2), 80. <https://doi.org/10.1007/s00203-023-03811-6>
- The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Foodborne Outbreaks in 2010 (2012). *EFSA Journal*, *10*(3). <https://doi.org/10.2903/j.efsa.2012.2597>
- Gao, Z., Daliri, E. B.-M., Wang, J., Liu, D., Chen, S., Ye, X., & Ding, T. (2019). Inhibitory Effect of Lactic Acid Bacteria on Foodborne Pathogens: A Review. *Journal of Food Protection*, *82*(3), 441–453. <https://doi.org/10.4315/0362-028X.JFP-18-303>
- Ghanbari, M., Jami, M., Domig, K. J., & Kneifel, W. (2013). Seafood biopreservation by lactic acid bacteria – A review. *LWT - Food Science and Technology*, *54*(2), 315–324. <https://doi.org/10.1016/j.lwt.2013.05.039>
- Gourama, H. (2020). Foodborne Pathogens. In A. Demirci, H. Feng, & K. Krishnamurthy (Eds.), *Food Engineering Series. Food Safety Engineering* (pp. 25–49). Springer International Publishing. https://doi.org/10.1007/978-3-030-42660-6_2
- Hoffmann, S., Bryan Macculloch, & Michael Batz (2015). Economic Burden of Major Foodborne Illnesses Acquired in the United States. United States Department of Agriculture, Economic Research Service. www.ers.usda.gov/publications/eib-economic-information-bulletin/eib140
- Keter, M. T., El Halfawy, N. M., & El-Naggar, M. Y. (2022). Incidence of virulence determinants and antibiotic resistance in lactic acid bacteria isolated from food products. *Future Microbiology*, *17*, 325–337. <https://doi.org/10.2217/fmb-2021-0053>

- Lakicevic, B., Nastasijevic, I., & Raseta, M. (2015). Sources of *Listeria Monocytogenes* Contamination in Retail Establishments. *Procedia Food Science*, 5, 160–163. <https://doi.org/10.1016/j.profoo.2015.09.046>
- Lira, F. M. de, Tanaka, F. Y. R., Rios, E. A., Carrilho, S. M., Abreu, S. S. de, Ferreira, G. F., Gonzaga, N., Pereira, U. d. P., Tamanini, R., Fagnani, R., & Beloti, V. (2023). Identification of lactic acid bacteria with anti-listeria activity. Characterization and application of a bacteriocinogenic strain in the control of *Listeria monocytogenes* in cheese. *The Journal of Dairy Research*, 90(3), 318–323. <https://doi.org/10.1017/S0022029923000584>
- Londero, A., Costa, M., Galli, L., Brusa, V., Linares, L., Prieto, M., & Leotta, G. (2019). Characterization and subtyping of *Listeria monocytogenes* strains from butcher shops. *LWT - Food Science and Technology*, 113, 108363. <https://doi.org/10.1016/j.lwt.2019.108363>
- Mazaheri, T., Cervantes-Huamán, B. R. H., Bermúdez-Capdevila, M., Ripolles-Avila, C., & Rodríguez-Jerez, J. J. (2021). *Listeria monocytogenes* Biofilms in the Food Industry: Is the Current Hygiene Program Sufficient to Combat the Persistence of the Pathogen? *Microorganisms*, 9(1). <https://doi.org/10.3390/microorganisms9010181>
- Mishra, C., & Lambert, J. (1996). Production of anti-microbial substances by probiotics. *Asia Pacific Journal of Clinical Nutrition*, 5(1), 20–24.
- Patrignani, F., Siroli, L., Gardini, F., & Lanciotti, R. (2016). Contribution of Two Different Packaging Material to Microbial Contamination of Peaches: Implications in Their Microbiological Quality. *Frontiers in Microbiology*, 7, 938. <https://doi.org/10.3389/fmicb.2016.00938>
- Pessione, E. (2012). Lactic acid bacteria contribution to gut microbiota complexity: Lights and shadows. *Frontiers in Cellular and Infection Microbiology*, 2, 86. <https://doi.org/10.3389/fcimb.2012.00086>
- Piras, C., Soggiu, A., Greco, V., Di Ciccio, P. A., Bonizzi, L., Procopio, A. C., Urbani, A., & Roncada, P. (2025). Lactic Acid Bacteria (LAB) and Their Bacteriocins for Applications in Food Safety Against *Listeria monocytogenes*. *Antibiotics (Basel, Switzerland)*, 14(6). <https://doi.org/10.3390/antibiotics14060572>
- Saranraj, P., Naidu, M., & Sivasakthivelan, P. (2013). Lactic Acid Bacteria and its Antimicrobial Properties: A Review. *International Journal of Pharmaceutical & Biological Archives*, 4(6), 1124–1133. https://www.researchgate.net/profile/Saranraj-P/publication/261366482_Lactic_Acid_Bacteria_and_its_Antimicrobial_Properties_A_Review/links/5a28e93fa6fdcc8e8671cd77/Lactic-Acid-Bacteria-and-its-Antimicrobial-Properties-A-Review.pdf
- Schöbitz, R., González, C., Villarreal, K., Horzella, M., Nahuelquín, Y., & Fuentes, R. (2014). A biocontroller to eliminate *Listeria monocytogenes* from the food processing environment. *Food Control*, 36(1), 217–223. <https://doi.org/10.1016/j.foodcont.2013.08.017>
- Silva, B. N., Teixeira, J. A., Cadavez, V., & Gonzales-Barron, U. (2023). Mild Heat Treatment and Biopreservatives for Artisanal Raw Milk Cheeses: Reducing Microbial Spoilage and Extending Shelf-Life through Thermisation, Plant Extracts and Lactic Acid Bacteria. *Foods (Basel, Switzerland)*, 12(17). <https://doi.org/10.3390/foods12173206>

- Smoot, L. M., & Pierson, M. D. (1998). Effect of environmental stress on the ability of *Listeria monocytogenes* Scott A to attach to food contact surfaces. *Journal of Food Protection*, *61*(10), 1293–1298. <https://doi.org/10.4315/0362-028x-61.10.1293>
- Webb, L., Ma, L., & Lu, X. (2022). Impact of lactic acid bacteria on the control of *Listeria monocytogenes* in ready-to-eat foods. *Food Quality and Safety*, *6*, Article fyac045. <https://doi.org/10.1093/fqsafe/fyac045>