Food Biology 2025, 14: 1-5
doi: 10.25081/fb.2025.v14.9224
https://updatepublishing.com/journal/index.php/fb

Research Article

ISSN: 2249-4790

Antibacterial activity of *Lactobacillus* species against some pathogenic and food spoilage bacteria

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ABSTRACT

The present study was conducted to examine the antimicrobial activity of different *Lactobacillus* species against pathogenic and food spoilage bacteria. Four species of *Lactobacillus* were isolated from homemade cheese, raw milk, dosa paste, sauce, and curd. *Lactobacillus* species isolated from the sampled food source include; *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, and *Lactobacillus casei*. The antimicrobial activity of *L. plantarum* (P2), *L. brevis*, *L. plantarum* (P1), *L. casei*, and *L. fermentum* was investigated against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus cereus* using well diffusion method of the indicator isolate. The results reveal that the *L. plantarum* (P2) has highest inhibition zones towards *E. coli* (25.7±1.35 mm), *S. aureus* (27.6±0.94 mm), *P. aeruginosa* (24.4±1.05 mm), and *B. cereus* (04.2±0.17 mm). The lowest zones of inhibition towards the sampled pathogenic and food spoilage bacteria were reported by *L. casei*. Our results confirm that the *L. plantarum* (P2), *L. brevis*, *L. plantarum* (P1), *L. casei*, and *L. fermentum* had efficient antimicrobial inhibitory effect on *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. cereus*. Thus these *Lactobacillus species* could be effectively used for the protection of food from different pathogenic and food Spoilage bacteria.

KEYWORDS: Lactic Acid Bacteria (LAB), Pathogenic and food spoilage bacteria, Antimicrobial activity

Received: October 11, 2024
Revised: December 14, 2024
Accepted: December 14, 2024
Published: February 07, 2025

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INTRODUCTION

Lactic Acid Bacteria (LAB) also known as lactobacillales belong to the order of gram-positive bacteria showing characteristics of acid tolerance, rigorously fermentative, non-respiring, non-sporulative bacteria with rod/or spherical in shape. They favour anaerobic conditions and are deficient in cytochromes. They typically produce lactic acid and are non-sporulating in nature, and non-moving bacteria. LAB has the ability to ferment carbohydrates into lactic acid, this property of LAB has made wide utilization food industry. *Aerococcus*, *Streptococcus*, *Lauconostoc*, *Enterococcus*, *Pediococcus*, *Lactobacillus*, *Corynebacterium*, and *Vagococcus* are a few examples of lactic acid bacteria species that have adapted to grow in a variety of environmental conditions. They can be discovered on certain plant surfaces, in soil, dairy products, shellfish, and certain animal digestive tracts (Gatesoupe, 1998). Although LAB does not make up the majority of the species in the normal gut microbiota, numerous efforts have been carried out to artificially increase their dominance (Verschuere *et al.*, 2000). Depending on how they break down carbohydrates, LAB is divided into two groups. The homo-fermentative group uses the Embden-Meyerhof-Parnas (glycolytic) pathway to transform a carbon source mostly into lactic acid. By using the phosphoketolase route, hetero-fermentative bacteria can use glucose to create equimolar levels of lactate, CO_2, ethanol, or acetate. *Lactococcus*, *Enterococcus*, *Pediococcus*, and *Streptococcus* are members of the homo-fermentative group. Vasiljevic and Shah (2008) reported that *Leuconostoc* and *Weisella* are examples of hetero-fermentative organisms.

Maintaining food safety and quality is a crucial challenge faced by the food industry. Pathogenic and spoilage bacteria can have a significant impact on the safety and shelf-life of food products, leading to potential health risks and economic losses (Thongphichai *et al.*, 2023). Food spoilage is a major issue in the food supply chain, as food products continuously undergo changes in quality as they move from farms to processors, retailers, and consumers. It is estimated that up to one-third of all food produced for human consumption is wasted each year due to spoilage (He *et al.*, 2018). Pathogenic bacteria, such as *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella spp.*, can contaminate food at various stages of production, processing, storage, and transportation, leading to potential food borne illness outbreaks (Thongphichai *et al.*, 2023). Additionally, some bacteria and fungi can produce toxins, contributing to chemical and biological food poisoning.

Lactic Acid Bacteria (LAB) have been intensively used in food preservation and research across the globe has reported that LAB stimulates the nutrient level of different fermented foods and its derived products (Gad *et al.*, 2016; Raman *et al.*, 2022). LAB improves the flavor, taste, texture, and shelf-life of different products (Korcari *et al.*, 2021). The LAB creates lactic acid, which makes the environment more acidic and decreases the number of pathogenic microorganisms. LAB has the ability to create organic acids, hydrogen peroxide, diacetyl, and bacteriocins which are some of the antibacterial substances. These substances are known to lessen food deterioration and the growth or proliferation of harmful bacteria. As a result, the food industry has begun to pay more attention to the utilization of these naturally occurring chemicals as food bio-preservative agents, which currently offers a viable alternative to chemical food preservation, particularly for ready-to-use products (Uškoviće *et al.*, 2010; Lappa *et al.*, 2022). Bacteriocins could also be used to prepare products that aren't being sufficiently thermally sterilized at the time of their production, as there is a risk of product becoming contaminated with pathogenic microorganisms like *Listeria monocytogenes*, which has been linked to numerous outbreaks around the world (O'Sullivan *et al.*, 2002; Arbulu *et al.*, 2022). Keeping in view the role of LAB the present study was conducted to evaluate the antimicrobial role of different species of *Lactobacillus* towards different pathogenic and food spoilage bacteria.

MATERIALS AND METHODS

Isolation and Identification of LAB

Homemade cheese, raw milk, dosa paste, sauce, curd, was serially diluted and spread on MRS agar medium. Plates were incubated anaerobically for 24-48 hrs at 30 °C. When required for the isolation of specific bacteria and incubated aerobically and anaerobically at various temperatures. A total of 13 isolates of bacterial species, namely *Aeromonas*, *Bacillus*, *Clostridium*, *Escherichia*, *Klebsiella*, *Listeria*, *Salmonella*, *Streptococcus*, *Staphylococcus*, *Enterobacter*, *Enterococcus*, and *Lactobacillus* were isolated from the sampled dairy products. On the basis of cultural, biochemical, physiological and morphological identification the *Lactobacillus* species were identified. Four species of *Lactobacillus* species were identified they include; *Lactobacillus plantarum* (P2), *Lactobacillus brevis*, *Lactobacillus plantarum* (P1), *Lactobacillus casei*, and *Lactobacillu fermentum*.

Bacterial Culture and Growth Conditions

Four bacterial strains *Escherichia coli* (Gram negative), *Staphylococcus aureus* (Gram positive), *Pseudomonas aeruginosa* (Gram Negative), and *Bacillus cereus* (Gram positive) were obtained from the American type culture collection. In this study 2-3 isolates of each of these bacterial pathogens were sub-cultured onto four different media (Nutrient Agar media, Mueller Hinton agar, Tryptone Soya Broth and Potato dextrose agar, obtained from Hi-Media laboratory Ltd, Mumbai, India) under aerobic conditions for 24 hours before testing. These four bacterial strains serve as the pathogenic and food spoilage bacteria for antimicrobial assay.

Antimicrobial Activity of LAB

The antimicrobial activity of *L. plantarum* (P2), *L. brevis*, *L. plantarum* (P1), *L. casei*, and *L. fermentum* was investigated against *E. coli*, *S. aureus*, *P. aeruginosa* and *B. cereus* using well diffusion method of the indicator isolate. For this method purpose cell free filtrate is obtained by centrifugation by adding 10 M solution of NaOH for neutralizing bacterial extract which remove the effect of organic acids. The produced extract was filtered on sterile Milli pore filters. The collected pathogenic bacteria were then incubated in peptone water at 370 °C for 24 hrs. After 24 hrs of incubation, 0.1 mL of each pathogenic bacteria was inoculated into Petri dishes containing 20 mL of Muller Hinton Agar. After the inoculation of each pathogenic bacteria strain the Petri dishes were left for an hour in the refrigerator. After these four wells were made in the obtained involution, these wells were filled with the isolated Lactobacillus bacteria species and these Petri dishes were incubated at 370 °C for 24 hrs. After 24 hrs the diameter of the inhibition zone by each Lactobacillus species towards the pathogenic bacteria species was measured in mm. Then the antimicrobial activity was determined by measuring the clear zones around the wells.

Statistical Analysis

Data was subjected to analyses of variance (ANOVA) using XLSTAT software. Treatment means were compared using the Duncan Multiple Range Test at ($p \leq 0.05$) level of significance using XLSTAT software (Duncan, 1955).

RESULTS

Lactobacillus is a genus of Gram-positive, non-spore-forming, rod-shaped bacteria that are commonly found in various fermented food products and the gastrointestinal tract of humans and animals. This genus is the largest within the group of lactic acid bacteria and currently contains over 200 recognized species. The present study was conducted to determine the antimicrobial activities of *Lactobacillus species* against the pathogenic and food spoilage bacteria. Four species of *Lactobacillus* were isolated from homemade cheese, raw milk, dosa paste, sauce, and curd. *Lactobacillus species* isolated from the sampled food source includes *L. brevis*, *L. plantarum*, *L. fermentum*, and *L. casei*. These species are widely used in the food industry and as probiotics due to their beneficial properties. Table 1 and Table 2 shows the morphological, physiological and biochemical attributes of L. *brevis*, *L. plantarum*, *L. fermentum*, and *L. casei* isolated from cheese, raw milk, dosa paste, sauce, and curd. *Lactobacillus brevis* is a facultative anaerobic, hetero-fermentative species that is commonly found in fermented foods, such as beer, wine, and vegetables. It is a Gram-positive, non-spore-forming, rod-shaped bacterium that can occur singly, in pairs, or in short chains. The cells typically measure 0.7-1.0 μm in width and 2.0-4.0 μm in length, with rounded ends. *L. plantarum* is a Gram-positive, non-motile, non-spore-

Table 1: Morphological characteristics of the bacterial strain obtained from different sources

Strain code	Cell's form	Type	Colour	Motility test	Gram staining
L. brevis	Rod shaped	Bacilli	Yellow	Non-motile	Gram positive
L. plantarum	Slender rods	Coccobacilli	Yellow	Non-Motile	Gram positive
L. fermentum	Rod Shaped	Cocci	Creamy White	Non-Motile	Gram positive
L. casei	Rod-shaped	Smooth	Opaque with pigment	Non-Motile	Gram positive

Table 2: Physiological and Biochemical characteristics of Isolated *Lactobacilli*

Characteristics	Raw milk	Curd	Cheeses	Sauce	Dosa Batter
Species	*L. plantarum* P1	*L. plantarum* P2	*L. casai*	*L. brevis*	*L. fermentum*
Morphology	Cream colony Gram+ve Rod	Cream Colony Gram+ve Rod	Cream Colony Gram+ve Rod	White colony Gram+ve Rod	White colony Gram+ve Rod
15°C only	+	+	+	+	-
45°C only	-	-	-	-	-
15 and 45°C only	+	+	+	+	+
Acid and gas from glucose	-	-	-	+	+
NH_3 from Arginine	-	-	-	+	+
Arabinose	-	-	+	+	+
Cellobiose	+	+	-	-	+
Mannitol	+	+	-	-	+
Mannose	+	+	+	-	+
Melebiose	+	+	-	-	+
Raffinose	+	+	+	-	+
Ribose	+	+	+	+	+
Salicin	+	+	+	-	+
Lactose	-	-	-	+	+
Rhamnose	-	-	-	-	-
Sorbitol	-	-	-	-	+
Xylose	-	-	-	+	+
Trehalose	-	-	-	-_	+

forming, micro-aerophilic, and mesophilic bacterium. The cells are straight rods with rounded ends, measuring 0.9-1.2 μm in width and 3.0-8.0 μm in length, and can occur singly, in pairs, or in short chains. Some strains of *L. plantarum* can possess true catalase and manganese-containing pseudo-catalase activities, as well as nitrate- and hematin-dependent nitrite reductases. *L. fermentum* is a Gram-positive, non-spore-forming, rod-shaped bacterium that is commonly found in fermented foods and the human gastrointestinal tract. The cells are typically 0.5-0.8 μm wide and 2.0-4.0 μm long, with rounded ends, and can occur singly, in pairs, or in short chains. *L. casei* is a Gram-positive, non-spore-forming, rod-shaped bacterium that is widely used in the production of fermented dairy products and as a probiotic. The cells are typically 0.5-0.8 μm wide and 1.0-10.0 μm long, with rounded ends, and can occur singly, in pairs, or in short chains.

LAB produces different metabolites that have the ability to restrict the growth of different pathogenic and food spoilage bacteria. Some species of LAB have good ability of antimicrobial characteristics against pathogenic and food spoilage bacteria. In the present study four different bacterial strains of lactobacillus species viz., *L. plantarum* (P2), *L. brevis*, *L. plantarum* (P1), *L. casei*, and *L. fermentum* were tested for their antimicrobial activity towards four pathogenic and food spoilage bacteria *viz.*, *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. cereus*. On the basis of the inhibition zones of *L. plantarum* (P2), *L. brevis*, *L. plantarum* (P1), *L. casei*, and *L. fermentum* towards *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. cereus* the antimicrobial activity of LAB was detected (Figure 1).

Table 3 shows the detail of inhibition zones of different diameters of different LABs towards selected pathogenic and food spoilage bacteria. It could be reported from the table that the *L. plantarum* (P2) has highest inhibition zones towards *E. coli* (25.7±1.35 mm), *S. aureus* (27.6±0.94 mm), *P. aeruginosa* (24.4±1.05 mm), and *B. cereus* (04.2±0.17 mm). The lowest zones of inhibition towards the sampled pathogenic and food spoilage bacteria were reported by *L. casei*.

In order to determine the level of significance for the antimicrobial activity of *Lactobacillus species* against some pathogenic and food spoilage bacteria analysis of variance (ANOVA) was subjected. Table 4 shows the summary of ANOVA. It can be observed that a significant level ($p = 0.0009$) difference in zones of inhibition was observed across different pathogenic and food spoilage bacteria. It was also observed that a significant level ($p = 0.011$) difference in zones of inhibition was reported by *Lactobacillus species* for their antimicrobial activity. Our results confirm that the *L. plantarum* (P2), *L. brevis*, *L. plantarum* (P1), *L. casei*, and *L. fermentum* had efficient antimicrobial inhibitory effect on *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. cereus*. Thus these *Lactobacillus species* could be effectively used for the protection of food from different pathogenic and food Spoilage bacteria.

Table 3: Shows the antibacterial activity of *Lactobacillus species* against some pathogenic and food spoilage bacteria

S. No.	Pathogenic and Food spoilage Bacteria	*L. plantarum* (P2)	*L. plantarum* (P1)	*L. brevis*	*L. casei*	*L. fermentum*
1	*E. coli*	25.7±1.35	23.06±0.88	19.5±1.18	13.9±0.36	16.7±0.69
2	*S. aureus*	27.6±0.94	20.4±1.17	18.2±0.47	16.1±0.29	18.9±0.81
3	*P. aeruginosa*	24.4±1.05	17.28±0.64	20.7±1.00	18.6±0.97	22.4±0.88
4	*B. cereus*	04.2±0.17	03.3±0.25	02.5±0.09	2.1±0.21	2.9±0.11

Table 4: ANOVA Summary

Source of Variation	SS	df	MS	F	P-value	F crit
Among Pathogenic and Food Spoilage bacteria	1115.23	3	371.74	60.68	0.0009	3.49
Among LAB spps	129.01	4	32.25	05.26	0.0110	3.26
Error	73.51	12	6.13			
Total	1317.75	19				

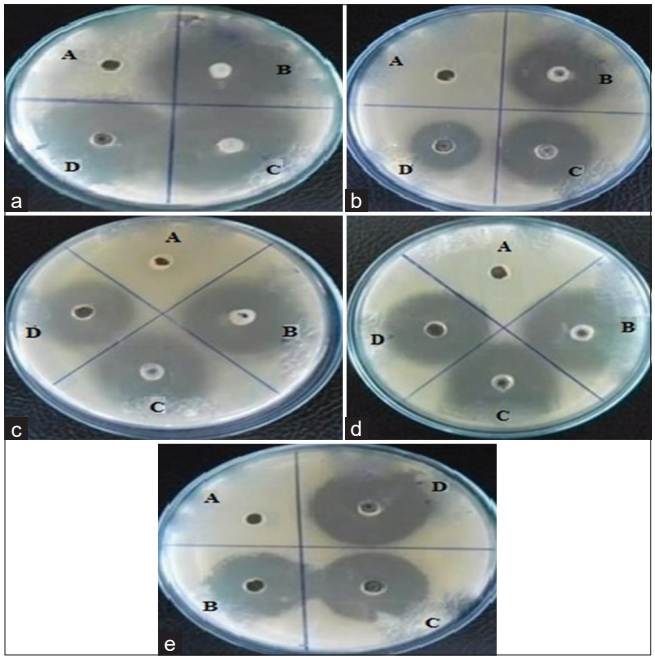

Figure 1: Antibacterial activity of *Lactobacillus species* against some pathogenic and food spoilage bacteria. a) *L. plantarum* (P2), b) *L. plantarum* (P1), c) *L. brevis*, d) L. casei, and e) *L. fermentum* (A=*B. cereus*, B=*E. coli*, C=*S. aureus*, D=*P. aeruginosa*)

DISCUSSION

Lactic acid bacteria (LAB) are known to produce various antimicrobial compounds, including organic acids, hydrogen peroxide, diacetyl, and bacteriocins, which can inhibit the growth of pathogenic and food spoilage bacteria (Jones *et al.*, 2011; Vignolo *et al.*, 2012; Kalhoro *et al.*, 2023). These antimicrobial compounds can act as natural biopreservatives, reducing the risk of foodborne illnesses and extending the shelf-life of food products (Jones *et al.*, 2011; Vignolo *et al.*, 2012). Lactobacillus species, in particular, have been extensively studied for their antibacterial activity against a wide range of microorganisms (Jones *et al.*, 2011; Hsiu *et al.*, 2016).

Studies have shown that Lactobacillus species can effectively inhibit the growth of various pathogenic and food spoilage bacteria, such as *L. monocytogenes*, *E. coli*, *Salmonella*, and *S. aureus* (Jones *et al.*, 2011; Sabo *et al.*, 2014; Hsiu *et al.*, 2016; Kalhoro *et al.*, 2023). The results of present study confirm that the *L. plantarum* (P2), *L. brevis*, *L. plantarum* (P1), *L. casei*, and *L. fermentum* had efficient antimicrobial inhibitory effect on *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. cereus*. These inhibitory effects are attributed to the production of antimicrobial compounds by *Lactobacillus species*, which can disrupt the cell membrane, interfere with cellular processes, and ultimately lead to the death of the target microorganisms (Jones *et al.*, 2011; Sabo *et al.*, 2014; Hsiu *et al.*, 2016; Kalhoro *et al.*, 2023).

The antibacterial activity of *Lactobacillus species* can be influenced by various factors, such as the specific strain, growth conditions, and the target microorganism. Furthermore, the use of Lactobacillus species as natural preservatives in food products has gained increasing attention, as it offers a viable alternative to synthetic preservatives and can contribute to the overall safety and quality of the final product (Jones *et al.*, 2011; Sabo *et al.*, 2014; Hsiu *et al.*, 2016; Kalhoro *et al.*, 2023).

CONCLUSION

The antibacterial activity of Lactobacillus species against pathogenic and food spoilage bacteria has been well-documented. These lactic acid bacteria can produce a range of antimicrobial compounds, such as organic acids, hydrogen peroxide, and bacteriocins, which can effectively inhibit the growth of various microorganisms. The use of Lactobacillus species as natural biopreservatives in food products is a promising approach to enhance food safety and extend shelf-life, thereby reducing the reliance on synthetic preservation methods. The results of the present study reveal that *L. plantarum* (P2), *L. brevis*, *L. plantarum* (P1), *L. casei*, and *L. fermentum* had efficient antimicrobial inhibitory effect on pathogenic and food spoilage bacteria like *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. cereus*. Thus these Lactobacillus species could be effectively used for the protection of food from different pathogenic and food spoilage bacteria.

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