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Original Research Article

The Probiotic Potential of Lactic Acid Bacteria Sourced From Plants, Animals, and Food-Based Samples Against Selected Bacterial Pathogens

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ABSRTACT

Lactic acid bacteria (LAB) are increasingly being studied for their potential to address the growing challenge of antimicrobial resistance. This study examined the antibacterial properties of LAB isolated from plant, animal, and food-related sources against selected pathogenic bacteria. Samples were processed, cultured in de Man–Rogosa–Sharpe (MRS) medium, and incubated at 37 °C for 48 hrs under anaerobic conditions to promote bacterial growth. Isolates were identified using biochemical characterization and the Vitek MS system, while their inhibitory effects were evaluated using the agar spot method. A total of nineteen Gram-positive, catalase-negative isolates were obtained, of which twelve were confirmed as LAB. The predominant species identified was *Lactobacillus plantarum* (41.6%), alongside other species including *Lactobacillus fermentum*, *Lactobacillus paracasei*, and *Bifidobacterium* spp. Antibacterial activity was assessed by measuring zones of inhibition and analyzed using non-parametric statistical methods. Isolates derived from animal sources demonstrated the highest median inhibition (25.00 mm; IQR: 14.50–32.50), followed by those from food (21.66 mm; IQR: 19.17–24.00), while plant-derived isolates showed comparatively lower activity (16.33 mm; IQR: 13.66–20.33). However, these differences were not statistically significant ($p > 0.05$). The findings indicate that LAB obtained from diverse natural sources possess measurable antibacterial effects, although activity levels may vary between strains. These results support the continued investigation of LAB as candidates for antimicrobial applications and their possible role in promoting human health through probiotic use.

Key words: Probiotic, Lactic acid Bacteria, Antimicrobial, Bacterial pathogens, Lactobacillus

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Introduction

Lactic acid bacteria (LAB) comprise a diverse group of Gram-positive, catalase-negative anaerobes that produce lactic acid as a metabolite of carbohydrate fermentation. They are well known for their role in fermentation and their presence in a wide range of natural environments. In addition to their traditional use in food processing, increasing attention has been directed toward their potential therapeutic benefits for human health.[1] In particular, their ability to produce antimicrobial compounds has positioned them as candidates for addressing challenges associated with resistant microbial strains.

LAB produce various bioactive substances, including organic acids and protein-based inhibitory compounds,[2–4] which can suppress the growth of competing foodborne and spoilage pathogens.[1–3] These properties have supported their use in food preservation and suggest possible applications in controlling foodborne pathogens.[5] According to the World Health Organization (WHO) estimates, the global death toll from foodborne illnesses could reach 10 million people annually by 2050, if no drastic interventions are implemented.[6]

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The widespread incidence of foodborne infections requires a systematic approach to urgently eliminate, prevent, and reduce pathogenic bacteria in foods. In addition, LAB could also impact the health and well-being of a population when administered in the right quantity as probiotics.[7] At the same time, their interaction with host systems has been linked to beneficial physiological effects,[8,9] including support of microbial balance and host defense mechanisms.[10–12] In this regard, the lactic acid bacteria can be an important source for novel antimicrobial agents.[5] Given the continued rise in antimicrobial resistance and the need for safer biological alternatives, naturally occurring LAB from commonly consumed sources represent a promising area of study. These organisms are frequently found in plant materials, fermented foods, and animal-derived products, making them accessible for investigation and potential application. A study on lactic acid bacteria found in natural products like fruits, vegetables, milk, milk-based and fermented foods and drinks, which are widely consumed by the population, could provide valuable insights into the positive effects of dietary consumption on overall public health. This study therefore aimed to isolate LAB from plant-, food-, and animal-based samples and evaluate their inhibitory activity against selected pathogenic bacteria, with the goal of assessing their relevance for antimicrobial and probiotic use.

Materials and Methods

Culture Media, Solvents and Equipment

Man-Rogosa-Sharpe (MRS) Agar, Man-Rogosa-Sharpe (MRS) Broth, Tryptic Soy (TSA) Agar, Mueller-Hinton Agar, Clotrimazole 500mg. Incubator chamber (Remi®), Laminar flow chamber (Esco®, Singapore), Vitek machine (bioMerieux®, France), Anaerobic jar (Oxoid®, UK), Autoclave, Hot water bath (Uniscope®, UK),

Microscope (Olympus®, USA), Top load balance (Ohaus®, USA). All the media used are products of Hi Media®), India

Collection and Preparation of Samples
Plant-based samples
Fresh random samples of cabbage, cucumber, lettuce, bell pepper, and red and green apples were purchased from random sellers in the Mushin market in Lagos state, Nigeria. Each sample was thoroughly rinsed, chopped, weighed (27.5g), and blended separately with an electric blender. A small quantity of distilled water was added to each sample, just enough to ease the blending process. After each sample was blended, the blending compartment was thoroughly washed, rinsed, and cleaned with alcohol before blending the subsequent sample. Each sample was blended until an even paste was obtained.

Food-based samples
Four different brands of yoghurt (Hollandia® yoghurt, Fresh® yoghurt, Habib® yoghurt, Greek® yoghurt), Danino® tigernut drink, and two samples of fermented pap water were purchased from commercial sellers in Mushin, Lagos. Each sample was diluted 1 in 10 in distilled water (10%v/v) and used for the experimental procedure.

Animal-based samples
Milk samples from cows, sheep, and goats were collected with sterile sampling equipment and containers. Before sample collection, the teats were thoroughly cleaned with sterile water and disinfectant wipes to remove any surface contaminants. The first few streams of milk were discarded to limit contamination from the teat canal. Milk samples were collected directly in sterile containers without contact with non-sterile surfaces, and sealed immediately to prevent external contamination. Samples were then transported to the laboratory in a cooler with ice packs within a few hours of collection to avoid any potential changes in the microbial population due to prolonged exposure to higher temperatures

Enrichment of broth with the samples
One milliliter of all the diluted samples was added to 19 mL sterile MRS broth supplemented with 10 µg/mL of clotrimazole under a biosafety cabinet and incubated anaerobically at 37°C for 48 hours to promote the growth and enrichment of Lactobacillus strains.[9]
Inoculation of enriched broth culture on agar plates
After incubation, a loopful of the 48hr broth culture was aseptically subcultured into a sterile MRS agar plate and incubated anaerobically at 37ºC for 48 hours.

Identification of the isolated bacteria
The bacterial isolates were conventionally identified using the standard biochemical methods such as colonial morphology, Gram staining reaction, catalase test, production of acid from glucose),[13] and the Vitek MS system (bioMerieux®).[14] The Vitek MS system evaluates a broad range of biochemical reactions, including carbohydrate fermentation and enzyme production, which enables the precise identification of LAB species.[14] The identified bacterial isolates were stored in deep MRS agar at 4°C.

Antibacterial activity of LAB against specific bacterial pathogens
The bacterial pathogens used for the antibacterial assay, *Pseudomonas fluorescens, Aeromonas hydrophila, Enterobacter cloaceae, Klebsiella pneumoniae, Staphylococcus aureus,* and *Shigella spp.* were obtained from the Department of Pharmaceutical Microbiology and Biotechnology Laboratory, University of Lagos. Each test bacterial pathogen was sub-cultured on sterile tryptic soya agar plates and incubated at 37ºC for 24 hours. After incubation, a culture of test bacteria was prepared in a 0.5 MacFarland standard solution. A loopful of 0.5 MacFarland standard solution of test bacteria culture was spread to cover the surface of a previously prepared, labelled sterile Mueller-Hinton agar plate with a spot mark. A loopful of the diluted (a colony was aseptically inoculated into 10mL of the dilution medium containing 2 mL of dextrose saline and 8 mL of normal saline) lactobacillus-identified bacteria isolate to be tested was placed as a spot on the marked spot and allowed to dry. This was done for all the LAB isolates against all the test organisms. A loopful of sterile distilled water was inoculated on a labeled Mueller-Hinton agar plate instead of test LAB as control. The plates were incubated at 37ºC for 48 hours, and the zone of inhibition was measured. All the experiments were conducted in duplicates.

Statistical Analysis
A one-way analysis of variance (ANOVA) conducted to compare the antimicrobial activity of LAB isolates from different sources showed that isolates from animal-derived samples exhibited a higher mean inhibition zone (24.25 ± 11.4 mm) compared to food (21.51 ± 3.6 mm) and plant-based isolates (18.66 ± 10.6 mm), but there was no statistically significant difference between groups, $F(2, 24) = 0.74$, $p = 0.49$ ($p > 0.05$). A Kruskal–Wallis H (non-parametric ANOVA) test performed due to unequal variance of data and small sample sizes showed no statistically significant difference between groups ($H(2) \approx 1.98$, $p > 0.05$), indicating comparable inhibitory potentials across the different sources. The non-parametric analysis of the antimicrobial activity expressed as median and interquartile range (IQR) showed that animal-derived isolates exhibited the highest median inhibition zone (25.00 mm; IQR: 14.50–32.50), followed by food isolates (21.66 mm; IQR: 19.17–24.00), while plant-derived isolates showed the lowest median (16.33 mm; IQR: 13.66–20.33). Despite these differences, variability within groups was substantial, particularly among animal isolates.

Results and Discussion

The study isolated 19 bacteria from 19 samples which include plant-based (cabbage, cucumber, lettuce, bell pepper, red and green apples), food-based (yoghurt, tiger nut, fermented pap water), and animal-based (Goat, sheep, and cow milk) samples.
Extensive morphological, biochemical, and physiological characterization of isolated strains that showed small, circular colonies with smooth and convex surfaces on MRS agar, Gram-positive rod-shaped cocci bacilli that appeared purple or crystal violet was consistent with the characteristics of *Lactobacillus*.[15] Other identifiable and defining trait of LAB exhibited by the strains were negative catalase activity and anaerobic growth under microaerophilic conditions. Among the 19 bacterial isolates, 12 were confirmed as LAB, one was a non-LAB anaerobe, while the Vitek system did not identify 6. The Vitek MS system identified 5 of the bacterial isolates as Lactobacillus spp. (*Bifidobacterium spp, Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus paracasei),* and one isolate as *Clostridium spp* as presented in Table 1. *Lactobacillus paracasei* was found mostly in milk and milk-based foods; *L. plantarum* was isolated from plants, milk, and milk-based foods; *L. fermentum* was isolated from plants and milk-based foods. *Bifidobacterium spp* was isolated only from plant-based samples (Table 1). The most isolated LAB from the samples was *Lactobacillus plantarum* (41.6%), while *Bifidobacterium spp* was the least.
The LAB strains isolated from animal-based samples inhibited a larger number of pathogens (12) than isolates from food (9) and plant-based samples (6). Three of the LAB isolates inhibited 4 of the 6 pathogens, while 2 inhibited 3 of the 6 pathogens. A higher number of the LAB strains had inhibitory activity on *Staphylococcus aureus* (10) and *Enterobacter cloaceae (7)* than on *Pseudomonas fluorescens* (3), *Klebsiella pneumoniae* (3), *Aeromonas hydrophila* (2), and *Shigella dysenteriae* (2) (Table 2)
The distribution (shown in the box plot, Figure 1) and the evaluation of the antibacterial activity of lactic acid bacteria (LAB) isolates (Table 3) from plant, food, and animal sources expressed as median and interquartile range (IQR) due to the non-normal data distribution showed that animal-derived isolates exhibited the highest median inhibition zone (25.00 mm; IQR: 14.50–32.50), followed by food-derived isolates (21.66 mm; IQR: 19.17–24.00), while plant-derived isolates showed the lowest activity (16.33 mm; IQR: 13.66–20.33)

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(Table 1). However, a Kruskal–Wallis H test indicated that the observable variation in antibacterial activity among the three groups was not statistically significant ($H(2) \approx 1.98$, $p > 0.05$).

Table 1: Identification of bacteria isolates by Vitex MS

Identified Anaerobe by Vitex Analysis	Sources of Sample	Number of isolates	Proportion
Lactobacillus plantarum	Cucumber, Goat milk, Yoghurt	5	41.6%
Lactobacillus fermentum	Green Apple, Yoghurt	2	16.6%
Bifidobacterium spp	Rotten Cabbage	1	0.083%
Lactobacillus paracasei	Sheep milk, Cow milk, Yoghurt, Tigernut	4	33.3%
Clostridium spp	Fresh Cabbage	1	NA
Unidentified	Bell pepper, Sheep milk, Fermented pap water	6	NA

Table 2: Inhibitory activity of LAB against bacterial pathogens

S\N	Sample ID	Name of Organism	Zone of inhibition(mm)					
			Aeromonas hydrophila	*Staphylococcus aureus*	*Enterobacter cloaceae*	*Pseudomonas flourescens*	*Klebsiella pneumoniae*	*Shigella dysenteriae*
1	HLD	*Lactobacillus plantarum*	-	17.333±1.528	-	-	25.667±0.577	-
2	HBB	*Lactobacillus fermentum*	-	26.667±1.155	-	-	-	-
3	FY	*Lactobacillus plantarum*	-	22.333±1.155	-	-	-	-
4	TGN	*Lactobacillus paracasei*	-	21±1.732	-	-	-	21.667±1.155
5	GY	*Lactobacillus paracasei*	-	22.333±2.309	21±1	-	-	15.667±1.155
6	FPW1	Unidentified organism	-	25.333±4.041	26.333±3.215	-	20±1	18±1
7	FPW2	Unidentified organism	-	15±4.583	22±2.646	-	-	15±1
8	Cu.	*Lactobacillus plantarum*	-	7.00±1.0000	15.00±1.000	-	-	-
9	FCa.	*Clostridium spp*	-	-	-	-	-	-
10	Let.	*Unidentified*	-	-	-	-	-	-
11	R.A.	*Unidentified*	-	13.66±2.3334	18.00±1.000	23.33±2.3334	-	-
12	G.A.	*Lactobacillus fermentum*	-	-	-	-	-	-
13	B.P.	*Unidentified*	-	8.33±0.3334	-	-	-	-
14	RCa.	*Bifidobacterium spp*	38.33±0.3334	13.66±0.3334	20.33±0.3334	17.66±0.3334	-	-

15	*GM2*	*Lactobacillus plantarum*	-	-	22.0±0	-	-	-
16	*GM3*	*Lactobacillus plantarum*	10.00±0	40.00±0	30.00±0	-	-	-
17	*SM1*	*Lactobacillus paracasei*	-	30±0	40±0.3	15±0	25±0	--
18	*CM1*	*Lactobacillus paracasei*	-	35±0	25.00±0.5	5±0	14±0	-
19		*Unidentified*	-	40.00±0	35.00±0.7	-	18.00±0	-
			2	10	7	3	3	2

Table 3: Antibacterial activity of LAB isolates from different sources

Source	N	Median(mm)	IQR(Q1–Q3) (mm)
Plant	6	16.33	13.66-20.33
Food	9	21.66	19.17-24.00
Animal	12	25	14.50–32.50

Values are expressed as median and interquartile range (IQR). No statistically significant difference was observed ($p > 0.05$, Kruskal–Wallis test).

In recent times, considerable interest has been focused on LAB due to their antimicrobial properties, which make them relevant in food bio-preservation and as probiotics in human health.[15,16] In addition, the potential application of the LAB strains as natural antimicrobial agents offers them as an alternative to synthetic preservatives that could enhance product safety and shelf-life extension, especially in pharmaceutical formulations.

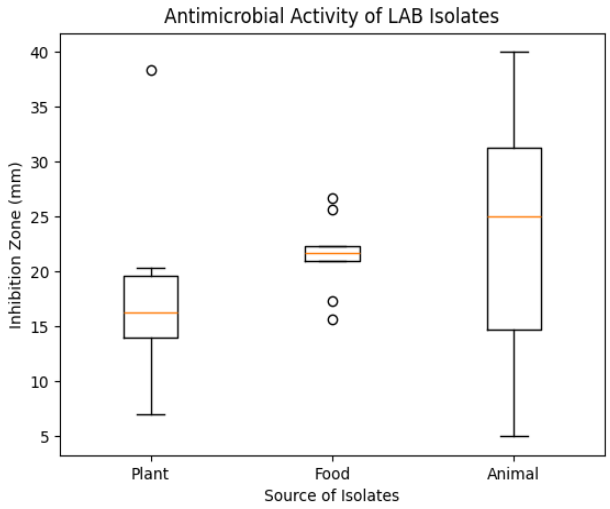

Figure 1: Boxplot showing the distribution of antibacterial activity (inhibition zone, mm) of lactic acid bacteria (LAB) isolates from plant, food, and animal sources. Animal-derived isolates exhibited a higher median inhibition zone with greater variability, while plant isolates showed lower central tendency. However, differences among groups were not statistically significant ($p > 0.05$).

In this study, 11(57%) of the isolated organisms were LAB, indicating that plant, food, and animal-based samples are good sources of the bacteria. This finding aligns with multiple studies in the literature that have reported isolation of LAB from fermented food, dairy, and plant-based fermented foods in Malaysia, Thailand, China, and Ethiopia.[17–20]

Antibacterial activity testing demonstrated that the isolated LAB has a wide range of inhibitory activity on bacterial pathogens. Broad-spectrum antibacterial activity was also found in LAB isolates in some other studies.[19,20] *Lactobacillus paracasei* displayed the broadest spectrum of antimicrobial activity of all the LAB strains. Two of its isolates inhibited 4 of the 6 pathogens, and another 2 strains of the same organism inhibited 3 of the 6 pathogens. A higher number of the LAB strains had inhibitory activity on *Staphylococcus aureus* (10) and *Enterobacter cloaceae (7)* than on *Pseudomonas fluorescens* (3), *Klebsiella pneumoniae* (3), *Aeromonas hydrophila* (2), and *Shigella dysenteriae* (2). This pattern of antimicrobial activity indicates Gram-positive bacteria may be more susceptible to the inhibitory effect of LAB than Gram-negative bacteria consistent with a study in Malaysia that reported that LAB isolates from human milk, infant faeces, and fermented grapes and dates showed better antimicrobial activity against Gram-negative pathogens.[17] However, the result contrasts with similar research where the LAB isolated from fermented Thai foods exhibited better inhibitory activity against Gram-negative bacteria than against Gram-positive pathogens.[18]

The results of this study demonstrate that LAB isolated from different natural sources exhibit measurable antibacterial activity against selected pathogens, although the magnitude of inhibition varied among isolates. In this study, LAB strains isolated from animal samples inhibited a larger number of pathogens (12) than isolates from plant-based (6) and food-based samples (9), suggesting the impact of the environment from where LAB is obtained on its potential antimicrobial activity. Although samples obtained from animal sources showed higher median inhibition values, the absence of statistical significance indicates that antimicrobial performance may not be strongly determined by source alone.

This pattern suggests that functional activity is likely influenced more by individual strain characteristics than by the origin of isolation. Differences in metabolite production, growth dynamics, and environmental adaptation could account for the variability observed within groups. The relatively wide dispersion of inhibition values, particularly among animal-derived isolates, further supports this interpretation.

The findings align with existing evidence that LAB possess inhibitory capabilities against a range of microorganisms,[5] largely due to the production of organic acids and other antimicrobial substances. However, the lack of clear separation between groups highlights the importance of targeted strain selection when considering practical applications.

From an applied perspective, these results reinforce the potential of LAB as biological agents in both food safety and health-related contexts. Their presence in commonly consumed materials also supports their feasibility for integration into probiotic formulations. Further work focusing on molecular identification, metabolite profiling, and in vivo validation would help clarify their functional potential and optimize their use.

However, this study has certain limitations. Firstly, it did not extract and characterize the metabolites responsible for the antimicrobial

activity of the LAB isolates. Further research could establish the nature and basis of the observed antimicrobial activity. This can involve extracting, identifying, and characterizing the antimicrobial metabolites responsible for bioactivity. It could also involve genomic analysis to detect genes involved in bacteriocin biosynthesis and metabolomic analysis to identify organic acids and other secondary metabolites. Secondly, the small sample size also places a limitation on the inference that could be drawn from the study.

Conclusion

The antibacterial activity of LAB isolates from plant, food, and animal-based samples indicates their bioactive potential. Their bacterial pathogen-inhibiting activity suggests they hold significant potential for food preservation, probiotic development, and therapeutic applications in infectious diseases. They could be characterized and integrated into food and pharmaceutical products to extend shelf-life by inhibiting pathogenic bacteria and preventing spoilage. All these possible applications emphasize the need for further investigation into their inhibitory mechanisms and potential commercial uses.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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