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Characterization of Lactic Acid Bacteria Isolated from Selected Fermented Foods and Evaluation of Their Antimicrobial Activity against Food-Borne Pathogens

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Abstract: **Lactic acid bacteria (LAB) are important microorganisms associated with fermented foods and are recognized for their probiotic and antimicrobial properties. This study aimed to characterize LAB isolated from selected fermented food (pap and yoghurt) and evaluate their antimicrobial activity against food-borne pathogens. Yellow maize grains were fermented to produce pap and the commercially produced yoghurt was obtained from a retail outlet. Samples were serially diluted and cultured on de Man, Rogosa and Sharpe (MRS) agar for LAB isolation. Pure isolates were characterized using phenotypic and molecular techniques. Antimicrobial activity was assessed against *Staphylococcus aureus* FFBI, *Salmonella typhi* SMIII, and *Escherichia coli* WBI using the point inoculation method. The isolates were Gram-positive, rod-shaped bacteria that were catalase-negative, oxidase-negative, coagulase-negative, indole-negative, methyl red-negative, and citrate-negative. Molecular characterization based on 16S rRNA gene sequencing identified both isolates as *Lactobacillus plantarum.* The pap isolate showed 92.90% sequence similarity with *Lactobacillus plantarum* strain MiLAB14, while the yoghurt isolate showed 80.60% similarity with *Lactobacillus plantarum* strain CRL 1480. The yoghurt isolate produced inhibition zones of 55 mm, 20 mm, and 25 mm against *S. aureus, S. typhi*, and *E. coli*, respectively, whereas the pap isolate produced inhibition zones of 42 mm, 18 mm, and 10 mm. The highest antimicrobial activity was observed against *S. aureus*. These findings indicate that pap and yoghurt are valuable sources of *Lactobacillus plantarum* with promising antimicrobial potential and possible applications as probiotics and natural food preservatives.**

Keywords: **Keywords: Antimicrobial activity, food-borne pathogens, Lactic acid bacteria, *Lactobacillus plantarum,* pap (ogi), yoghurt.**

I. INTRODUCTION

Food-borne diseases remain a major public health concern worldwide due to their impact on human health and food security. These diseases are caused by the consumption of food contaminated with pathogenic microorganisms, toxins, or harmful chemicals. Common food-borne bacterial pathogens such as *Escherichia coli, Staphylococcus aureus,* and *Salmonella* species are frequently implicated in outbreaks of food poisoning and gastrointestinal infections (Khalid, 2011). The increasing prevalence of antimicrobial resistance among these pathogens has stimulated interest in the search for natural and safe alternatives for their control in foods and food-processing environments.

Lactic acid bacteria (LAB) are a diverse group of Gram-positive, non-spore-forming, catalase-negative microorganisms that produce lactic acid as the major end product of carbohydrate fermentation. They are naturally associated with a wide variety of fermented foods and have long been recognized for their beneficial roles in food preservation, flavor development, and improvement of nutritional quality (Mokoena, 2017). Common genera of LAB include *Lactobacillus, Lactococcus,* *Pediococcus, Leuconostoc,* and *Streptococcus*. Due to their Generally Recognized as Safe (GRAS) status, LAB are widely utilized in the food industry as starter cultures and probiotic organisms.

One of the most important characteristics of LAB is their ability to inhibit the growth of pathogenic and spoilage microorganisms. Their antimicrobial activity is mainly attributed to the production of organic acids, hydrogen peroxide, diacetyl, carbon dioxide, and bacteriocins, which create unfavorable conditions for pathogenic bacteria (Ouwehand and Vesterlund, 2004). The production of lactic acid lowers the pH of the surrounding environment, thereby restricting the growth and survival of many harmful microorganisms. In addition, some LAB strains produce bacteriocins, which are proteinaceous compounds capable of inhibiting closely related and food-borne pathogenic bacteria.

Fermented foods constitute an important source of LAB and have attracted considerable scientific attention due to their health-promoting properties. Pap, also known as ogi, is a traditional fermented cereal-based food commonly consumed in Nigeria and other West African countries. It is produced through the natural fermentation of maize, sorghum, or millet and serves as an important source of beneficial microorganisms, particularly LAB. During fermentation, LAB become dominant and contribute to the development of the characteristic taste, texture, and microbiological safety of the product (Achi and Ukwuru, 2015). In the present study, pap was produced through controlled fermentation to obtain indigenous LAB isolates for antimicrobial evaluation.

Yoghurt is another fermented food rich in LAB and is produced through the fermentation of milk by specific bacterial cultures. It is widely consumed due to its nutritional value and probiotic benefits. Commercial yoghurts available in markets contain viable LAB populations capable of exerting antimicrobial effects against various pathogenic microorganisms. The presence of LAB in yoghurt contributes not only to product preservation but also to improved gut health and enhanced resistance against enteric pathogens (Marco *et al.*, 2017). Therefore, yoghurt represents a valuable source of LAB with potential applications in food safety and bio-preservation.

The growing demand for natural antimicrobial agents has increased research interest in the isolation and characterization of LAB from fermented foods. LAB-derived antimicrobial compounds offer promising alternatives to synthetic preservatives and may contribute to reducing the reliance on conventional antibiotics in food systems. Several studies have demonstrated the effectiveness of LAB isolates against important food-borne pathogens, suggesting their potential use as protective cultures and biocontrol agents in food production (Mokoena, 2017).

Therefore, this study was undertaken to characterize lactic acid bacteria from selected fermented foods, namely fermented pap and commercially available yoghurt, and to evaluate their antimicrobial activity against selected food-borne pathogens. The findings of this study may contribute to the identification of LAB strains with potential applications in food preservation, food safety, and the development of natural antimicrobial products.

II. MATERIALS AND METHODS

Sample Collection

Fresh maize grains were obtained from a local market in Agbani, Enugu State, and used for the preparation of fermented pap (ogi). Commercial yoghurt was purchased from a retail outlet and transported to the microbiology laboratory under aseptic conditions for analysis.

Preparation and Fermentation of Pap (Ogi)

Pap (ogi) was prepared using the traditional fermentation method. The maize grains were carefully sorted to remove debris, washed thoroughly, and steeped in sterile water for 72 h at room temperature (28 ± 2°C). During fermentation, natural microbial activity softened the grains. After fermentation, the grains were wet-milled and sieved to obtain a smooth slurry. The slurry was allowed to sediment, after which the supernatant was decanted. The resulting fermented pap was collected aseptically and used for microbiological analysis.

Isolation of Lactic Acid Bacteria (LAB)

Ten milliliters (10 mL) of yoghurt and 10 g of fermented pap were separately homogenized in 90 mL of sterile peptone water to obtain initial suspensions. Serial ten-fold dilutions were prepared up to 10^{-4}. From appropriate dilutions, 0.1 mL aliquots were aseptically spread-plated onto de Man, Rogosa and Sharpe (MRS) agar plates.

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The inoculated plates were incubated at 37°C for 48 h under anaerobic conditions. Distinct colonies exhibiting typical characteristics of lactic acid bacteria were selected and repeatedly sub-cultured on fresh MRS agar to obtain pure isolates.

Identification of LAB Isolates

A. Phenotypic Characterization

Pure isolates were characterized based on colonial morphology, cell morphology, Gram staining reaction, and biochemical properties. Colony characteristics such as shape, size, colour, elevation, margin, and surface appearance were recorded.

Gram staining was performed using standard microbiological procedures. Biochemical tests, including catalase, oxidase, coagulase, indole, methyl red, and citrate utilization tests, were carried out for presumptive identification.

B. Molecular Characterization

Genomic DNA was extracted from selected LAB isolates using a commercial bacterial DNA extraction kit following the manufacturer's instructions. The quality and concentration of extracted DNA were assessed using agarose gel electrophoresis and spectrophotometry.

The 16S rRNA gene was amplified using Polymerase Chain Reaction (PCR) with universal bacterial primers. The PCR mixture contained template DNA, forward and reverse primers, PCR master mix, and nuclease-free water. Amplification was carried out in a thermal cycler under standard cycling conditions, including initial denaturation, denaturation, annealing, extension, and final extension steps.

PCR products were resolved on a 1.5% agarose gel stained with an appropriate nucleic acid stain and visualized under ultraviolet light. Successful amplicons were purified and subjected to DNA sequencing.

The obtained sequences were edited and compared with reference sequences in the National Center for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Search Tool (BLAST). Identification was based on sequence similarity with known bacterial strains. Phylogenetic analysis was conducted to determine evolutionary relationships between the isolates and related reference strains. The confirmed LAB isolates were then used for antimicrobial activity assays against selected food-borne pathogens.

Antimicrobial Activity Assay of LAB Isolates

Collection of Foodborne Pathogens

The test organisms *Staphylococcus aureus* FFBI, *Salmonella typhi* SMIII, and *Escherichia col*i WBI were obtained from the Microbiology Laboratory of Enugu State University of Science and Technology (ESUT).

Activation of Test Organisms

The test pathogens were activated using their respective selective media: Mannitol Salt Agar for *Staphylococcus aureus*, Salmonella Shigella Agar for *Salmonella typhi*, and Eosin Methylene Blue Agar for *Escherichia coli*, prior to use.

Antimicrobial Activity Assay

The antimicrobial activity of the LAB isolates was evaluated using the point inoculation method. Mueller-Hinton agar plates were prepared and uniformly inoculated with standardized suspensions of the test pathogens using sterile swab sticks.

Twenty-four-hour cultures of LAB isolates were then point inoculated onto the inoculated Mueller-Hinton agar plates. The plates were incubated at 37°C for 24 h.

After incubation, zones of inhibition around each LAB inoculation point were measured in millimeters using a transparent ruler. Antimicrobial activity was determined based on the diameter of the inhibition zones produced against each test organism.

Statistical Analysis

All experiments were performed in triplicate. Data obtained were expressed as mean ± standard deviation (mean ± SD). Results were presented in tables and charts where appropriate.

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III. RESULTS AND DISCUSSION

The phenotypic characterization of the lactic acid bacteria (LAB) isolates recovered from yoghurt and pap (ogi)

The phenotypic characterization of the lactic acid bacteria (LAB) isolates recovered from yoghurt and pap (ogi) revealed characteristics commonly associated with members of the genus *Lactobacillus*. Both isolates exhibited circular colonies with convex elevation and entire margins, while the yoghurt isolate produced creamy colonies and the pap isolate produced white colonies. The smooth and moist appearance observed in both isolates is typical of LAB grown on de Man Rogosa Sharpe (MRS) agar. Similar colonial characteristics have been reported for *Lactobacillus plantarum* isolated from fermented dairy and cereal products (Adebayo-Tayo and Onilude, 2008).

Microscopic examination showed that both isolates were Gram-positive and rod-shaped. These characteristics are important taxonomic features of the genus *Lactobacillus* and have been widely used in the preliminary identification of LAB from fermented foods (Holzapfel and Wood, 2014).

The biochemical profiles of the isolates further supported their classification as *Lactobacillus* species. Both isolates were negative for catalase, oxidase, coagulase, indole, methyl red, and citrate utilization tests. Catalase negativity is considered a major characteristic of LAB because these organisms generally lack the enzyme catalase. These result agree with the findings of Axelsson (2004) and Khalid (2011) that reported *Lactobacillus plantarum* to exhibit these properties.

Molecular Characteristics of LAB Isolates from Yoghurt and Pap (Ogi)

Molecular characterization using 16S rRNA gene sequencing provided more definitive identification of the isolates. The pap isolate (PI) exhibited a pairwise identity of 92.90% with *Lactobacillus plantarum* strain MiLAB14, while the yoghurt isolate (YI) showed 80.60% similarity with *Lactobacillus plantarum s*train CRL 1480. Although the percentage identities varied, the closest related organisms identified through sequence analysis belonged to *Lactobacillus plantarum.* The high bit score and low E-values obtained for both isolates indicate a statistically significant alignment between the query sequences and the reference sequences in the database. According to Janda and Abbott (2007), 16S rRNA gene sequencing remains one of the most reliable methods for bacterial identification because of its conserved and variable regions, which enable differentiation among bacterial taxa.

The agarose gel electrophoresis image (Figure 1) confirmed successful amplification of the 16S rRNA gene from both isolates. Distinct DNA bands observed in lanes A and B indicate the presence of amplified genetic material corresponding to the target gene. Similar observations have been reported in studies involving molecular identification of LAB isolated from fermented foods (Temmerman *et al.*, 2004). The successful amplification further validates the suitability of the extracted genomic DNA for sequence analysis.

The phylogenetic trees generated for the isolates (Figures 2a and 2b) showed clustering of both isolates with previously identified strains of *Lactobacillus plantarum.* The clustering pattern observed in the present study indicates that the isolates share a common evolutionary origin with established *Lactobacillus plantarum* strains reported in international nucleotide databases.

The occurrence of *Lactobacillus plantarum* in both yoghurt and pap is not surprising because this species is frequently associated with fermented foods of both dairy and plant origin. *L. plantarum* possesses remarkable adaptability to different ecological niches due to its ability to metabolize a wide range of carbohydrates and tolerate acidic conditions (Siezen and Van Hylckama Vlieg, 2011). Its presence in yoghurt and pap suggests its active involvement in the fermentation process, where it contributes to acid production, flavour development, product preservation, and enhancement of food safety through inhibition of undesirable microorganisms.

Table 1: Phenotypic Characteristics of LAB Isolates from Yoghurt and Pap (Ogi)

Characteristic	Yoghurt Isolate(YI)	Pap Isolate(PI)
Colony Colour	Creamy	White
Colony Shape	Circular	Circular
Elevation	Convex	Convex
Margin	Entire	Entire

Surface Appearance	Smooth, moist	Smooth, moist
Gram Reaction	Positive	Positive
Cell Morphology	Rod-shaped	Rod-shaped
Catalase Test	Negative	Negative
Oxidase Test	Negative	Negative
Coagulase Test	Negative	Negative
Indole Test	Negative	Negative
Methyl red test	Negative	Negative
Citrate utilization Test	Negative	Negative

Table 2: Molecular Characteristics of LAB Isolates from Yoghurt and Pap (Ogi)

Sample Code	% Pairwise Identity	Accession	Bit-Score	Description of related Organism	E Value	Hit end	Hit start
PI	92.90%	AY383631	959.531	*Lactobacillus plantarum* strain MiLAB14	0	810	136
YI	80.60%	AY865605	331.671	*Lactobacillus plantarum* strain CRL 1480	1.41E-85	555	110

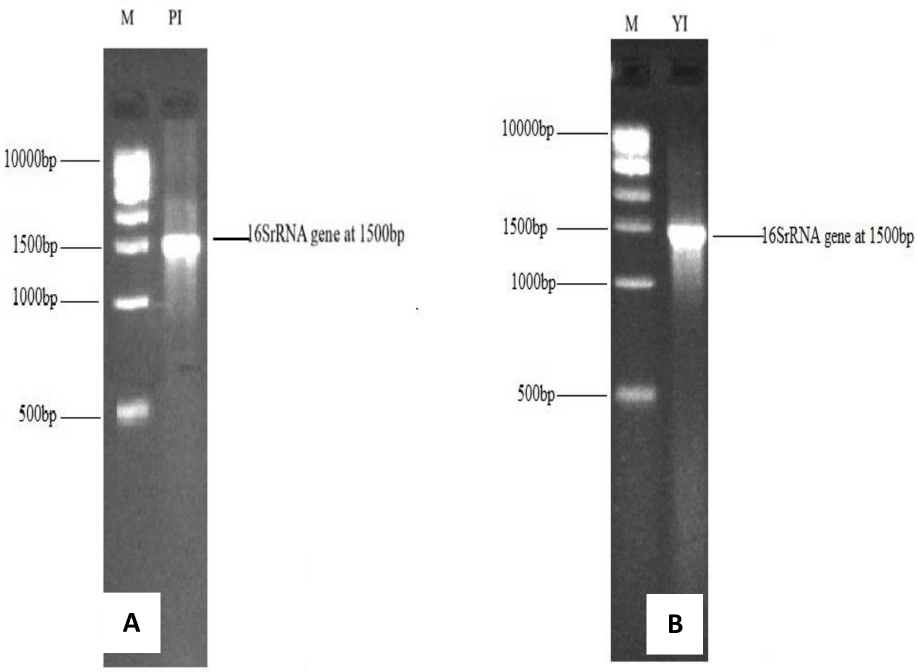

Figure 1: Gel Image Showing Amplification of the 16rRNA gene of the LAB Isolates

Legend: Lane M is a 1Kbp ladder

A= *Lactobacillus plantarum* PI

B= *Lactobacillus plantarum* YI

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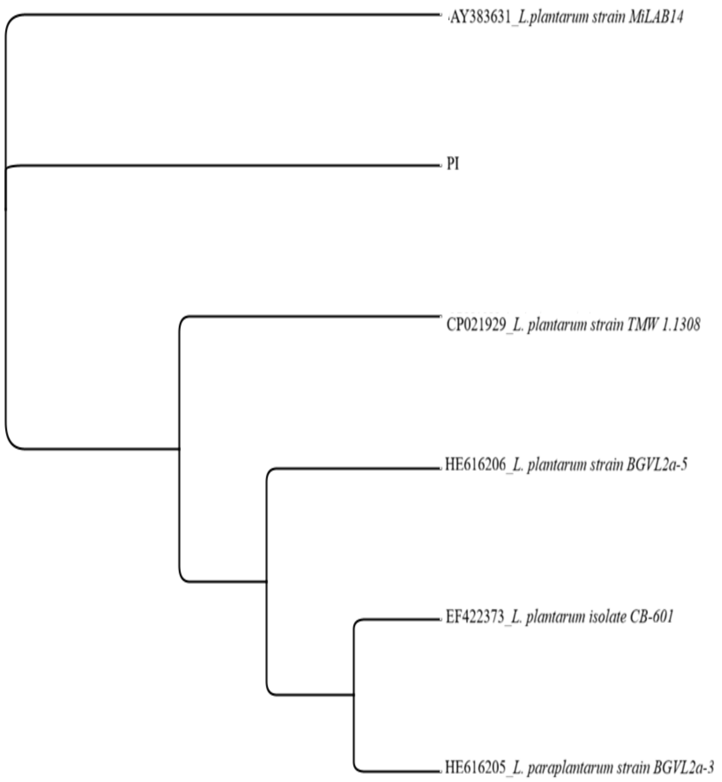

Figure 2a: Phylogenetic Tree of *Lactobacillus plantarum* PI

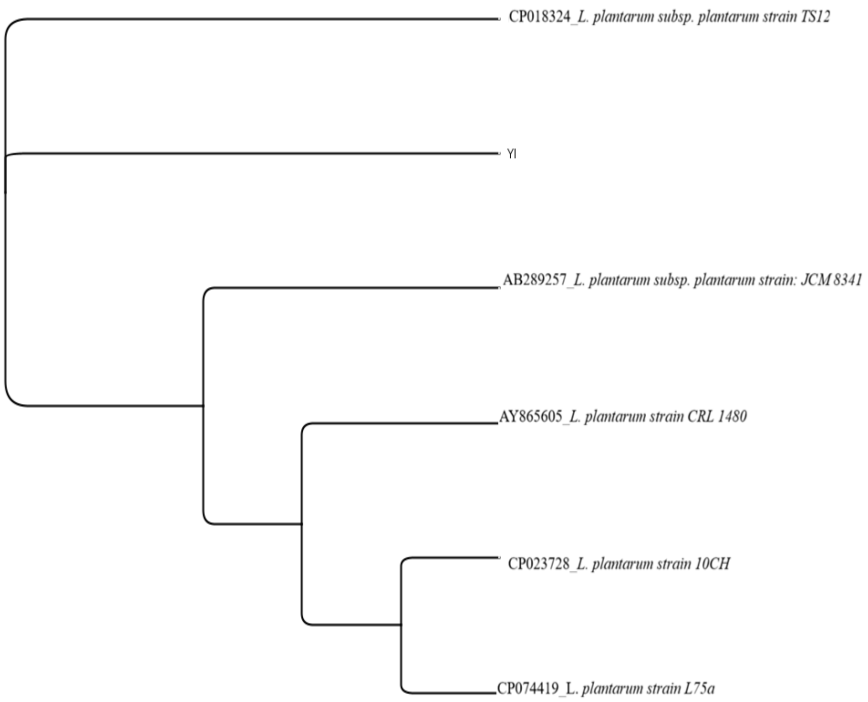

Figure 2b: Phylogenetic Tree of *Lactobacillus plantarum* YI

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Antimicrobial Activity of *Lactobacillus plantarum* Strains PI and YI from Pap and Yoghurt Against Selected Food-Borne Pathogens

The antimicrobial activity of the two *Lactobacillus plantarum* strains isolated from pap (PI) and yoghurt (YI) against selected food-borne pathogens is presented in Figure 3a &b. The results demonstrated that both isolates exhibited inhibitory effects against *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*, although the degree of inhibition varied among the test organisms.

The yoghurt isolate (*Lactobacillus plantarum* YI) showed the highest antimicrobial activity against *Staphylococcus aureus* with a zone of inhibition of 55 mm, while the pap isolate (*Lactobacillus plantarum* PI) produced a zone of 42 mm. These inhibition zones indicate a strong antagonistic effect against the Gram-positive pathogen. The greater susceptibility of *S. aureus* may be attributed to the action of antimicrobial metabolites such as organic acids, hydrogen peroxide, and bacteriocins produced by *Lactobacillus plantarum.* Similar findings have been reported by Todorov and Dicks (2007), who observed significant inhibition of *Staphylococcus aureus* by bacteriocin-producing strains of *L. plantarum.*

Against *Salmonella typhi*, both isolates demonstrated moderate antimicrobial activity, with inhibition zones of 18 mm and 20 mm for PI and YI, respectively. Although the inhibitory effect was lower than that observed against *S. aureus,* the results indicate that both strains possess the ability to suppress the growth of this important food-borne pathogen. The relatively lower susceptibility of *S. typhi* may be due to its Gram-negative cell wall structure, which contains an outer membrane that can limit the penetration of antimicrobial substances (Gálvez *et al.*, 2007).

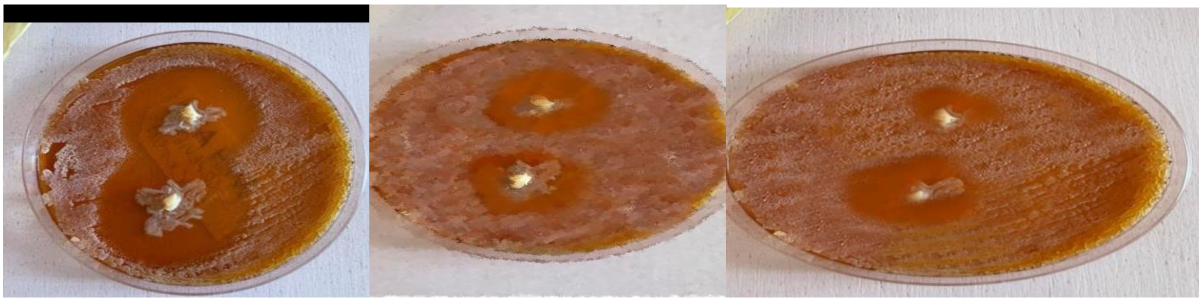
The lowest antimicrobial activity was observed against *Escherichia coli*. The pap isolate produced a zone of inhibition of 10 mm, whereas the yoghurt isolate produced a substantially larger inhibition zone of 25 mm. This difference suggests that the yoghurt-derived strain may possess a higher capacity for producing antimicrobial compounds or may synthesize more potent inhibitory substances than the pap isolate. The reduced susceptibility of *E. coli* compared to *S. aureus* is consistent with previous studies that have shown Gram-negative bacteria to be more resistant to bacteriocins and other antimicrobial metabolites due to the protective function of their outer membrane (Cleveland *et al.*, 2001).

However, contrary findings have been reported by Hussein *et al.* (2025), who observed inhibition zones of 20 mm against *Staphylococcus aureus*, 17.1 mm against *Salmonella Typhi*, and 23 mm against *Escherichia coli* by *Lactobacillus plantarum* KR3 isolated from fermented food. In their study, *Escherichia coli* was the most susceptible pathogen, whereas *Salmonella typhi* showed the least susceptibility. This contrasts with the present findings, where *S. aureus* exhibited the highest susceptibility and *E. coli* the lowest. Such variations may be attributed to strain-specific differences in antimicrobial metabolite production, differences in pathogen sensitivity, growth conditions, fermentation substrates, and assay methodologies employed by different researchers.

The strong inhibitory effects demonstrated by both *Lactobacillus plantarum* strains suggest their potential application as natural bio-preservatives and probiotic cultures in food systems. Their ability to suppress common food-borne pathogens supports their possible use in improving food safety and extending shelf life of fermented food products. Furthermore, the pronounced activity against *Staphylococcus aureus* highlights their potential role in controlling pathogenic contamination in food products.

Staphylococcus aureus FFBI (42mm) **Salmonella typhi SMIII (18mm)** **Escherichia coli WBI (10mm)**

Figure 3a: Antimicrobial Activity of *Lactobacillus plantarum* Strains PI against Selected Foodborne Pathogens



Staphylococcus aureus FFBI **Salmonella typhi SMIII** **Escherichia coli WBI**

(55mm) **(20mm)** **(25mm)**

Figure 3b: Antimicrobial Activity of Lactobacillus plantarum Strains YI against Selected Foodborne Pathogens

IV. CONCLUSION

This study demonstrated that fermented pap (ogi) and yoghurt are important reservoirs of beneficial lactic acid bacteria with significant antimicrobial potential. The isolation and characterization of bacterial strains from both fermented products confirmed the presence of *Lactobacillus plantarum*. The successful identification of this organism from both traditional and commercial fermented foods highlights the microbiological value of these products and their potential role in improving food quality and safety.

The antimicrobial activity exhibited by the isolates against *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli* demonstrates their capacity to inhibit food-borne pathogens and suggests their suitability for use as protective cultures in food systems. The greater susceptibility of *Staphylococcus aureus* to the isolates indicates that the antimicrobial metabolites produced by *Lactobacillus plantarum* may be particularly effective against certain pathogenic bacteria commonly associated with food contamination.

The findings underscore the potential application of these isolates as natural bio-preservatives and probiotic microorganisms. Their incorporation into food products could enhance microbiological safety, improve shelf life, and reduce reliance on synthetic preservatives. Such applications are increasingly important in the development of safer, healthier, and more sustainable food systems.

Furthermore, this study contributes to global efforts aimed at achieving the United Nations Sustainable Development Goals (SDGs). By identifying naturally occurring microorganisms capable of suppressing food-borne pathogens, the study supports SDG 3 (Good Health and Well-being) through the promotion of safer foods and the reduction of food-related illnesses. It also aligns with SDG 2 (Zero Hunger) by contributing to improved food quality, preservation, and availability. In addition, the potential use of naturally derived antimicrobial agents in place of synthetic preservatives supports SDG 12 (Responsible Consumption and Production), which encourages sustainable approaches to food production and preservation.

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