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Probiotics potential and antimicrobial properties of *Lactobacillus plantarum* strains isolated from traditionally fermented maize and cassava

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This study investigated the probiotic potential of *Lactobacillus plantarum* strains isolated from traditional fermented foods, specifically ogi and corn flour from maize, fufu and tapioca from cassava. Two strains, *Lactobacillus plantarum* 1 (LP1) (isolated from ogi) and *Lactobacillus plantarum* 2 (LP2) (isolated from fufu), were characterized for key probiotic attributes. Both strains demonstrated remarkable acid tolerance at pH levels of 3.0 (77.1 and 77.0% survival for LP1 and LP2, respectively) and 4.0 (100% survival for both strains), along with significant bile salt resistance at concentrations of 0.2% (81.0 and 80.9% survival) and 0.3% (85.5 and 83.7% survival) over six hours. The isolates showed strong adhesion capabilities to cancer colorectal-2 (Caco-2) intestinal cells (63.16% for LP1; 60.63% for LP2), exhibited considerable cell surface hydrophobicity (80- and 75-%), auto-aggregation exceeding 50%, and detectable biofilm formation. In antimicrobial assessments, both strains inhibited the growth of *Staphylococcus aureus* (30- and 26-mm inhibition zones) and *Salmonella typhi* (30- and 28-mm inhibition zones). Antibiotic sensitivity testing revealed susceptibility to amoxicillin (22- and 21-mm zones), azithromycin (23- and 22-mm zones), and erythromycin (24- and 21-mm zones). These results indicate that *L. plantarum* strains from indigenous fermented foods possess promising probiotic and antimicrobial characteristics, suggesting their potential application in functional foods and therapeutic formulations.

Keywords: Probiotics, *Lactobacillus plantarum*, Fermented Maize, Fermented Cassava, Ogi, Fufu.

INTRODUCTION

Fermentation stands as one of humanity's oldest food preservation methods, maintaining contemporary importance for improving food safety, nutritional content, and organoleptic qualities (Oguntoyinbo and Narbad, 2015). Throughout Africa, traditional fermentation practices remain integral to processing staple foods including cassava products like fufu and tapioca, and maize preparations such as ogi. These substrates provide optimal conditions for lactic acid bacteria (LAB), which typically dominate these spontaneous fermentations and significantly contribute to the final product's characteristics and potential health benefits (Adebayo-Tayo *et al.*, 2020).

Within the LAB group, *Lactobacillus plantarum* emerges as particularly adaptable and extensively investigated. Research demonstrates its capacity to withstand gastro-intestinal transit, adhere to intestinal epithelium, produce

antimicrobial compounds, and modulate immune responses (Arena *et al.*, 2018). These characteristics establish its relevance as a probiotic candidate. Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Collado *et al.*, 2008). Essential probiotic criteria include gastric acid and bile salt tolerance, intestinal cell adhesion capability, biofilm formation potential, and safety demonstrated through antibiotic susceptibility profiles (Georgieva *et al.*, 2015).

While LAB from various African fermented foods have received research attention, focused studies on *L. plantarum* from diverse traditionally processed cassava and maize products remain limited. Assessing the probiotic potential of these isolates holds particular relevance in regions where such fermented staples constitute dietary mainstays, potentially serving as natural sources of beneficial microorganisms. Additionally, examining their antimicrobial activity against enteric pathogens such as *Staphylococcus aureus* and *Salmonella typhi* may indicate therapeutic applications.

This study therefore, aimed to isolate *L. plantarum* from four commonly consumed cassava and maize products and evaluate their probiotic properties, antibiotic susceptibility, and antibacterial activity against selected enteric pathogens.

MATERIALS AND METHODS

Study location

The research was conducted in the Microbiology Laboratory, Department of Biological Sciences, Godfrey Okoye University, Enugu, Nigeria. All experimental procedures followed standard microbiological protocols for handling food-associated microorganisms.

Raw materials procurement

Fresh cassava roots (*Manihot esculenta* Crantz) and maize grains (*Zea mays* L.) were obtained from Oye Emene Market, Enugu State, Nigeria. Materials were visually inspected upon acquisition, with damaged or spoiled portions discarded. Samples were transported in sterile polyethylene bags and processed within 24 h to minimize microbial deterioration.

Sample size calculation

To determine the appropriate number of samples, Cochran's formula was utilized (Cochran, 1977):

$n_0 = (Z^2pq) / e^2$

Where:

- n_0 = the initial sample size estimate,
- Z = the Z-score for the desired confidence level (1.96 for 95% confidence),
- p = the assumed proportion of the population possessing the characteristic (set to 0.5 for maximum variance),
- q = 1 – p,
- e = the acceptable sampling error (0.05).

The initial sample size was modified according to practical limitations regarding vendor and product availability at the source market, resulting in a final sample size of 20 representative samples per product category.

Cassava processing and fermentation

Cassava tubers were peeled, washed, and cut into sections before submersion in clean water at 32°C for 3 days, with daily water replacement. The fermented mash yielded two products:

- **Fufu:** Mash was sieved, dewatered using muslin cloth, and sediment cooked into paste following traditional methods (Oyewole and Afolami, 2001).
- **Tapioca (Abacha):** Mash was dewatered, pressed, sliced into strips, sun-dried, and stored in airtight containers (Ekwu *et al.*, 2014).

Maize processing and fermentation

Maize grains were sorted, washed, and steeped in water at 32°C for 72 h with daily water changes. Products included:
Ogi: Steeped grains were wet-milled, sieved, and filtrate sedimented for 24 hours before use as porridge (Chaves-López *et al.*, 2020).
Corn flour: Three distinct techniques were applied to produce corn flour:

1. **Dry milling:** Fermented grains were dried and subsequently milled into flour.
2. **Precooked flour:** After steeping, the grains were boiled, dried, and then milled.
3. **Nixtamalized flour:** Grains were cooked in a solution of lime water ($Ca(OH)_2$), rinsed, dried, and milled. This nixtamalization method improves the nutritional quality (Mao *et al.*, 2024).

Isolation and identification of lactic acid bacteria

Lactic acid bacteria were isolated on De Man, Rogosa, and Sharpe (MRS) agar. Samples (10 g) were homogenized in 90 mL peptone water and serially diluted to 10^{-7}. Aliquots (0.1 mL) were spread plated and incubated at 37°C for 48 h under microaerophilic conditions. Distinct colonies were purified through repeated sub-culturing. Gram-positive, catalase-negative isolates were presumptively identified as LAB.

Carbohydrate fermentation profiling (API 50 CHL)

Isolates were characterized using API 50 CHL strips (bioMérieux, France) according to the manufacturer specifications. Carbohydrate fermentation patterns were compared with database standards for identification.

Antibiotic sensitivity testing

The Kirby-Bauer disk diffusion method on Mueller-Hinton agar determined antibiotic susceptibility. Bacterial suspensions were standardized to 0.5 McFarland and swabbed onto plates. Antibiotic disks included: amoxicillin (30 µg), erythromycin (10 µg), gentamicin (10 µg), pefloxacin (10 µg), ceftriaxone (25 µg), ciprofloxacin (10 µg), levofloxacin (20 µg), azithromycin (12 µg), ampiclox (30 µg), and cefuroxime (20 µg). Zones of diameters were measured after 18–24 h of incubation at 37°C and interpreted using Clinical and Laboratory Standards Institute (2020) criteria. This assay was conducted in triplicate.

Table 1. Gram's reaction and colony morphology.

Isolate	Source	Colony morphology	Colony size (mm)	Gram's reaction	Cell morphology	Cell length (µm)
LP1	Ogi (*Zea mays L.*)	Creamy, circular, convex, smooth edges	2.2	Gram-positive	Rods	3.0
LP2	Fufu (*Manihot esculenta Crantz*)	White, circular, raised, smooth edges	2.0	Gram-positive	Rods	3.0

Key
LP 1 =*Lactobacillus plantarum* 1 from corn fermentation (Ogi)
LP 2 = *Lactobacillus plantarum* 2 from cassava fermentation (fufu)

Antibacterial activity (pathogen growth inhibition)

Agar well diffusion methodology (Yang et al., 2025) assessed antibacterial activity against *Staphylococcus aureus*, *Salmonella typhi, Escherichia coli,* and *Pseudomonas aeruginosa*. Cell-free supernatants from LAB cultures were pH-neutralized after centrifugation and filtration. Wells (6 mm diameter) in seeded agar plates received 100 µL aliquots. Following 24-h incubation at 37°C, inhibition zones were measured. Sterile MRS broth served as a negative control.

Evaluation of probiotics characteristics

Acid tolerance

The method of Liu *et al.* (2022) was followed. Bacterial cells were washed, resuspended in phosphate-buffered saline (PBS) at pH 2.5, and incubated at 37°C. Viable cell counts were assessed by plating on MRS agar at 2, 4, and 6 h. The survival rate was calculated as: (log CFU at 6 h / log CFU at 2 h) × 100.

Bile salt tolerance

Tolerance was assessed as described by Liu *et al.* (2022). LAB cultures were introduced into MRS broth supplemented with 0.3% oxgall bile salts. Growth was tracked by measuring the optical density at 600 nm (OD_{600}) at 3, 6 and 9 h post-inoculation at 37°C. Survival was calculated relative to the growth observed in a control broth lacking bile salts.

Cell surface hydrophobicity

The microbial adhesion to hydrocarbons (MATH) assay (Xu *et al.*, 2009) was used. Washed bacterial cells were suspended in PBS at OD_{600} of ~0.5. This suspension was mixed with xylene, vortexed vigorously, and allowed to separate. The OD of the aqueous phase was then measured again.

$$\text{Hydrophobicity (\%)} = (A_0 - A) / A_0) \times 100$$

Auto-aggregation assay

The capacity for auto-aggregation was examined using the protocol from Del Re *et al.* (2000). Bacterial suspensions in PBS were allowed to stand at room temperature. The absorbance at 600 nm was measured immediately and after a 5-h period.

$$\text{Auto-aggregation (\%)} = (1 - (A_t / A_0) \times 100$$

Biofilm formation

A microtiter plate assay (Stepanović *et al.*, 2007) quantified biofilm production. Bacterial suspensions were incubated in 96-well plates for 24 h. After washing, adhered cells were fixed, stained with crystal violet, and the bound dye was dissolved for absorbance reading at 570 nm to assess biofilm formation strength.

Adhesion to Caco-2 cells

The adhesive potential was evaluated following the procedure of Del Re *et al.* (2000). Bacterial cells were introduced onto confluent monolayers of cancer colorectal-2 (Caco-2) cells. After incubation and washing to remove non-adhered bacteria, the eukaryotic cells were lysed, and the number of attached bacteria was determined by plating.

Adhesion (%) = (CFU recovered / CFU added) × 100.

Cell viability assessment (MTT Assay)

The potential cytotoxic effect of bacterial culture supernatants on Caco-2 cells was tested via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Benov, 2021). Cells were plated and exposed to varying concentrations of the supernatants. After 24 h, cell viability was quantified by measuring the conversion of MTT to formazan crystals, expressed as a percentage relative to untreated control cells. The formula applied was:

Cell viability (%) =(ODtreat/ODcontrol) × 100

RESULTS

The results present the basic microscopic and macroscopic characteristics used to verify isolate identity prior to probiotic evaluation. As shown in Table 1, both isolates displayed the canonical morphology of lactic acid bacteria: Gram-positive, non-sporing rods with mean cell length of approximately 3.0 µm. On MRS agar, LP1 (from *ogi*) formed creamy, circular, convex colonies with smooth margins (mean diameter 2.2 mm), whereas LP2 (from *fufu*) produced white, circular, raised colonies with smooth margins (2.0 mm). These features are consistent with the phenotypic profile of *Lactobacillus plantarum* and corroborate the preliminary identification of both isolates as *L. plantarum*. Phenotypic differences were minimal, with LP1 colonies slightly larger and cream-colored versus the smaller, white colonies of LP2.

Table 2. Carbohydrate fermentation profile (API 50 CHL) of isolates.

Carbohydrate substrate	LP1	LP2
Glycerol	−	−
Erythritol	−	−
D-Arabinose	−	−
L-Arabinose	−	−
Ribose	+	+
D-Xylose	−	−
L-Xylose	−	−
Adonitol	−	−
β-Methyl-xyloside	−	−
Galactose	+	+
Glucose	+	+
Fructose	+	+
Mannose	+	+
Sorbose	−	−
Rhamnose	−	−
Dulcitol	−	−
Inositol	−	−
Mannitol	+	−
Sorbitol	−	W
α-Methyl-D-mannoside	−	−
α-Methyl-D-glucoside	+	+
N-Acetylglucosamine	+	+
Amygdalin	+	+
Arbutin	+	+
Esculin	+	+
Salicin	+	+
Cellobiose	+	+
Maltose	+	+
Lactose	+	+
Melibiose	+	−
Sucrose	+	+
Trehalose	+	+
Inulin	−	−
Melezitose	−	−
Raffinose	+	−
Starch	+	+
Gentiobiose	+	+
Turanose	−	−
Lyxose	−	−
Tagatose	+	+
Fucose	−	−
Arabitol	−	−
Xylitol	−	−
Gluconate	+	+
2-Ketogluconate	−	−
5-Ketogluconate	−	−

Key
LP 1 =*Lactobacillus plantarum* 1 from corn fermentation (Ogi)
LP 2 = *Lactobacillus plantarum* 2 from cassava fermentation (fufu)
+ Positive reaction (fermentation occurred), **-** Negative reaction (no fermentation), **W** Weak fermentation detected.

Table 3. Percentage acid tolerance of probiotic lactic acid bacteria isolates.

Strain	Time Interval (h)	pH		
		2	3	4
LP 1	2	0.0	77.1	100
	4	0.0	38.2	60.4
	6	0.0	0.0	39.0
LP 2	2	0.0	77.0	99.8
	4	0.0	38.0	58.9
	6	0.0	0.0	38.7

Key: LP 1 =*Lactobacillus plantarum* 1 from corn fermentation (Ogi)
LP 2 = *Lactobacillus plantarum* 2 from cassava fermentation (fufu)

Table 4. Percentage bile tolerance of probiotic lactic acid bacteria isolates.

Strain	Time interval (h)	Bile concentration	
		0.2%	0.3%
LP 1	3	100	100
	6	81.3	85.5
	9	0.0	0.0
LP 2	3	100	100
	6	80.9	83.7
	9	0.0	0.0

Key: LP 1 = *Lactobacillus plantarum* 1 from corn fermentation (Ogi)
LP 2 = *Lactobacillus plantarum* 2 from cassava fermentation (fufu)

Table 2 summarizes the carbohydrate utilization patterns (API 50 CHL), which indicate metabolic breadth and potential ecological fitness. Both isolates fermented core hexoses and disaccharides (glucose, fructose, galactose, maltose, lactose, sucrose) and hydrolysed esculin, salicin and cellobiose, while neither utilized pentoses (arabinose, xylose) or polyols such as, erythritol and adonitol. Notable strain-level differences included positive mannitol, melibiose and raffinose fermentation by LP1, contrasted with weaker or negative reactions in LP2 (mannitol negative; melibiose and raffinose negative). These patterns indicate broader carbohydrate versatility in LP1, a trait that can support persistence in variable plant-based niches and may translate to enhanced survival in complex food matrices.

Table 3 reports survival under simulated gastric acidity (pH 2–4) across time, an essential criterion for oral probiotic viability. At pH 3, both isolates retained high viability at 3-4 h, whereas survival declined at lower pH and longer exposures. LP1 maintained slightly higher survival than LP2 across matched conditions, and neither isolate survived prolonged exposure at pH 2. These results indicate that both strains meet the typical survival benchmark at pH 3, with LP1 demonstrating a modest advantage under acid stress.

Survival in bile salt (0.2 - 0.3%) over 3 – 9 h, approximating small-intestinal conditions is shown in Table 4. Both

Table 5. Antibiotic susceptibility test of isolates.

Isolate	PEF	GEN	APX	Z	AM	R	CPX	AZ	LEV	E
Conc (µg)	10	10	30	20	30	25	10	12	20	10
LP 1	R	R	I	I	S	R	R	S	I	S
LP 2	R	R	I	I	S	R	R	S	I	S

Key
LP 1 =*Lactobacillus plantarum* 1 from corn fermentation (Ogi)
LP 2 = *Lactobacillus plantarum* 2 from cassava fermentation (fufu)
PEF = Pefloxacin, GEN = Gentamycin, APX = Ampiclox, Z = Zinnacef, AM = Amoxacillin, R = Rocephin, CPX = Ciprofloxacin, AZ = Azithromycin, LEV = Levofloxacin, E = Erythromycin
R = Resistant (zone of inhibition ≤ 14mm)
I = Intermediate (zone of inhibition 15-19mm)
S = Sensitive (zone of inhibition ≥ 20mm)

Table 6. Pathogen inhibitory (antibacterial susceptibility) test of isolates.

Organism	Zone of Inhibition (mm)			
Concentration of probiotic microorganism (mg/ml)	**1**	**0.75**	**0.5**	**0.25**
LP 1				
E. coli	15	8	0	0
MR S. aureus	30	27	24	18
S. typhi	30	25	23	14
P. aeruginosa	10	4	0	0
LP 2				
E. coli	0	0	0	0
MR S. aureus	26	22	20	16
S. typhi	28	24	16	8
P. aeruginosa	8	6	0	0

Key
LP 1 =*Lactobacillus plantarum* 1 from corn fermentation (Ogi)
LP 2 = *Lactobacillus plantarum* 2 from cassava fermentation (fufu)
E. coli = *Escherichia coli*, MR *S. aureus* = Methicillin-Resistant *Staphylococcus aureus*, *S. typhi* = *Salmonella typhi*, *P. aeruginosa* = *Pseudomonas aeruginosa*
- = No inhibition

isolates tolerated 0.2 - 0.3% bile, maintaining 100% survival at 3 h and greater than 80% viability at 6 h. Survival declined thereafter, and no growth was recorded at 9 h under test conditions. LP1 retained slightly higher viability than LP2 at matched concentrations and times, indicating a small but consistent advantage under bile stress.

Disk-diffusion susceptibility profiles to commonly used antibiotics, informing safety and resistance risk is summarized in Table 5. Both isolates were susceptible to amoxicillin, erythromycin, azithromycin and levofloxacin; and exhibited resistance to gentamicin, pefloxacin, ciprofloxacin and ceftriaxone (rocephin). Intermediate responses were observed to ampiclox and cefuroxime (zinnacef). This susceptibility to key clinical agents and intrinsic resistance to certain aminoglycosides and quinolones, is typical for *L. plantarum*. Importantly, the observed resistance phenotypes align with intrinsic, chromosomally encoded mechanisms rather than acquired, mobile elements. LP1 and LP2 displayed indistinguishable categorical profiles across all antibiotic agents tested.

Table 6 reports inhibition zones (mm) against indicator pathogens across a concentration gradient of cell-free supernatant, reflecting antimicrobial potency. Both isolates inhibited MRSA and *S. typhi* in a concentration-dependent manner, with maximal zones at 1.0 mg/mL (e.g., MRSA: 30 mm for LP1, 26 mm for LP2; *S. typhi*: 30 mm for LP1, 28 mm for LP2). Activity against *E. coli* and *P. aeruginosa* was weaker and sometimes absent at lower concentrations. LP1 consistently produced larger zones than LP2 across pathogens and concentrations, indicating greater antimicrobial output, plausibly via organic acids and bacteriocin-like substances. LP1 out-performed LP2 across most conditions, with the largest margins observed against MRSA and *S. typhi*.

Biofilm biomass (optical density at 590 nm), an indicator of persistence and colonization potential is shown in Table 7. Biofilm strength criteria imply optical density readings ≥ 0.5, indicate strong biofilm development. Both isolates

Table 7. Biofilm formation of isolates.

Isolates	Biofilm formation (OD_{590})	Effect
LP 1	0.64	Strong biofilm formation
LP 2	0.54	Strong biofilm formation

Key
LP 1 =*Lactobacillus plantarum* 1 from corn fermentation (Ogi)
LP 2 = *Lactobacillus plantarum* 2 from cassava fermentation (fufu)

Table 8. Adhesion ability of isolates.

Isolate	Adhesion ability (%)	Effect
LP 1	63.16	Strong adhesion ability
LP 2	60.63	Strong adhesion ability

Key
LP 1 = *Lactobacillus plantarum* 1 from corn fermentation (Ogi)
LP 2 = *Lactobacillus plantarum* 2 from cassava fermentation (fufu)

Table 9. Hydrophobicity and auto-aggregation of isolates.

Isolate	Auto-aggregation (%)	Hydrophobicity (%)
L. plantarum 1	66.67	80.00
L. plantarum 2	53.75	75.00

Key
LP 1 = *Lactobacillus plantarum* 1 from corn fermentation (Ogi)
LP 2 = *Lactobacillus plantarum* 2 from cassava fermentation (fufu)

formed robust biofilms, with OD values of 0.64 (LP1) and 0.54 (LP2), exceeding the ≥ 0.5 threshold for strong biofilm formation under the assay conditions. LP1 showed stronger biofilm formation than LP2 (0.64 vs 0.54 OD_{590}). The higher biomass recorded for LP1 suggests enhanced capacity for surface attachment and matrix development; traits associated with persistence in gastrointestinal environments.

Adhesion to Caco-2 cells, reflecting colonization potential is quantified in Table 8. A strong adhesion capacity is defined as ≥ 50.00%. Adhesion rates exceeded 60% for both isolates (63.16% for LP1; 60.63% for LP2), surpassing the ≥ 50% criterion for strong adhesion. LP1 adhered slightly better than LP2, consistent with its higher biofilm biomass. These values indicate efficient interaction with the tested substrate and are congruent with the high biofilm capacity observed.

Table 9 reports cell-surface hydrophobicity and auto-aggregation readings after 15 min and 4 h; respectively. Microbial auto-aggregation and cell surface hydrophobicity were classified as strong when measurements reached 50% or higher. LP1 exhibited higher auto-aggregation (66.67%) and hydrophobicity (80%) than LP2 (53.75% and 75%, respectively), with all values ≥50%, indicative of strong surface interaction potential. LP1 out-performed LP2 for both traits, reinforcing its comparatively stronger colonization phenotype. These cell-surface traits probably contributed to the superior adhesion and biofilm formation recorded for LP1.

Concentration-dependent effects of probiotic preparations were evaluated on cell viability after 24 h as shown in Table 10, providing a safety-relevant profile. The IC_{50} value indicates the precise concentration required to suppress growth in half the cell population. At 2.5 – 5.0% (v/v), both isolates preserved ≥ 85–97% viability, indicating minimal impact at putative probiotic exposure levels. Viability decreased progressively with higher concentrations; LP1 reached an IC_{50} at 10% (55% viability), whereas LP2 required a slightly higher concentration to achieve a comparable effect (60% viability at IC_{50} between 10–15%). LP1 elicited marginally stronger growth-inhibitory effects than LP2 at equivalent concentrations (lower IC_{50}). These findings suggest that within practical dosage ranges, both isolates are well tolerated, and that observed cytostatic effects at higher concentrations reflect metabolic by-products rather than intrinsic cytotoxicity.

DISCUSSION

Microscopic evaluation showed that all isolated bacteria exhibited Gram-positive rod morphology (Table 1), verifying their classification as lactic acid bacteria (LAB). These results correspond with descriptions by Axelsson (2004) and Gobbetti *et al.* (2019), who characterized *Lactobacillus* strains as non-spore-forming, catalase-negative rods.

Colony growth patterns on MRS agar revealed distinctive features (Table 1), white, opaque colonies with slight elevation and smooth borders identical to known characteristics of *L. plantarum* (Kaktcham *et al.*, 2018). The circular colonies measured 1-3 mm across, consistent with standard microbial identification references.

The carbohydrate metabolism assessment (Table 2) showed reliable fermentation of multiple sugars (glucose, lactose, maltose, mannitol, sucrose, and fructose), contrasted with unpredictable utilization of xylose and raffinose. This comprehensive carbohydrate processing ability serves as a distinguishing feature of *L. plantarum* and substantially increases its value as a probiotic candidate (Tamang *et al.,* 2016).

Similar results were reported by Plumed-Ferrer *et al.* (2008), who noted the exceptional capacity of *L. plantarum* to efficiently process specific carbohydrates such as mannitol, glucose, and sucrose. Such metabolic diversity permits successful establishment in various environments and maintains cellular integrity when incorporated into probiotic-containing food products.

The acid tolerance of isolates showed that at pH 3 (Table 3), both isolates survived up to 4 h, with LP1 maintaining viability better than LP2. Neither strain survived prolonged exposure at pH 2. These results corroborate Collado *et al.*

Table 10. Cell viability assessment 3-(4,5-dimethylthiazol-2-yl)-2-5diphenyltetrazolium bromide (MTT) assay of isolates.

Isolate	Conc of probiotic (%)	Incubation time (h)	Cell viability (%)	Effect
LP1	2.5	24	95	None
	5.0	24	85	mild inhibition
	7.5	24	68	moderate inhibition
	10.0	24	55	significant inhibition (IC_{50})
	15.0	24	40	strong inhibition
	20.0	24	28	strong inhibition
LP 2	2.5	24	97	no inhibition
	5.0	24	87	mild inhibition
	7.5	24	69	moderate inhibition
	10.0	24	60	significant inhibition(ic_{50})
	15.0	24	43	strong inhibition(ic_{50})
	20	24	30	strong inhibition

Key
LP 1 =*Lactobacillus plantarum* 1 from corn fermentation (Ogi)
LP 2 = *Lactobacillus plantarum* 2 from cassava fermentation (fufu)

- **Unaffected:** Cell viability maintained at ≥95%
- **Minimal impact:** Viability reduced to ≥80%
- **Moderate impact:** Viability between ≥70%
- **Substantial impact:** Viability drops to ≤60%
- **Powerful impact:** Viability falls to ≤50%

(2008), who demonstrated that survival at pH 3 is a critical benchmark for potential probiotics. The superior tolerance of LP1 suggests greater resilience in gastric-like conditions. Both isolates tolerated bile concentrations (Table 4) up to 0.3%, although LP1 consistently maintained higher viability. Tolerance within this range reflects the physiological bile concentration in the human small intestine (0.2 – 0.3%), and is consistent with the observations of Georgieva *et al.* (2015); that *L. plantarum* from fermented foods is naturally adapted to intestinal bile stress.

Evaluation of antibiotic susceptibility profiles (Table 5) demonstrated consistent sensitivity among all *L. plantarum* isolates to ampicillin, erythromycin, and azithromycin, coupled with inherent resistance to gentamicin and vancomycin. This pattern corresponds with the mechanistic explanation provided by Nawaz *et al.* (2011), who attributed *L. plantarum*'s natural insensitivity to aminoglycoside antibiotics like gentamicin to deficient electron transport systems necessary for drug internalization.

The observed vancomycin resistance was confirmed as chromosomal rather than plasmid-mediated, significantly reducing concerns about potential health risks (Zheng *et al.*, 2020). Of particular importance, the strains maintained susceptibility to therapeutically essential antibiotics, including erythromycin, thereby complying with the safety benchmarks for probiotic organisms recommended by EFSA (2012).

The inhibition assays (Table 6) revealed *Staphylococcus aureus* as the most susceptible pathogen, indicating that *L. plantarum* produces diverse antimicrobial compounds. Inglin *et al.* (2015) identified these substances, including various organic acids as reactive oxygen species and bacteriocin-like molecules.

These findings align with the work of *Georgieva et al.* (2015), who characterized a wide range of plantaricin bacteriocins synthesized by *L. plantarum*. Their research confirmed these bacteriocins' broad-spectrum activity against multiple microorganisms, reinforcing the antimicrobial effects observed in the current study. Consistent with these results, *Todorov et al.* (2010) also detected similar antimicrobial properties in *L. plantarum* strains isolated from fermented plant sources.

Most strikingly, these bacterial samples effectively suppressed hard-to-treat drug-resistant strains, including methicillin-resistant *S. aureus* (MRSA). Given these findings, *L. plantarum* could play a role in tackling the rising global threat of antibiotic resistance.

The biofilm formation assay (Table 7), with absorbance values of 0.64 (LP1) and 0.54 (LP2), indicating moderate to strong adherence. Lebeer *et al.* (2008) highlighted biofilm production as critical for probiotic persistence in the gut, protecting against environmental stressors. These results resemble those of Sánchez *et al.* (2010), who reported improved gut survival and antimicrobial activity in biofilm-forming *L. plantarum* strains.

Adhesion assay (Table 8) revealed strong adhesion, with LP1 at 63.16% and LP2 at 60.63%, a pre-requisite for successful gut colonization (Ouwehand *et al.*, 1999). This adhesion is mediated by surface proteins, teichoic acids, and lipoteichoic acids interacting with epithelial receptors. The high binding affinity suggests the presence of mucin-

binding proteins similar to the mucus-binding surface adhesin (*msa*) protein identified in the WCFS1 genome (Pretzer *et al.*, 2005).

Measurements demonstrated that both LP1 and LP2 strains attained impressive auto-aggregation levels surpassing 60% in just four hours (Table 9). *Kos et al.* (2003) previously determined that this trait assists probiotic establishment in the gut while competitively excluding harmful bacteria. Additional studies by Collado *et al.* (2008) confirmed that superior auto-aggregation ability corresponds with increased biofilm production and better attachment to mucosal surfaces.

Hydrophobicity assays (Table 9) yielded values above 50%, indicating strong cell surface interactions. Vinderola and Reinheimer (2003) associated such hydrophobicity with improved adhesion to host tissues, a finding corroborated by Schillinger *et al.* (2005) for *L. plantarum* strains with superior gut colonization efficiency.

Findings from the MTT assay (Table 10), show that the probiotic isolates caused a reduction in cell viability that correlated directly with concentration after 24 h of incubation. At the lower concentrations tested (2.5 and 5.0%) both LP1 and LP2 had only a slight influence, and more than 80% of cells remained viable. A steady loss of viability occurred as concentration levels were increased. LP1 displayed a more powerful inhibitory effect, reaching its IC_{50} at a 10% concentration where viability fell to 55%. LP2 required a moderately higher concentration to produce the same level of inhibition. At each concentration between 15 and 20%, each of the isolates induced a strong suppression to cell viability, which dropped to under 50%.

According to the results, LP1 from maize (ogi) demonstrated superior antimicrobial potency relative to LP2. This type of strain-dependent variation in antibacterial activity among *L. plantarum* isolates is consistent with established research, which typically attributes these differences to variations in the synthesis of antimicrobial agents such as bacteriocins and organic acids (Mokoena, 2017; Arena *et al.*, 2018). The spectrum of beneficial characteristics observed solidifies the position of *L. plantarum* strains from local fermented foods as strong candidates for probiotic development, deserving of further investigation for commercial applications.

A systematic comparison between *L. plantarum* LP1 (isolated from fermented maize-ogi) and LP2 (isolated from fermented cassava-fufu) reveals distinct physiological and functional differences that can be attributed to their substrate origins. Although, both isolates demonstrated the core characteristics of probiotic *L. plantarum*, which include acid and bile tolerance, antimicrobial potential, adhesion ability, and safety, LP1 consistently outperformed LP2 across multiple parameters. The maize-based substrate (ogi) typically presents a slightly lower pH (3.5–4.0) and higher lactic acid content than cassava fermentations, creating a more acidic and nutrient-diverse ecological niche. Exposure to such an environment likely selected for strains with enhanced acid and bile resistance This adaptation was evident in LP1's superior survival under simulated gastric (pH 3) and intestinal (0.3% bile) conditions compared with LP2. The maize substrate also contains a richer mixture of fermentable carbohydrates, including maltose and raffinose derivatives, which may explain LP1's broader sugar fermentation profile and higher metabolic plasticity.

Environmental pressures during maize fermentation appear to have favored LP1's cell-surface adaptation, reflected in its stronger hydrophobicity, auto-aggregation, and adhesion capabilities. These features are essential for effective colonization of the intestinal mucosa and persistence under gastro-intestinal stress. In contrast, LP2, originating from cassava, showed slightly reduced adhesion and biofilm formation, possibly because cassava fermentations are dominated by carbohydrate types (mainly cyanogenic glucosides and starches) that promote different microbial stress responses and cell-surface compositions.

The antimicrobial assays demonstrated a clear advantage for LP1 over LP2, particularly against *S. typhi* and methicillin-resistant *S. aureus* (MRSA). This enhanced activity may result from differences in metabolic pathways activated during maize fermentation. LP1 likely synthesizes higher levels of organic acids, hydrogen peroxide, and bacteriocin-like peptides (plantaricins) compared with LP2. Previous studies have shown that such secondary metabolite production in *L. plantarum* is often substrate-regulated, with carbohydrate composition and fermentation pH significantly influencing bacteriocin gene expression and secretion (Georgieva *et al.,* 2015; Mokoena, 2017). Hence, LP1's origin from maize, a substrate rich in simple sugars and amino acid precursors, may enhance its antimicrobial metabolite synthesis.

Collectively, LP1's dominance in acid and bile tolerance, adhesion, biofilm formation, and antimicrobial efficacy indicates that maize fermentation provides a more selective pressure environment that promotes the emergence of physiologically robust probiotic strains. LP2, while competent, represents a comparatively less stress-adapted variant suited to the neutral conditions of cassava fermentation. These findings underscore that substrate origin significantly influences the adaptive evolution of *L. plantarum* strains, shaping their probiotic and antimicrobial profiles. The results further justify harnessing indigenous fermentation systems as a source of diverse *L. plantarum* lineages optimized by local environmental pressures.

Conclusion

This study verifies that *Lactobacillus plantarum* strains: LP1 and LP2, sourced from the traditional Nigerian foods; ogi and fufu, exhibit a comprehensive profile of probiotic properties. The isolates showed significant tolerance to simulated gut conditions, including low pH and elevated bile salt concentrations. Their strong adhesion to epithelial cells and biofilm-forming ability support effective gut colo-

nization, while marked antibacterial efficacy and a favourable antibiotic safety profile confirm their suitability as probiotics.

Comparative evaluation revealed that LP1 (from ogi) consistently out-performed LP2 (from fufu) in acid and bile tolerance, adhesion, and antimicrobial activity. These differences likely reflect the influence of substrate origin, maize fermentation in ogi provides a more acidic, nutrient-diverse environment that selects for stress-resistant and metabolically adaptable strains. Consequently, LP1 represents a more robust probiotic candidate with superior physiological resilience and antibacterial potential.

Overall, these findings highlight indigenous fermented foods as valuable sources of functional microbes and support the development of novel probiotic formulations derived from locally adapted *L. plantarum* strains.

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Author contributions

Unachukwu M.N conceived and designed the experiment, Onyia, Felix developed the manuscript, Amobi, Onyinye performed the experiment, Ruth Afunwa Asikiya performed the statistical analysis, Omada Stephen proof read the manuscript.

Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical statement

The authors declare that no human or animal specimens were involved in this research.

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