Journal of Applied Biosciences 222: 24683 – 24694
ISSN 1997-5902

Investigation of probiotics potential of *Lactobacillus plantarum* strains isolated from traditionally fermented maize and cassava.

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Submitted 15/05/2026, Published online on 31/07/2026 in the https://www.m.elewa.org/journals/journal-of-applied-biosciences-about-jab/ https://doi.org/10.35759/JABs.222.4

ABSTRACT

Background: Probiotics are beneficial microorganisms that confer health benefits to the host when consumed in adequate amounts. Traditionally fermented foods serve as important natural sources of these organisms. Indigenous fermented foods such as *ogi,* fermented corn flour, fufu and tapioca from maize and cassava harbour diverse lactic acid bacteria with potential probiotic and antimicrobial properties.

Objectives: This study was carried out to isolate and identify *Lactobacillus plantarum* from fermented maize and cassava products, and to characterize some genetic profiles of the isolated *Lactobacillus plantarum* strains using Whole Genome Sequencing

Methods and Results: Two strains of *Lactobacillus plantarum* LP1 (isolated from fermented maize) and LP2 (from fermented cassava), were characterized for key probiotic attributes using whole genome sequence. Both strains demonstrated remarkable presence of essential genes such as mucin 22, *fbp, dltD, bsh,* and *pln* genes which are associated with probiotic functionality, particularly those involved in cellular adhesion, bile salt resistance, bacteriocin production and immune regulation. Both strains possessed plantaricin associated genes (*plnA – plnH*) indicating bacteriocin producing potentials. Genomic analysis also identified seven prophage regions in both isolates, while CRISPR analysis showed one CRISPR array in LP1 and two CRISPR array in LP2.

Conclusion and application of results: 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.

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.*, 2017). Within the LAB group, *Lactobacillus plantarum* emerges as particularly adaptable and extensively investigated. Research demonstrates its capacity to withstand gastrointestinal 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 (*ogi*, fermented corn flour, fufu and tapioca) and evaluate their probiotic properties using genomic analysis.

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 hours 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 the terms are defined as:

- 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 *et al.*, 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 hours 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 (10g) 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 hours under microaerophilic conditions. Distinct colonies were purified through repeated subculturing. 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 manufacturer specifications. Carbohydrate fermentation patterns were compared with database standards for identification.

Evaluation of Probiotics Characteristics

Genome Sequencing: Two *L. plantarum* strains, called LP 1 and LP 2, were selected for whole-genome sequencing. The method followed techniques reported by Tanizawa *et al.* (2015).

RESULTS

Table 1 presents 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 summarizes the carbohydrate utilization patterns (API 50 CHL), which indicate metabolic breadth and potential ecological fitness.

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).

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.

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 compares the genomic characteristics of the two *Lactobacillus plantarum* isolates (LP1 and LP2) with the reference strain (WCFS1). It shows their sources, genome sizes, GC content, and key genetic features such as the number of genes, coding genes, prophage regions, pseudogenes, and RNA genes, highlighting similarities and differences among the strains.

Table 3: Genomic analysis of *Lactobacillus plantarum* 1 and 2

STRAIN	LP 1	LP 2	REFERENCE STRAIN WCFS1
Source	Maize (*Ogi*)	Cassava (Fufu)	Human Saliva
Genome Size (bp)	3394299	3398178	3308274
G+C Content (%)	45.8%	45.8%	45.6%
Total number of genes	3363	3387	3174
Coding genes	3292	3220	3063
No. of prophage region	7	7	4
Pseudogenes	64	62	23
Total number of RNA	81	84	88
Number of tRNA	62	62	70
No. of Crisper Array	1	2	-
Number of rRNA	16	16	16
Number of nc RNA	3	6	3

Key *LP* 1= *Lactobacillus plantarum* 1, *LP* 2 = *Lactobacillus plantarum* 2

Table 4 summaries probiotic-related genes identified in *Lactobacillus plantarum* strains LP1 and LP2, and compares them with a reference strain (WCFS1). It shows the genes involved, their functions (such as adhesion, immune modulation and bacteriocin production), and whether each gene is present in the strains.

Table 4: Probiotic genes found in *Lactobacillus plantarum strains* Lp 1 and Lp 2

GENES	FUNCTIONS	RESPONSE	*Lactobacillus plantarum strains*		REFERENCE STRAIN
			LP 1	LP 2	WCFS1
ADHESION ABILITY					
Mucin 22	Mucin binding	adhesion ability	+	+	+
Fbp	Fibronectin binding	Adhesion ability	+	+	+
IMMUNE MODULATION					
dltD	Incorporation of D-alanine into LTA	Resistance to human b-defensin-2	+	+	+
dltB	Incorporation of D-alanine into LTA	Anti-inflammatory potential in vitro in PBMCs and in vivo in a murine model of colitis or in a rat model for visceral pain perception	+	+	+
ACTIVE REMOVAL OF STRESSORS					

bsh	Bile salt hydrolase	Bile salt resistance	+	+	+
GadB	GABA transporter	Acid tolerance	+	+	+
DNA AND PROTEIN PROTECTION REPAIR					
clpc		Persistent capacity *in vivo*	+	+	+
ClpL	clpATPase (chaperon)	Acid and bile tolerance	+	+	+
Dps	DNA protection during starvation	DNA protection during starvation	+	+	+
STRESS RESISTANCE GENES					
DltA	d- anylation of LTA	Acid and defensin Resistance	+	+	+
ANTI-PATHOGENIC EFFECT					
LuxS	Production of AI-2. AI-3	Autoinduction ability	+	+	+
Pln GENES					
plnA	Regulatory and signalling	Induction peptide (pheromone)	+	+	+
PlnB	Regulatory	Histidine protein kinase	+	+	+
PlnC	Regulatory	Response regulator protein	+	+	+
PlnD	Regulatory	Response regulator protein	+	+	+
PlnE	Structural	Bacteriocin peptide (plantaricin E)	+	+	+
PlnF	Structural	Bacteriocin peptide (plantaricin F)	+	+	+
PlnJ	Structural	Bacteriocin peptide (plantaricin J)	+	+	+
PlnK	Structural	Bacteriocin peptide (plantaricin K)	+	+	+
PlnN	Structural	Bacteriocin peptide	+	+	+

PlnW	Structural	Bacteriocin peptide	+	+	+
PlnI	Immunity	Immunity protein	+	+	+
PlnL	Immunity	Immunity protein	+	+	+
PlnG	Transport	ABC transporter protein	+	+	+
PlnH	Transport	ABC transporter accessory protein	+	+	+

Key: *LP* 1= *Lactobacillus plantarum* 1*, LP* 2 = *Lactobacillus plantarum* 2
+ = Present, - = Absent

DISCUSSION

Microscopic evaluation showed all isolated bacteria exhibited Gram-positive rod morphology (Table 1), verifying their classification as 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 *Lactobacillus plantarum* (Kaktcham *et al.*, 2017). The circular colonies measured 1-3 mm across, consistent with standard microbial identification (Withman,2015). 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.*, 2020). 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 genomic analysis, (Table 3) definitively classified the isolates as *Lactobacillus plantarum*. As Felis *&* Dellaglio (2007) have stressed, whole genome sequencing provides the highest level of accuracy and repeatability for microbial identification. Comparative genomic analysis showed strong alignment with reference strains, confirming earlier biochemical and phenotypic results. These genomic findings correspond with Siezen *et al.* (2011), who noted extensive genetic diversity within *L. plantarum* strains, particularly in genes involved in carbohydrate metabolism and stress adaptation. Molecular verification provides a stronger foundation for considering these strains as prospective probiotics, especially in light of their demonstrated functional attributes under laboratory conditions. *Lactobacillus plantarum* LP1, a strain isolated from fermented maize, was found to have a circular chromosome spanning 3,394,299 base pairs. Analysis of its base composition revealed a guanine and cytosine (G+C) content of 45.8%. Gene prediction and annotation identified 3,363 genes in total.

Among these, 3,292 are functional protein-coding genes, and 64 are pseudogenes. The genome also specifies 62 tRNA genes, 16 rRNA genes, and 3 non-coding RNA genes. The investigation also detected one clustered regularly interspaced short palindromic repeats (CRISPR) array and seven different prophage regions. A genetically similar strain, *Lactobacillus plantarum* LP2, was obtained from fermented cassava. Its sequenced DNA is 3,398,178 bp long and shares the same 45.8% G+C ratio as LP1. We determined that LP2 contains 3,387 genes, comprising 3,220 that code for proteins and 62 that are pseudogenes. The assortment of RNA genes is exactly the same as in LP1, with 62 tRNAs, 16 rRNAs, and 3 ncRNAs. A principal structural variation is that LP2 was found to have two CRISPR arrays, one more than the sole array present in LP1. It is significant that both strains were confirmed to have seven prophage regions. Table 4 Examined the functional capabilities of LP1 and LP2. LP1 revealed genes that encode the Muc22 and Fbp proteins. These act as adhesins, enabling the bacterium to bind to intestinal mucin and fibronectin. This aligns with earlier work that recognizes such adhesion molecules as vital for the probiotic function of *L. plantarum* (Oh *et al.*, 2022). Also present is the *dltD* gene, which contributes to the D-alanylation of lipoteichoic acid. This specific modification has been tied to the regulation of the host's immune activity (Genomic Characterization of *L. plantarum* Strains, 2023). Other noteworthy genetic elements are a bile salt hydrolase (*bsh*) gene, granting resistance to bile, and the *clpC* gene, which aids in stress response and maintaining protein integrity, *pln* genes which code for bacteriocins (plantaricins) and aids in pathogen inhibition, making these isolate potentially good probiotic candidates. The results from table 4 also revealed that both isolates harbor key plantaricin (*pln*) genes, including structural genes (*plnE, plnF, plnJ, plnK*), regulatory genes (*plnA, plnB, plnC* and *plnD*), as well as transport (*plnG* and *plnH*), immunity genes (*plnI*). This indicates the genetic potential of the isolates to produce bacteriocins and exert antimicrobial activity. This result is also in line with whole genome sequencing of reference strain WCFS1 which showed the presence of these plantaricin genes (Genomic Characterization of *L. plantarum* Strains, 2023). LP2 was shown to possess the same core probiotic genes found in LP1. This common genetic toolkit encompasses the mucin- and fibronectin-binding adhesins, along with *dltD*, *bsh*, *clpC* and *pln* genes. The pair of CRISPR arrays in LP2's genome is inferred to grant it a superior defensive capability against bacteriophage predation. A comprehensive analysis of *L. plantarum* genomes supports this view, demonstrating that these systems enhance genomic integrity (The CRISPR-Cas System in *L. plantarum* Strains, 2024). 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. A systematic comparison between *Lactobacillus 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. The maize-based substrate (*ogi*) typically presents the fundamental genomic characteristics of LP1 (from maize) and LP2 (from cassava) are closely aligned; however, their different sources are mirrored in several crucial genetic variations. The detection of a full complement of probiotic genes in strains from cereal and tuber origins significantly widens the recognized ecological distribution of *L. plantarum*, a bacterium that has been more frequently investigated in dairy or vegetable

settings. One especially important discovery is the presence of two CRISPR arrays in LP2, compared to just one in LP1. Given that multiple CRISPR systems are not common in this species, this most likely indicates a stronger, more adaptable antiviral defense system (The CRISPR-Cas System in *L. plantarum* Strains, 2024). Another shared trait is that both strains carry seven prophage regions, a number that exceeds the three to five typically reported for other isolates (Whole Genome Sequencing of FCa3L, 2023). This provides compelling evidence that both strains developed in fermentation environments teeming with bacteriophages. At the genetic level, LP1 and LP2 both exhibit an advantageous blend of properties that support attachment to the intestinal lining, immune regulation, bile acid tolerance, and cellular stress handling. While these individual attributes have been noted in other strains, their co-existence in these particular indigenous isolates underscores the unique value of these traditional fermented foods as sources of new *L. plantarum* strains with probiotic capabilities. The results further justify harnessing indigenous fermentation systems as a source of diverse *L. plantarum* lineages optimized by local environmental pressures.

CONCLUSION AND APPLICATION OF RESULTS

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. Their genomic analysis also provides a stronger foundation for considering these strains as prospective probiotics, especially in light of their demonstrated functional attributes under laboratory conditions. Comparative evaluation revealed that LP1 (from *ogi*) and LP2 (from fufu) showed great probiotic properties, although they also exhibited key differences in their genomic profiles, 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. Although, the pair of CRISPR arrays in LP2's genome is inferred to grant it a superior defensive capability against bacteriophage predation. This strengthened immunity was probably naturally selected for in the cassava fermentation environment, where phage encounters are assumed to be frequent. 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.

ACKNOWLEDGMENTS

The entire staff of the Department of Biological Science, Godfrey Okoye University Enugu, Department of Microbiology Nnamdi Azikiwe University, Akwa and National Open University, Jabi, Abuja was acknowledged.

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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