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Probiotic potential and functional properties of lactic acid bacteria isolated from gut of mouse and their ability to produce fermented milk

Fatima Mojisola Akanbi , Majekodunmi Racheal Adedayo, Bashirat Olamide Yusuf-Salihu

Department of Microbiology, Faculty of Pure and Applied Sciences, Kwara State University, Malete, Nigeria.

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ABSTRACT

Lactic acid bacteria (LAB), are among the organisms that are generally considered safe, and have been used as probiotic agents due to their ability to produce a variety of beneficial compounds in food during processing and storage, in addition to their biological-safety as non-pathogenic and non-toxic. The present study investigated the probiotic potential and fermentative property of LAB obtained from the gut of mouse. Lactic acid bacteria were isolated from the gut of mouse, characterized and identified through molecular technique. The optimal growth conditions for the isolates and fermentative ability on milk were assessed using standard methods. Four species of LAB were characterized through their DNA and identified as *Lactobacillus casei* (PV355690), *Lactobacillus fermentum*, (PV355691), *Lactococcus lactis* (PV355692), and *Pediococcus pentosaceus* (PV355693). Optimum culture conditions for the LAB isolated were 0-4 % of NaCl, pH of 4-11, 0-0.5 % bile concentration, and 0.00 to 0.8 % phenol tolerance, all isolates were gamma hemolytic. *Lactobacillus casei* (PV355690), *Lactobacillus fermentum*, (PV355691), *Lactococcus lactis* (PV355692), and *Pediococcus pentosaceus* (PV355693) were able to fermented milk. As a result, the obtained isolates have probiotic potential, and are able to reduce the pH of milk, thus ferment. Thus, they can be considered as probiotic bacteria in the food processing industry, and for fermentation purposes.

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1. Introduction

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer health benefits on the host. These beneficial microorganisms are mostly classified as lactic acid bacteria (LAB), including genera such as *Lactobacillus, Bifidobacterium,* and *Streptococcus.*

Over recent decades, probiotic consumption has surged due to growing awareness of potential health advantages, such as strengthened immunity, better gut health, and disease prevention (1). Substantial research

*Corresponding author.
E-mail address: zakswonder@gmail.com

highlights that probiotics play a critical role in preserving a balanced gut flora, which is essential for general health and wellbeing (2).

LAB are a key probiotic subset, renowned for fermenting carbohydrates into lactic acid. This fermentation process contributes to the flavor and preservation of food like yogurt, kefir, and sauerkraut, while playing a vital role in gut health. LAB maintain optimal gastrointestinal pH balance, suppressing harmful pathogen growth, and contribute to synthesis of vital nutrients including B-group vitamins and vitamin K (3, 4). Furthermore, Bioactive substances like bacteriocins are produced by LAB. and short-chain fatty acids (SCFAs), which exhibit antimicrobial properties and modulate immune response (5). This intricate interplay between LAB and gut health emphasizes the significance of understanding these microorganisms both individually and collectively in their contribution to gut microbiome functioning.

The gut microbiota is made up of trillions of bacteria in the gastrointestinal tract and is essential for several physiological functions, including immunological control, metabolism, and digestion (6). Gut microbiota imbalance, termed dysbiosis, can result in a number of health problems, including as metabolic syndrome, obesity, and inflammatory bowel disease (7). Probiotics, particularly LAB, restore microbial balance and support gut health by enhancing helpful microorganism diversity and suppressing dangerous pathogen growth (8). Developing successful dietary plans and interventions that promote health and prevent disease requires an understanding of the processes by which probiotics exert favorable gastrointestinal effects.

Studies using animal models, particularly mice, have been instrumental in elucidating probiotic impacts on gut health. For example, *Lactobacillus reuteri* administration in mouse models promoted regeneration of intestinal epithelium and repaired injured mucosa by stimulating Wnt/β-catenin signaling and maintaining intestinal stem cell activity (9). Studies demonstrated that LAB strain administration could significantly alter gut microbiota composition in mice, resulting in increased immunological responses, decreased inflammation, and improved intestinal barrier function (10).

Specific strains of *Lactobacillus* and *Bifidobacterium* have been demonstrated to increase beneficial bacteria abundance while decreasing pathogenic species, thereby promoting a healthy microbiota profile (11). These findings underscore the relevance of mouse models in probiotic research, providing insights into potential applications in human health.

The relationship between probiotics and the intestinal environment is intricate and multifaceted. Probiotics must survive harsh gastrointestinal tract conditions, including exposure to gastric acid and bile salts, to exert beneficial effects (12). LAB's capacity to stick to the gut mucosa is crucial for establishment and functionality within the gut ecosystem (13). This adherence facilitates stable microbial community establishment and enhances production of metabolites contributing to gut health, such as short-chain fatty acids (SCFAs) which have anti-inflammatory qualities and provide intestinal epithelial cells with energy (14).

This research addresses critical gaps at the intersection of food science, microbiology, and public health by characterizing indigenous LAB with dual functionality

as both starter cultures and probiotic agents. The study advances scientific understanding of probiotic mechanisms through systematic evaluation of various functional properties which include bile salt tolerance, pH tolerance, hemolytic safety, antimicrobial properties, and gut health modulation capabilities, while directly responding to industrial challenges in maintaining LAB viability and meeting consumer demands for minimally processed, health-promoting foods. Considering to the increasing global prevalence of digestive disorders and dysbiosis-related complications, this work, by identifying novel indigenous starter cultures that simultaneously possess fermentative, preservative, and probiotic properties, could provide evidence-based solutions through fermented dairy products development with proven probiotic efficacy.

2. Materials and Methods

2.1. Collection of samples

An axenic mouse (a mouse was used because sufficient isolates were obtained) was procured from Kwara State University Biochemistry Department and aseptically moved to the Microbiology lab for experimental procedures. The mouse was euthanized using chloroform, a method ensuring rapid and humane paralysis. The gut was aseptically removed and processed to isolate LAB after euthanasia.

2.2. Preparation of growth media, reagents and equipment used

De Man, Rogosa, and Sharpe (MRS) broth and agar were used for the cultivation of LAB. After dissecting the mouse, intestine contents were collected aseptically, homogenized, and inoculated into MRS broth for 24 to 48 h under anaerobic conditions at 37° C to enrich LAB populations. To separate individual LAB colonies, the enriched samples were serially diluted and plated on MRS agar. Chemical reagents, including glucose, bile salts, and various buffers (pH 6.5-7.5), were prepared for microbiological and biochemical assays. Ethanol and other solvents were used for sample preparation and analysis. Essential laboratory equipment included incubators, centrifuges, spectrophotometers, and PCR machines for molecular examination.

2.3. Isolation of LAB

Isolation of LAB from the gut of mouse was done based on the approach outlined by El Kahlout et al (6). Emerging colonies on plates were taken as presumptive LAB based on the approach outlined by El Kahlout et al. The isolate's identity was further confirmed by subjecting them to molecular characterization using their extracted DNA.

2.4. Molecular characterization and identification of LAB isolate

The strains of promising probiotic LAB were identified by 16S rRNA (16). Overnight cultures were used to harvest genomic DNA of this strain using the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's instructions, which included lysozyme treatment and proteinase K digestion. DNA concentration and purity were assessed using a NanoDrop spectrophotometer (17). The universal primers 27F were used to amplify the 16S rRNA gene. (5′-AGAGTTTGATCCTGGCTCAG-3′) and U1492R (5′-GGTTACCTTGTTACGACTT-3′) with GoTaq Green Master Mix (Promega, USA) in a conventional thermocycler (Veriti, Applied Biosystems, USA). The PCR cycling conditions consisted of an initial denaturation at 94 °C for 3 min, followed by 29 cycles

of 94 °C for 45 s, 53 °C for 60 s, and 72 °C for 90 s, with a final extension at 72 °C for 5 min. Amplified products were visualized on a 2% agarose gel. BioEdit software was used to evaluate the resultant 16S rRNA gene sequences. (version 7.0). Consensus sequences were compared against the GenBank database using NCBI BLAST to confirm the species identification of the isolates (18).

2.5. Functional property/ characteristics assay of the isolated LAB

2.5.1. NaCl tolerance test

NaCl concentrations were used to test the isolates tolerance to NaCl. of 0.5, 1, 2, and 4 % w/v added to the MRS broth. After inoculating fresh broth, it was incubated at 37 °C for 48 h. Optical densities were measured after 24 and 48 h using a spectrophotometer (Photolab 7600, UV-VIS, EU) at 620 nm. Results were obtained by looking at the optical density of the culture media (19).

2.5.2. pH tolerance test

The MRS broth's pH was changed to 2.5 using 3 N HCl and 1 N NaOH. New bacterial cultures were added to the appropriate MRS broth in test tubes and incubated at 37 °C for 48 h. Optical density at 620 nm was measured using a spectrophotometer after 24 and 48 h. Results were acquired by looking at the optical density of the culture media (19).

2.5.3. Bile Salt tolerance test

For inoculation, precisely 0.1 mL of cultures grown overnight were put to a sterile MRS broth medium with 0.1, 0.3, and 0.5% bile salt. The incubation commenced at 37 °C, and optical density was measured at 620 nm after 24 and 48 h using a spectrophotometer. The optical density of the culture media was examined to obtain the results (19).

2.5.4. Phenol tolerance test

In order to evaluate the four selected LAB isolates phenol tolerance, overnight LAB cultures were moved to a fresh MRS broth that contained0, 0.2, 0.4, 0.6 and 0.8 % phenol. A spectrophotometer was used to measure optical density at 620 nm after 24 and 48 h of incubation at 37 °C. According to (19), optical density was observed and recorded.

2.5.5. Hemolytic assay

On blood agar plates, the isolated LAB strains' hemolytic activity was assessed (20). The hemolytic activity of the isolated strains was evaluated based on the lysis of red blood cells in the media surrounding the colonies. The bacteria were tested for hemolytic activity on tryptone soy agar with sheep blood (TSA-SB) by streaking 24 h cultures on the blood agar plates followed by incubation at 37 °C under anaerobic conditions (Anaerogen, Oxoid) for 24 h. The appearance of clear zones around the bacteria colonies indicated the presence of β-haemolysis whereas green zones around the colonies suggested α-hemolysis (21).

2.5.6. Diacetyl production test

The test was carried out by measuring 1 mL of α-naphtol with 5 mL of peptone water and 1 mL KOH were measured and added to sterile, clean test tubes. The tubes were injected with the test organisms and incubated at 37 °C for 48 h. Positive responses are indicated by the formation of a bright red ring (22).

2.6. Determination of the fermentative ability of LAB isolated

The ability of the isolated LAB to make fermented milk products was assessed. The milk preparation and

fermentation procedures were adapted from the methodologies outlined by Nova et al (14), which involved pasteurizing pure cow milk, inoculating it with selected LAB strains, then incubation for 24 h at 37 °C. This process caused the pH to drop and the concentration of lactic acid to rise, indicating successful fermentation. A measure of 100 g of milk powder was mixed with 1 L of distilled water to get a concentration of 4 % (w/v) milk. The mixture was pasteurized at 70 °C in a water bath for 30 min. Afterward, it was cooled down to the room temperature. Next, a 2 % bacterial culture was added to the milk as inoculum, and fermentation was allowed for 24 h at 37 °C in sealed containers.

3. Results

3.1. Characteristics of the isolated LAB

There were four distinct LAB isolates identified based on the displayed biochemical properties and fermentation potential as *Lactobacillus* sp. (AK 002), *Lactobacillus* sp. (AK 003), *Lactococcus* sp. (AK 004) and *Pediococcus* sp. (AK 005).

3.2. Molecular Identification of the LAB that were isolated

The isolates were verified by molecular identification; AK-002 as *Lactobacillus casei* (PV355690), AK 003 as *Lactobacillus fermentum* (PV355691), AK 004 as *Lactococcuslactis* (PV355692) and AK 005 as *Pediococcus pentosaceus* (PV355693) according to the 16S rRNA and the bacteria's reported accession number (Table 1). Fig. 1 shows A maximum likelihood phylogenetic tree indicating a genetic connection between RNA sequences of LAB strains derived from the mouse gut and reference strains available in NCBI. This phylogenetic dendogram shows evolutionary relationships among LAB isolates based on 16S rRNA gene sequences. The tree displays distinct clustering by genus, with *Lacticaseibacillus* casei strains (HAVAL4 and MED5) which are indicated through very short branch lengths (0.0000) caused by minimal genetic divergence thereby forming a closely related upper cluster, *Pediococcus pentosaceus* and *Lactococcus lactis* strains occupying the middle section, and *Limosilactobacillus fermentum* strains clustering tightly together. The numerical values on branches indicate evolutionary distances or bootstrap support values, while the scale bar represents genetic distance units of 2.00. The dendogram confirms that experimental isolates (AK-003, AK-004, AK-005, and AK-002) cluster appropriately with their corresponding GenBank reference strains, validating their species-level identification through phylogenetic analysis.

3.3. Functional property of the LAB

3.3.1. Sodium chloride (NaCl) tolerance by the LAB

The LAB isolates were able to grow well in NaCl concentration ranging from 0.5 to 4.0 %. However, the growth rate of all the isolates decreased as NaCl concentration increased as shown in Table 2. *Lactobacillus lactis* (PV355692) exhibited maximum growth (1.86 ± 0.01^{d}) followed by *Lactobacillus fermentum* (PV355691) (1.47 ± 0.01^{d}), then by *Lactobacillus casei* (PV355690) (1.31 ± 0.02^{d}) and (PV355693) (1.15 ± 0.02^{d}). The highest tolerance value of 0.80 ± 0.02^{a} (OD_{600}nm) at the highest NaCl level of 4.0% was maintained by *Lactobacillus lactis* (PV355692) but *Lactobacillus casei* (PV355690) and *Pediococcus pentosaceus* (PV355693) showed minimal threshold at 0.52 ± 0.05^{a} and 0.52 ± 0.02^{a}. *Lactococcus lactis* (PV355692) was the most salt-tolerant isolate at all the experimental

conditions. Significant variations between isolates were revealed by statistical analysis ($p<0.05$) Result is presented in Table 2.

3.3.2. Effect of pH on LAB's rate of growth

All isolates of LAB were able to grow across a broad range of pH. They grew well at pH 4.3 and pH 11, though growth was observed to be optimal at around neutral. *Lactobacillus fermentum* (PV355691) had the least growth at the extreme acidic conditions of pH 4.3 (0.32 ± 0.02^{a}) while *Lactobacillus lactis* (PV355692) had the highest acid-tolerance with growth of 0.59 ± 0.03^{a}. *Lactococcus lactis* (PV355692) demonstrated the highest survival capacity at pH 11.0 through its ability to withstand alkaline conditions with a growth rate of 0.67 ± 0.04^{b}. Among the four LAB strains *Lactococcus lactis* (PV355692) performed best in terms of pH tolerance and the ability to grow at a wide range of pH. Result is presented in Table 3.

3.3.3. Effect of bile salt on LAB growth

The isolates could proliferate in the presence of of 0.1 to 0.5 % concentrations of bile salt. The growth rate of *Pediococcus pentosaceus* (PV355693) remained highest at 0.1% ($0.83 \pm 0.03)^{b}$ and it also had the highest growth record at highest bile salt concentration used (0.5%) ($0.76 \pm 0.05)^{b}$. *Lactobacillus* casei (PV355690) and *Lactococcus lactis* (PV355692) displayed moderate tolerance to bile salt across all concentrations used (0.59 ± 0.03^{b} and 0.53 ± 0.02^{a}) but *Lactobacillus fermentum* (PV355691) showed the lowest growth performance at 0.3 and 0.5 %. In all concentrations, *Pediococcus pentosaceus* (PV355693) demonstrated highest bile salt tolerance. The result is presented in Table 4.

3.3.4 . Effect of phenol on growth rate of the LAB

The effect of phenol on growth is displayed in Table 5; the LAB isolates growth was dose-dependent. All of the LAB isolates' development was generally inhibited by the rise in phenol content. The optimum growth and highest phenol tolerance was recorded by *Lactobacillus casei* (PV355690) at all concentrations used (0% at a value of 1.47 ± 0.01^{e} and 0.20 ± 0.00^{a} at 0.8% phenol concentrations). Phenol tolerance by isolates at different concentrations were considerably different at ($p<0.05$).

3.3.5. Hemolytic ability of the LAB

The LAB isolates' hemolytic capacity is shown as in Table 7. The data gathered reviewed that *Lactobacillus casei* (PV355690), *Lactobacillus fermentum* (PV355691), *Lactococcus lactics* (PV355692) were gamma γ-haemolytic but *Pediococcus pentosaceus* (PV355693) has a clear halo around the colonies, indicating β-haemolytic.

3.3.6.LAB's production of Diacetyl

The isolates capacity to generate diacetyl is presented in Table 7, among the four isolates only *Lactococcus lactis* (PV355692) could produce diacetyl. A positive result confirmed by slightly reddish coloration was only seen in the medium containing *Lactococcus lactics* thus, indicating that it could produce diacetyl. Other isolates showed negative reaction.

Table 1. Molecular characteristics of selected lactic acid producing LAB strains

0	Query (%)	Cover Percent Identity	Accession Number
Lactobacillus casei	100	100	PV355690
Lactobacillus fermentum	100	100	PV355691
Lactobacillus lactis	100	100	PV355692
Pediococcus pentosaceus	99	99.67	PV355693

Table 2. Sodium chloride (NaCl) tolerance of LAB (OD_{600} nm)

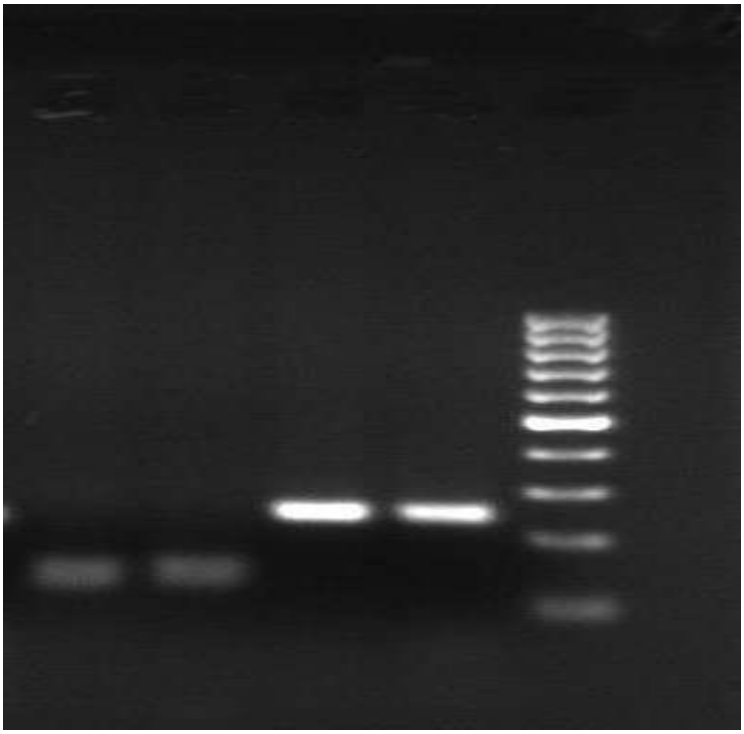
	Lactic Acid Bacteria			
NaCl Conc. (%)	***Lactobacillus casei***	***Lactobacillus fermentum***	***Lactobacillus lactis***	***Pediococcus pentosaceus***
0.5	1.31±0.02^{d}	1.47±0.01^{d}	1.86±0.01^{d}	1.15±0.02^{d}
1.0	1.04±0.03^{c}	0.97±0.02^{c}	1.34±0.00^{c}	0.86±0.03^{c}
2.0	0.81±0.02^{b}	0.91±0.03^{b}	0.94±0.03^{b}	0.64±0.03^{b}
4.0	0.52±0.05^{a}	0.71±0.03^{a}	0.80±0.02^{a}	0.52±0.02^{a}

Note: Data are the mean of duplicates ± SD
Data within a column having different superscripts are significantly different at p≥0.05 using the DUNCAN multiple range

Table 3. pH tolerance of LAB (OD_{600} nm)

	Lactic Acid Bacteria			
pH	***Lactobacillus casei***	***Lactobacillus fermentum***	***Lactobacillus lactis***	***Pediococcus pentosaceus***
4.3	0.36±0.02^{a}	0.32±0.02^{a}	0.59±0.03^{a}	0.34±0.01^{a}
5.0	1.01±0.03^{d}	1.34±0.04^{d}	1.65±0.04^{d}	1.15±0.03^{c}
7.0	1.72±0.02^{e}	1.89±0.03^{e}	2.05±0.04^{e}	1.52±0.02^{e}
9.0	0.90±0.02^{c}	1.05±0.04^{c}	1.34±0.03^{c}	0.85±0.03^{e}
11.0	0.54±0.03^{a}	0.48±0.03^{b}	0.67±0.04^{d}	0.48±0.02^{d}

Note: Data are the mean of duplicates ± SD
Data within a column having different superscripts are significantly different at p≥0.05 using the DUNCAN multiple range



Picture 1. Pictorial presentation of PCR gel

Picture 2. Pictorial representation of fermented milk from *Pediococcus pentosaceus*

Picture 3. Pictorial representation of fermented milk from *Lactobacillus fermentum*

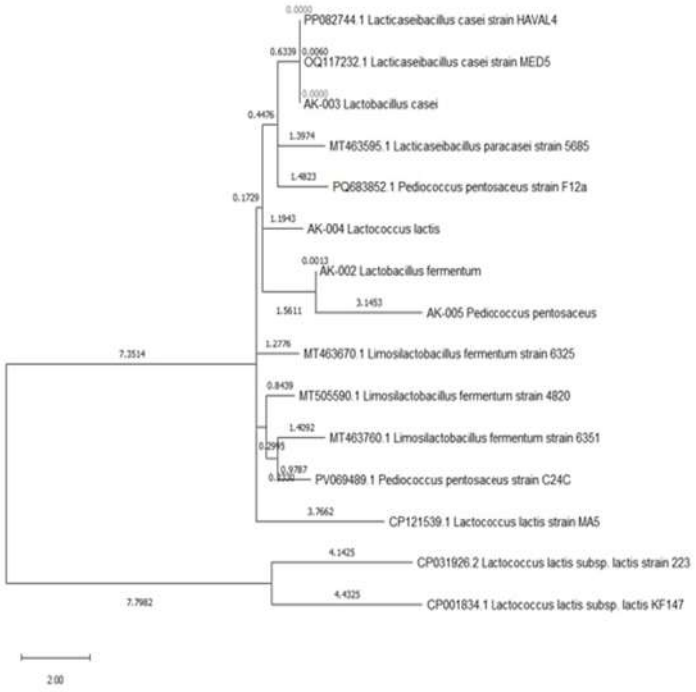


Figure 1. Dendogram of Lactic Acid Bacteria Isolates Based on 16S rRNA Gene Sequences

Table 4. Effect of bile salt on the growth of LAB (OD_{600} nm)

Bile Salt (%)	Lactic Acid Bacteria			
	Lactobacillus casei	***Lactobacillus fermentum***	***Lactobacillus lactis***	***Pediococcus pentosaceus***
0.1	0.63±0.03[b]	0.70±0.02[c]	0.56±0.04[b]	0.83±0.03[b]
0.3	0.77±0.02[c]	0.59±0.03[b]	0.79±0.01[c]	0.58±0.03[a]
0.5	0.39±0.02[a]	0.53±0.02[a]	0.42±0.01[a]	0.76±0.05[b]

Note: Data are the mean of duplicates ± SD
Data within a column having different superscripts are significantly different at p≥0.05 using the DUNCAN multiple range

Table 5. Effect of phenol on the growth of of LAB (OD_{600} nm)

Phenol (%)	Lactic Acid Bacteria			
	Lactobacillus casei	***Lactobacillus fermentum***	***Lactobacillus lactis***	***Pediococcus pentosaceus***
0	1.47±0.01[e]	1.05±0.01[e]	1.23±0.00[e]	1.19±0.00[e]
0.2	1.03±0.00[d]	0.78±0.00[d]	0.95±0.00[d]	0.99±0.00[d]
0.4	0.80±0.00[c]	0.54±0.00[c]	0.64±0.00[c]	0.60±0.00[c]
0.6	0.42±0.00[b]	0.32±0.00[b]	0.35±0.00[b]	0.30±0.00[b]
0.8	0.20±0.00[a]	0.15±0.00[a]	0.19±0.00[a]	0.14±0.00[a]

Note: Data are the mean of duplicates ± SD
Data within a column having different superscripts are significantly different at p≥0.05 using the DUNCAN multiple range

Table 6. Hemolytic activity of LAB isolated

Organism	Hemolysis	Interpretation
Lactobacillus casei	γ-hemolysis	absence of hemolysis.
Lactobacillus fermentum	γ-hemolysis	absence of hemolysis.
Lactococcus lactis	γ-hemolysis	absence of hemolysis.
Pediococcus pentosaceus	β-hemolysis	clear halos around colonies

Key:
γ = Gamma
β = Beta

Table 7. Diacetyl production of LAB

Lactic Acid Bacteria Isolate	Diacetyl Production Ability	Interpretation
Lactobacillus casei	_	No red or pink colouration
Lactobacillus fermentum	_	No red or pink colouration
Lactococcus lactis	+	Slight reddish colouration
Pediococcus pentosaceus	_	No red or pink colouration

3.4. Fermentative capacity of the LAB

All the LAB were capable of producing enough acid to cause the curdling effect on milk by lowering the pH. The resulting fermented milk thickened and show properties of fermented milk.

4. Discussion

Four LAB strains with possible probiotic qualities were successfully isolated from the mouse gut in this investigation. The isolates were identified as *Lactobacillus casei* (AK-002, PV355690), *Lactobacillus fermentum* (AK-003, PV355691), *Lactococcus lactis* (AK-004, PV355692), and *Pediococcus pentosaceus* (AK-005, PV355693).

The isolation of *Lactobacillus* species from mouse gut is well-established in scientific literature. LAB demonstrate a universal lifestyle across diverse ecosystems, including food and the gastrointestinal tract of animals with warm blood. Duar et al. (30) documented the isolation of *Lactobacillus* species (*L. acidophilus* and *L. fermentum*) which were isolated from a variety of hosts including humans, pigs, hamsters, and horses, noting that *L. fermentum* is a member of the nomadic species group of *lactobacilli*.

LAB isolated from mouse gut hold special importance for creating probiotics and probiotic-enriched meals for the same species due to their host-adaptation and high ecological fitness. These bacteria can also demonstrate effectiveness as probiotics in other organisms possessing related gut microbial communities. Specific LAB species have been associated with improved gut health and function (25, 26). Mouse model studies have established important correlations between probiotic bacteria and enhanced gut health. These live bacteria modulate gut health through multiple mechanisms: lowering pH, producing antibacterial substances, and competing for space and nutrients on the epithelial mucosa. These mechanisms enable the trapping and elimination of foreign organisms from the gut, thereby preventing associated infections (25). *Lactobacillus casei, Lactobacillus fermentum, Lactococcus lactis*, and *Pediococcus pentosaceus* are examples of LAB that are essential for food fermentation, preservation, and functional enhancement. *L. casei* is widely used in dairy products like yogurt and cheese for its probiotic potential, acid tolerance, and ability to enhance flavor and texture (6). The study of Adimpong et al. (1) shows that *L. fermentum*, commonly found in cereal and plant-based fermentations, contributes to improved nutritional quality by producing exopolysaccharides, degrading antinutritional factors, and enhancing

antioxidant properties. In contrast, *L. lactis* serves as a classical starter culture in dairy fermentation, particularly cheese production, due to its rapid acidification and production of nisin, a natural bacteriocin effective against foodborne pathogens (27). According to Jiang et al. (20) *P. pentosaceus*is notable for its strong acidification ability and antimicrobial activity in vegetable and cereal fermentations, contributing to shelf-life extension and safety of fermented products.

Bile tolerance is essential for LAB to thrive and perform effectively in the small intestine where bile aids fat emulsification during digestion (8). The isolated LAB tolerated and grew in bile presence. The degree to which bile inhibits gut defense mechanisms depends on its concentration. The LAB's tolerance to tested bile salt concentrations suggests potential gut survival ability if used as probiotics, corroborating previous findings on bile tolerance by probiotic bacteria (10).

pH tolerance across wide ranges is another critical probiotic feature (28). Gut microbiomes must grow in acidic stomach pH and alkaline small intestine conditions. All four isolates grew well across alkaline and acidic pH ranges, enhancing their probiotic potential by enabling survival and activity throughout the gastrointestinal tract without denaturation or functionality loss.

All isolates survived and grew well, remaining uninhibited after 24 h of incubation with 0, 0.2, 0.4, and 0.8% phenol. PanapparambilSooraj et al. (33) showed that aromatic amino acids from dietary or endogenous proteins are deaminated by gut bacteria to produce phenols. Ammonia, phenols, indoles, and amines products of colonic protein degradation are said to have harmful effects in vitro and in animal models. Phenol tolerance is a necessary probiotic characteristic since their presence in fecal samples suggests that they impact gut mucosa and can have bacteriostatic effects against certain lactobacilli strains. The recorded phenol tolerance demonstrates these isolates' potential to survive gut environments and confer health benefits despite phenol or other aromatic compounds from protein digestion (30).

The gamma hemolytic reaction of three LAB isolates suggests safety on epithelial mucosa layers (33). Absence of hemolytic activity is a probiotic selection criterion, indicating non-virulence. Similarly, Gajjar et al. (21) reported that all LAB strains assayed for probiotic function showed no positive hemolytic activity, with no color change in medium surrounding colonies on blood agar plates. *L. fermentum* isolated from the guts of cows in Cameroon's western highlands were hemolytic-negative (31). However, *Pediococcus pentosaceus* exhibited beta-hemolytic effect and may not be suitable for probiotic function.

Diacetyl production is a major LAB metabolic function, important when choosing functional probiotics. Diacetyl plays an essential role in fermented food taste and has been investigated for antimicrobial and beneficial effects on gut ecosystems (32). However, *Lactobacillus casei, Lactobacillus fermentum*, and *Pediococcus pentosaceus* showed no diacetyl production; only *Lactococcus lactis* demonstrated this ability. This agrees with studies reporting that diacetyl production by LAB varies, depending mainly on species and substrate types (4). While diacetyl presence alone doesn't determine probiotic effectiveness, it can enhance flavor and improve food acceptance (22). Additionally, specific diacetyl metabolic pathways

impact beneficial traits, including inhibiting harmful bacteria and modulating immune function (33).

LAB produce various organic acids that reduce pH, induce milk fermentation, and create sour taste. The acid coagulates or curdles milk and inhibits many spoilage and pathogenic bacteria. Fermentative ability, evidenced by milk curdling and thickening, was confirmed in all four LAB isolates. These benefits of organic acid production and other antibacterial compounds like bacteriocins (ribosomally synthesized bacterial proteins acting similarly to antibiotics on closely related strains) can be leveraged as natural agents in food processing and storage (3). The acceptability and shelf life of microbially processed foods depend directly on engaged organism cultures, often called starter cultures. Starter cultures influence proximate composition, dictate organoleptic properties, confer health benefits, and prolong shelf life (28). Local food requires indigenous starter cultures for processing. Benefits of food processing using starter cultures are numerous, necessitating continuous search for indigenous starter cultures (16).

5. Conclusion

This study successfully isolated and identified four species of LAB from the gut of mouse: *Lactobacillus casei* (PV355690), *Lactobacillus fermentum* (PV355691), *Lactococcuslactis* (PV355692), and *Pediococcuspentosaceus* (PV355693). These isolates exhibited important probiotic properties, especially the production of bacteriocins with different levels of antibacterial activity. Notably, the crude bacteriocin from *Lactococcuslactis* (PV355692) demonstrated showed strongest inhibitory effect against various pathogenic bacteria which includes *Pseudomonas aeruginosa*, *Escherichia coli, Bacilluscereus,* and *Staphylococcus aureus.* The ability of isolates to grow under conditions that mimic the intestinal environment confirms their probiotic potential. This study also supports the fermentative capability of LAB produced from mouse intestine and supports their bioprocessing applications. The documented functional properties make these isolates desirable for food processing and preservation, and have the potential to improve gastrointestinal health by eliminating pathogens. Overall, these results offer insightfull information about the probiotic capabilities of LAB isolated from the mouse intestine, their potential as starter cultures, and their usability as bio-preservatives in food processing.

Authorship Contribution

Fatima Akanbi: Conceptualization, Methodology, Data curation, Writing original draft, Visualization, Funding acquisition.

Racheal Adedayo: Conceptualization, Review and Editing, Supervision.

Yusuf-Salihu: Review and editing, Project Administration.

Declaration of competing interest

The authors declare no competing interesting be it financial or personal relationships that could have influenced the work reported in this article.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Findings

This study advances probiotic research by isolating and investigating the very rare source of LAB in the

gastrointestinal tract of the mouse. The discovery and identification of four LAB strains; *Lactobacillus casei, Lactobacillus fermentum, Pediococcuspentosaceus*, and *Lactococcus lactis* adds to microbial diversity known to possess probiotic potential.

These strains exhibited strong resilience under various stress factors including acidic and alkaline pH, bile salts, phenol, and salt concentrations, while displaying non-hemolytic behavior. Their ability to ferment milk and thrive under gastrointestinal-like conditions indicates both functional relevance and safety for potential application in food systems.

Overall, this work introduces novel LAB strains with desirable probiotic and preservative properties, offering new perspectives for their use in functional food production and biotherapeutic development.

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