

Original Research Article

Antimicrobials of lactic acid bacteria isolated from artisanal nutraceuticals mitigated foodborne and multidrug-resistant pathogens

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Abstract

Purpose: To investigate the potential antimicrobial activities of lactic acid bacteria isolated from artisanal fermented nutraceuticals in combating multidrug-resistant and food-borne pathogens (MDR-FBP).

Methods: Serial dilution and enrichment techniques were employed to isolate the Lactic Acid Bacteria (LAB). The isolates were subjected to biochemical characterization using standard microbiological procedures, and identities confirmed through sequencing of the 16S rDNA gene. The survival of LAB was assessed in media with a low pH of 2.5 and 0.3% bile salt. The antibacterial properties, as well as the production of hydrogen peroxide and bacteriocins by the cell-free supernatant (CFS) standardized to 0.5 McFarland, were evaluated against MDR-FBPs using the agar well diffusion assay.

Results: The identified probiotics were classified into five taxa of LAB, which included *Lactobacillus* spp, *Bacillus* spp, *Enterococcus* spp, *Pediococcus* spp, and *Streptococcus thermophilus*. All LAB isolates exhibited a < 2-log reduction in cell viability when exposed to pH 2.5 and 0.3 % w/v bile salt after 4 hours. The LAB consortia isolated in this study demonstrated effectiveness against *Listeria*, *Pseudomonas*, *E. coli*, *Staphylococcus*, and *Salmonella* activities. The bacteriocins and hydrogen peroxides produced by the LAB showed marked activity and significantly inhibited the growth of multidrug-resistant and foodborne pathogens ($p < 0.05$).

Conclusion: Artisanal fermented nutraceuticals are cost-effective and serve as a good source for a variety of beneficial bacteria with strong antimicrobial properties, naturally addressing the challenges posed by multidrug-resistant and food-borne pathogens.

Keywords: Probiotics, Antimicrobials, Bacteriocin, Hydrogen peroxide, Drug-resistant, foodborne pathogens

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INTRODUCTION

The escalating concern around antimicrobial resistance (AMR) and consumers' increasing

demand for a safe food supply has sparked heightened interest in utilizing probiotics as a natural biocontrol strategy to diminish foodborne pathogens along the food continuum [1].

Antimicrobial resistance, food security, and healthy living have all been seriously threatened in recent decades by the emergence of multidrug-resistant and foodborne pathogens (MDR-FBPs). Lactic Acid Bacteria (LAB), primarily known for their probiotic benefits, play a crucial role in enhancing organoleptic properties in fermented food while protecting against harmful pathogens. These beneficial bacteria produce a variety of antimicrobial substances, such as hydrogen peroxide (H₂O₂), ethanol, diacetyl, carbon dioxide, and bacteriocins, which serve as preservatives in food [2].

The challenge posed by MDR-FBPs is a pressing global concern. Lactic acid bacteria effectively generate various antimicrobial agents that are instrumental in food preservation and countering the negative impacts of MDR-FBPs. Specifically, the hydrogen peroxide produced by LAB is lethal to certain pathogenic bacteria that lack catalase, and functions through a superoxide anion chain reaction with enhanced oxidative toxicity under various conditions of pH and temperature [3]. Previous studies suggest that producing hydrogen peroxide and organic acids contributes significantly to the antagonism of LAB towards Gram-negative pathogens [4]. This study aims to evaluate the antimicrobial capabilities of LAB isolated from artisanal fermented nutraceuticals in the fight against MDR-FBPs.

EXPERIMENTAL

Isolation of LAB

For the LAB screening, various artisanal fermented nutraceuticals were procured from three markets (Ogige, Ikpa, and Beach) in Nsukka town, Enugu State, Nigeria. The food samples were coded as OK (Okpeye), O (Ogiri), and P (pap, Akamu, or Ogi). U (Ukpaka) and Y (yoghurt). LAB strains were isolated through enrichment and subsequent serial dilution [5].

Biochemical characterization of LAB

The selected LAB isolates were subjected to biochemical characterization such as hemolysis tests, oxidase tests, and sugar (carbohydrate) fermentation patterns using standard microbiological procedures [6].

Pathogens and cultural conditions

The MDR-FBPs were obtained from the stock culture collection of the Department of Pharmaceutical Microbiology and Biotechnology laboratory at the University of Nigeria, Nsukka, Nigeria. The MDR-FBPs used for the study

include *Bacillus* spp., *Escherichia coli*, *Pseudomonas aeruginosa* ATCC 27883, *Salmonella typhimurium*, *Listeria monocytogenes* ATCC 13932, and *Staphylococcus aureus*. Before use, the pathogens were preserved on Brain Heart Infusion (BHI) agar (HiMedia, India) and enriched with BHI broth.

Viability of LAB at low pH and bile salt concentrations

The *Lactobacilli* strains were tested for their survival ability in low pH and bile salt. The viability of the LAB was determined as described elsewhere [7].

Lactobacillus identification using 16S rRNA gene sequencing

Lactobacillus strains with appropriate probiotic potential were identified by 16S rRNA gene sequencing. Genomic extraction of *Lactobacillus* isolates was performed using the boiling method. The PCR reaction was carried out using the universal primers 27F (5' AGT TTG ATC CTG GCT CAG 3') and 1492R (5' ACG GCT ACC TTG TTA CGA CTT 3'). The PCR reaction conditions were reported in our previous study [5]. The genetic identity of various 16S rDNA sequences of isolated probiotics was compared to sequences from typed LAB strains held in GenBank.

Preparation and treatment of cell-free supernatant (CFS)

Each LAB strain was initially grown in 20 mL MRS broth at 37 °C for 24–48 h under anaerobic conditions. The culture was then divided into three portions and centrifuged at 15,000 rpm for 15 minutes to obtain cell-free supernatant (CFS), which was filtered through a 0.45 µm membrane filter paper (Millipore, India). The resulting filtrate was standardized to a 0.5 McFarland standard.

Evaluation of the antipathogen (antibacterial), hydrogen peroxide and bacteriocin activities of probiotics

The antimicrobial activities of the selected LAB isolates against *Bacillus* spp., *Escherichia coli*, *Pseudomonas aeruginosa* ATCC 27883, *Salmonella typhimurium*, *Listeria monocytogenes* ATCC 13932, and *Staphylococcus aureus* were determined by the agar well diffusion assay.

Statistical analysis

All experiments were conducted in triplicate, and results are presented as mean ± standard error

of the mean (SEM). Statistical analysis was performed using SPSS® version 20.0 and GraphPad Prism® version 7. One-way ANOVA was adopted and statistical significance ($p < 0.05$) was determined using Duncan's multiple range test.

RESULTS

Viability of LAB at low pH and bile concentrations

The viabilities of probionts at pH 2.5 and 0.3 % w/v bile concentration are shown in Table 1. A 121, 515 and 628 % reduction in cell number at pH 2.5 for an exposure time of 4 h was observed. Similarly, a 515, 545 and 595 % reduction was recorded in *B. thuringiensis*, *E. durans*, and *B. cereus* in 0.3 % w/v bile condition for the same period of 4 h.

Antibacterial activities of LAB

The antibacterial activity results in Table 2 show that *P. pentosaceus*, *L. brevis*, and *L. plantarum* exhibited strong activity against *S. aureus* and moderate activity against *L. monocytogenes* ATCC 13932. Statistically significant differences ($p < 0.05$) were observed in the anti-*Salmonella*

activity of *P. pentosaceus* and *L. brevis*, and in the anti-*Listeria* activity of *L. plantarum*.

Tables 3 and Table 4 summarize the hydrogen peroxide (H_2O_2) and bacteriocin activities of lactic acid bacteria (LAB) isolates against multidrug-resistant foodborne pathogens (MDR-FBPs). Bacteriocin activities of *L. brevis* and *L. plantarum* significantly inhibited *L. monocytogenes*. *Pediococcus pentosaceus* and *L. plantarum* significantly inhibited *Salmonella typhimurium*. *Pediococcus acidilactici* significantly inhibited *L. monocytogenes*, *S. typhimurium*, and *S. aureus*. *Lactobacillus brevis*, *P. pentosaceus*, and *L. paracasei* significantly inhibited *E. coli*. *Bacillus cereus*, *S. thermophilus*, and *L. ingluviei* showed the strongest activity against *L. monocytogenes*. Anti-*Pseudomonas* activity was observed in bacteriocins from *L. brevis*, *B. cereus*, *L. ingluviei*, *E. durans*, and *L. plantarum*. Similarly, bacteriocins from *L. brevis* and *L. plantarum* significantly inhibited *S. aureus*. Interestingly, only *P. pentosaceus* produced H_2O_2 against *E. coli*. *Lactobacillus brevis* (IZD = 12.5 ± 1.5 mm) significantly inhibited *S. aureus* via H_2O_2 production. *Lactobacillus brevis* and *L. plantarum* had no activity against *E. coli* via H_2O_2 production. Overall, the LAB isolates demonstrated significant antimicrobial properties via bacteriocin and H_2O_2 production against key MDR-FBPs.

Table 1: Mean viability of LAB at pH 2.5 and 0.3 % w/v bile salt

Code	LAB	Percentage reduction in cell viability at pH 2.5 ($\log_{10}$ CFU/mL)	Percentage reduction in cell viability to 0.3 % w/v bile salt concentration ($\log_{10}$ CFU/mL)
OK1	<i>P. pentosaceus</i>	32	32
OK3	<i>L. casei</i>	75	35
OK8	<i>P. acidilactici</i>	49	6.04
OK14	<i>L. brevis</i>	121	0.00 ^a
OK15	<i>L. plantarum</i>	78	31
O1	<i>L. plantarum</i>	69	0.00 ^a
O8	<i>E. durans</i>	43	51
O12	<i>B. thuringiensis</i>	63	515
O19	<i>B. cereus</i>	515	9
O20	<i>B. cereus</i>	37	46
P2	<i>S. thermophilus</i>	89	0.00 ^a
P7	<i>L. brevis</i>	34	8
P9	<i>L. plantarum</i>	79	19
P10	<i>L. paracasei</i>	18	0.00 ^a
P16	<i>P. pentosaceus</i>	79	37
U4	<i>L. ingluviei</i>	0.00 ^a	8
U10	<i>E. durans</i>	23	545
U13	<i>B. cereus</i>	31	10
U15	<i>B. cereus</i>	89	595
U20	<i>b. fermentum</i>	33	11
Y11	<i>P. pentosaceus</i>	31	19
Y12	<i>L. brevis</i>	41	0.00
Y13	<i>P. pentosaceus</i>	15	19
Y16	<i>B. cereus</i>	31	0.00 ^a
Y18	<i>P. pentosaceus</i>	628	30

^avalues are negligible

Table 2: Antibacterial activity of LAB against selected pathogens

LAB	Indicator microorganism					
	<i>L. monocytogenes</i> ATCC 13932	<i>P. aeruginosa</i> ATCC 27883	<i>E. coli</i>	<i>Bacillus</i> <i>sp</i>	<i>S. typhimurium</i>	<i>S. aureus</i>
<i>P. pentosaceus</i>	12.5±1.5 ^a	12.0±2.0 ^a	10.0±0.0 ^a	10.5±0.5 ^a	21.5±0.5 ^b	14.5±0.5 ^a
<i>L. casei</i>	12.5±0.5 ^b	10.0±0.0 ^a	9.0±0.0 ^a	10.0±0.0 ^a	19.0±0.0 ^a	20.5±1.5 ^b
<i>P. acidilactici</i>	16.0±0.0 ^c	9.0±0.0 ^a	10.0±0.0 ^a	13.0±1.0 ^b	17.0±0.0 ^a	32.5±2.5 ^b
<i>L. brevis</i>	14.0±1.0 ^a	20.0±1.0 ^a	9.0±0.0 ^a	10.5±1.5 ^a	10.0±1.0 ^a	23.5±1.5 ^b
<i>L. plantarum</i>	15.5±0.5 ^b	15.0±1.0 ^a	9.0±0.0 ^a	9.0±0.0 ^a	21.5±1.5 ^b	20.5±0.5 ^b
<i>L. plantarum</i>	10.0±0.0 ^a	22.0±2.0 ^b	9.0±0.0 ^a	11.5±0.5 ^a	11.5±0.5 ^a	10.5±0.5 ^a
<i>E. durans</i>	14.5±1.5 ^a	15.0±1.5 ^b	9.0±0.0 ^a	14.5±0.5 ^b	17.5±1.5 ^b	11.0±1.0 ^a
<i>B. thuringiensis</i>	12.0±0.0 ^a	9.5±1.5 ^a	9.0±0.0 ^a	9.0±0.0 ^a	16.0±1.0 ^b	10.5±0.5 ^a
<i>B. cereus</i>	12.5±0.5 ^a	9.5±1.5 ^a	10.0±0.0 ^a	13.0±1.0 ^a	17.5±1.5 ^a	18.0±1.0 ^b
<i>B. cereus</i>	10.0±0.0 ^a	10.0±0.0 ^a	10.0±0.0	10.5±0.5 ^a	10.0±0.0 ^a	16.5±2.5 ^b
<i>S. thermophilus</i>	16.0±2.0 ^a	9.0±0.0 ^a	9.0±0.0 ^a	10.5±0.5 ^b	9.0±0.0 ^a	16.0±1.0 ^a
<i>L. brevis</i>	11.5±0.5 ^a	9.0±0.0 ^a	9.0±0.0 ^a	11.0±0.0 ^a	13.5±1.5 ^a	22.5±0.5 ^b
<i>L. plantarum</i>	11.5±0.5 ^a	10.0±0.0 ^a	9.0±0.0 ^a	11.0±1.0 ^a	12.5±1.5 ^a	9.0±0.0 ^a
<i>L. paracasei</i>	15.5±1.5 ^a	11.0±0.0 ^a	15.0±0.0 ^b	13.5±0.5 ^a	10.0±0.0 ^a	10.0±0.0 ^a
<i>P. pentosaceus</i>	11.0±1.0 ^a	11.5±1.5 ^a	9.0±0.0 ^a	9.0±0.0 ^a	10.0±0.0 ^a	9.0±0.0 ^a
<i>L. ingluviei</i>	14.0±1.0 ^a	11.0±1.0 ^a	10.0±0.0 ^a	11.5±0.5 ^a	9.0±0.0 ^a	10.0±0.0 ^a
<i>E. durans</i>	14.3±0.3 ^a	16.5±0.5 ^a	9.0±0.0 ^a	10.5±0.5 ^a	10.0±0.0 ^a	10.5±0.5 ^a
<i>B. cereus</i>	12.0±0.6 ^a	14.5±0.5 ^a	10.5±0.5 ^b	14.0±1.0 ^a	10.0±0.0 ^a	11.0±0.0 ^a
<i>B. cereus</i>	11.0±1.0 ^a	13.5±2.0 ^{a,b}	9.0±0.0 ^a	11.5±0.5 ^a	15.5±1.5 ^a	17.0±2.0 ^b
<i>L. fermentum</i>	12.0±1.0 ^a	10.5±1.5 ^a	9.0±0.0 ^a	12.5±0.5 ^a	9.0±0.0 ^a	19.0±0.0 ^b
<i>P. pentosaceus</i>	15.0±0.0 ^b	15.5±2.0 ^a	10.0±0.0 ^a	11.0±1.0 ^a	12.5±0.5 ^b	10.5±0.5 ^{a,b}
<i>L. Brevis</i>	14.0±1.0 ^a	13.5±0.5 ^b	15.5±0.5 ^b	10.5±0.5 ^a	21.0±2.0 ^b	12.5±1.5 ^a
<i>P. pentosaceus</i>	14.5±1.5 ^a	13.5±1.5 ^a	20.0±0.0 ^a	9.0±0.0 ^a	23.0±2.0 ^a	10.5±0.5 ^a
<i>B. cereus</i>	12.5±0.5 ^a	13.5±0.5 ^a	9.5±0.5 ^a	14.5±0.5 ^b	20.0±1.0 ^c	10.0±0.0 ^a
<i>P. pentosaceus</i>	14.5±0.5 ^a	12.5±0.5 ^a	10.0±0.0 ^a	9.5±0.5 ^a	10.0±0.0 ^a	10.0±0.0 ^a

Table 3: Activities of hydrogen peroxide (H₂O₂) of LAB isolates against selected pathogens

LAB	Indicator microorganisms					
	<i>L. monocytogenes</i> ATCC 13932	<i>P. aeruginosa</i> ATCC 27883	<i>E. coli</i>	<i>Bacillus</i> <i>sp</i>	<i>S. typhimurium</i>	<i>S. aureus</i>
<i>P. pentosaceus</i>	12.5±0.5 ^a	10.0±0.0 ^a	9.5±0.5 ^a	9.5±0.5 ^a	9.5±0.5 ^{a,b}	11.0±2.0 ^a
<i>L. casei</i>	9.5±0.5 ^a	10.0±0.0 [*]	10.0±0.0 ^a	9.5±0.5 ^a	17.5±0.5 ^a	14.5±0.5 ^a
<i>P. acidilactici</i>	12.5±0.0 ^a	9.0±0.0 [*]	9.0±0.0 [*]	10.0±0.0 ^a	21.5±1.5 ^{a,b}	22.5±1.0 ^a
<i>L. brevis</i>	12.0±0.0 ^a	15.0±2.0 ^a	9.0±0.0 [*]	11.0±0.0 ^a	11.5±0.5 ^a	9.5±0.5 ^a
<i>L. plantarum</i>	10.0±0.0 ^a	12.5±1.5 ^a	9.0±0.0 [*]	11.0±0.0 ^a	14.5±0.5 ^a	12.5±1.5 ^a
<i>L. plantarum</i>	10.5±0.5 ^a	12.0±2.0 ^a	9.0±0.0 [*]	12.5±0.5 ^a	11.5±0.5 ^a	10.0±0.0 ^a
<i>E. durans</i>	12.5±0.5 ^a	9.0±0.0 ^a	9.0±0.0 [*]	12.0±0.0 ^{a,b}	13.5±0.5 ^{a,b}	16.5±0.5 ^b
<i>B. thuringiensis</i>	11.5±0.5 ^a	9.0±0.0 ^a	9.5±0.5 ^a	11.0±1.0 ^a	11.0±1.0 ^a	13.0±0.5 ^a
<i>B. cereus</i>	12.5±1.5 ^a	9.0±0.0 ^a	10.0±0.0 ^a	12.5±0.5 ^a	15.0±2.0 ^a	20.5±1.5 ^b
<i>B. cereus</i>	11.5±0.5 ^a	10.0±0.0 ^a	10.0±0.0 [*]	11.0±0.0 ^a	14.5±1.5 ^b	9.0±0.0 ^a
<i>S. thermophilus</i>	14.5±0.5 ^a	9.0±0.0 [*]	9.0±0.0 [*]	11.5±0.0 ^a	10.0±0.0 ^a	18.5±0.5 ^a
<i>L. brevis</i>	12.0±1.0 ^a	9.0±0.0 [*]	9.0±0.0 [*]	10.0±0.0 ^{a,b}	9.0±0.0 [*]	16.5±1.5 ^a
<i>L. plantarum</i>	13.5±1.5 ^a	10.0±0.0 [*]	10.0±0.0 [*]	10.0±0.0 ^a	10.5±0.5 ^a	9.0±0.0 [*]
<i>L. paracasei</i>	12.5±0.5 ^a	11.0±1.0 ^a	11.0±1.0 ^a	13.0±1.0 ^a	10.5±0.5 ^a	9.0±0.0 ^a
<i>P. pentosaceus</i>	11.0±1.0 ^a	9.0±0.0 ^a	9.0±0.0 [*]	10.0±0.0 [*]	11.5±0.5 ^a	9.0±0.0 [*]
<i>L. ingluviei</i>	14.5±0.5 ^a	13.5±1.5 ^a	10.0±0.0 [*]	11.5±0.5 ^a	9.0±0.0 [*]	10.0±0.0 [*]
<i>E. durans</i>	11.5±1.5 ^a	12.5±1.5 ^a	10.0±0.0 [*]	11.0±0.0 ^a	10.0±0.0 [*]	9.0±0.0 ^a
<i>B. cereus</i>	10.5±0.0 ^a	12.5±0.0 ^a	10.0±0.0 ^{a,b}	11.0±0.5 ^a	10.5±0.5 ^a	10.0±0.0 [*]
<i>B. cereus</i>	9.0±0.0 ^a	10.0±0.0 ^a	9.0±0.0 [*]	11.0±2.0 ^a	15.0±2.0 ^a	10.0±0.0 ^a
<i>L. fermentum</i>	10.5±1.0 ^a	10.0±0.0 ^a	9.0±0.0 [*]	13.0±2.0 ^a	10.0±0.0 [*]	26.0±2.0 ^c
<i>P. pentosaceus</i>	13.5±0.5 ^a	11.5±0.5 ^a	10.0±0.0 [*]	9.5±0.5 ^a	10.0±0.0 ^a	10.0±0.0 ^a
<i>L. brevis</i>	13.0±0.0 ^a	10.0±0.0 ^a	11.0±0.0 ^a	9.5±0.5 ^a	15.0±1.0 ^a	9.0±0.0 ^a
<i>P. Pentosaceus</i>	11.0±0.0 ^a	12.0±1.0 ^a	11.0±0.0 ^a	9.5±0.5 ^a	16.0±2.0 ^a	9.5±0.5 ^a
<i>B. cereus</i>	12.0±0.0 ^a	16.5±0.5 ^a	9.0±0.0 ^a	10.5±0.5 ^a	16.5±0.5 ^a	9.5±0.5 ^a
<i>P. pentosaceus</i>	12.5±0.5 ^a	10.0±0.0 ^a	13.5±1.5 ^a	10.5±0.5 ^a	10.0±0.0 ^a	9.5±0.5 ^a

*, no result for Duncan statistic. Values are mean ± standard error of mean (SEM)

Table 4: Bacteriocin activity of LAB isolates against selected pathogens

LAB	Indicator microorganisms					
	<i>L. monocytogenes</i> ATCC 13932	<i>P. aeruginosa</i> ATCC27883	<i>E. coli</i>	<i>Bacillus</i> <i>sp</i>	<i>S. typhimurium</i>	<i>S. aureus</i>
<i>P. pentosaceus</i>	11.5±0.0 ^a	11.0±1.0 ^a	10.0±0.0 ^a	10.5±0.5 ^a	14.5±1.5 ^a	12.5±0.5 ^a
<i>b. casei</i>	11.5±0.5 ^b	9.0±0.0 [*]	9.0±0.0 ^a	10.0±1.0 ^a	22.0±1.0 ^b	14.5±0.5 ^a
<i>P. acidilactici</i>	14.5±0.5 ^b	9.0±0.0 [*]	9.0±0.0 [*]	9.5±0.5 ^a	24.5±1.5 ^b	14.5±1.5 ^a
<i>L. brevis</i>	11.5±0.5 ^a	15.5±1.5 ^a	9.0±0.0 [*]	11.0±1.0 ^a	12.5±1.5 ^a	16.0±2.0 ^a
<i>L. plantarum</i>	10.5±0.5 ^a	15.0±1.0 ^a	9.0±0.0 [*]	9.5±0.5 ^a	12.5±1.5 ^a	20.0±1.0 ^a
<i>L. plantarum</i>	9.5±0.5 ^a	15.0±2.0 ^{a,b}	9.0±0.0 [*]	12.0±0.0 ^a	14.5±2.0 ^a	9.5±0.5 ^a
<i>E. durans</i>	14.5±0.0 ^a	13.5±1.5 ^{a,b}	9.0±0.0 [*]	10.0±1.0 ^a	12.0±0.0 ^a	10.0±0.0 ^a
<i>B. thuringiensis</i>	12.0±1.0 ^a	9.0±0.0 ^a	9.0±0.0 ^a	9.5±0.5 ^a	10.0±0.0 ^a	10.0±0.0 ^a
<i>B. cereus</i>	15.0±0.0 ^a	9.0±0.0 ^a	13.0±2.0 ^a	13.0±1.0 ^a	11.5±1.5 ^a	10.0±0.0 ^a
<i>B. cereus</i>	12.0±1.0 ^a	10.5±0.5 ^a	10.0±0.0 [*]	12.0±1.0 ^a	10.5±0.5 ^{a,b}	11.0±1.0 ^{a,b}
<i>S. thermophilus</i>	16.0±1.0 ^b	9.0±0.0 ^a	9.0±0.0 [*]	10.0±0.0 [*]	10.0±0.0 [*]	18.5±0.5 ^a
<i>L. brevis</i>	11.0±1.0 ^a	9.0±0.0 ^a	9.0±0.0 [*]	9.0±0.0 ^a	9.0±0.0 ^a	14.5±0.5 ^a
<i>L. plantarum</i>	12.0±2.0 ^a	10.0±0.0 [*]	9.0±0.0 [*]	10.0±0.0 ^a	10.0±0.0 ^a	9.0±0.0 [*]
<i>L. paracasei</i>	17.5±1.5 ^a	12.5±1.5 ^a	13.0±0.0 ^{a,b}	12.5±0.5 ^a	10.0±0.0 ^a	11.5±1.5 ^a
<i>P. pentosaceus</i>	10.5±0.5 ^a	9.0±0.0 ^a	9.0±0.0 [*]	9.0±0.0 [*]	9.0±0.0 ^a	9.0±0.0 ^a
<i>L. ingluviei</i>	14.5±0.5 ^a	13.0±0.0 ^a	10.0±0.0 [*]	11.5±0.5 ^a	9.0±0.0 [*]	10.0±0.0 [*]
<i>E. durans</i>	11.0±0.0 ^a	14.0±1.0 ^a	9.0±0.0 [*]	11.5±0.5 ^a	10.0±0.0 [*]	11.0±0.0 ^a
<i>B. cereus</i>	9.5±0.0 ^a	13.0±1.0 ^a	9.0±0.0 ^a	11.0±2.0 ^a	10.0±0.0 ^a	11.0±0.0 [*]
<i>B. cereus</i>	10.0±1.0 ^a	17.0±1.0 ^c	9.0±0.0 [*]	10.0±0.5 [*]	13.5±0.5 [*]	10.0±0.0 ^a
<i>L. fermentum</i>	10.5±0.5 ^a	9.0±0.0 ^a	9.0±0.0 [*]	11.5±0.5 ^a	9.0±0.0 [*]	10.5±0.5 ^a
<i>P. pentosaceus</i>	16.0±0.0 ^b	10.5±0.5 ^a	10.0±0.0 [*]	10.5±0.5 ^a	10.0±0.0 ^a	13.0±1.0 ^b
<i>L. brevis</i>	13.0±0.0 ^a	11.5±0.5 ^a	11.0±1.0 ^a	9.0±0.0 ^a	19.0±0.0 ^{a,b}	10.5±0.5 ^a
<i>P. pentosaceus</i>	10.0±1.0 ^a	10.0±1.0 ^a	9.0±0.0 [*]	11.5±1.5 ^a	18.5±1.5 ^a	10.0±0.0 ^a
<i>B. cereus</i>	11.5±0.5 ^a	15.0±1.0 ^a	10.0±0.0 ^a	11.0±1.0 [*]	9.0±0.0 ^a	10.0±0.0 ^a
<i>P. pentosaceus</i>	14.5±2.5 ^a	10.0±0.0 ^a	9.5±0.5 ^a	11.5±0.5 ^a	11.0±1.0 ^a	10.5±0.5 ^a

* - no result for Duncan statistic. Values are mean ± SEM. Within a row, values with different superscripts are significantly different at $P < 0.05$

DISCUSSION

Lactic acid bacteria were isolated from five traditionally fermented Nigerian food products (*Okpeye*, *Ogiri*, *Akamu*, *Ukpaka*, and yoghurt) sold in three different markets in Nsukka province of Enugu State, Nigeria. The isolated LAB from the nutraceuticals were members of *Lactobacillus* spp., *Bacillus*, *Enterococcus*, *Pediococcus* spp., and *S. thermophilus* [8,9]. Interestingly, all the LAB isolates exhibited a < 2-log reduction in cell viability when challenged at pH 2.5 and with 0.3 % w/v bile salt after 4 h, indicating a high tolerance to acidic and bile conditions.

The tolerance of LAB to very low pH reported in the study is similar to that observed for LAB isolated from other regions [10,11]. The viability of various isolates at acidic pH of 2.5 and with 0.3 % w/v bile salt confirmed their potential to survive the GIT assault required for probiotics intended for human or animal use. Furthermore, the pH of the stomach may be balanced by food or other carrier matrix molecules after intake, thereby making it unlikely that the probiotic strains will be directly exposed to it [12]. The results of bile tolerance reported in this study for

L. plantarum, *P. pentosaceus*, and *L. brevis* align with those of other researchers [13].

The LAB bacteriocins disrupt cell membrane integrity and inhibit protein and nucleic acid synthesis in sensitive pathogens, challenging resistance development [14]. Bacteriocins from *L. brevis* and *L. plantarum* were bacteriostatic against *L. monocytogenes* ATCC 13932, *Salmonella typhimurium*, and *E. coli* [15]. They also exhibited anti-pseudomonas activities, inhibiting *S. aureus* and producing H₂O₂ against *E. coli*. These probiotics also exhibited anti-staphylococcal activities. Bacteriocins from *P. acidilactici* inhibited *L. monocytogenes*, *S. typhimurium*, and *S. aureus*, while *L. brevis*, *P. pentosaceus*, and *L. paracasei* inhibited *E. coli*. *B. cereus*, *S. thermophilus*, and *L. ingluviei* showed anti-pseudomonas activities [16]. The consortia of LAB isolated in this study exhibited antimicrobial activities against *Listeria*, *pseudomonas*, *E. coli*, *Staphylococcus*, and *Salmonella*. This result corroborates other authors [17,18].

CONCLUSION

This study has demonstrated the potential of lactic acid bacteria (LAB) isolated from artisanal

fermented nutraceuticals to combat foodborne and multidrug-resistant infections by highlighting their robust antibacterial qualities. These LAB produce bacteriocins and hydrogen peroxide, making them promising alternatives to conventional antibiotics amid rising antibiotic resistance. Additionally, these fermented products may serve as rich sources of novel LAB for gut health improvement and industrial probiotic development.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities on claims relating to the content of this article will be borne by the authors.

REFERENCES

1. Ayala DI, Cook PW, Franco JG, Bugarel M, Kottapalli KR, Loneragan GH, Brashears MM, Nightingale KK. A systematic approach to identify and characterize the effectiveness and safety of novel probiotic strains to control foodborne pathogens. *Front Microbiol* 2019; 10: 1108.
2. de Souza de Azevedo PO, Mendonca CMN, Moreno ACR, Bueno AVI, de Almeida SRY, Seibert L, Converti A, Watanabe IS, Gierus M, de Souza Oliveira RP. Antibacterial and antifungal activity of crude and freeze-dried bacteriocin-like inhibitory substance produced by *Pediococcus pentosaceus*. *Sci Rep* 2020; 10(1): 12291.
3. Vieco-Saiz N, Belguesmia Y, Raspoet R, Auclair E, Gancel F, Kempf I, Drider D. Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. *Front Microbiol* 2019; 10: 57.
4. Chiu CH, Lin TY, Chu C, Huang YC, Lin MC. *Lactobacillus casei rhamnosus* Lcr35 in the treatment of enteric pathogens: Antimicrobial effects and clinical relevance. *Int J Antimicrob Agents* 2007; 30(1): 62–66.
5. Berebon DP, Attama AA, Ofokansi KC, Evurani SA, Gugu TH, Onyi N, Eze CO, Onah AI, Onwusoba RC, Nwodo U. Molecular identification of lactic acid bacteria isolated from Nigerian traditional fermented foods and beverage with in vitro probiotic potentials. *Trop J Nat Prod Res* 2022; 6(2): 236-243.
6. Banik A, Anjum H, Habib H, Abony M, Begum A, Ahmed Z. Characterization of lactic acid bacteria isolated from street pickles of Dhaka, Bangladesh. *Heliyon* 2023; 9(6).
7. Ren D, Li C, Qin Y, Yin R, Du S, Ye F, Liu C, Liu H, Wang M, Li Y, et al. In vitro evaluation of the probiotic and functional potential of *Lactobacillus* strains isolated from fermented food and human intestine. *Anaerobe* 2014; 30: 1-10.
8. Obioha P, Ouoba LII, Anyogu A, Awamaria B, Atchia SM, Ojimelukwe PC, Sutherland JP, Ghoddsu HB. Identification and characterization of the lactic acid bacteria associated with the traditional fermentation of dairy fermented products. *Braz J Microbiol* 2021; 52(2), 869. <https://doi.org/10.1007/s42770-021-00461-y>
9. Berebon DP, Evurani SA, Attama AA, Ofokansi KC, Eze CO, Onah AI, Onwusoba RC, Ugwu CT, Ezeibe EN. Antimicrobial profiles of probiotic lactic acid bacteria isolated from Nigerian fermented spice condiment Okpeye (*Prosopis africana*). *Trop J Pharm Res*, 2025; 24(2): 241-248
10. Amenu D, Bacha K. Probiotic potential and safety analysis of lactic acid bacteria isolated from Ethiopian traditional fermented foods and beverages. *Ann Microbiol* 2023; 73, 37.
11. Khushboo, Karnwal A, Malik T. Characterization and selection of probiotic lactic acid bacteria from different dietary sources for development of functional foods. *Front Microbiol* 2023; 14:1170725.
12. Argyri AA, Zoumpopoulou G, Karatzas K-AG, Tsakalidou E, Nychas G-JE, Panagou EZ. Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. *Food Microbiol* 2013; 33(2): 282–291.
13. Mulaw G, Sisay Tessema T, Muleta D, Tesfaye A. In vitro evaluation of probiotic properties of lactic acid bacteria isolated from some traditionally fermented Ethiopian food products. *Int J Microbiol* 2019; 7179514.
14. Qiao Z, Sun H, Zhou Q, Yi L, Wang X, Shan Y, Yi Y, Liu B, Zhou Y, Lü X. Characterization and antibacterial action mode of bacteriocin BMP32r and its application as an antimicrobial agent for the therapy of multidrug-resistant bacterial infection. *Int J Biol Macromol* 2020; 164, 845–854.

15. Mokoena MP, Omatola CA, Olaniran AO. Applications of lactic acid bacteria and their bacteriocins against food spoilage microorganisms and foodborne pathogens. *Molecules* 2021; 26(22): 7055.
16. Pegoraro K, Sereno MJ, Cavicchioli VQ, Viana C, Nero LA, Bersot L-S. Bacteriocinogenic potential of lactic acid bacteria isolated from artisanal colonial-type cheese. *Arch Vet Sci* 2020; 25(1).
17. Hussein AO, Khalil K, Mohd Zaini NA, Al Atya AK, Aqma WS. Antimicrobial activity of *Lactobacillus* spp. isolated from fermented foods and their inhibitory effect against foodborne pathogens. *PeerJ* 2025; 13
18. Ogunbanwo ST, Sanni AI, Onilude AA. Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. *Afr J Biotechnol* 2023; 2(8): 219-227.