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(Research Article)

Probiotic activities of lactic acid bacteria from Cassava and Ogi effluents

Inyogu James Chukwuemeka [1], Orji Jerry Okechukwu [1], Dumbuya Mohamed [2], Chiamaka Maria-Goretti Adinnu [3], Uchechukwu Okekeaji [4], Obike Onyedikachi Chijindu [5], Raphael Leke Emmanuel [6], Uzoefuna Chima Casmir [7] and Christiana Inuaesiet Edemekong [8, 9, *]

[1] *Department of Applied Microbiology, Faculty of Sciences, Ebonyi State University, Abakaliki, Nigeria, PMB 53, Nigeria.*
[2] *Department of Environmental Chemistry, Faculty of Basic Sciences, Njala University, Sierra Leone.*
[3] *Department of Food Science and Technology, College of Applied Food Sciences and Tourism, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.*
[4] *Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Science, Gregory University, Uturu.*
[5] *Department of Clinical Pharmacy, Faculty of Pharmaceutical Science, University of Nigeria, Nsukka*
[6] *Department of Biology, Faculty of Natural and Applied Science, Ignatius Ajuru University of Education, Rumuolomeni, Port-Harcourt, Nigeria.*
[7] *Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, State University of Medical and Applied Sciences, Igbo Eno, Enugu State, Nigeria.*
[8] *Department of Public Health, Faculty of Health Care Services, Federal University of Allied Health Science, Trans-Ekulu, P.M.B. 01473, Enugu, Nigeria.*
[9] *Department of Biotechnology, Faculty of Science, Ebonyi State University, Abakaliki, P.M.B. 53, Nigeria.*

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Abstract

The Cassava and Ogi effluents include substantial quantities of bacteria, particularly lactic acid (LABs), which has garnered much attention as probiotics to improve human health. The potential desirable characteristics of the LAB as a probiotic have necessitated the screening of various fermentative compounds to identify a good source of LAB for probiotic use. This was designed to evaluate the probiotic activities of 10 Lactic Acid Bacteria (LAB) strains previously isolated from Cassava and Ogi effluents. Probiotic activities, including pH, titratable acidity, bile salt tolerance, and safety (DNase, hemolysis, and antibacterial activity) were examined using standard laboratory techniques. The pH and total titratable acidity (TTA) of last stage effluents samples generated during Ogi, and Cassava fufu production revealed that the pH of the samples ranges from 3.1 to 5.3 and the TTA ranges from 0.21 to 0.26 %. The pH and TTA of the samples clearly showed that they were acidic. It revealed that the maximum optical density values at 0.2% and 0.3% bile tolerance were 2.40±0.75 and 3.62±0.11 for LAB 9. All the isolates showed appropriate growth in the presence of bile and acid tolerance pH_3-pH_7. The safety evaluation of the LAB strains revealed negative hemolysis (Gamma hemolysis) and negative DNase activity. Results of antibacterial activity indicate that the LAB strains from Ogi and Cassava last stage effluent samples were found to inhibit the growth of most of the pathogenic clinical strains of *S. aureus*, *E. coli*, *K. pneumoniae*, and *B. subtilis* with the highest inhibition zones exhibited from the LAB strains was 23 mm. LAB1, LAB 2, LAB 3, LAB5, LAB6, LAB9, and LAB10 showed no inhibition activity against E. coli. Our overall findings indicate that 10 LAB strains displayed varying species-specific inhibition and their varying *in vitro* bioassay should not undermine the potential probiotics' desirable characteristics but rather substantiate their role *in vivo* evaluation of the strain for their potential as desirable probiotics that could be harnessed and used for the formulation of nutriceutical supplement to improve human wellbeing.

Keywords: Probiotic; Cassava; Ogi; LAB strains

* Corresponding author: Christiana Inuaesiet Edemekong

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1. Introduction

Probiotics are live microbial cultures that have a beneficial effect on human health by improving intestinal microbial balance [1]. Lactic acid bacteria (LAB) are essential microbial groups consisting of several probiotic bacteria, among which *Lactobacilli* has been reported to be the most active and non-pathogenic [1]. Lactic acid bacteria constitute an important group of these organisms and have been associated with the production of fermented foods and feeds for many centuries [2, 3, 4]. In recent times, screening of fermented foods with mixed cultures has proven to be a reliable approach to obtaining useful and genetically stable bacterial strains [1]. In many instances, these microbes exhibit stable properties as well as the ability to survive under stress conditions due to the complex environment they were isolated from.

Some of the reasons for their widespread use are the ability to retard spoilage, preserve food as well as improve flavor and texture of foods. They also play a fundamental role in the microbial ecology of foods by synthesizing numerous antimicrobial compounds such as organic acids, hydrogen peroxide, diacetyl, and bacteriocins [4, 5]. These synthesized antimicrobial compounds are implicated in the inhibition of many microorganisms including spoilage and pathogenic organisms [3, 4].

As human infection persists, antimicrobial resistance (AMR) occurs naturally, but the overuse of antibiotics in humans is accelerating the process of drug resistance. Bacteria can become resistant, and consequently increase the probability of clinical failure [6, 7, 8, 9].

As the search for potential drug compounds continues, probiotics are preferred when compared to antibiotics in the treatment of infections because prolonged use of antibiotics has resulted in many pathogenic bacteria developing resistance. Probiotic bacteria produce various compounds, such as organic acids (lactic and acetic acids), bacteriocins, and reuterin, which are inhibitory to the pathogen's growth. Also, these compounds produced reduce the pH, thereby retarding the growth of pathogens [1, 4, 5]. Additionally, for probiotic bacteria to be effective they must possess several specific properties. One such property is the ability to survive in acidic and bile-containing media as they have to undergo these conditions during their passage through the gastrointestinal tracts [1, 2, 3, 4].

Lactic acid bacteria (LAB) from cassava and Ogi last-stage effluents can have probiotic properties and can be used as natural barriers to pathogens that cause intestinal disease. Cassava and Ogi are traditional staple-based Nigerian dishes, both obtained through fermentation processes [1]; therefore, both products and/or by-products can be used for LAB isolation. Notably, LAB strains are also found in fermented vegetables, meat, and cereals [1, 10]. For example, LAB such as *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus delbrueckii*, and *Lactobacillus casei* can be obtained from the carbohydrate-based fermentation product retted cassava (a wet-paste made from fermented cassava) [1, 11].

The probiotic activity of LAB strains has been extensively studied in fermented African food such as Cassava and Ogi effluents [5, 12, 13] but there are a handful of unevaluated novel LAB strains from Ogi and Cassava effluent generated in other parts of the world for probiotic activity, which may provide a lead to strain with safe and beneficial effect to human health.

2. Material and methods

2.1. Sample Collection and Processing

As previously isolated by Chukwuemeka *et al*. [1], ten (10) strains of LAB (1 *Pediococcus pentosaceus, 2 Lactobacillus plantarum, 3 Leuconostoc mesenteroides, 4 Lactobacillus plantarum strain, 5 Lactiplantibacillus plantarum, 6 Leuconostoc pseudomesenteroides, 7 Lactiplantibacillus argentoratensis, 8 Lactiplantibacillus plantarum, 9 Lactococcus lactis 10 Lactobacillus plantarum* strain) earlier cultured on De Man, Rogosa, and Sharpe (MRS) agar (BIOMERIEUX, France) from effluents generated by sampling 10ml each Ogi and cassava during the final processing stage collected from Ndieguazu, Okposi, Oriuzor, Umuezeokoha, and Umuezeoka). The strains from Chukwuemeka *et al*. [1], were processed using standard microbiological techniques including biochemical techniques and further validated using the Vitex 2 compact 60 next-generation automated system (BIOMERIEUX, France).

Clinical Bacteria: The following non-redundant pathogenic bacterial isolates (*E. coli, Bacilus subtilis, S. aureus* and *Klebsiella pneumoniae*) were obtained from the Medical Microbiological laboratory unit of Alex Ekwueme Federal University Teaching Hospital Abakaliki located at latitude 6.3231° N and longitude 8.1121° E, Ebonyi State [6]. These test organisms were further confirmed using Vitex 2 compact 60 next-generation automated system (BIOMERIEUX, France).

2.2. Preparation of solutions

2.2.1. Buffer preparation

A buffer concentration of 0.1 M was prepared using 1M monobasic sodium phosphate and 1M dibasic sodium phosphate. 1M monobasic (Hopebio, Qingdao, China) and dibasic phosphate (Hopebio, Qingdao, China) was prepared thus.

- **Monobasic stock**: A 24 g of sodium phosphate monobasic (Hopebio, Qingdao, China) was weighed out and dissolved in 1000ml of distilled water.
- **Dibasic stock**: A 28.4 g of sodium phosphate dibasic (Hopebio, Qingdao, China) was weighed out and dissolved in 1000ml of deionizer water. Then, the monobasic buffer (which lowers the pH) was turned on a beaker and placed on the magnetic stirrer (Gulfex medical and Scientific, England) after which the dibasic (which increases pH) was added also. The two salts (monobasic and dibasic) were continually added until it got to the required volume needed [14].

2.3. Assessement of pH and titratable acidity

2.3.1. Determination of pH

The pH of the samples was determined according to the method described by Igbabul *et al.* [15]. Briefly, 1ml each of the samples effluents from Ogi production and effluents from fufu production were homogenized with 9ml of distilled water. The mixture was allowed to equilibrate at room temperature. The pH of the samples was then determined by a pH meter (Gulfex medical and Scientific, England).

2.3.2. Determination of titratable acidity

Titratable acidity was determined by the method described by Oladipo *et al*. [16]. Briefly, 10 ml of the sample was dissolved in 30 ml of distilled water and mixed thoroughly. A few drops of phenolphthalein indicator (BDH Chemicals Ltd, Poole England) were added in the mixed solution. It was titrated against standard 0. 1N sodium hydroxide solution (Labpak Chemicals Ltd Conventry, England) until a pale pink colour was persisted for about 10-15 seconds for complete neutralization. The titratable acidity was calculated based on lactic acid in the samples and is given in the equation below.

$$\text{Titratble acidity (\%)} = \frac{Vol. NaOH\ used\ (ml) \times\ 0.1N\ NaOH\ \times milliequvalent\ factor\ \times 100}{volume\ of\ the\ sample\ used\ (ml)}$$

2.4. Probiotic Potentials of Lactic acid Bacteria Isolates

2.4.1. Bile Salt assimilation from Culture Medium

The lactic acid bacterial isolates were evaluated for bile salt assimilation (rapidity of growth) in a broth medium with and without bile salts. 18 hr old lactic acid bacteria cultures were inoculated into MRS broth ((Hopebio, Qingdao, China) containing 0.2 and 0.3% (w/v) of bile salt (Sodium taurocholate) (Tianjin kermel Chemical Reagent Co., Ltd, China) and were then incubated at 37°C for 24 h. The control was prepared without bile salt. After 24 h incubation, the optical density (OD) at 600nm were measured and compared to the control culture [17, 18].

2.4.2. Acid Tolerance by Lactic acid Bacteria Isolates

By applying the method of Noor Nawaz *et al.*[19] an 18hr old pure culture of lactic acid bacteria isolates were inoculated (1% /v) into acidified MRS broth (Hopebio, Qingdao, China) previously adjusted to pH of 1.5 and 7 using IM HC1 (Tianjin kermel Chemical Reagent Co., Ltd, China) and were then incubated at 37 °C for 48 hr. After incubation, 1 ml of the inoculated broth was serially diluted with peptone water (0.1 % w/v) and pour plated. The plates were incubated at 37 °C for 24 h to ascertain the viability of the lactic acid bacteria. The cultures were designated positive (+) for growth and negative (-) for no growth. The controls have the lactic acid bacteria isolates incubated in MRS broth without acidification.

2.5. Safety Evaluation of LAB Strains

2.5.1. Hemolytic Activity

The hemolytic activities of the LAB isolates were determined using the procedure described by Zhang *et al.* [20]. All the isolates tested were streaked onto blood agar plates containing 5% (w/v) sheep blood and incubated at 37^{0}C for 48 h.

After the incubation, the plates were examined for signs of β-haemolysis (clear zones around the colonies), α-haemolysis (a green-hued zone around the colonies) or ϒ-haemolysis (no halo around the colonies).

2.5.2. DNase Activity

The LAB isolates were streaked onto a deoxyribonuclease (DNase) agar medium (Lifesave Biotech, USA) to test for production of the DNase enzyme. The plates were then incubated at 37°C for 48 h and observed for the zone of DNase activity. Clear pinkish zones around the colonies were considered as positive DNase activity [21].

2.6. Antibacterial activity of Isolates

Antibacterial activity of LAB isolates against clinical test bacteria assessed using agar well diffusion method [22, 23]. First, the test organisms were subcultured for confirmation after 24hrs before use. Test bacteria were *E. coli, S. aureus, K. pneumoniae* and *Bacillius subtilis*. The pathogenic bacteria (100 µl) was added to soft agar, mixed, and overlaid on Mueller-Hinton agar (Thermo Fisher Scientific, U.S.A). Wells were made on Mueller-Hinton agar (Thermo Fisher Scientific, U.S.A) plates using a sterile 6mm cork borer (supertek®, U.S.A). A 100 µl of overnight grown isolates culture were poured into a well on plates. Plates were allowed to dry and incubated at 37°C for 24-48 h. After overnight incubation, the zone of inhibitions was measured and recorded in millimeters (mm) [22, 23].

3. Results

3.1. pH and Total Titratable Acidity (TTA) of Samples from Effluents Generated during Ogi, and Cassava Fufu Production

The pH and total titratable acidity (TTA) of Samples from effluents generated during ogi, and cassava fufu production are shown in Table 1. It revealed that the pH of the samples ranges from 3.1 to 5.3 and the TTA ranges from 0.21 to 0.26 %. The pH and TTA of the samples clearly showed that they were acidic.

3.2. The Probiotic Properties of the Lactic Acid Bacteria Isolated from Effluents Generated During Ogi, and Cassava Fufu Productions

The probiotic properties of the lactic acid bacteria isolated from effluents generated during ogi, and cassava fufu production are shown in the Table 2a and 2b. It revealed that the maximum optical density values at 0.2% and 0.3% bile were 2.40±0.75 and 3.62±0.11 which were both for LAB 9. All the isolates showed appropriate growth in the presence of bile (Table 2a) and also were tolerant to different pH values as there was growth after incubation (table 2b) hence showing a good probiotic property.

3.3. Safety Evaluation of LAB isolates from Effluents Generated from Effluents Generated During Ogi, and Cassava Fufu Productions

The hemolytic activities of the LAB isolates are shown in Table 3. It revealed that all the isolates exhibited a gamma type of hemolysis. Also, the DNAse activities of the LAB isolates are shown in Table 4. It revealed that all the isolates showed no pinkish coloration (negative result) after incubation on the DNA agar by the isolates. Hence this revealed that all the isolates tested do not exhibit pathogenicity and are safe for consumption.

3.4. Screening of Lactic Acid Bacteria Isolated for antagonistic activity against Clinical test Bacterial Isolates

The antibacterial activities of LAB against four clinical test bacteria are shown in Table 5 below. It revealed that LAB 1, 2, 3, 5, 6, 9, and 10 showed negative activities against *E. coli.* LAB 1 and 6 showed negative activities against *S. aureus.* LAB 1, 4, 5, 8, and 10 showed negative activities against *K. pneumoniae* and Isolates 2, 5, and 6 showed negative activities against *B. subtilis.*

Table 1 pH and Total Titratable Acidity (TTA) of Samples from Effluents Generated during Ogi, and Cassava Fufu Production

S/N	Locations	Sources/ Effluents	Range of pH	Range of TTA (%)
1	Ndieguazu	Ogi	3.1 - 4.9	0.21 - 0.26
		Cassava Fufu	3.5 - 4.8	0.21 - 0.26
2	Okposi	Ogi	3.2 - 4.3	0.21 - 0.26
		Cassava Fufu	3.8 - 5.1	0.21 - 0.25
3	Oriuzor	Ogi	3.1 - 4.1	0.23 - 0.26
		Cassava Fufu	3.9 - 5.3	0.23 - 0.26
4	Umuezeokoha	Ogi	3.2 - 4.3	0.22 - 0.26
		Cassava Fufu	3.9 - 5.1	0.21 - 0.26
5	Umuezeoka	Ogi	3.3 - 4.8	0.22 - 0.26
		Cassava Fufu	3.9- 5.1	0.21- 0.26

Table 2a The Probiotic Properties of the Lactic Acid Bacteria Isolated from Effluents Generated during Ogi, and Cassava Fufu Productions showing Bile salt assimilation from culture medium

Source	LAB isolates	0.2%	0.3%	Control
Ogi	LAB 1	1.20±0.18	1.40±0.02	0.23
Ogi	LAB 2	1.40±0.02	1.30±0.50	0.51
Ogi	LAB 3	1.10±0.07	2.30±0.26	0.23
Ogi	LAB 4	1.30±0.26	2.40±0.14	0.35
Ogi	LAB 5	1.30±0.09	2.30±0.26	0.34
Fufu	LAB 6	1.10±0.04	2.30±0.22	0.48
Fufu	LAB 7	1.10±0.05	2.30±0.27	0.40
Fufu	LAB 8	1.10±0.19	2.82±0.33	0.23
Fufu	LAB 9	2.40±0.75	3.62±0.11	0.44
Fufu	LAB 10	1.20±0.04	2.72±0.14	0.43

Key: LAB = Lactic Acid Bacteria, 1-*Pediococcus pentosaceus*, 2-*Lactobacillus plantarum*, 3- *Leuconostoc mesenteroides*, 4-*Lactobacillus plantarum* strain, 5-*Lactiplantibacillus plantarum*, 6-*Leuconostoc pseudomesenteroides*, 7-*Lactiplantibacillus argentoratensis*, 8-*Lactiplantibacillus plantarum*, 9-*Lactococcus lactis* 10-*Lactobacillus plantarum* strain

Table 2b The Probiotic Properties of the Lactic Acid Bacteria Isolated from Effluents Generated During Ogi, and Cassava Fufu Productions showing Acid tolerance by LAB isolates

Source	LAB isolates	At pH_3	At pH_4	At pH_5	At pH_7 (control)
Ogi	LAB 1	+	+	+	+
Ogi	LAB 2	+	+	+	+
Ogi	LAB 3	+	+	+	+
Ogi	LAB 4	+	+	+	+
Ogi	LAB 5	+	+	+	+
Fufu	LAB 6	+	+	+	+
Fufu	LAB 7	+	+	+	+
Fufu	LAB 8	+	+	+	+
Fufu	LAB 9	+	+	+	+
Fufu	LAB 10	+	+	+	+

Key: LAB = Lactic Acid Bacteria, + = positive, 1-*Pediococcus pentosaceus*, 2-*Lactobacillus plantarum*, 3- *Leuconostoc mesenteroides*, 4-*Lactobacillus plantarum* strain, 5-*Lactiplantibacillus plantarum*, 6-*Leuconostoc pseudomesenteroides*, 7-*Lactiplantibacillus argentoratensis*, 8-*Lactiplantibacillus plantarum*, 9-*Lactococcus lactis* 10-*Lactobacillus plantarum* strain

Table 3 Hemolytic activities

LAB isolates	Types of haemolytic activities
LAB 1	Gamma haemolysis
LAB 2	Gamma haemolysis
LAB 3	Gamma haemolysis
LAB 4	Gamma haemolysis
LAB 5	Gamma haemolysis
LAB 6	Gamma haemolysis
LAB 7	Gamma haemolysis
LAB 8	Gamma haemolysis
LAB 9	Gamma haemolysis
LAB 10	Gamma haemolysis

Key: LAB = Lactic Acid Bacteria, 1-*Pediococcus pentosaceus*, 2-*Lactobacillus plantarum*, 3- *Leuconostoc mesenteroides*, 4-*Lactobacillus plantarum* strain, 5-*Lactiplantibacillus plantarum*, 6-*Leuconostoc pseudomesenteroides*, 7-*Lactiplantibacillus argentoratensis*, 8-*Lactiplantibacillus plantarum*, 9-*Lactococcus lactis* 10-*Lactobacillus plantarum* strain

Table 4 DNAse activities

LAB isolates	Clear pinkish coloration around the colonies
LAB 1	-
LAB 2	-
LAB 3	-
LAB 4	-
LAB 5	-
LAB 6	-

LAB 7	-
LAB 8	-
LAB 9	-
LAB 10	-

Key: LAB = Lactic Acid Bacteria, **= (-)** Negative 1-*Pediococcus pentosaceus*, 2-*Lactobacillus plantarum*, 3- *Leuconostoc mesenteroides*, 4-*Lactobacillus plantarum* strain, 5-*Lactiplantibacillus plantarum*, 6-*Leuconostoc pseudomesenteroides*, 7-*Lactiplantibacillus argentoratensis*, 8-*Lactiplantibacillus plantarum*, 9-*Lactococcus lactis* 10-*Lactobacillus plantarum* strain

Table 5 Antibacterial activity of LAB strain against the Four Clinical Test Bacterial Isolates

Source	LAB isolates	Clinical Isolate and Zone of inhibition (mm)			
		E. coli	***S. aureus***	***K.pneumoniae***	***B. subtillis***
Ogi	LAB 1	-	-	-	10
Ogi	LAB 2	-	23	15	-
Ogi	LAB 3	-	11	13	9
Ogi	LAB 4	9	21	-	12
Ogi	LAB 5	-	15	-	-
Fufu	LAB 6	-	-	11	-
Fufu	LAB 7	17	13	10	17
Fufu	LAB 8	15	18	-	9
Fufu	LAB 9	-	10	10	16
Fufu	LAB 10	-	13	-	9

Key: + = positive inhibition, - = negative (no inhibition), mm-Millimeter, LAB = Lactic Acid Bacteria, **=** (-) Negative, 1-*Pediococcus pentosaceus*, 2-*Lactobacillus plantarum*, 3- *Leuconostoc mesenteroides*, 4-*Lactobacillus plantarum* strain, 5-*Lactiplantibacillus plantarum*, 6-*Leuconostoc pseudomesenteroides*, 7-*Lactiplantibacillus argentoratensis*, 8-*Lactiplantibacillus plantarum*, 9-*Lactococcus lactis* 10-*Lactobacillus plantarum* strain

4. Discussion

The VITEX 2 system characteristics of the previously identified 10 isolates were observed to confirm the type of LAB isolate which were; 1 *Pediococcus pentosaceus*, 2 *Lactobacillus plantarum*, 3 *Leuconostoc mesenteroides*, 4 *Lactobacillus plantarum* strain, 5 *Lactiplantibacillus plantarum*, 6 *Leuconostoc pseudomesenteroides*, 7 *Lactiplantibacillus argentoratensis*, 8 *Lactiplantibacillus plantarum*, 9 *Lactococcus lactis* 10 *Lactobacillus plantarum* strain [1].

As important parameters in the probiotic evaluation, the viability of the ten LAB isolates from Ogi and Cassava effluent samples were assessed considering their acid bile tolerance and pH. It is worth noting that in an *in vivo* assay, the probiotics will have to face all the antimicrobial factors in the stomach (pepsin, gastric acid, and low pH) and intestines (bile salts, trypsin, and high pH), as well as mild heat stimulus caused by the internal body temperature (approximate 37.5°C), which forces the probiotics must have acid and bile tolerance or other exclusion mechanisms to survive in the gut [24].

The result of this study revealed that the maximum optical density values at 0.2% and 0.3% bile tolerance had mean values of 2.40±0.75 and 3.62±0.11, which were both for LAB 9. It also revealed that the pH of the samples ranged from 3.1 to 5.3, and the TTA ranged from 0.21 to 0.26 %. The pH and TTA of the samples clearly showed evidence of acidity.

Moreover, the findings showed a consistently higher tolerance to bile acids similar to earlier studies [25]. Also, Roldan-Perez *et al.* [26] reported bile salt conditions (0.3, 0.6, and 1.0 % w/v) and the pH of 6.5, 3.0, and 8.0 corresponding to the oral cavity, stomach, and large intestine, respectively. Several researchers have reported tolerance to these pH conditions for these species [27, 28, 29]. Also, Doğan and Ay. [30], and Sarkar *et al.* [31] reported that *Pediococcus pentosaceus* and *Pediococcus acidilactici* survived in acidic gastric juice with a pH of 2–3, making them potential

probiotic LAB strains. *Lactococcus lactis* from various food items showed potential survival rates in simulated gastric juice at pH 3 [32, 33]

Previous studies revealed that the tolerant abilities of LAB strains are strain-specific [34, 35]. Similarly, in the present study, the 10 selected LAB isolates showed varying survival rates demonstrating heterogeneous tolerance for bile salts and acidic conditions (Table 2b). This high tolerance allows them to proliferate in gastrointestinal tracts, and remain effective when administered.

The distinct strategies used by each strain to survive pH and bile salts may account for the variation in strain survival in the gastrointestinal system [1, 36]. *Lactobacillus*, for example, has mechanisms for acid resistance such as proton pumps, the ability to alkalinize the external environment, modification of the membrane's lipid content, and the formation of biofilms, which facilitate auto-aggregation and protection from extreme pH conditions [37].

Additionally, the tolerance of LAB to bile salts is related to the microbial enzyme Bile Salt Hydrolase (SBH) that deconjugates the active efflux of salts and primary bile acids, and the alteration of the membrane and cell wall [38, 39]. These enzymes enhance the survival and colonization of LAB in the mammalian intestine.

Although there are several parameters for evaluating the safety of the LAB. We conducted DNase activity tests, hemolytic assays, and antibacterial assays. The DNase test was also used to determine the pathogenicity of bacteria that produce the DNase enzyme, which hydrolyses DNA. The absence of DNase in the tested isolates was thus confirmed, indicating their potential safety for use in fermentation. The isolated LAB strains were also proven to be safe after a hemolytic test revealed no hemolysis (ϒ- hemolysis). Similar studies have demonstrated non-hemolytic properties of LAB in fermented butter, Sardinian dairy products, chicken guts, and Ethiopian traditional fermented foods and beverages [25, 40, 41, 42] while the effects of LAB from Egyptian fermented food on the general health condition of rats were investigated, no abnormal behavior, body weight loss, or changes in organ indices were observed after 21 days of feeding rats with the LAB strains [43]. Thus, consistent with previous research, these strains can be considered safe and attractive candidates for probiotics. On the other hand, *L. rhamnosus* has been discovered to damage the structure of cellular membranes and cause ATP efflux, which results in pore development and reduces the growth of *M. luteus* [44].

As increased emphasis has been given to the spread and convergence of antibiotic resistance, antimicrobial activity against pathogenic bacteria has garnered increasing attention [8, 9, 45, 46, 47]. To combat both Gram-positive and Gram-negative bacteria, putative LAB probiotics should have antibacterial qualities. Our LAB strains from Ogi and Cassava were found to inhibit the growth of all the pathogenic clinical strains (Table 5).

In the present study, the highest inhibition zone diameter obtained from our LAB strains was 23 mm but different inhibition zones have been reported from LAB strains by different authors; evidence that *P. pentosaceus* had a higher inhibition (>4.0 mm) against all evaluated pathogens [26]; Amenu and Bacha. [42] found that eleven LABs demonstrated the most significant antagonistic effect, with a zone of inhibition ranging from 18 to 29.96 mm. In line with this, Tadesse *et al*. [48] demonstrated that LAB isolated from Borde and Shamita inhibited *Staphylococcus aureus*, *Salmonella Typhimurium*, and *Escherichia coli* growth with inhibition zones ranging from 15 to 17 mm in diameter. Similarly, Mulaw *et al*. [49] found 34 probiotic LABs had effective antagonistic activity, with inhibition zone diameters ranging from 12 to 22 mm. On the other hand, Negasi *et al*. [50] discovered that LAB from Ethiopian fermented dairy product, *Ergo*, had potential antibacterial action against *Salmonella* Typhimurium, with inhibitory zones ranging from 10 to 14.5 mm in diameter; Afolayan *et al.* [51] in Nigeria reported LAB from Ogi displayed large zone of inhibition ($11 \leq x \leq 20$) was observed against *Salmonella* species (SS11) and *Shigella* species.

Notably, the antibacterial ability of LAB strains is mainly produced by the secreted compounds (such as organic acids [lactic, acetic, and propionic], hydrogen peroxide, diacetyl, ethanol, and bacteriocin) [3, 4, 52], and the special microenvironment in the gastrointestinal tract (enzymes, adverse pH, and mild heat shock), further enhances the antibacterial potency of these compounds [27, 53], indicating that they might exhibit higher antibacterial abilities if they are orally taken. In fact, the antibacterial activity is relevant to coaggregation activity [44, 53]. Probiotic bacteria produce antimicrobial substances during their intimate contact with pathogenic bacteria to eliminate pathogens [43]. Antibacterial activity and safety characteristics were considered the most important properties of LABs as probiotic but our study was limited to other parameter such as Co-aggregative ability with pathogens, Antioxidant screening, Cell surface hydrophobicity, tolerance for phenol, auto-aggregation activity, exopolysaccharide (EPS) production phenotype which these parameters are recommended for further evaluation.

5. Conclusion

The antibacterial activity and excellent tolerance to pH and bile conditions of probiotic isolates are indicative of their essential role in the elimination of pathogens and their safety for human health. The demonstration of extensive antibacterial activity against *S. aureus*, *E. coli*, *K. pneumoniae*, and *B. subtilis* suggests their potential as probiotics for the formulation of nutriceutical foods. These findings also revealed that the LAB from Ogi and Cassava effluent samples showed elaborate probiotic properties and may require further *in vivo* evaluations.

Compliance with ethical standards

Disclosure of conflict of interest

Authors have declared that no competing interests exist.

Statement of ethical approval

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Authors' contributions

This work was carried out in collaboration among all authors. All authors investigated the study, did literature searches and did data Validation and Visualization. All the authors reviewed and approved the final draft, and are responsible for all aspects of the work.

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