Microbiology Research Journal International

Volume 33, Issue 3, Page 50-60, 2023; Article no.MRJI.99781
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Probiotic Properties of Lactic Acid Bacteria Isolated from Spontaneously Fermented Kunun-Zaki

A. Daniel Aderolake [a*], O. Egbebi Adeola [a] and A. Onasanya Amos [b]

[a] Department of Biological Sciences, Afe Babalola University, Ado-Ekiti, Nigeria.
[b] Department of Chemical Sciences, Afe Babalola University, Ado-Ekiti, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2023/v33i31371

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/99781

Original Research Article

Received: 08/03/2023
Accepted: 10/05/2023
Published: 22/05/2023

ABSTRACT

This study was conducted to investigate the probiotic properties of twenty lactic acid bacteria (LAB) previously isolated and identified from naturally fermented kunun-zaki (a Nigerian fermented cereal beverage). The probiotic properties of the twenty lactic acid bacteria (LAB) isolates were assessed using different standard methods and agar well diffusion method. The results showed that most of the isolates tolerated acidic pH and survived at 30-40^{o}C. All the tested LAB isolates grew at 1% NaCl concentration and remarkably, six LAB isolates {*L. brevis* (3) and *L. plantarum* (3)} exhibited good growth at higher NaCl concentrations (10-15%). The highest percentage of cellular auto-aggregation was observed in *L plantarum* (84.86%) and the lowest was obtained in *L. brevis* (27.44%). All the twenty LAB isolates tested in-vitro in this study grew in bile salt; fermented glucose; produced good aroma; produced no hemolysis revealing they were not pathogenic and produced antagonistic activity against selected pathogens (*Staphylococcus aureus*, *Salmonella typhi, Shigella. dysenteriae* and *Escherichia coli*) which made them suitable and safe for human consumption as potential probiotics and for industrial purposes.

**Corresponding author: E-mail: preciousrollydan@gmail.com;*

Keywords: Probiotic properties; lactic acid bacteria; Kunun-zaki.

1. INTRODUCTION

"Kunun-zaki is one of popular the indigenous fermented non-alcoholic beverages prepared from millet (*Pennisetum glaucum* L.), maize (*Zea mays*), or rice (*Oryza sativa*). Its wide acceptance has extended beyond the savannah region of Nigeria [1] and also, is consumed at any time of the day by both adults and children as breakfast drink, food complement, refreshing drink for visitors, appetizers and are commonly served in social gatherings" [2]. "The most promising microorganisms selected as starter culture are those that are isolated from the native microbiota of traditional products [3] since they are well adapted to the environmental conditions of food and are capable of controlling spoilage and pathogenic microbiota of food" [4]. "To select a microorganism as a starter or starter culture, it is necessary to carry out a proper study regarding its metabolism and activities, since in some cases, its effects and/or properties may vary between laboratory conditions and food products" [5]. "Also, starter culture must be recognized as safe, capable of being produced on a large scale and remain viable and stable during storage" [6]. "The starter cultures that are mostly used to produce fermented foods and beverages, particularly acidic fermented products, belong to lactic acid bacteria (LAB). Such bacteria include members of *Lactobacillus, Leuconostoc*, *Enterococcus, Streptococcus, Oenococcus*, and *Pediococcus*, and some of them may exert direct beneficial effects on health as live probiotic microbes" [7].

"Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer health benefits on the host" [8,9]. *"*Probiotic organisms used in foods must have the ability to resist gastric juices, exposure to bile, and be able to proliferate and colonize the digestive tract" [10]. "The beneficial effects of probiotic foods on human health and nutrition are constantly increasing popularly being used as bio-ingredients in many functional fermented foods" [11,12]. "The most commonly used probiotic bacteria belong to the heterogeneous group of LAB (*Lactobacillus spp, Enterococcus spp*) and to the genus *Bifidobacterium spp*, however, yeasts and other microbes have also been developed as potential probiotics in recent years" [13]. "Products containing probiotic bacteria generally include foods and supplements" [14]. "Fermented milk products are the most traditional source of probiotic strains of lactobacilli [15]; however, commercial probiotics have been added for the fermentation and production of meat products, snacks, fruit juice, *etc"* [16].

"The most commonly used *Lactobacillus* species in probiotic settings include *L. acidophilus*, *L. plantarum*, *L. casei*, *L. paracasei*, *L. johnsonii*, *L. reuteri*, and *L. rhamnosus"* [17]. "Lactic acid bacterial fermentation is used to improve sensory and nutritional properties of foods [18] and to produce products of high and consistent qualities, the fermentation process has to be controlled using tailor made starter culture; this would improve the products' shelf-life quality thereby reducing microbial risks associated with traditionally food process" [19]. "Metabolic activities are associated with production of many beneficial compounds such as organic acids, polyols, exopolysaccharides and antimicrobial compounds" [19]. "Probiotic strains of *Lactobacilli* particularly adhere to the GI tract of the host cells, which is dependent on their aggregation ability" [20]. "They also act as a barrier to prevent the adherence of pathogenic bacteria, by interfering with colonization and proliferation of pathogens, thereby preventing the manifestation of infections" [21]. "*Lactobacilli* species also produces antimicrobial peptides (bacteriocins), organic acids (acetic acid and lactic acid), and other metabolites (hydrogen peroxide), which are reported to inhibit the growth of pathogenic bacteria" [22]. "Probiotic strains of *Lactobacillus plantarum* also participate in the absorption of water and sodium in the colon and decrease diarrhoea symptoms. They also exert holistic health benefits for medical conditions such as psoriasis, allergies, and nervous system diseases" [20]. "Among the potential benefits of probiotic lactic acid bacteria (LAB) is their practical use for bio-preservation of foods, fungal decontamination, and novel biotherapy" [23]. The aim of this study was to evaluate the probiotic and safety properties of lactic acid bacteria isolated from spontaneously fermented kunun-zaki and to assess their use in the food industry.

2. MATERIALS AND METHODS

Twenty LABs {*L. brevis* (7); *L plantarum* (12) and *L acidophilus* (1)} isolated from another study [24] from spontaneously fermented kunun-zaki and identified using RAPD-PCR analysis were used in this study. These twenty lactic acid

bacteria were isolated from various kunun-zaki prepared from different cereal substrates (millet, sorghum, maize, wheat and paddy rice) [24].

Test for pH tolerance: The modified methods described by Ayo-Omogie and Okorie [25] was used to determine the tolerance of the lactic acid bacteria (Table 1) obtained from another study to acidic pH when tested *in vitro*. 1 ml of each lactic acid bacterial culture at 1.5×10^8 Cfu/ml (fresh cultures of the isolates grown on MRS agar plates were harvested with sterile cotton swab and suspended separately in 1ml of sterile saline, 0.85% NaCl; to prepare a suspension equivalent to 0.5 McFarland turbidity standard) was inoculated into sterile de Man Rogosa and Sharpe (MRS) broth tubes of pH 2, 3 and 4. The pH was adjusted with HCl or NaOH using a pH meter (Hanna pocket-sized pH meter, model H1-98128) and incubated anaerobically at 37^{o}C for 24h. After incubation,1 ml inoculum from each tube was inoculated into MRS agar medium using pour plating technique and incubated anaerobically in an anaerobic jar at 37^{o}C for 48 h. Presence or absence of growth of lactic acid bacteria on agar MRS agar was used to designate isolate as pH tolerant.

Sensitivity to temperature: Test LAB culture (24h old) was inoculated into 10 ml sterile MRS broth for each of the isolate and incubated anaerobically at varying temperatures from 25 to 40^{o}C for 48 to 72h. Thereafter, 1ml each of inoculum was pour plated on MRS agar and incubated at 37^{o}C for 48h. The growth of LAB on MRS agar plates was used to designate isolates as temperature tolerant [25].

NaCl tolerance test: MRS agar plates were prepared in eight different concentrations of NaCl (0, 0.5, 1, 5, 7.5, 10, 12.5, and 15 **% respectively).** Test LAB isolates grown on MRS broth for 24h were streaked on hardened MRS agar plates and these were incubated at 37^{o}C for 24 h. The influence of NaCl concentrations on the degree of inhibition of bacterial growth was recorded [20].

Cellular auto-aggregation assay: The method described by Nath et al. [20] was used; LAB grown on MRS broth overnight was centrifuged at 5000 rpm for 10 min to harvest the cell pellets. Pellets were repeatedly washed with phosphate-buffered saline solution (PBS) (pH 7.2), re-suspended in PBS , and the initial absorbance was noted at 600 nm, these bacterial suspensions was then incubated at 37^{o}C for 2h, following which the final absorbance of the supernatant were measured at 600 nm. The percentage of cellular auto-aggregation was measured by the formula:-

Auto-aggregation rate (%) = $\frac{OD\ intial - OD\ final}{OD\ initial}$ x100 [20].

Bile tolerance test: One hundred microlitres (100µl) of overnight grown LAB cultures were inoculated in freshly prepared MRS broth containing 0.3% bile salt (Himedia Pvt. Ltd). Test isolates were also inoculated in MRS broth without bile (as control). Both test tubes (with and without bile salts) containing test isolates were incubated at 37^{o}C for 4h, and growths at different time interval (0h,1h,2h,3h,4h) and percentage resistances recorded by measuring the absorbance of MRS broth at 600 nm. After the incubation period of 4 h, the viability of the LABs in 0.3% bile was also evaluated by spreading 100 µl of the bacterial samples onto the MRS agar plate [20].

Glucose fermentation test: Eighteen-hour-old LAB cultures grown on MRS broth were centrifuged at 4000 rpm for 15 min, and the bacterial pellets were recovered. The pellets were washed twice by PBS buffer and re-suspended in the PBS buffer. Thereafter, 500 µl of PBS buffer containing the bacterial cells were inoculated into MRS broth supplemented by 1% glucose and 0.5% phenol red (dye) and incubated at 37^{o}C for 24 h. Change in color from purple to yellow indicates positive glucose fermentation, whereas no change in color indicates negative glucose fermentation [20].

Hemolytic activity: Hemolytic activity is a determining factor for probiotic bacteria, and the absence of hemolytic activity indicates that the particular bacteria were non-virulent. Sheep blood agar was prepared by weighing 2.8g of nutrient agar into 95mls of sterile distil water; sterilized at 121^{o}C for 15 minutes in an autoclave and allow to cool to 45^{o}C. 5% (vol/ vol) sterile defibrinated blood that has been warmed to room temperature was added and mix gently but well to avoid air and this was dispensed into sterile plates and allow to solidify. 24h LAB cultures were streaked on sheep blood agar and incubated at 37^{o}C for 48 h. The zone formed around the colonies was observed, which is categorized as β-haemolysis (clear zone), α-haemolysis (green-hued zone), or γ-haemolysis (no zone) [20].

Extraction of antibacterial agents and evaluation of their antagonistic activity: About 5 ml of 48h old LAB isolates were mixed with an equal volume of ethyl acetate and shaken at 20 rpm for 10 min in a rotary shaker (Table Top Model, PLT 207). The sample was centrifuged, and the supernatant was transferred into a fresh tube to evaporate the ethyl acetate. The remaining content was used to study the antagonistic effect on the selected test pathogen. The test pathogens were *Escherichia coli*, Salmonella species, *Shigella dysenteriae* and *Staphyloccoccus aureus* obtained from Afe Babalola University Ado-Ekiti (ABUAD) clinic. The pathogens were reactivated at 37^{o}C for 24h on agar plates under aerobic condition as follows: Eosin methylene blue agar for *Escherichia coli*; Salmonella-Shigella agar for *Salmonella typhi* and *Shigella dysenteriae*; manitol salt agar for *Staphylococcus aureus*. The effectiveness of bacterial metabolites was studied by agar well diffusion method [26]. MHA plates of each test pathogen were prepared, and wells were made using a sterile cork borer (5 mm). The wells were loaded with of 60µl aliquots of the supernatant and incubated at 37^{o}C for 24h. Dimethyl sulfoxide was used as a control. The diameters of the zones of growth inhibition were measured and classified as sensitive or +++ (<20 mm), moderate or ++ (10–20 mm), low or + (>10 mm) and resistant or – (0mm) [27].

Potential use in fermentation: The acidification ability of isolated LAB cultures was evaluated on their potential use in food fermentation according to the methods described by Wang et al. [28] in triplicates. LAB cultures were inoculated in reconstituted sterile skimmed milk powder (10% w/v) at 1%. A pH meter was used to determine the pH value changes in the milk at different time (h) intervals (3, 6 and 24) of incubation at 37^{o}C. The acidification rate was calculated as:-

$$\Delta pH = pH_o - \Delta pH_t$$

Values of ΔpH after 3h (ΔpH3), 6h (ΔpH6) and 24h (ΔpH24) were used to compare the strain-acidifying activity [28].

Pure cultures of the twenty identified LABs were tested singly for their potential use as starter culture for kunun-zaki production. Fresh cultures of the isolates grown on MRS agar plates were harvested with sterile cotton swabs and suspended (separately) in 1ml of sterile saline (0.85% NaCl) to prepare a dense suspension (equivalent to a no. 2 McFarland turbidity standard; Agarry et al., [29]); this was used to inoculate singly or in various combinations of two or three LAB isolates into 10ml of sterile Hydrolyzed cereal starch Broth (HCS-broth: 500g gelatinized cereal starch was hydrolyzed with 200g ground malted rice to which 2g of soy bean flour was added and sterilized at 121^{o}C for 10 min) and incubated at ambient temperature (30±2^{o}C) for 24h [29]. After incubation, the samples were subjected to an in-house sensory evaluation by a trained 5 member expert profile panelist that can distinguish between the intensity of aroma differences of a product; therefore production of good aroma (buttery characteristics) was used as a quality indicator to establish their potential use in inoculum development [29].

3. RESULTS AND DISCUSSION

pH tolerance of LAB: Tolerance to low pH is one of the most important criteria in the selection of probiotic species and of the twenty lactic acid bacteria (LAB) isolates used in this study. Eight(8) of the isolates comprising of *Lactobacillus brevis* (3)*; Lactobacillus plantarum* (4) and *Lactobacillus acidophilus* (1) were grew at pH 2, this was in accordance with the reports of Ayo-Omogie et al. [25] that *Lactobacillus acidophilus* and *Lactobacillus plantarum* tolerated pH 2.0*.* Fourteen of the isolates comprising of *Lactobacillus brevis* (5); *Lactobacillus plantarum* (8) and *Lactobacillus acidophilus* (1) grew at pH 3 (Table 1). Interestingly, most of the LAB isolates studied were observed to grow at pH 4, however, two LAB isolates comprising of *L. brevis* (1) and *L. plantarum* (1) did not grow at any of the pH (2, 3, 4) used in this study (Table 1). Therefore, tolerance of these LAB isolates is an indication that they are good probiotics and can survive the acidic conditions of the intestinal system.

Sensitivity to temperature result: Most of the lactic acid bacteria isolates studied survived the selected temperature range of 25-40^{o}C. Nine isolates of *L. plantarum* and isolates of *L. brevis* grew at 25^{o}C. All the twenty LAB isolates were able to grow and survive at temperatures of 30^{o}C and 35^{o}C (Table 1). This was in conformity with the result obtained by Ayo-Omogie and Okorie [25] that most of the isolates grew at 25^{o}C-40^{o}C. These results may be an indication of their potential to survive temperature of the human gut and the selected temperature ranges was chosen to simulate the normal human body temperature.

Table 1. Characteristics of LAB isolates at various pH, temperature and NaCl concentrations

		Growth pH			Growth temperature (^{o}C)				Growth in NaCl (%)							
S/N	**LAB isolates used**	**2**	**3**	**4**	**25**	**30**	**35**	**40**	**0**	**0.5**	**1**	**5**	**7.5**	**10**	**12.5**	**15**
1	*L plantarum*	-	-	-	+	+	+	-	+	+	+	-	-	-	-	-
2	*L. brevis*	-	-	+	-	+	+	+	+	+	+	-	-	-	-	-
3	*L. brevis*	-	+	+	-	+	+	+	+	+	+	+	-	+	-	-
4	*L. brevis*	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
5	*L. brevis*	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
6	*L plantarum*	-	+	+	+	+	+	+	+	+	-	-	+	-	-	-
7	*L. brevis*	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-
8	*L plantarum*	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-
9	*L plantarum*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	*L. brevis*	-	+	+	-	+	+	+	+	+	+	+	+	-	-	-
11	*L plantarum*	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-
12	*L plantarum*	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-
13	*L plantarum*	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-
14	*L plantarum*	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-
15	*L plantarum*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
16	*L plantarum*	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-
17	*L plantarum*	-	+	+	-	+	+	+	+	+	+	-	-	-	-	-
18	*L. brevis*	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
19	*L.acidophilus*	+	+	+	-	+	+	+	+	+	+	+	-	-	-	-
20	*L plantarum*	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-

These lactic acid bacteria were probably of different strains even though they were from the same species. +: Growth;- No growth

Sodium chloride tolerance test result: As shown in Table 1, nineteen of the LAB isolates studied were able to tolerate growth on 0, 0.5 and 1% NaCl, only one isolate of *L plantarum* that did not grow in 1% NaCl and likewise at 5% NaCl. Fifteen out of the twenty lactic acid bacteria studied grew at 5% NaCl while 8 isolates survived and grew in 7.5% NaCl (Table 1). Furthermore, 6 of the LAB isolates comprising of *L. brevis* (3) and *L. plantarum* (3) tolerated 10% NaCl. Nath et al. [20] reported no growth above 10% NaCl. Remarkably, the isolates in this study tolerated higher salt concentrations than those reported by Ayo-Omogie and Okorie [25] and Nath et al. [20] Ayo-Omogie and Okorie [25] reported that all their isolates grew at 4-6% NaCl concentrations and Nath et al. [20] reported that no growth was observed above 10% NaCl concentration. However, at 12.5% NaCl concentration, two of the *L. plantarum* grew well and similarly at 15% NaCl concentration, one *L. brevis* and one *L. plantarum* exhibited good growth in this study (Table 1) which establish the fact that these isolates are good probictics and can survive in the gut.

Glucose fermentation test result: Also shown in Table 2, all the twenty LAB isolates fermented glucose. The color of the medium changed from red to yellow which indicated that this sugar was fermented to produce organic acids thereby reducing the pH of the medium. This was in conformity with the report of Nath et al. [20] that the *Lactobacillus plantarum* isolated was capable of fermenting glucose.

Hemolytic activity: The hemolytic activity of the twenty selected Lactic acid bacteria isolates were evaluated on sheep blood agar plates and no hemolytic effects were observed (γ-hemolysis).This result implies that all the LAB isolates were non-virulent (Table 2). This was in conformity with the report of Nath et al. [20] that the in-vitro screening of probiotic properties of *Lactobacillus plantarum* from fermented milk product did not show any hemolysis when tested. Similarly, Wang et al. [28] reported that all the ten selected strains of lactic acid bacteria screened showed no hemolytic activity.

Extraction of antibacterial agents and evaluation of their antagonistic activity: As shown in Table 2, the twenty LAB isolates obtained in this study were observed to have antagonistic activity against four pathogenic bacteria (*Escherichia coli, Staphylococcus aureus, Salmonella typhi* and *Shigella dysenteriae*). Twelve lactic acid bacteria isolates comprising of *L. brevis* (3), *L. plantarum* (8) and *L. acidophilus* (1) showed a strong antagonistic activity of 20-30 mm against *Staphylococcus aureus*. However, *L. plantarum* showed the highest zone of inhibition (30mm) against *S. aureus* and another *L. plantarum* showed the least (<10 mm) against the same organism (Table 2). A high antagonistic activity of 20mm was also observed in *L. plantarum* against *Shigella dysenteriae* whereas the same isolate did not show any antagonistic activity against *Escherichia coli* (0 mm). Furthermore, *L. brevis* showed the highest antagonistic activity of 30mm against *Escherichia coli* (Table 2). Ayo-Omogie and Okorie [25] reported that the highest inhibition of 39mm was displayed by *Lactobacillus acidophilus* against *Salmonella enterica subsp. typhi* while the most significant inhibition against *Escherichia coli* was shown by *Lactobacillus plantarum* (35mm).

Potential use in fermentation: The salt tolerance, acidification ability as well as the LAB's ability to produce good aroma when inoculated into sterile HCS broth were examined on selected probiotic LAB strains to evaluate their potential use in food fermentation (Table 2). For acidification ability (Table 4), *Lactobacillus plantarum* (1) produced the highest value of ΔpH after 3h incubation (ΔpH_3= 1) followed by three other *Lactobacillus plantarum* isolates (ΔpH_3= 0.7 for the three isolates). However, after 6h of incubation, the highest acidification rate $\Delta pH_{6\ of}$ (1.3) was observed in *L. plantarum* while after 24h of incubation, the highest acidification rate ($\Delta pH_{24)}$ of 3 was still observed in *L. plantarum*. It was also observed that as the hour of fermentation is increasing, acidification rate increased (Table 4). All these isolates were probably different strains of *Lactobacillus plantarum*, the degree of aroma produced by the LAB isolates differed (Table 2). Fourteen LAB isolates comprising of *Lactobacillus brevis* (3), *Lactobacillus plantarum* (10), *Lactobacillus acidophilus* (1) were able to produce strong (good) buttery aroma while six isolates produced weak aroma (Table 2). Onyimba et al. [30] reported that *Lactobacillus plantarum, Lactobacillus acidophilus* and *Lactobacillus fermentum* produced the best aroma while fermenting hydrolysed sorghum starch (HSS). Production of good aroma (buttery) was used as quality indicator to establish their potential use in inoculum development.

Bile tolerance test: Bile tolerance is one of the most crucial properties for probiotic bacteria, as it

Table 2. Hemolytic activity, glucose fermentation, degree of aroma and antagonistic activity of lactic acid bacteria species against selected pathogens

					LAB isolates inhibition characteristics			
S/N	**LAB used**	**Hemolytic activity**	**Glucose fermentation**	**Degree of aroma**	***E coli***	***S aureus***	***S typhi***	***S dysentariae***
1	*L plantarum*	-	+	+	+++	+++	+	-
2	*L. brevis*	-	+	+	+	++	-	+
3	*L. brevis*	-	+	+	+	+++	+	++
4	*L. brevis*	-	+	+++	+	+++	+	++
5	*L. brevis*	-	+	+	-	++	+	-
6	*L plantarum*	-	+	+++	++	++	++	+
7	*L. brevis*	-	+	+++	-	++	+	++
8	*L plantarum*	-	+	+++	++	+++	++	+++
9	*L plantarum*	-	+	+++	++	+++	++	++
10	*L. brevis*	-	+	+	++	+++	++	++
11	*L plantarum*	-	+	+++	+	+++	++	++
12	*L plantarum*	-	+	+++	++	+++	+	+++
13	*L plantarum*	-	+	+++	++	+	-	++
14	*L plantarum*	-	+	+	+	+++	+	+++
15	*L plantarum*	-	+	+++	+	++	++	+
16	*L plantarum*	-	+	+++	-	++	++	++
17	*L plantarum*	-	+	+++	-	+++	+	+++
18	*L. brevis*	-	+	+++	+++	+++	++	++
19	*L.acidophilus*	-	+	+++	+	+++	++	++
20	*L plantarum*	-	+	+++	++	++	+	

These lactic acid bacteria were probably of different strains even though they were from the same species but from different cereal substrates.=Hemolytic activity: -no hemolysis; Glucose fermentation: + ability of isolates to ferment glucose; Degree of aroma: +++-strong buttery aroma present,+ -weak aroma; LAB isolates inhibition characteristics:- +++ =20-30 mm zone of inhibition, ++ =10-20mm zone of inhibition, + =1-10mm zone of inhibition, - = no zone of inhibition.

Table 3. Lactic acid bacteria isolates bile salts tolerance test

		Absorbance of isolates grown with bile salts					Absorbance of isolates grown without bile salts					Growth on MRS agar	
S/N	**LAB isolates**	**0**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**	**With bile salt**	**Without bile salt**
1	*L plantarum*	0.7	0.8	0.9	1.0	1.2	0.7	0.6	0.8	0.9	1.4	+	+
2	*L. brevis*	0.8	0.7	0.9	1.9	1.5	0.7	0.6	1.0	1.1	1.7	+	+
3	*L. brevis*	0.7	0.8	1.0	1.2	1.5	0.8	0.6	0.2	1.7	2.2	+	+
4	*L. brevis*	0.7	0.8	0.9	1.1	1.2	0.8	0.7	0.9	0.9	1.1	+	+
5	*L. brevis*	1.0	1.1	0.9	1.0	1.4	1.1	0.8	0.9	1.3	2.0	+	+
6	*L plantarum*	0.5	0.6	1.0	0.1	1.9	0.5	0.4	1.2	1.4	2.0	+	+
7	*L. brevis*	1.6	1.7	1.0	1.2	1.7	1.1	1.2	1.3	1.8	2.1	+	+
8	*L plantarum*	0.7	0.8	1.0	1.3	1.5	0.5	0.5	0.9	1.2	1.8	+	+
9	*L plantarum*	0.8	0.8	1.6	1.5	1.9	0.6	0.6	1.1	1.8	2.0	+	+
10	*L. brevis*	0.7	0.7	1.0	1.2	1.6	0.7	0.6	1.0	1.3	2.0	+	+
11	*L plantarum*	0.7	0.8	0.8	1.0	1.2	0.6	0.6	0.9	1.0	1.1	+	+
12	*L plantarum*	0.7	0.8	0.9	0.9	1.6	0.6	0.6	0.9	1.0	1.6	+	+
13	*L plantarum*	1.2	1.2	0.9	1.1	1.3	0.8	0.6	0.9	1.0	1.4	+	+
14	*L plantarum*	1.3	1.3	0.9	1.6	1.9	1.4	1.0	0.9	1.3	1.2	+	+
15	*L plantarum*	1.5	1.5	1.4	1.4	2.0	1.0	0.9	0.9	1.2	2.0	+	+
16	*L plantarum*	1.7	0.7	1.5	1.4	2.0	0.9	0.7	0.9	1.4	2.2	+	+
17	*L plantarum*	0.8	0.8	1.0	1.3	1.7	0.7	0.6	0.9	1.1	2.2	+	+
18	*L. brevis*	0.6	0.6	1.0	1.3	1.7	0.6	0.6	0.9	1.3	2.2	+	+
19	*L.acidophilus*	0.8	1.0	0.8	1.3	1.9	0.7	0.6	1.0	1.5	2.2	+	+
20	*L plantarum*	1.3	1.1	1.0	1.3	1.7	1.2	1.0	0.9	1.0	1.8	+	+

These lactic acid bacteria were probably of different strains even though they were from the same species but from different cereal substrates.

Table 4. LAB isolates cellular auto-aggregation assay and growth properties in skimmed milk

S/N	Lab Code	Cellular Auto-Aggregation Assay			LAB Growth (h) in 10% Skimmed Milk						
		Initial OD	Final OD	%	0	3	ΔpH_3 (3)	6	ΔpH_6 (6)	24	ΔpH_{24} (24)
1	*L plantarum*	0.5	0.3	41.4	7.2	6.9	0.3	6.9	0.3	6.8	0.4
2	*L. brevis*	0.4	0.3	27.4	7.2	6.8	0.4	6.6	0.6	6.6	0.6
3	*L. brevis*	0.5	0.3	38.2	7.2	6.9	0.3	6.8	0.4	6	1.2
4	*L. brevis*	0.4	0.2	46.1	7.2	6.8	0.4	6.7	0.5	6.6	0.6
5	*L. brevis*	0.9	0.3	66.5	7.2	6.6	0.6	6.5	0.7	6.2	1
6	*L plantarum*	0.4	0.2	41.3	7.2	6.8	0.4	6.7	0.5	6.3	0.9
7	*L. brevis*	0.5	0.3	44.8	7.2	6.9	0.3	6.8	0.4	5.8	1.4
8	*L plantarum*	0.4	0.3	30.0	7.2	6.2	1	5.9	1.3	5.5	1.7
9	*L plantarum*	0.4	0.2	49.7	7.2	6.5	0.7	6.1	1.1	5.2	2
10	*L. brevis*	0.5	0.3	45.6	7.2	6.7	0.5	6.7	0.5	6.3	0.9
11	*L plantarum*	0.5	0.3	43.3	7.2	6.5	0.7	6	1.2	4.2	3
12	*L plantarum*	0.4	0.2	51.0	7.2	6.6	0.6	6.2	1	4.4	2.8
13	*L plantarum*	0.7	0.4	42.8	7.2	6.8	0.4	6.6	0.6	5.4	1.8
14	*L plantarum*	0.7	0.4	45.5	7.2	6.8	0.4	6.6	0.6	6.1	1.1
15	*L plantarum*	1.5	0.2	84.9	7.2	6.8	0.4	6.1	1.1	5.1	2.1
16	*L plantarum*	1.5	0.3	79.2	7.2	6.7	0.5	6.7	0.5	5.4	1.8
17	*L plantarum*	0.5	0.3	40.8	7.2	6.5	0.7	6.1	1.1	5.3	1.9
18	*L. brevis*	0.5	0.3	48.2	7.2	6.6	0.6	6.3	0.9	4.9	2.3
19	*L.acidophilus*	0.5	0.2	58.3	7.2	6.6	0.6	6.5	0.7	5	2.2
20	*L plantarum*	0.9	0.5	50.3	7.2	6.7	0.5	6.6	0.6	5.7	1.5

These lactic acid bacteria were probably of different strains even though they were from the same species but from different cereal substrates; OD=optical density; ΔpH=acidification rate; +=presence; -=absence

determines its ability to survive in the small intestine, and consequently its capacity to play its functional role as a probiotic [20]. The results obtained in this study (Table 4) show that the twenty lactic acid bacteria (LAB) were able to grow in the medium containing 0.3% bile salt. Nath et al. [20] reported a survival rate of 83.70% after 3 hours of incubation.

Cellular auto-aggregation assay result: The ability to aggregate is a desirable property for probiotics in health promoting foods and in this study, highest percentage of cellular auto aggregation was observed in a strain of *Lactobacillus plantarum,* LAB-46MB-1, which was 84.86% followed by another strain of *Lactobacillus plantarum*, LAB-49MB-1, which was 79.23% and the lowest cellular auto-aggregation of 27.44% was recorded in one strain of *Lactobacillus brevis*, LAB-10SB-6 (Table 4).

4. CONCLUSION

In the present study, the lactic acid bacteria (*Lactobacillus brevis, Lactobacillus plantarum* and *Lactobacillus acidophillus*) tested in-vitro possessed good probiotic characteristics. They were able to survive at low pH, they survived the selected temperature range (25-40ºC), they survived at high NaCl concentrations; they exhibited good antimicrobial activities and produced no hemolysis. These organisms can be good candidates for food industries as prospective probiotic cultures.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nwokolo C. 7 Superb Health Benefits of Kunu Drink Health Guide NG; 2019.
2. Aleru CP, Ollor AO, Wachukwu CK. Microbial Quality Assessment of kunun zaki Beverage Sold in Port Harcourt, Rivers State, Nigeria. Int J Pharm Life Sci. 2017;3(9):18-22.
3. Pereira GVM, De Carvalho-Neto DP, Junqueira ACDO, Karp SG, Letti LA, Magalhães Junior AI et al. A review of selection criteria for starter culture development in the food fermentation industry. Food Rev Int. 2020;36:135-67.
4. Laranjo M, Potes ME, Elias M. Role of starter cultures on the safety of fermented meat products. Front Microbiol. 2019;26:583.
5. García-Díez JG, Saraiva C. Use of starter cultures in foods from animal origin to improve their safety. Int J Environ Res Public Health. 2021;18(5):2544.
6. Taskila S. Industrial production of starter cultures. In: Speranza B, Bevilacqua A, Corbo MR, Sinigaglia M. (Eds): Starter Cultures in Food Production. John Wiley & Sons, Ltd: West Sussex, UK. 2017;79–100.
7. Moneim A, Sulieman E. Microbial Starter Cultures. LAP Lambert Academic Publishing- 17 Meldrum Street, Beau Bassin- 17504- Mauritius; 2017. ISBN: 978- 620-2-05961-9 Available:https://www.researchgate.net/publication/320710806.
8. Puebla-Barragan S, Reid G. Probiotics in cosmetic and personal care products: trends and challenges. Molecules. 2021; 26(5):1249:2605124.
9. Jeygowri N, Parahitiyawa, N, Jeyatilake, S, Ranadheera S et al. Study on isolation of potentially probiotic Lactobacillus species from fermented rice. Trop Agric Res. 2015;26(3):428-40.
10. Bari ML, Mamun AA. Microbial safety issues related to probiotic beverages and the organisms used in their manufacture;2021. Available:https://www.sciencedirect.com/science/article/pii/B9780128185889000012 pp365-388
11. Maldonado Galdeano C, Cazorla SI, Lemme Dumit JM, Vélez E, Perdigón G. Beneficial effects of probiotic consumption on the immune system. Ann Nutr Metab. 2019;74(2):115-24.
12. Tamang JP, Shin DH, Jung SJ, Chae SW. Functional properties of microorganisms in fermented foods. Front Microbiol. 2016; 7:578.
13. Sen S, Mansell TJ. Yeasts as probiotics: mechanisms, outcomes, and future potentials. Fungal Genet Biol. 2020;137: 103333.
14. Soemarie YB, Milanda T, Barliana MI. Fermented foods as probiotics: a review. J Adv Pharm Technol Res. 2021;12(4):335-9.
15. Khorshidian N, Yousefi M, Mortazavian AM. Fermented milk: the most popular

probiotic food carrier. Adv Food Nutr Res. 2020;94:91-114.
16. Fenster K, Freeburg B, Hollard C, Wong C, Rønhave Laursen RR, Ouwehand AC. The production and delivery of probiotics: A review of a practical approach. Microorganisms. 2019;7(3):83.
17. Ahmed Z, Vohra MS, Khan MN, Ahmed A, Khan TA. Antimicrobial role of Lactobacillus species as potential probiotics against enteropathogenic bacteria in chickens. J Infect Dev Ctries. 2019;13(2):130-6.
18. Mathur H, Beresford TP, Cotter PD. Health benefits of lactic acid bacteria fermenters. Nutrients. 2020;12(6):1679.
19. Bintsis T. Lactic acid bacteria as starter cultures: an update in their metabolism and genetics. AIMS Microbiol. 2018;4(4):665-84.
20. Nath S, Sikidar J, Roy M, Bibhas D. In vitro screening of probiotic properties of Lactobacillus plantarum isolated from fermented milk product. Food Qual Saf. 2020;4:213-23.
21. Patrignani F, Siroli L, Parolin C, Serrazanetti DI, Vitali B, Lanciotti R. Use of Lactobacillus crispatus to produce a probiotic cheese as potential gender food for preventing gynaecological infections. PLOS ONE. 2019;14(1):e0208906.
22. Li P, Gu Q. Antimicrobial effects of probiotics and novel probiotic based approaches for infectious diseases. In: Probiotics-current knowledge and future prospects. Intech Open. London. 2018;1-19.
23. Egbere OJ, Odu CE, Onyimba I, ANgene AC, Dashen MM, Ogaji OA, Hemen WN, Entonu EE. Inhibitory effect of lactic acid bacteria on aflatoxinB1 producing Aspergillus flavus isolated from garri. European Journal of Biology and Biotechnology. 2022;3(1):242.
24. Daniel AA, Egbebi AO, Onasanya AA. Isolation and identification of lactic acid bacteria from spontaneously fermented kunun-zaki using RAPD-PCR Analysis. AIJNAS. 2023;3(1):47-55.
25. Ayo-Omogie HN, Okorie EI. In vitro probiotic potential of autochthonous lactic acid bacteria and microbiology of kunun made from mixed grains British Microbiology Research [journal]. 2016; 14(4):1-10.
26. Shakhatreh M, Jacob HJ, Hussein E, Masadah M. Microbiological analysis, antimicrobial activity and heavy metals content of Jordanian Ma'in hot-springs water. J Infect Public Health. 2017;10(6):1-10.
27. Prabhurajeshwar C, Chandrakanth RK. Probiotic potential of lactobacilli with antagonistic activity against pathogenic strains: an in vitro validation for the production of inhibitory substances. Biomed J. 2017;40(5):270-83.
28. Wang J, Yang K, Miaomiao L, Zhang J, Xinyuan W, Mingtao F. Screening for potential probiotic from spontaneously fermented nondairy foods based on *In vitro* probiotic and safety properties. Ann Microbiol. 2018;68:803-13.
29. Agarry OO, Nkama I, Akoma O. Production of kunun- zaki (A Nigerian fermented cereal beverage) using starter culture. Int Res J Microbiol. 2010;1(2):18-25.
30. Onyimba IA, Itelima JU, Job MO, Ogbonna AI, Ode CO. Production of sorghum based kunun zaki using selected starter cultures. Int. J Sci. 2017;6(9):1370.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/99781