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Full Length Research Article

Probiotic Potential of Lactic Acid Bacteria Isolated from Local Foods in Ado-Ekiti, South West Nigeria

[1]A.O. Ajayi and [2]O.A. Ajenifuja

[1]Department of Microbiology, Federal University Oye-Ekiti, Ekiti State, Nigeria
[2]Department of Science Technology, Microbiology Unit, School of Science and Computer Studies, Federal Polytechnic, Ado-Ekiti, Ekiti State, Nigeria
[*Corresponding Author; Email: ayodele.ajayi@fuoye.edu.ng]

ABSTRACT

Probiotics are live microorganisms acclaimed to provide health benefits when consumed and are generally considered safe for consumption. The lactic acid bacteria (LAB) are common groups of bacteria with certain health benefits, including the ability of some strains to play probiotic roles. Some indigenous fermented foods localized in Ado-Ekiti carry LAB that may have some probiotic properties. The aim of this study was to determine the probiotic potential of some LAB isolated from some local fermented foods in Ado-Ekiti. Samples of locally fermented foods: "*garri*", "*iru*", "*ogi*", and "*fura de nono*" were obtained from the local market in Ado-Ekiti for isolation of LAB. All samples were cultured using standard methods. A total of sixteen (16) isolates were recovered from the samples and screened for their antibacterial activity against four human pathogenic bacterial strains which were *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus*. The LAB with enhanced antibacterial activity were further screened for potential probiotic characteristics using pH tolerance test, bile salt tolerance test, NaCl tolerance test, temperature sensitivity, and lactose utilization. The LAB isolates identified from the local food samples included: *Lactobacillus fermentum*, *Acetobacter* spp., *Bacillus subtilis*, *Lactobacillus plantarum*, and *Lactococcus lactis*. Out of the 16 strains obtained, five strains that showed considerable antibacterial activity were evaluated for their probiotic potential. The five strains were susceptible to conventional antibiotics. It was also shown that the five strains were able to grow at the selected range of pH 4.0 – 7.0, they were able to survive at the temperature ranges between 20 ^{o}C and 45 ^{o}C, they were lactose-tolerant, and they were able to tolerate 1 – 5% sodium chloride (NaCl) concentration. Some isolated LAB exhibited excellent probiotic characteristics and thus can be recommended as a potential use as probiotics. The findings of this study suggest that popular local foods in Ado Ekiti, such as "*garri*", "*iru*", "*ogi*", and "*fura de nono*", harbor some LAB with potential probiotic properties, and can be explored for possible health benefits.

Keywords: Lactic acid bacteria (LAB), Local foods, Pathogens, Probiotics, Ado Ekiti.

INTRODUCTION

The LAB are a diverse group of microorganisms that play a vital role in food fermentation and some of them have been recognized for their potential probiotic potential (Doron and Snydman, 2015). These beneficial bacteria produce lactic acid as their primary metabolic byproduct, contributing to the preservation of locally fermented foods and improved organoleptic properties of fermented foods. The LAB are diverse and widely distributed in nature and can be isolated from various sources, including dairy products, vegetables, fruits, and the human gastrointestinal tract (Rijkers *et al*., 2011). Popular examples of the LAB include *Lactobacillus*, *Enterococcus* spp., *Lactobacillus plantarum, L. brevis*, *L. paraplantarum*, *L. coryniformis*, *Pediococcus pentosaceus, Leuconostoc citreum*, *L. mesenteroides*, *L. argentinum,* and *Weissella* spp. (Plengvidhya *et al*., 2007). The bacteria *Leuconostoc* spp., *Weissella* spp., and *Lactobacillus* spp. were present in Kimchi (Swain *et al*., 2014). *Lactobacillus acidophilus, Bifidobacterium bifidum, Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus helveticus, Lactobacillus kefiranofaciens, Lactococcus lactis*, and *Leuconostoc* species were present in Kefir (Guzel-Seydim *et al*., 2011). Despite the diversity of the LAB, only a fraction of them is known for their probiotics (Haghshenas *et al*., 2015, 2017).

Probiotics are living microorganisms that are acclaimed to confer certain health benefits, are considered safe, and are usually consumed alongside different types of foods, especially fermented foods of local origins. These groups of organisms are gaining massive popularity globally, with patented versions earning good incomes. Common health benefits of probiotics include increased digestion in the gut of humans, modification of immunological response in hosts, prevention of infection in the digestive tract, prevention of irritable bowel syndrome, etc. (Bodke and Jogdand, 2022). The exploration of indigenous LAB strains from local sources holds significant promise for developing novel probiotic candidates tailored to specific populations and dietary patterns. However, most patented versions of probiotics are less accessible to people in rural and sub-urban areas due to a lack of awareness. This increases the need to actively and continuously explore other sources of bacteria in local foods and environments to find bacterial potentials for LAB (Zawistowska-Rojek *et al.,* 2022). According to Zoumpopoulou *et al*. (2017), bacteria considered probiotic candidates must satisfy certain conditions which include the safety of the bacteria, lack of virulence factors, lack of antibiotic resistance, ability to survive bile and stomach acids, increased affordability, etc. Fortunately, some of the LAB explored in previous studies satisfy some of these conditions (FAO and WHO, 2002). Due to the immense benefits attributed to probiotics, the bacteria have the potential to contribute to food security and promote public health, as indicated in the sustainable development goals. Probiotic strains can be isolated directly from naturally fermented milk products or milk and can then be added as starter

cultures for fermentation in products such as cheese, yogurt, and butter (Haghshenas *et al*., 2015, 2017).

In the past few decades, the pattern of consumption of food products has changed considerably due to growing awareness of the need to include healthy foods in the diet. In this sense, the food industry has directed its attention toward the production of foods with functional properties that provide beneficial health effects in addition to their basic nutritional functions and probiotics are an important component of this effort (Zoumpopoulou *et al*., 2017). Furthermore, the search for novel strains of bacteria with probiotic potential is a deliberate and continuous exercise. Different foods are usually screened on a routine basis to increase the chance of getting such organisms with probiotic properties. In this context, Ado-Ekiti, a town in Southwestern Nigeria, with a population of approximately 1 million people, harbors a rich culinary tradition that includes a variety of locally fermented foods such as *garri*, *iru*, *ogi*, *fura de nono,* offering a potential reservoir of novel LAB strains.

The *Garri* is processed and obtained from cassava by garri producers, especially the farmers. *Iru* is a popular local food condiment produced from the seeds of *Parkia biglobosa* through fermentation. *Ogi* can be produced from maize and millet but the one produced from fermented maize (*Zea mays*) is more common. The *Fura de nono* is a locally made drink from fermented milk and ground millet grains; this is a drink with northern Nigeria origins, which is produced and consumed mostly by the Fulani, *Fura* is the millet, while *Nono* is locally fermented cow milk which was this was obtained from Hausa community in Ado-Ekiti. However, the available studies do not delve into the probiotic potential of organisms in these fermented foods – *garri*, *iru*, *ogi*, *fura de nono*. The findings of this study will contribute to the identification and characterization of novel probiotic LAB strains from these local foods in Ado-Ekiti, Nigeria. These strains would further expand the array of probiotic organisms available that have potential applications in the development of functional foods, dietary supplements, and therapeutic interventions for various health conditions. Therefore, the aim of this study is to determine the probiotic potential of some LAB isolated from some local foods in Ado-Ekiti, Nigeria.

MATERIALS AND METHODS

Study Area

The study area for this study was Ado-Ekiti metropolis, the State capital of Ekiti State, Southwestern Nigeria. The city is located within the tropical rainforest belt. It lies at an elevation of approximately 250 meters above sea level, with coordinates of 7°35'N, 5°15'E. The city is situated in the central part of Ekiti State, approximately 148 kilometers east of Ibadan. It is known for its rich cultural heritage and its traditional markets. The residents of the city are a typical working-class population with a mixture of farmers who come from neighboring rural areas and sell foods and agricultural produce in the city. Local foods are a major source of diets to the residents and such foods have cultural inclinations to the people.

Collection of Food Samples

Samples of *garri*, *iru* (fermented African locust bean), *ogi*, and *fura de nono* were obtained from the Irona market in Ado-Ekiti. The samples were collected in sterile containers and immediately transported to the Microbiology Laboratory of the Department of Science Laboratory Technology, Federal Polytechnic, Ado-Ekiti for further analysis.

Isolation and Purification of Lactic Acid Bacteria from Local Food Samples

The method of sample preparation described by Barbosa *et al*. (2022), and the method of isolation of lactic acid bacteria described by Pundir *et al*. (2013) were adopted in this study. Test tube serial dilution technique was used. Five gram (5 g) of the local food samples (*garri*, *iru*, *ogi*, and *fura de nono*) were weighed and mixed thoroughly in 10 mL of sterile distilled water. Twenty milliliter (10 mL) of each sample was diluted into 90 mL of MRS broth. The test tube containing the mixture was shaken homogeneously, and the test tubes were placed in GasPak jars to achieve anaerobic conditions and then incubated at 37°C for 24 hrs. These initial test tubes for each food sample were taken as the stock. The stock mixtures were thereafter subjected to serial dilution using the agar dilution method. In the serial dilution, agar plate technique, 10 mL of a stock mixture was suspended and agitated in 90 mL distilled water blanks to form a microbial suspension. Serial dilutions with the following dilution factors: 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} were made by pipetting 10 mL into 90 mL distilled water blanks. Ten milliliter (10 mL) of each dilution were inoculated onto De Man–Rogosa–Sharpe (MRS) agar plates prepared by pouring 15 mL of sterile and cooled molten media in sterile petri dishes and then incubated at 37 ^{o}C for 24 hours for bacterial growth. The petri dishes were observed for the appearance of colonies and the number of colonies produced on each plate of different dilutions was recorded. Bacteria were purified by streak plate method on MRS agar and anaerobically incubated at 37 ^{o}C for 24hrs. Distinct and well-isolated colonies were later transferred to MRS agar slants and then maintained in the refrigerator at 4 ^{o}C till further analysis.

Biochemical Characterization of Lactic Acid Bacterial Isolates

The lactic acid bacteria (LAB) isolated were biochemically characterized using the following methods; gram's reaction, catalase test, oxidase test, indole production, motility test, spore staining, and sugar fermentation.

Assessment of Antibacterial Activity of LAB

Method of assessment of antibacterial activity of LAB isolates described by Pundir *et al*. (2013) was adopted. The antibacterial activity of LAB isolates was determined against *E. coli*, *P. aeruginosa*, *S. typhi*, and *S. aureus*. These test strains were obtained from the Microbiology Laboratory of the Department of Science Laboratory Technology, Federal Polytechnic, Ado-Ekiti. For screening of isolated LAB cultures, each culture was inoculated to MRS broth incubated at 37 ^{o}C for 24 hours on a shaker. After incubation, 2 mL of each fermented culture broth of LAB isolates in the test tube was centrifuged and supernatant (0.5 mL) was taken to test the antibacterial activity by agar well diffusion method against the four test bacterial strains. Each isolated culture was screened against the test bacteria. For standardization of the bacterial inoculum, an overnight culture of bacterial test strains that were grown in Muller Hinton broth at 37 °C. They were diluted to a turbidity equivalent to that of a 0.5 McFarland equivalent to 10^{-5} cfu/ML standard as described by Khunajakr *et al.* (2008).

Antibacterial Susceptibility testing

The antibacterial susceptibility of the LAB isolates was determined using the Kirby–Bauer disk diffusion method as previously described by CLSI (2013). Susceptibility pattern was assessed using paper disks containing the following antibiotics: penicillin (10 µg), cephalothin (30 µg), oxacillin (1 µg), clindamycin (2 µg), erythromycin (1 5µg), and amoxyclav (30 µg). Broth cultures of the LAB were prepared using MRS broth and adjusted to 0.5 McFarland standards. A 100 µL suspension of freshly grown bacterial cultures was spread on MRS agar plates and left to dry. The antibiotic discs (Maxi-care Medical Laboratory, Nigeria) were placed on the surface of the agar and the plates were incubated at 37^{o}C for 24 hours. After incubation, the diameter of the inhibition zone was measured accordingly.

Evaluation of Probiotic Potentials of LAB Cultures

Lactic acid bacterial cultures with good antimicrobial activity were selected for further determination of probiotic potential using the method described by Pundir *et al*. (2013) as follows: pH tolerance, bile salt tolerance, temperature sensitivity, lactose utilization, and NaCl tolerance.

pH Tolerance: The isolated bacterial cultures were inoculated into sterile MRS broth tubes of varying pH 1 – 7 were incubated at 37 ^{o}C for 24 hours according to Menconi *et al*. (2014). The pH was varied by diluting 0.1 M HCl in 1000 mL distilled water, from which a drop was taken and added to the inoculated broth culture of LAB isolates; the probe was dipped into the inoculum to take the pH reading. Then 0.1 mL inoculums from each tube was cultured onto MRS agar medium by pour plate method and incubated at 37 ^{o}C for 24 hrs. The growth of LAB on MRS agar was used to designate isolates as pH tolerant.

Bile Salt Tolerance: As described by Tambekar and Bhutada (2010), MRS broth containing 0.5, 1.0, 1.5 and 2.0% bile salts (*cholestyramine*) were inoculated with each selected bacterial culture and incubated at 37^{o}C for 48hrs. Then 0.1 mL inoculums were transferred to MRS agar by pour plate method and incubated at 37^{o}C for 48hrs. The growth of LAB cultures on agar plates was used to designate isolates as bile salt tolerant.

Temperature Sensitivity: The selected bacterial cultures were incubated at varying temperatures, i.e. 20, 35, 37, 40 and 47^{o}C in a digital water bath (Dragon Lab Scientific, Nigeria) or 48 – 72 hrs. Freshly prepared 0.1ml LAB inoculum was transferred to MRS plates by pour plate method and incubated at 37^{o}C for 48hrs. The growth of LAB on MRS agar plates was used to designate the bacteria as temperature tolerant.

Lactose Utilization: The method described by Ahmed and Kanwal (2014) was used for lactose utilisation. The acid production by selected bacterial cultures was detected by observing the change in color of the medium. Sterilized fermentation medium (10 g peptone, NaCl 15 g, phenol red 0.018 g, lactose 5 g, for 1 L distilled water and final pH 7.0) were inoculated with different LAB and incubated at 35^{o}C for 24 hrs. The change in color from red to yellow indicated the production of acid.

NaCl tolerance: Method described by Hoque *et al*. (2010) was adopted. Salt tolerance of selected bacterial cultures was assessed after 3 days of incubation at concentrations of 1%, 2%, 3%, 4% and 5% NaCl in MRS broth.

RESULTS

The cultural, morphological, and biochemical characteristics of the isolated LAB from local food samples are depicted in Table 1. The morphological characteristics of the colonies elevation ranged from flat to raised. The colour of the colonies obtained were creamy, white, yellow and purple. The edges of the colonies ranged from circular to irregular, and some colonies showed smooth and rough surfaces. All colonies were Gram-stained and viewed under the microscope and were Gram-positive and rod shapes appeared as single chains. All isolates were identified using standard biochemical tests as indicated in Table 1. Six isolates showed positive reactions while ten showed negative reactions to catalase. The identified LAB isolates identified were *L. fermentum*, *Acetobacter* spp., *B. subtilis*, *L. plantarum*, and *Lactococcus lactis.* The occurrence of LAB isolates from the local food samples is shown in Table 2. *Fura de nono* had the highest number of LAB isolates, which were *Lactococcus lactis*, *Lactobacillus plantarum,* and *Streptococcus* spp. The *Lactobacillus fermentum* and *Acetobacter* spp. were isolated from *ogi*, *Lactobacillus plantarum* and *Bacillus subtilis* were isolated from *garri*, while only *Bacillus subtilis* was isolated from *iru*,

Antibacterial activity of LAB recovered from local food samples is presented in Table 3. *Lactococcus lactis* from *fura de nono* showed maximum zone of inhibition against *E. coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and minimum inhibition zone against *Staphylococcus aureus*. *Lactobacillus fermentum* from *ogi* and *Lactobacillus plantarum* from *garri* exhibited maximum zone of inhibition against *E. coli*, *Staph. aureus*, *S. typhi*, and minimum activity against *Ps. aeruginosa*. However, *Bacillus subtilis* from *iru* exhibited maximum zone of inhibition against *E. coli*, *Ps. aeruginosa,* and *Staph. aureus*, while it is minimum against *S. typhi*. The antibiotic susceptibility pattern of selected lactic acid bacteria (LAB) isolates from local food samples is depicted in Figure 2. The isolates *Lactobacillus fermentum* and *Lactobacillus plantarum* were sensitive to the entire antibiotics but were only resistant to amoxicillin. *Acetobacter* spp. and *Bacillus subtilis* were resistant to oxacillin but sensitive to other antibiotics. *Lactococcus lactis* was sensitive to penicillin, cephalosporin, clindamycin, and erythromycin at varying zones of inhibition of 22.50, 19.00, 14.50, and 27.00 mm respectively. Out of 16 isolates tested, some isolates were found to exhibit antibacterial activity against test bacteria (*E. coli*, *Ps. aeruginosa*, *S. typhi*, and *Staph aureus*). The isolates Og1 (*Lactobacillus fermentum*), Og3 (*Acetobacter* spp.), Ir2 (*Bacillus subtilis*), Gr3 (*Lactobacillus plantarum*) and Fd1 (*Lactococcuslactis*) showed inhibitory activity against the entire tested bacteria whereas isolate Ir4 and Fd4 exhibited activity against 3 tested bacteria out of 4 and rest of the isolates showed activity against 1 or 2 tested bacteria strains. Five isolates (Og1, Og3, Ir2, Gr3, and Fd1) were selected for further evaluation of probiotic properties based on the maximum zone of inhibition and inhibition against all tested bacteria.

Probiotic potential levels of the isolates from local food samples are shown in Table 4. The selected LAB isolates were able to grow in pH 7.0, 6.0, 5.0, and 4.0 but were unable to grow at pH 3.0, 2.0 and 1.0. They were able to survive in 0.5, 1.0, 1.5 and 2.0 % bile salt concentrations as revealed in Table 4. LAB isolates were able to survive at temperature 20, 35, 37, 40 and 47^{o}C. The entire selected LAB isolates were grown in a fermentation medium supplemented with lactose and were observed for a change in colour from red to yellow which indicates the production of lactic acid. It was observed that every selected LAB isolate was able to produce lactic acid from lactose. The lactic acid bacterial isolates were able to tolerate 1 – 5% NaCl concentration as shown in Table 4

DISCUSSION

The isolation and characterization of probiotic LAB from local foods in Ado-Ekiti, Nigeria, hold significant potential for discovering novel probiotic strains tailored to specific populations and dietary patterns. The rich culinary tradition of Ado-Ekiti, characterized by a variety of fermented foods, serves as a potential reservoir of diverse and well-adapted LAB strains. The probiotic potential of the isolated LAB strains was evaluated through a battery of in vitro assays, including antimicrobial activity, acid and bile tolerance tests. These assays have provided insights into the ability of the strains to inhibit pathogenic bacteria, colonize the intestinal tract, survive the harsh acidic and bile salt environment of the gastrointestinal tract, and produce health-promoting substances.

In this study, a total of 75 colonies were observed on the agar plates. Sixteen colonies were selected based on their characteristic morphological and physiological characteristics, and they were initially subjected to morphological and biochemical tests. The LAB isolates were identified by cultural, morphological, and biochemical characteristics. The cultural, morphological, and biochemical characteristics of the LAB isolates as presented in Table 1 revealed the characteristics of LAB isolates belonging to genus *Lactobacillus* as Gram-positive rod-shaped, non-endospore forming, catalase negative, acid-producing and gas formation from sugars. Also, the biochemical characterization revealed that the bacterial isolates were able to ferment all the tested sugars. The lactic acid bacteria (LAB) isolates identified from the local food samples include *Lactobacillus fermentum* from *Ogi*, *Acetobacter* spp from *Ogi*, *Bacillus subtilis* from *Iru*, *Lactobacillus plantarum and L. lactis* from *Fura de nono*. These morphological and biochemical features observed in this study further confirmed that these fermented foods served as good repositories of LAB. This important finding agrees with similar studies that have equally explored the possibility of recovering new probiotic strains from locally fermented foods peculiar to different regions of the world. The search for LAB and other organisms from local foods that may possess probiotics is a routine but important exercise that demands necessary priority (Alameri *et al*., 2022; Huligere *et al*., 2023; Somashekaraiah *et al*., 2019).

The *Fura de nono*, a traditional fermented cereal beverage consumed in northern Nigeria, was obtained from the Hausa community in Ado-Ekiti. This present study further confirmed the presence of LAB from the food product as a rich source of LAB, which can be used as probiotics. These beneficial bacteria can have a positive impact on gut health, immune function, and overall well-being. The LAB can produce bacteriocins, which are natural antibiotics that inhibit the growth of harmful bacteria. They can adhere to the lining of the intestines, forming a protective barrier against harmful bacteria. The bacteria obtained from the foods in this study survived acidic conditions which implies they will be able to function properly within the gut of humans.

Table 1: Morphological and biochemical characteristics of isolated lactic acid bacteria from local food samples

SAMPLES		Col Morp	Shape	Grm	Cat	Oxi	Ind	Mot	Spr	SUGAR FERMENTATION						PROBABLE ISOLATES
										Glu	Mal	Lac	Gal	Suc	Fru	
Ogi	10^{-3}	Smooth, round, purple, flat	Rod chain	+ ve	– ve	– ve		– ve	– ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	*Lactobacillus fermentum*
	10^{-4}	Smooth, round, purple, flat	Rod chain	+ ve	– ve	– ve		– ve	– ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	*Lactobacillus fermentum*
	10^{-5}	Smooth, spheroid, pink	Rod chain	– ve	+ ve	– ve	– ve	+ ve	– ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	*Acetobacter* spp.
	10^{-6}	Smooth, round, purple, raised	Rod chain	+ ve	– ve	– ve		– ve	– ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	*Lactobacillus fermentum*
Iru	10^{-3}	Creamy white colour, circular	Short rod	+ ve	+ ve	– ve	+ ve	– ve	+ ve	+ ve	+ ve	– ve	+ ve	+ ve	+ ve	*Bacillus subtilis*
	10^{-4}	Creamy white colour, circular	Short rod	+ ve	+ ve	– ve	+ ve	– ve	+ ve	+ ve	+ ve	– ve	+ ve	+ ve	+ ve	*Bacillus subtilis*
	10^{-5}	Creamy white colour, circular	Short rod	+ ve	+ ve	– ve	+ ve	– ve	+ ve	+	+ ve	– ve	+ ve	+ ve	+ ve	*Bacillus subtilis*
	10^{-6}	Creamy white colour, circular	Short rod	+ ve	+ ve	– ve	+ ve	– ve	+ ve	+ ve	+ ve	– ve	+ ve	+ ve	+ ve	*Bacillus subtilis*
Garri	10^{-3}	Creamy-white, circular, smooth	Rods with ellipsoidal spores	+ ve	– ve	– ve	– ve	– ve	– ve	+ ve	+ ve	– ve	+ ve	+ ve	+ ve	*Lactobacillus plantarum*
	10^{-4}	Creamy white colour, circular	Short rod	+ ve	+ ve	– ve	+ ve	– ve	+ ve	+ ve	+ ve	– ve	+ ve	+ ve	+ ve	*Bacillus subtilis*
	10^{-5}	Creamy-white, circular, smooth	Rods with ellipsoidal spores	+ ve	– ve	– ve	– ve	– ve	– ve	+ ve	+ ve	– ve	+ ve	+ ve	+ ve	*Lactobacillus plantarum*
	10^{-6}	Creamy-white, circular, smooth	Rods with ellipsoidal spores	+ ve	– ve	– ve	– ve	– ve	– ve	+ ve	+ ve	– ve	+ ve	+ ve	+ ve	*Lactobacillus plantarum*
Fura	10^{-3}	Yellowish, round moist	Cocci	+ ve	– ve			– ve	– ve	+ ve	– ve	+ ve	– ve	– ve	+ ve	*Lactococcus lactis*
	10^{-4}	Smooth, round, purple	Rod chain	+ ve	– ve	– ve		– ve	– ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	*Lactobacillus fermentum*
	10^{-5}	Yellowish, round moist	Cocci	+ ve	– ve			– ve	– ve	+ ve	– ve	+ ve	– ve	– ve	+ ve	*Lactococcus lactis*
	10^{-6}	Yellowish, round moist	Cocci	+ ve	– ve			– ve	– ve	+ ve	– ve	+ ve	– ve	– ve	+ ve	*Lactococcus lactis*

Col Morp – Colony morphology; Grm – gram reaction; Cat – catalase; Oxi – oxidase; Ind – Indole; Mot Motility; Spr – spore formation; Glu – glucose; Mal – maltose; Lac – lactose; Gal – galactose; Suc – sucrose; Fru – fructose

Table 2: Occurrence of LAB isolates from the local food samples

FOOD SAMPLES	LAB ISOLATES
Ogi	*Lactobacillus fermentum*, *Acetobacter* spp.
Iru	*Bacillus subtilis*
Garri	*Lactobacillus plantarum*, *Bacillus subtilis*,
Fura de nono	*Lactococcus lactis*, *Lactobacillus plantarum* and *Streptococcus* spp.,

Table 3: Antibacterial activity of lactic acid bacteria isolated from local food samples

SAMPLES	ISOLATES	TEST BACTERIA WITH DIAMETER OF ZONES OF INHIBITION (mm)			
		E. coli	***P. aeruginosa***	***S. typhi***	***Staph. aureus***
Og1	*Lactobacillus fermentum*	25.00	10.00	19.00	15.00
Og2	*Lactobacillus fermentum*	23.50	Nz	Nz	19.50
Og3	*Acetobacter* spp.	27.00	15.00	24.00	12.50
Og4	*Lactobacillus fermentum*	15.00	Nz	Nz	Nz
Ir1	*Bacillus subtilis*	Nz	20.00	Nz	Nz
Ir2	*Bacillus subtilis*	20.00	13.00	9.00	12.00
Ir3	*Bacillus subtilis*	11.00	Nz	Nz	10.00
Ir4	*Bacillus subtilis*	18.00	Nz	5.00	14.00
Gr1	*Lactobacillus plantarum*	Nz	Nz	12.00	Nz
Gr2	*Bacillus subtilis*	15.50	19.00	Nz	Nz
Gr3	*Lactobacillus plantarum*	28.00	12.00	18.00	23.50
Gr4	*Lactobacillus plantarum*	Nz	16.50	12.00	Nz
Fd1	*Lactococcus lactis*	32.00	17.50	19.00	12.00
Fd2	*Lactobacillus fermentum*	Nz	14.50	Nz	Nz
Fd3	*Lactococcus lactis*	Nz	13.50	15.00	Nz
Fd4	*Lactococcus lactis*	12.00	10.00	Nz	12.50

Nz – No zone of inhibition

(a) **(b)** **(c)** **(d)**

Figure 1: Antibacterial activity of LAB isolates against (a) *E. coli* (b) *Staphylococcus aureus* (c) *Salmonella typhi* (d) *Pseudomonas aeruginosa*

The *Ogi* that was sampled in this study contained two major organisms - *Lactobacillus fermentum* and the *Acetobacter* spp. This further confirms that both organisms are part of the array of LAB in Ogi. Some studies have reported a wider array of bacteria, including the LAB that are recovered from Ogi. However, it should be noted that the Ogi is commonly found in South West Nigeria. Are prepared from a variety of substrates like millet, corn, etc; and the varieties of substrates used in its preparation can influence the type of LAB found in the food product (Afolayan *et al.,* 2017). The L. plantarum is one of the prominent LAB that have been noted for their good potential as probiotics particularly due to their antimicrobial activity against pathogenic bacteria. In this study, the *B. subtilis* was the dominant organism that was recovered from *Iru.* This organism is one of the bacteria that are reputable for their probiotic property and they are reputable for their ability to assist digestion in the human gut through the breakdown of complex carbohydrates and proteins.

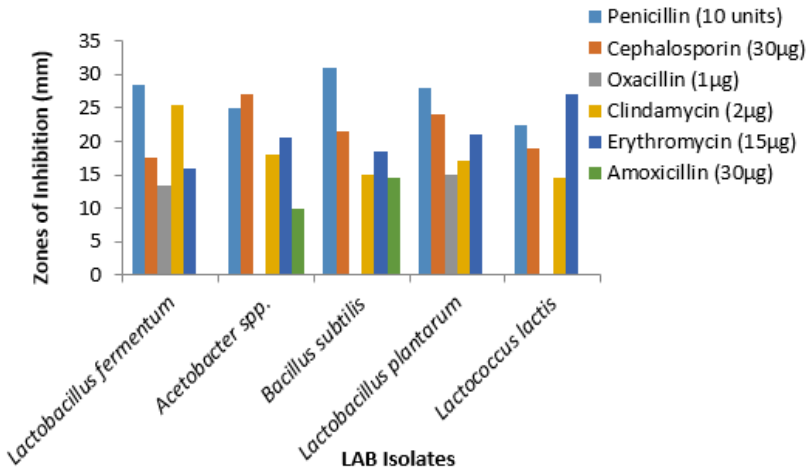

Figure 2: Antibiotic susceptibility pattern of selected lactic acid bacteria isolates

Table 4: Physicochemical test for the probiotic potential levels of the isolates from local food samples

PARAMETERS	LACTIC ACID BACTERIA ISOLATES				
	Og1	Og3	Ir2	Gr3	Fd1
pH tolerance					
7.0	+	+	+	+	+
6.0	+	+	+	+	+
5.0	+	+	+	+	+
4.0	+	+	+	+	+
3.0	–	–	–	–	–
2.0	–	–	–	–	–
1.0	–	–	–	–	–
Bile salt tolerance (%)					
2.0	+	+	+	+	+
1.5	+	+	+	+	+
1.0	+	+	+	+	+
0.5	+	+	+	+	+
Temperature sensitivity (°C)					
47	+	+	+	+	+
40	+	+	+	+	+
37	+	+	+	+	+
35	+	+	+	+	+
20	+	+	+	+	+
Lactose utilisation	+	+	+	+	+
NaCl tolerance (%)					
5	+	+	+	+	+
4	+	+	+	+	+
3	+	+	+	+	+
2	+	+	+	+	+
1	+	+	+	+	+

+ = positive; – = negative

They are also able to fight potentially pathogenic bacteria within the gut (Lee *et al.,* 2019). The dominant organisms found in *Garri* obtained in this study are the *L. pantarum* and *B. subtillis*. Previous studies have also reported an array of LAB in *Garri* samples. In addition to the benefits of *B. subtilis* already highlighted, *L. plantarum* is also a good probiotic candidate, especially for its antimicrobial activity (Arasu *et al.,* 2016, Islam *et al*., 2023)

The result of the antibacterial properties of LAB isolates of this study is similar to the work performed by Tambekar and Bhutada (2010), who tested the antibacterial properties of isolated LAB species from milk samples against *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhi*. The zone of inhibition of isolated LAB species in this study was 19 mm for *Escherichia coli* and 25 mm for *Salmonella typhi* whereas in present studies the zone of inhibition against

Escherichia coli was 15.00 mm and against *Salmonella typhi* was 8.00 mm on average. The antibacterial activity may be due to the production of acetic and lactic acids that lowered the pH of the medium or competition for nutrients, or due to the production of bacteriocins or antibacterial compounds. These results also agree with several studies that have also reported the antimicrobial effect of LAB recovered from various fermented foods against pathogenic bacterial strains (Ren *et al.,* 2018, Ibrahim *et al.,* 2021, Haryani *et al.,* 2023). One of the qualities of probiotic bacterial candidates is their ability to prevent the proliferation of pathogenic bacteria within the gut and this present study further confirms that the LAB comprising of the *Acetobacter* spp., *L. palntarum, L. bevis* that was all obtained in the foods sampled will be useful in that regard. These bacteria have been reported for the ability to produce bacteriocins and other antimicrobial peptides that confer antibacterial activity against the pathogenic bacteria that were used as test bacterial strains in this present study. These bacteria were carefully selected based on the endemicity of the infections they cause in Ado Ekiti, Nigeria.

Some of the selected LAB isolates showed varying degrees of susceptibility patterns to the antibiotics with zones of inhibition ranging from 10.00 – 31.00 mm. This is similar to the findings of Pundir *et al*. (2013) who reported a higher resistance antimicrobial activity of antibiotics for eight LAB isolates. In the study, they indicated that resistance among LAB and other bacteria to a wide spectrum of antibiotics is an indication that if probiotics isolate induced in patients, peradventure treated with antibiotic therapy may be helpful in faster recovery of the patients due to rapid establishment of desirable microbial flora. Resistance of the probiotic strains to some antibiotics could be used for both preventive and therapeutic purposes in controlling intestinal infections (El-Naggar, 2014). It has been noted that bacteria that show resistance to antibiotics carry plasmids and mobile genetic elements that can be transferred to other related bacteria in the same environments (Bennett, 2008, Svara and Rankin, 2011). A study by Zavišić *et al*. (2023) indicated that the presence of resistance among LAB with purported probiotic activity can potentially represent a safety hazard for food with probiotic strains, dietary supplements, as well as medicines. The susceptibility of the LAB in this present study to the selected antibiotics tested lowers the risk of transfer of antibiotic resistance within the gut.

The selected LAB isolates were incapacitated at pH <4.0, and were able to survive in 0.5, 1.0, 1.5 and 2.0 % bile salt concentrations as shown in Table 4. This is similar to the study of Pundir *et al*. (2013), where they isolated lactic acid bacteria from local fermented foods which were able to survive at pH 4.0 and could tolerate bile salt concentration of 0.3 and 0.5%; however, the samples in this study is different from theirs, hence the disparity in pH tolerance. Tolerance to bile salts is a prerequisite for colonization and maintenance of bacteria in the gut of the host (Havenaar *et al*., 2012). This will help *Lactobacilli* to reach the small intestine and colon and contribute in balancing the intestinal microflora according to Albuquerque *et al*. (2018). The entire selected LAB isolates were able to survive at the temperatures at which they were tested at temperatures between 20 – 45 ^{o}C. Temperature is considered an important factor that can affect the growth of bacterial probiotics and other LAB bacteria with probiotic properties. These temperature ranges were selected to determine whether the isolated cultures were able to grow within the range of normal body temperature (Granato *et al*., 2010).

In this study, the LAB isolates were able to tolerate 1 – 5% NaCl concentration as revealed in Table 4. NaCl is an inhibitory substance that may inhibit the growth of certain types of bacteria. If the lactic acid bacteria were sensitive to NaCl then it would not be able to show its activity in the presence of NaCl so it was essential to test the NaCl tolerance of lactic acid bacterial isolates. The present experimental results were similar to the work done by Adebayo-Tayo and Onilude (2008). However, Hoque *et al*. (2010) observed the NaCl (1 – 9%) tolerance of their *Lactobacillus* sp. isolated from yogurts. This illustrates that LAB from different food samples and different environments can demonstrate varying levels of tolerance. However, the higher the tolerance the better.

CONCLUSION

This study investigated the probiotic potential of lactic acid bacteria (LAB) isolated from local foods in Ado-Ekiti, South West Nigeria. The findings of this study demonstrate that local fermented foods such as *garri*, *ogi*, *iru*, and *fura de nono* harbor a diverse array of LAB strains with promising probiotic properties. However, *fura de nono* had the highest diversity of LAB isolates, which makes it the potential local fermented food for developing novel probiotic candidates tailored to the specific dietary patterns and health needs of populations in Ado-Ekiti, Nigeria. The isolated LAB strains exhibited strong antibacterial activity against pathogenic bacteria and showed tolerance to acidic and bile stress conditions. Further work can also be required to standardize the LAB isolated and identified in the present work with a view to escalating their use as probiotics in Ado Ekiti and beyond.

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