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Characterization of lactic acid bacteria from fermented cereal-based foods in Anyigba, Nigeria, for potential probiotic and bio-preservation applications

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ABSTRACT

Background: Traditional fermented cereal-based foods are dietary staples in Nigeria, as they provide essential nutrients and contribute to food security. These fermentations are primarily driven by Lactic Acid Bacteria (LAB), which are known for their health-promoting and bio-preservative properties. Despite their widespread consumption, a systematic phenotypic characterization of LAB from indigenous foods in specific regions is often overlooked.

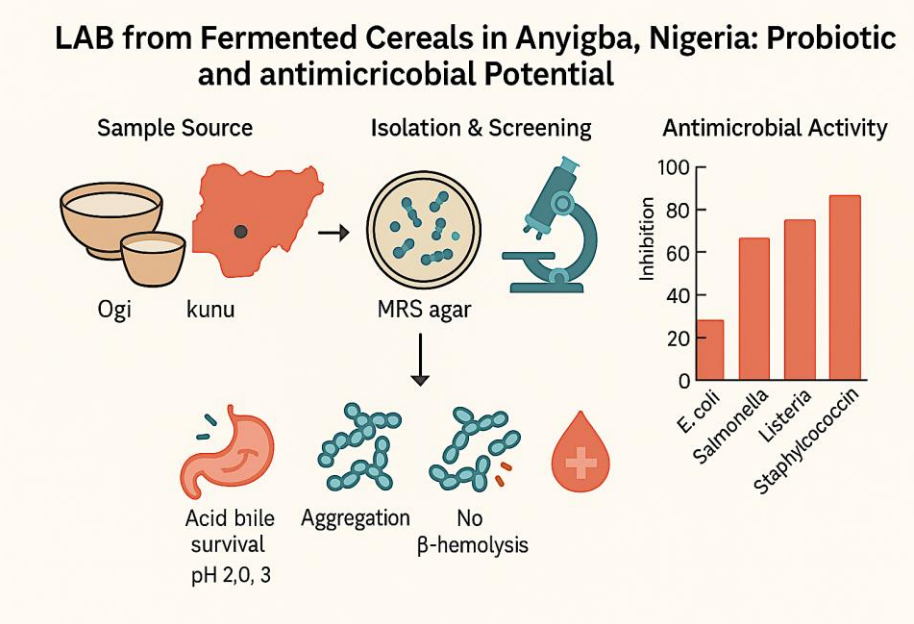
Objective: This study was based in Anyigba, Kogi State, Nigeria. It aimed to isolate, phenotypically characterize, and screen LAB strains from locally fermented cereal-based foods for their potential probiotic attributes and antimicrobial defenses against common foodborne pathogens. This study resorted to molecular methods to exploit microbial diversity for applied microbiological solutions.

Methods: A total of 15 samples of fermented cereal-based foods (*ogi* and *kunu*) were collected from local markets and households in Anyigba, Kogi State, Nigeria. LAB were isolated on de Man Rogosa Sharpe (MRS) agar. Presumptive LAB isolates were identified based on Gram staining, catalase negativity, and CO_2 production from glucose. Selected isolates were further characterized for acid tolerance (pH 2.0 or 3.0 for 3 hours), bile salt tolerance (0.3% oxgall for 3 hours), auto-aggregation, co-aggregation with selected pathogens (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Salmonella Typhimurium* ATCC 14028), and antimicrobial activity against these pathogens using the agar well diffusion method. Hemolytic activity was also assessed on blood agar.

Results: Out of 98 distinct LAB isolates obtained, 78.6% demonstrated high survival rates (> 50%) after 3 hours at pH 3.0, and 65.3% survived 3 hours in 0.3% oxgall. Among 20 selected promising isolates, strong auto-aggregation (> 40%) was observed in 35.0% (7/20) of strains. Co-aggregation with *E. coli*, *S. aureus*, and *S. Typhimurium* ranged from 18-55% among these selected strains. Furthermore, 70.0% (14/20) of the selected isolates showed significant antimicrobial activity against at least one of the tested pathogens, with inhibition zones ranging from 10 to 22 mm. None of the selected isolates exhibited β-hemolytic activity.

Conclusion: The fermented cereal-based foods from Anyigba, Nigeria, are rich sources of diverse LAB strains possessing desirable probiotic characteristics, including robust acid and bile salt tolerance, significant aggregation abilities, and broad-spectrum antimicrobial activity against critical foodborne pathogens. These findings highlight the substantial potential of these indigenous LAB as promising candidates for the development of novel probiotic products in medical microbiology, as well as natural biopreservatives in the food industry and applied microbiology. These LAB strains have the potential to greatly contribute to enhanced food safety and human health, particularly within the Anyigba region of Nigeria.

Keywords: Lactic Acid Bacteria, Fermented Cereals, Probiotics, Antibiotics, Bio-preservation, Anyigba, Nigeria



INTRODUCTION

Microorganisms play a pivotal role in diverse ecosystems, from environmental matrices to the human gut, and are central to numerous industrial processes [1]. Among these, Lactic Acid Bacteria (LAB) constitute a functionally diverse group of Gram-positive, non-spore-forming, catalase-negative bacteria, primarily recognized for their ability to produce lactic acid through carbohydrate fermentation [2]. Their ubiquitous presence in nature, particularly in fermented foods, underscores their immense significance in food microbiology, contributing to food preservation, flavor development, and nutritional enrichment [3].

Traditional fermented foods, especially cereal-based products, are integral to the dietary landscape of many communities worldwide, particularly in Africa. In Nigeria, staples like *ogi* (a fermented maize porridge) and *kunu* (a fermented millet or sorghum beverage) are not merely sustenance but are deeply embedded in cultural practices, providing affordable nourishment and a significant contribution to food security [4]. The spontaneous fermentation of these products is driven by complex microbial communities that are dominated by strains of LAB. The spontaneous fermentation process is what confers the distinct organoleptic properties and enhances the bioavailability of nutrients in these cereal-based products [5].

Beyond their role in food preservation, LAB are widely acclaimed for their potential probiotic attributes. Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [6]. This concept falls squarely within the domain of medical microbiology, as probiotic LAB have been implicated in various health benefits, such as improving gut health, modulating the immune system, preventing pathogen colonization, and alleviating symptoms of certain diseases [7,8]. Key probiotic properties include survival through the gastrointestinal tract (tolerance to low pH and bile salts), adherence to intestinal cells, and the production of antimicrobial compounds [9].

Furthermore, the antimicrobial capabilities of LAB extend to bio-preservation, a crucial aspect of food and applied microbiology. LAB produce a variety of inhibitory substances, including organic acids (lactic and acetic acids), hydrogen peroxide, diacetyl, and bacteriocins (antimicrobial peptides synthesized at the ribosome) [10,11]. These compounds effectively inhibit the growth of spoilage microorganisms and foodborne pathogens, thereby extending the shelf life and enhancing the safety of food products. This natural approach to food preservation is increasingly favored by consumers seeking alternatives to synthetic preservatives [12]. The potential to discover novel antimicrobial compounds from LAB is of high interest in applied microbiology and could have implications beyond food. For example, LAB could be utilized as a feed additive or for pathogen control in livestock [13].

Despite the widespread consumption and economic importance of traditional fermented foods in Nigeria, the specific characteristics and functional properties of the indigenous LAB strains that drive these fermentations, particularly in localized regions like Anyigba, Kogi State, remain under-explored. Such regional studies are vital for understanding local microbial biodiversity and identifying strains uniquely adapted to local food matrices and human populations, which could offer superior performance as probiotics or bio-preservatives [14]. This approach also has implications for environmental microbiology, as it recognizes the unique microbial ecosystems within the fermentation of these traditional foods.

This study was therefore designed to systematically isolate and comprehensively characterize LAB from traditional fermented cereal-based foods (*ogi* and *kunu*) consumed in Anyigba, Nigeria. The specific objectives

were to assess their phenotypic characteristics, including their tolerance to simulated gastrointestinal conditions (low pH and bile salts), evaluate their auto- and co-aggregation abilities with specific foodborne pathogens, and determine their in vitro antimicrobial activity against these pathogens. By focusing on traditional microbiological methods, this research aims to identify promising indigenous LAB strains for potential application as novel probiotics in medical microbiology and as natural bio-preservatives in the food industry, contributing valuable data to the broader fields of applied and food microbiology.

MATERIALS & METHODS

Sample collection: A total of 15 samples of traditional fermented cereal-based foods were collected from local markets and households within Anyigba, Kogi State, Nigeria, from June to August 2024. Of the samples collected, 8 samples were *ogi* (fermented maize paste) and 7 samples were *kunu* (fermented millet/sorghum beverage). All samples were collected aseptically in sterile containers and transported on ice to the Microbiology Laboratory, Prince Abubakar Audu University, Anyigba, for immediate processing.

Isolation and Preliminary Identification of Lactic Acid Bacteria: 10g or 10mL of each sample was homogenized in 90mL sterile peptone water. Serial tenfold dilutions were prepared, and 0.1mL aliquots of appropriate dilutions were spread-plated onto de Man Rogosa Sharpe (MRS) agar plates (Oxoid, UK). The plates were incubated anaerobically at 37°C for 48 hours using anaerobic gas packs (AnaeroGen, Oxoid, UK). After incubation, distinct colonies were selected. These colonies were grown iteratively, as subcultures, on MRS agar to obtain pure cultures. Pure isolates were examined by Gram staining and tested for catalase activity (using 3% hydrogen peroxide). Only Gram-positive, catalase-negative rods or cocci were considered presumptive LAB and selected for further characterization. Isolates were stored in MRS broth containing 20% glycerol at -20°C for long-term preservation.

Characterization of presumptive LAB isolates--sugar fermentation pattern: The ability of isolates to ferment various carbohydrates was determined using an API 50 CHL system (bioMérieux, France) according to the manufacturer's instructions. Briefly, bacterial suspensions were prepared in API 50 CHL medium, inoculated into each well containing a different carbohydrate, and incubated at 37°C for 48 hours. Color changes indicating acid production were recorded.

Characterization of presumptive LAB isolates--gas production from glucose (heterofermentative/ homofermentative differentiation): Heterofermentative LAB strains were distinguished from homofermentative strains by observing gas production in Durham tubes from glucose fermentation in MRS broth. Tubes were inoculated with fresh cultures and incubated at 37°C for 48 hours. Gas formation indicated heterofermentative metabolism.

Screening for probiotic properties in vitro--acid tolerance: The acid tolerance of selected LAB isolates was assessed by exposing them to simulated gastric conditions. Overnight cultures were harvested by centrifugation (5000 × g for 10 min), washed twice with sterile physiological saline, and resuspended in MRS broth adjusted to pH 2.0 or pH 3.0 using 1N HCl. A control group was maintained at pH 6.5. Bacterial counts were determined immediately after inoculation (0 hours) and after 3 hours of incubation at 37°C by plating serial dilutions on MRS agar. Survival rates were calculated as:

(1) **Survival Rate (%) = (CFU/mL at 3 hours / CFU/mL at 0 hours) × 100**

Screening for probiotic properties in vitro--bile salt tolerance: Tolerance to bile salts was determined by incubating bacterial suspensions in MRS broth

supplemented with 0.3% (w/v) oxgall (Sigma-Aldrich, USA), a concentration commonly used to simulate intestinal bile conditions. Overnight cultures were treated similarly to the acid tolerance test and resuspended in MRS broth with and without oxgall. Bacterial counts were determined at 0 hours and after 3 hours of incubation at 37°C. Survival rates were calculated using the same formula as for acid tolerance.

Screening for probiotic properties in vitro--auto-aggregation assay: Auto-aggregation ability was determined as described by Collado et al. [15] with slight modifications. Overnight cultures were centrifuged, washed, and resuspended in phosphate-buffered saline (PBS, pH 7.2) to an optical density (OD_{600}) of approximately 0.5. The suspensions were incubated at 37°C without shaking. The OD_{600} of the upper suspension was measured at 0, 2, and 4 hours. Auto-aggregation percentage was calculated as:

(2) **Auto-aggregation (%) = (1 - OD_t/OD_0) × 100**, where OD_t is the optical density at time t (2 or 4 hours) and OD_0 is the optical density at 0 hours.

Screening for probiotic properties in vitro--co-aggregation assay: The co-aggregation assay was performed by mixing equal volumes of selected LAB strains (OD_{600} = 0.5 in PBS) with target foodborne pathogens (Escherichia coli ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Salmonella Typhimurium* ATCC 14028 obtained from the Molecular Biology Unit of the Prince Abubakar Audu University Laboratory, Anyigba, Kogi state, Nigeria, all adjusted to OD_{600} = 0.5). A control for each strain was also prepared. The mixtures were incubated at 37°C for 2 hours. The OD_{600} of the supernatant was measured, and the co-aggregation percentage was calculated as:

(3) **Co-aggregation (%) = (2(OD_{LAB} + $OD_{pathogen}$) - OD_{mix}) / (2(OD_{LAB} + $OD_{pathogen}$)) × 100**, where OD_{LAB} and $OD_{pathogen}$ are the OD_{600} of the individual LAB and pathogen controls, respectively, and OD_{mix} is the OD_{600} of the mixed suspension.

Screening for probiotic properties in vitro--hemolytic activity: Hemolytic activity was assessed by streaking selected LAB isolates onto blood agar plates containing 5% (v/v) sheep blood. Plates were incubated at 37°C for 24-48 hours. Hemolytic activity was categorized as α-hemolysis (partial lysis with green discoloration), β-hemolysis (complete lysis with a clear zone), or γ-hemolysis (no lysis). Only γ-hemolytic strains were considered safe for probiotic application.

Antimicrobial activity against foodborne pathogens: The agar well diffusion method was used to evaluate the antimicrobial activity of cell-free supernatants (CFS) of selected LAB isolates against the following indicator pathogens: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and Salmonella Typhimurium ATCC 14028. Overnight LAB cultures were centrifuged (10,000 × g for 15min at 4°C), and the supernatants were filter-sterilized through 0.22µm pore-size filters (Millipore, USA). The pH of the CFS was adjusted to 6.0 with 1N NaOH to exclude the effect of lactic acid.

Mueller-Hinton agar plates were inoculated with 100µL of an overnight culture of each indicator pathogen (adjusted to 0.5 McFarland standard). Wells of 6mm diameter were bored into the agar, and 100µL of the adjusted CFS was added to each well. Plates were incubated at 37°C for 18-24 hours. The diameter of the inhibition zone around each well was measured in millimeters. MRS broth was used as a negative control.

Ethical statement: This study did not involve human or animal experimentation. The samples collected were food products from markets and households; therefore, no ethical approval was required.

Statistical analysis: All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical analysis was performed using IBM SPSS Statistics version 26.0. One-way ANOVA was used to

compare means, followed by Tukey's post-hoc test where appropriate. A P-value of less than 0.05 was considered statistically significant.

RESULTS

Isolation and preliminary identification of lactic acid bacteria: From the 15 fermented cereal-based food samples, a total of 98 distinct presumptive LAB isolates were obtained. All isolates were Gram-positive and exhibited negative catalase activity. Morphologically, 65 isolates were identified as rods (putative lactobacilli) and 33 as cocci (putative lactococci or enterococci). Further preliminary classification based on gas production from glucose metabolism revealed that 71 isolates were homofermentative (did not produce gas), while 27 were heterofermentative (produced gas). These results are consistent with the typical characteristics of LAB found in such fermented matrices.

Acid tolerance: The ability of LAB isolates to survive under low pH conditions, simulating gastric passage, varied among the strains (Table 1). A significant proportion of isolates demonstrated good acid tolerance. At pH 3.0, 78.6% (77/98) of the isolates showed a survival rate greater than 50% after 3 hours. At the more stringent pH, 2.0, 41.8% (41/98) of the isolates maintained a survival rate above 30%, though none achieved >50% survival. This indicates that a substantial number of LAB strains from these traditional fermented foods possess inherent resistance to acidic environments.

Table 1: Survival rates of LAB isolates at low pH conditions (n=98). Data represent mean ± standard deviation of triplicate experiments.

pH of Medium	Mean Survival Rate (%) ± SD	Range (%)	Number of isolates with >50% survival
6.5 (Control)	98.2 ± 1.5	95-100	98
3.0	68.5 ± 12.3	25-95	77
2.0	39.1 ± 9.8	15-70	0 (41 isolates >30%)

Bile salt tolerance: The results for bile salt tolerance are presented in Table 2. Of the 98 LAB isolates, 65.3% (64/98) demonstrated a survival rate of greater than 50% after 3 hours of incubation in the presence of 0.3% oxgall. This finding suggests that a considerable number of these indigenous LAB strains can survive the presence of bile salts in the small intestine, a crucial prerequisite for probiotic functionality.

Table 2: Survival rates of LAB isolates in the presence of 0.3% oxgall (n=98). Data represent mean ± standard deviation of triplicate experiments.

Condition	Mean Survival Rate (%) ± SD	Range (%)	Number of isolates with >50% survival
Control (no oxgall)	99.1 ± 0.9	97-100	98
0.3% Oxgall	58.7 ± 11.6	20-85	64

Auto-aggregation and co-aggregation: For aggregation assays, 20 isolates demonstrating good tolerance to both acid and bile were selected for further characterization. The auto-aggregation abilities of these selected LAB isolates varied considerably over time; percentages ranged from 15% to 62%, indicating diverse surface properties among the strains. Of the 20 selected LAB isolates, 7, or 35.0%, showed strong auto-aggregation (> 40%) after 4 hours of incubation (Table 3).

Table 3: Auto-aggregation percentages of 20 selected LAB isolates. Data represent mean ± standard deviation of triplicate experiments.

LAB Isolate	Auto-aggregation at 2h (%) ± SD	Auto-aggregation at 4h (%) ± SD
LAB-C1	28.5 ± 1.2	40.1 ± 1.8
LAB-C3	22.3 ± 0.9	35.5 ± 1.5
LAB-C4	18.7 ± 0.8	29.8 ± 1.1
LAB-C6	30.2 ± 1.1	45.3 ± 1.9
LAB-O2	15.1 ± 0.7	25.6 ± 1.0
LAB-O5	35.6 ± 1.5	52.4 ± 2.0
LAB-O7	20.9 ± 0.8	33.7 ± 1.3
LAB-O8	25.8 ± 1.0	38.9 ± 1.6
LAB-C9	31.9 ± 1.3	48.7 ± 2.1
LAB-C10	24.5 ± 1.0	36.8 ± 1.4
LAB-O11	29.1 ± 1.2	42.6 ± 1.7
LAB-O12	17.3 ± 0.6	28.5 ± 1.1
LAB-C13	38.4 ± 1.6	62.0 ± 2.5
LAB-C14	26.7 ± 1.1	39.2 ± 1.6
LAB-O15	23.6 ± 0.9	34.9 ± 1.3
LAB-O16	32.0 ± 1.3	49.5 ± 2.0
LAB-C17	19.8 ± 0.7	31.2 ± 1.2
LAB-C18	27.5 ± 1.1	41.6 ± 1.7
LAB-O19	21.4 ± 0.8	33.1 ± 1.3
LAB-O20	30.5 ± 1.2	47.8 ± 1.9

The co-aggregation abilities with the tested foodborne pathogens also varied among the selected LAB strains (Table 4). Co-aggregation with *E. coli* ATCC 25922 ranged from 20% to 55%, with *S. aureus* ATCC 25923 from 18% to 50%, and with *S. Typhimurium* ATCC 14028 from 25% to 48%. Several LAB strains (e.g., LAB-C1, LAB-O5, LAB-C13) exhibited significant co-aggregation with all three pathogens, indicating their potential to interfere with pathogen colonization.

Table 4: Co-aggregation percentages of 20 selected LAB isolates with foodborne pathogens. Data represent mean ± standard deviation of triplicate experiments.

LAB Isolate	Co-aggregation with *E. coli* (%) ± SD	Co-aggregation with *S. aureus* (%) ± SD	Co-aggregation with *S. Typhimurium* (%) ± SD
LAB-C1	48.2 ± 1.9	45.1 ± 1.8	42.8 ± 1.7
LAB-C3	35.5 ± 1.4	32.6 ± 1.3	30.1 ± 1.2
LAB-C4	25.8 ± 1.0	22.9 ± 0.9	20.5 ± 0.8
LAB-C6	40.1 ± 1.6	38.5 ± 1.5	36.7 ± 1.4
LAB-O2	20.4 ± 0.8	18.7 ± 0.7	25.3 ± 1.0
LAB-O5	55.0 ± 2.2	50.2 ± 2.0	48.1 ± 1.9
LAB-O7	30.9 ± 1.2	28.4 ± 1.1	26.0 ± 1.0
LAB-O8	38.6 ± 1.5	35.9 ± 1.4	33.2 ± 1.3
LAB-C9	49.5 ± 2.0	46.8 ± 1.9	44.0 ± 1.8

LAB Isolate	Co-aggregation with *E. coli* (%) ± SD	Co-aggregation with *S. aureus* (%) ± SD	Co-aggregation with *S. Typhimurium* (%) ± SD
LAB-C10	36.2 ± 1.4	33.1 ± 1.3	31.5 ± 1.2
LAB-O11	42.0 ± 1.7	39.5 ± 1.6	37.8 ± 1.5
LAB-O12	22.1 ± 0.9	20.0 ± 0.8	27.0 ± 1.1
LAB-C13	52.5 ± 2.1	49.0 ± 2.0	46.5 ± 1.9
LAB-C14	39.8 ± 1.6	37.2 ± 1.5	35.0 ± 1.4
LAB-O15	31.0 ± 1.2	29.1 ± 1.1	28.5 ± 1.1
LAB-O16	45.0 ± 1.8	42.5 ± 1.7	40.0 ± 1.6
LAB-C17	28.9 ± 1.1	26.5 ± 1.0	25.0 ± 1.0
LAB-C18	43.5 ± 1.7	40.8 ± 1.6	38.0 ± 1.5
LAB-O19	33.0 ± 1.3	30.5 ± 1.2	29.0 ± 1.1
LAB-O20	47.0 ± 1.9	44.0 ± 1.8	41.5 ± 1.7

Hemolytic activity: None of the 20 selected LAB isolates exhibited β-hemolytic activity (complete lysis of red blood cells) on sheep blood agar plates. Instead, all isolates showed γ-hemolysis (no hemolysis), which is a crucial safety criterion for potential probiotic strains.

Antimicrobial activity against foodborne pathogens: The cell-free supernatants (CFS) of the 20 selected LAB isolates were screened for antimicrobial activity against *E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *S. Typhimurium* ATCC 14028. After adjusting the pH of CFS to 6.0 to specifically assess non-acidic inhibitory compounds, 70.0% (14/20) of the isolates demonstrated significant antimicrobial activity against at least one of the tested pathogens (Table 5).

Table 5: Antimicrobial activity of selected LAB isolates (cell-free supernatant, pH adjusted to 6.0) against foodborne pathogens (mean inhibition zone diameter in mm ± SD). ND: No inhibition detected. Data represent mean ± standard deviation of triplicate experiments.

LAB Isolate	*E. coli* ATCC 25922	*S. aureus* ATCC 25923	*S. Typhimurium* ATCC 14028
LAB-C1	12 ± 0.5	15 ± 0.8	**11 ± 0.6**
LAB-C3	10 ± 0.3	12 ± 0.4	**10 ± 0.5**
LAB-C4	ND	10 ± 0.3	**ND**
LAB-C6	14 ± 0.6	16 ± 0.7	**13 ± 0.4**
LAB-O2	ND	11 ± 0.4	**ND**
LAB-O5	15 ± 0.7	18 ± 0.9	**14 ± 0.5**
LAB-O7	11 ± 0.4	13 ± 0.5	**10 ± 0.3**
LAB-O8	ND	10 ± 0.3	**ND**
LAB-C9	16 ± 0.8	20 ± 1.1	**15 ± 0.6**
LAB-C10	ND	12 ± 0.4	**ND**
LAB-O11	13 ± 0.5	15 ± 0.6	**12 ± 0.4**
LAB-O12	ND	10 ± 0.3	**ND**
LAB-C13	17 ± 0.8	22 ± 1.0	**16 ± 0.7**
LAB-C14	10 ± 0.3	13 ± 0.5	**10 ± 0.3**
LAB-O15	ND	11 ± 0.4	**ND**
LAB-O16	14 ± 0.6	17 ± 0.8	**13 ± 0.5**

LAB Isolate	*E. coli* ATCC 25922	*S. aureus* ATCC 25923	*S. Typhimurium* ATCC 14028
LAB-C17	ND	10 ± 0.3	**ND**
LAB-C18	15 ± 0.7	18 ± 0.9	**14 ± 0.6**
LAB-O19	ND	12 ± 0.4	**ND**
LAB-O20	16 ± 0.8	19 ± 0.9	**15 ± 0.7**
Control (MRS broth)	0	0	**0**

The strongest antimicrobial activity was consistently observed against *S. aureus*, with inhibition zones up to 22 mm, for isolates LAB-C13 and LAB-O5. Several isolates also showed significant antimicrobial activity against Gram-negative pathogens, like *E. coli* and *S. Typhimurium*. These results indicate the production of effective non-acidic antimicrobial compounds by these LAB strains, highlighting their potential for bio-preservation applications in various food matrices, thereby contributing to food safety.

DISCUSSION

This study presents a thorough phenotypic characterization of LAB strains isolated from traditional fermented cereal-based foods indigenous to Anyigba, Kogi State, Nigeria. The findings provide valuable insights into the functional diversity of these local microorganisms and highlight their significant potential for applications in medical, food, and applied microbiology.

The isolation of 98 distinct LAB strains, predominantly Gram-positive and catalase-negative, confirms that these traditional fermented products are rich reservoirs of these beneficial bacteria. The presence of both homofermentative and heterofermentative types suggests a diverse metabolic capability within the LAB population, which contributes to the complex sensory profiles and preservative qualities of *ogi* and *kunu* [16]. This microbial diversity, rooted in the specific environmental microbiology of local fermentation practices, is a valuable resource often overlooked in broader studies.

A key criterion for any potential probiotic strain is its ability to survive the harsh conditions of the mammalian gastrointestinal tract, including the low pH of the stomach and the presence of bile salts in the small intestine [17-18]. Our results demonstrate that a high percentage of the indigenous LAB isolates exhibited robust tolerance to acidic conditions (pH 3.0) and in the presence of bile salts (0.3% oxgall). These findings are highly encouraging for their potential application in medical microbiology as probiotics, as they indicate a strong likelihood of reaching the intestines alive in sufficient numbers to exert health benefits. Such survival rates are comparable to or exceed those reported for commercial probiotic strains [19]. This inherent robustness may be attributed to their long-term adaptation to the challenging fermentation environments of these traditional foods, reflecting principles of environmental microbiology and adaptation.

Beyond mere survival, the ability of LAB to adhere to intestinal surfaces and interact with host or pathogenic microbes is crucial for probiotic efficacy [20]. Our auto-aggregation assays revealed that a substantial number of selected isolates could self-aggregate, a trait often linked to adhesion and biofilm formation on host surfaces, which can contribute to gut colonization and competitive exclusion [21]. Even more importantly, the observed co-aggregation with virulent foodborne pathogens such as *E. coli*, *S. aureus*, and *S. Typhimurium* signifies a direct mechanism by which these LAB strains could contribute to medical microbiology, particularly in gut health and food safety initiatives. By forming

aggregates with pathogens, LAB can potentially prevent their adherence to the intestinal epithelium, thereby reducing pathogen colonization and subsequent infection [22]. This mechanism is also relevant in veterinary microbiology, where similar competitive exclusion principles are applied to control pathogens in animal guts [23].

The demonstration of antimicrobial activity by cell-free supernatants (CFS) against tested pathogens further underscores the potential of these LAB strains. By adjusting the pH of the CFS, we focused on non-acidic antimicrobial compounds, likely bacteriocins or hydrogen peroxide. Potent inhibition was observed, particularly against *Staphylococcus aureus*, which is a significant pathogen in medical and food microbiology, as it causes various infections and food poisoning. Inhibition was also observed against Gram-negative strains, including *E. coli* and *Salmonella Typhimurium*. This antimicrobial property further highlights the role of LAB in bio-preservation: they can serve as natural food preservatives, extending shelf life and enhancing food safety by controlling undesirable microbial growth [24-25]. This application holds significant promise in applied microbiology, offering a sustainable and natural alternative to chemical preservatives. The broad-spectrum activity of some isolates suggests their versatility across various food matrices and could even be explored for surface disinfection in food processing environments.

The safety assessment, confirming the absence of β-hemolytic activity, is a fundamental prerequisite for any microorganism designated for probiotic or food application. This finding reinforces the safety profile of the identified promising LAB strains, paving the way for their further investigation and potential commercial development.

This study significantly contributes to the understanding of indigenous microbial resources in Nigeria, a region with a rich tradition of fermented foods but often underrepresented in global microbiome research. By focusing on traditional microbiological methods, this research lays a robust, reproducible foundation for the phenotypic characterization of LAB in indigenous Nigerian foods, forming the basis for future molecular investigations. The phenotypic attributes demonstrated by these LAB isolates—including their resilience to harsh conditions, aggregation capabilities, and direct antimicrobial action—collectively position them as strong candidates for various applied microbiological purposes. This includes the development of novel probiotic functional foods for human health, innovative bio-preservative starter cultures for the food industry, and even potential applications in veterinary microbiology for animal health and feed safety. The exploration of such local biodiversity is crucial for discovering unique strains with superior characteristics, particularly those adapted to specific local dietary and environmental contexts.

CONCLUSION

The traditional fermented cereal-based foods (*ogi* and *kunu*) widely consumed in Anyigba, Kogi State, Nigeria, represent a valuable and largely untapped reservoir of diverse LAB. Our comprehensive phenotypic characterization revealed that a significant proportion of these indigenous LAB strains possess highly desirable attributes crucial for probiotic and bio-preservation applications. Specifically, these strains demonstrated robust survival under simulated gastrointestinal conditions (low pH and bile salt presence), exhibited promising auto- and co-aggregation abilities with clinically relevant foodborne pathogens, and produced non-acidic antimicrobial compounds effective against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella Typhimurium*. Furthermore, the confirmed absence of β-hemolytic activity assures their safety for use.

These findings underscore the substantial potential of these local LAB as novel candidates for functional food

development, contributing to medical microbiology through enhanced gut health, and as natural bio-preservatives to improve food safety and extend shelf life within the food industry. Their versatility also suggests promising avenues for applied microbiology and potential benefits in veterinary microbiology. This research highlights the critical importance of exploring indigenous microbial diversity for sustainable solutions in public health and food systems, encouraging further research into the specific mechanisms and *in vivo* efficacy of these promising Nigerian LAB strains.

List of Abbreviations: LAB: lactic acid bacteria, g: gram, mL: milliliter, mm: millimeter, MRS: de Man Rogosa Sharpe agar, ND: no inhibition detected

Authors' Contributions: All authors contributed to this article.

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