

**Original Article****Isolation and screening of yeast strains from locally fermented foods for probiotic potential**

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ABSTRACT

Fermented foods are rich reservoirs of diverse yeast strains with promising probiotic potential. This study investigated the isolation and probiotic properties of yeast isolates obtained from fermented foods. Five fermented foods (Fufu, locust beans, burukutu, palm wine, and ogi) were aseptically collected, transported to the laboratory, and analysed. Yeasts were enumerated and isolated on Potato Dextrose Agar (PDA) using the pour plate technique. Presumptive isolates were identified based on colonial morphology, microscopic features, and biochemical characteristics. Safety assessments included haemolytic activity and antifungal susceptibility testing. Probiotic potential was evaluated based on acid and bile salt tolerance, antimicrobial activity, hydrophobicity, auto-aggregation, co-aggregation, and antioxidant activity. Twenty-five yeast isolates belonging to the genera *Saccharomyces*, *Rhodotorula*, *Pichia*, *Candida*, *Hanseniaspora*, and *Geotrichum* were recovered. Sixteen (64.0%) isolates exhibited γ -haemolysis, while nine (36.0%) showed haemolytic activity. Antifungal susceptibility testing revealed resistance rates of 16.0% to fluconazole, 28.0% to ketoconazole, and 40.0% to itraconazole and nystatin. Based on safety criteria, 16 (64.0%) isolates were considered suitable for probiotic evaluation. Antimicrobial activity varied among isolates, with three (18.8%) inhibiting *Escherichia coli* and four (25.0%) inhibiting *Salmonella* spp. (5.3 ± 0.88 - 11.0 ± 1.15 mm). Acid tolerance was low, whereas bile salt tolerance showed higher survival. Hydrophobicity was generally low, auto-aggregation was high (median = 81.25%). Antioxidant assays showed significant differences among isolates ($p < 0.05$), with isolate F1 showing the highest DPPH radical scavenging activity ($60.0 \pm 0.13\%$), followed by P3 ($53.0 \pm 0.14\%$), compared to ascorbic acid ($97.5 \pm 0.65\%$). These findings demonstrate the potential of yeast isolates as probiotic candidates for functional food applications.

Keywords: Food biotechnology, Isolation, locally fermented foods, Yeast

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INTRODUCTION

The use of probiotics for promoting human health has gained considerable attention, particularly due to the growing demand for functional foods and dietary supplements [1]. Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits on the host [2]. Traditionally, most commercial probiotics belong to lactic acid bacteria (LAB), particularly members of the genera *Lactobacillus*, *Enterococcus*, and *Bifidobacterium* [3]. However, increasing scientific interest has expanded the search for alternative probiotic microorganisms beyond bacteria, including yeasts [1].

Although only a limited number of yeast strains are currently recognized as probiotics, yeasts possess several advantages over bacteria [4]. They exhibit greater tolerance to harsh gastrointestinal (GI) conditions, including fluctuations in pH and bile concentration, and are inherently resistant to antibacterial antibiotics, enabling their use during antibiotic therapy [5]. Additionally, yeast cell walls contain immunomodulatory components such as β -glucans and mannoproteins, which may contribute to host immune stimulation [6]. Their relatively larger cell size (~10 times larger) compared to bacteria may also provide competitive advantages in pathogen exclusion thus enhancing their prospects to be a probiotic candidate [7]. Among the yeast genera reported to exhibit probiotic potential are *Pichia*, *Saccharomyces*, *Kluyveromyces*, *Yarrowia*, *Schizosaccharomyces*, and *Torulaspora* [8].

Probiotic functionality is closely linked to specific phenotypic and genetic traits [9]. Key characteristics include the ability to adhere to intestinal epithelial cells, tolerate gastric acidity and bile salts, produce antimicrobial substances, exhibit antioxidant activity, and survive under stressful environmental conditions [10]. Traditional fermented foods are important reservoirs of diverse microbial communities, including potentially beneficial yeast strains. Fermentation enhances the nutritional quality of foods by increasing bioavailability of nutrients, reducing antinutritional factors, and eliminating certain toxins and allergens [11]. In Nigeria and other parts of Africa, commonly consumed fermented foods such as ogi, pito, fufu, palm wine, local cheese, and other cereal- and legume-based products harbour rich and diverse yeast populations [12]. However, the probiotic potential of these indigenous yeast strains remains underexplored.

Globally, the probiotic industry continues to expand rapidly, driven by increased awareness of gut health and immune support). Nevertheless, most commercial probiotic strains originate from temperate regions, with limited representation of strains isolated from tropical ecosystems such as Africa [13]. In Nigeria, the probiotic market remains largely dependent on imported products [14]. This reliance not only increases cost and limits accessibility but also overlooks the potential advantages of locally adapted strains that may be better suited to regional diets, microbiota composition, and environmental conditions [15].

The exploration of indigenous yeast strains for probiotic development

presents both scientific and socio-economic opportunities. Locally derived probiotics could offer improved efficacy for the target population, contribute to the management of prevalent gastrointestinal disorders, and stimulate local biotechnology industries [16]. This study focused on isolating yeast strains from locally fermented foods and evaluating their probiotic potential.

MATERIALS AND METHODS

Sample Collection

Fresh samples of locally fermented foods (ogi, fufu, locust beans) were purchased from Tunga market, Minna, Niger State while the palm wine and burukutu were purchased from Dama Minna, Niger State. Samples were collected in sterile containers, placed in an ice box and transported to the Department of Microbiology of the Federal University of Technology Minna, Niger State for further analysis.

Sample Processing and Isolation of Yeast Isolates

Each sample was serially diluted. For solid samples, 1 gram of each sample was homogeneously diluted with 9 mL of sterile water to get the stock solution. After which 1mL of stock solution was transferred into test tube containing 9 mL of sterile distilled water to give 10^{-1} dilution factor. This process of transfer from preceding tube to the next continued until 10^{-5} dilution factor was obtained [17]. Inoculation was carried out by plating 1 mL aliquots from 10^{-3} and 10^{-4} dilution factor using pour plate method on potato dextrose agar plate and incubated at 37 °C for 24 hours. The characteristic yeast colonies on agar

plate were purified by repeated subculturing on fresh potato dextrose agar plates and incubated at 37 °C for 24 hours. The purified isolates obtained were stored in agar slants and kept in the refrigerator at 4 °C for further microbiological analysis [18].

Identification of Isolates

Colony characteristics such as shape, elevation, texture, and color were recorded, while Gram staining was used for microscopic identification [19]. Biochemical tests including sugar fermentation assays were performed using standard protocols.

Antifungal susceptibility test:

Antifungal susceptibility of the yeast isolates was determined using the agar well diffusion method as described by [20], with slight modifications. Overnight yeast cultures were adjusted to 0.5 McFarland standard. The standardized suspensions were uniformly swabbed onto Mueller-Hinton agar plates. Commercial antifungal agents Fluconazole, Nystatin, Itraconazole, and Ketoconazole were prepared to desired concentration (10µg/ml) and inoculated into agar well. The plates were incubated at 37 °C for 24-48 hours. After incubation, zones of inhibition were measured in millimetres, and susceptibility was interpreted as Susceptible (S), Intermediate (I), or Resistant (R) according to established interpretative criteria.

Haemolytic test: Haemolytic activity was assessed following the method described by [21]. Yeast isolates were streaked onto blood agar plates supplemented with 5% defibrinated blood and incubated at 37 °C for 24-48

hours. Isolate that showed no effect were considered non-haemolytic, while isolates showing greenish zones and clear zones were classified haemolytic.

Acid Tolerance Test: Acid tolerance was evaluated using the method described by [22] with slight modifications. Selected yeast isolates were cultured in Yeast Peptone Dextrose (YPD) broth at 37 °C, harvested by centrifugation at 8000 rpm for 15 minutes, and washed with phosphate-buffered saline (PBS, pH 7.0). Cell suspensions were adjusted to approximately 10⁶ CFU/mL. Acid resistance was tested by inoculating aliquots of each suspension into sterile PBS adjusted to pH 3.0 using 5 N HCl and then switching to 0.1 N HCl. The suspensions were incubated at 37 °C, and viable counts were determined at 0 and 3 hours using the plate count method on YPD agar. The percentage survival was calculated using the equation 1:

$$\% \text{survival rate} = \frac{\log \text{CFU/ml}(t_0)}{\log \text{CFU/ml}(t_3)} \quad (1)$$

Where:

$\log \text{CFU/ml}(t_0)$ = yeast cell count (log CFU/ml) at the initial time

$\log \text{CFU/ml}(t_3)$
= yeast cell count (log CFU/ml) after a given incubation time.

Bile salt tolerance: Bile tolerance was determined following the method of [22] with slight modifications. Yeast suspensions prepared as described above were inoculated into YPD broth supplemented with 0.3% (w/v) bile salts and incubated at 37 °C. Viable counts were determined at 3 and 6 hours. Percentage survival was calculated using the same equation 1 described for acid tolerance.

Antimicrobial activity Assay:

Antimicrobial activity was assessed using the agar well diffusion method as described by [23]. Yeast isolates were cultured in YPD broth at 37 °C for 24 hours and centrifuged at 4000 rpm for 5 minutes to obtain cell-free supernatant (CFS). Test pathogens (*Escherichia coli*, *Salmonella* spp., and *Pseudomonas aeruginosa*) were adjusted to 0.5 McFarland standard and uniformly spread onto Mueller-Hinton agar plates. Wells (6-8 mm diameter) were bored aseptically into the agar, and 100 µL of CFS was dispensed into each well. Plates were incubated at 37 °C for 24 hours, after which inhibition zones were measured.

Autoaggregation test: Autoaggregation ability was evaluated according to [24]. Yeast cells were harvested, washed, and resuspended in PBS to a final density of approximately 1.5 x 10⁸ CFU/mL (OD₆₀₀). Four millilitres of each suspension were vortexed and incubated at room temperature for 4 hours. Absorbance was measured at 600 nm at 0 and 4 hours. Autoaggregation percentage was calculated using equation 2:

$$100 - \frac{(A_t/A_0) \times 100}{1} \quad (2)$$

Where:

A_t = Absorbance readings at time point ($t = 4$)

A_0 = Absorbance readings taken initially.

Coaggregation test: Coaggregation with pathogenic bacteria was determined using the method of [25] with modifications. Yeast and bacterial pathogen suspensions (1.5 x 10⁸ CFU/mL, OD₆₀₀) were prepared separately. Equal volumes (2 mL each) of yeast and bacterial suspensions were

mixed and incubated for 4 hours. Absorbance was measured at 600 nm. Control tubes containing individual yeast and bacterial suspensions were also prepared. Coaggregation percentage was calculated using equation 3:

$$Ax + Ay/2 \times 100 = \frac{(Ax + Ay/2 - A(x+y))}{(3)} \quad (3)$$

Where x and y represent yeast strains in the control tubes, and (x+y) the mixture.

Hydrophobicity test: Cell surface hydrophobicity was determined using the xylene adhesion method described by [26] with modifications. Yeast cultures were grown overnight in YPD broth, centrifuged at 8000 rpm for 10 minutes at 4 °C, washed twice, and resuspended in PBS. Three millilitres of yeast suspension were mixed with 1 mL of xylene in sterile test tubes. The mixture was vortexed and incubated at 37 °C for 1 hour to allow phase separation. The aqueous phase was carefully removed, and absorbance was measured at 600 nm. Hydrophobicity was calculated using equation 4:

$$\frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} \times 100 \quad (4)$$

Antioxidant activity: The antioxidant activity of the selected yeast isolates was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, following the method described by [27] with slight modifications. Fresh yeast cultures were grown in Yeast Peptone Dextrose (YPD) broth at 37 °C for 24 h. The cultures were centrifuged at 8000 rpm for 10 min, and the supernatants were collected for analysis. A 0.1 mM DPPH solution was prepared in methanol and stored in the dark until use. 1.0 mL of yeast cell-free supernatant was mixed

with 2.0 mL of DPPH solution and incubated in the dark at room temperature for 30 min to allow for reaction. Methanol served as the blank, while a mixture of DPPH solution and methanol without sample served as the control. Ascorbic acid was used as the standard antioxidant reference. After incubation, the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. All assays were carried out in triplicate. The percentage of DPPH radical scavenging activity was calculated using the equation 5:

$$\% \text{ Inhibition} = \frac{A_c - A_s}{A_c} \times 100 \quad (5)$$

Where:

A_c = Absorbance of the control

A_s = Absorbance of the sample

RESULTS

Yeast Load of Selected Locally Fermented Foods

The yeast load of the selected fermented foods is presented in Figure 1. The yeast counts of the varied significantly ($p < 0.05$). Palm wine recorded the highest yeast count ($3.0 \pm 0.06 \times 10^7$ CFU/ml), followed by Locust beans ($2.4 \pm 0.17 \times 10^7$ CFU/g), Burukutu ($2.3 \pm 0.03 \times 10^7$ CFU/ml), while Fufu and Ogi recorded lower counts of $1.7 \pm 0.12 \times 10^7$ CFU/g and $1.3 \pm 0.20 \times 10^7$ CFU/g, respectively. A total of 25 yeast isolates were obtained from all the fermented food samples.

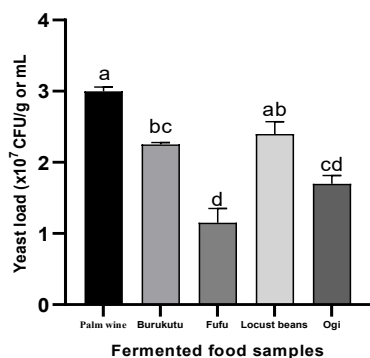


Figure 1: Yeast Load in Selected Fermented Foods

Table 1: Macroscopic and Microscopic characteristics of Yeast isolates

Isolate code	Morphology on PDA	Microscopy	Glucose	Mannitol	Lactose	Fructose	Maltose	Sucrose	Suspected species
F1	Creamy colonies	Oval budding cells	+	-	-	+	+	+	<i>Saccharomyces</i> sp.
F2	Cream coloured, rough with starburst-like appearance	Oval shaped cells	+	-	-	+	+	+	<i>Saccharomyces</i> sp.
F3	Creamy, smooth colonies	Oval budding cells	+	-	-	+	+	+	<i>Saccharomyces</i> sp.
F4	Spreading white colonies	Barrel shaped arthroconidia arrange in chains	+	-	-	+	+	+	<i>Geotrichum</i> sp.
F5	Creamy colonies	Oval budding cells	+	-	-	+	+	+	<i>Candida</i> sp.
P1	Creamy colonies	Oval cells	+	-	-	+	-	-	<i>Saccharomyces</i> sp.
P2	Creamy colonies	Oval budding cells	+	-	-	+	-	-	<i>Saccharomyces</i> sp.
P3	Smooth, Creamy colonies	Oval cells	+	-	-	+	-	-	<i>Pichia</i> sp.

Morphological and Biochemical Characterization of Isolated Yeast Species

The macroscopic, microscopic and biochemical characteristics of isolated yeasts is presented in Table 1. Based on the macroscopic, microscopic and biochemical observation *Saccharomyces* sp. *Rhodotorula* sp., *Pichia* sp., *Candida* sp., *Hanseniaspora* sp. and *Geotrichum* sp. were identified.

P4	Creamy colonies	Oval cells	+	-	-	+	+	+	<i>Saccharomyces</i> sp.
P5	Dull, dry colonies	Oval cells	-	-	-	+	+	+	<i>Saccharomyces</i> sp.
PW1	Cream, glossy	Oval cells	+	-	-	+	-	+	<i>Saccharomyces</i> sp.
PW2	Creamy colonies	Oval cells	+	-	-	+	-	+	<i>Saccharomyces</i> sp.
PW3	Creamy colonies	Oval cells	+	-	-	+	-	+	<i>Saccharomyces</i> sp.
PW4	Cream with dull edges	Oval cells	+	-	-	+	-	+	<i>Pichia</i> sp.
PW5	Creamy colonies	Oval cells	+	-	-	+	-	+	<i>Saccharomyces</i> sp.
BR1	Sticky creamy colonies	Oval cells	+	-	-	+	+	+	<i>Saccharomyces</i> sp.
BR2	Creamy colonies	Oval cells	+	-	-	+	+	+	<i>Saccharomyces</i> sp.
BR3	Cream colonies	Oval cells	+	-	-	+	+	+	<i>Candida</i> sp.
BR4	Creamy surface	Oval cells	+	-	-	+	+	+	<i>Saccharomyces</i> sp.
BR5	Dull, pinkish colonies	Oval cells	-	+	-	+	+	+	<i>Rhodotorula</i> sp.
LB1	Cream with dull edges	Oval cells	-	-	-	-	-	-	<i>Pichia</i> sp.
LB2	Dull surface	Oval cells	+	-	-	-	-	-	<i>Hanseniaspora</i> sp.

LB3	Cream with dull edges	Oval cells	+	-	-	-	+	-	<i>Pichia</i> sp.
LB4	Creamy colonies	Oval cells	+	-	-	-	-	-	<i>Saccharomyces</i> sp.
LB5	Creamy colonies	Oval cells	+	-	-	-	-	-	<i>Saccharomyces</i> sp.

KEY: PDA= Potato dextrose agar

Safety Assessment of Yeast Isolates: The safety of the isolated yeast species was evaluated based on haemolytic activity and antifungal susceptibility (Table 2). Potential pathogenicity was determined based on the haemolytic activity of the isolates. Out of the 25 yeast isolates, 16 (64.0%) exhibited γ -haemolysis (no haemolysis), while 9 (36.0%) isolates showed haemolytic activity.

In the antifungal susceptibility screening 4 (16.0%) of the isolates were resistant to Fluconazole, 7 (28.0%) to Ketoconazole while 10 (40.0%) were

resistant to Itraconazole and Nystatin. Analysis of multi-antifungal resistance showed that none (0.0%) of the isolates were resistant to all four antifungal agents, 1 (4.0%) was resistant to three agents, 6 (24.0%) were resistant to two agents, 15 (60.0%) were resistant to only one agent, and 3 (12.0%) were resistant to none of the tested antifungals. Based on the tested safety parameters, 16 (64.0%) of the Yeast isolates were considered safe for potential probiotic use, exhibiting no haemolytic activity and limited antifungal resistance.

Table 2: Haemolytic Activity and Antifungal Susceptibility of isolated Yeast species

Isolate Code	Haemolysis	Antifungal agents (10 µg/mL)			
		Fluconazole	Ketoconazole	Itraconazole	Nystatin (100 IU/mL)
F1	Γ	S	S	R	S
F2	Γ	S	R	S	R
F3	Γ	S	S	S	S
F4	B	S	R	S	R
F5	B	S	S	R	R
P1	Γ	S	S	R	S
P2	Γ	S	S	S	R
P3	Γ	S	S	S	R
P4	Γ	S	S	S	S
P5	Γ	S	R	S	S
PW1	Γ	S	R	S	S
PW2	Γ	S	S	S	R
PW3	Γ	S	S	R	R
PW4	B	R	R	S	R
PW5	Γ	S	R	S	S
BR1	Γ	S	R	S	S
BR2	Γ	S	S	R	R
BR3	B	R	S	R	S
BR4	Γ	S	S	R	S
BR5	A	S	S	S	R
LB1	B	R	S	S	R
LB2	A	S	S	S	R
LB3	B	R	S	S	S
LB4	Γ	S	S	S	R
LB5	B	S	R	S	S

KEY: γ: Gamma haemolysis (none); α: Alpha (partial) haemolysis; β: Beta (complete) haemolysis; S: Sensitive; R: Resistant

Antibacterial activity of Yeast Isolates

The antibacterial activity of selected yeast isolates against *Pseudomonas*

aeruginosa, *Escherichia coli*, and *Salmonella* sp. is presented in Table 3. The isolates displayed varying level of activity against the test isolates. Against *Pseudomonas aeruginosa*, 15 (93.8%)

of the tested isolates exhibited measurable zones of inhibition ranging from 10.0 ± 0.58 to 21.3 ± 0.67 mm. The highest activities were observed in isolates Br4 (21.3 ± 0.67 mm), Pw3 (20.7 ± 0.88 mm), Pw1 (20.0 ± 0.58 mm), and Br2 (19.0 ± 1.15 mm). Only 3 (18.8%) of the isolates exhibited

activity against *Escherichia coli*, with inhibition zones recorded ranging from 8.7 ± 0.33 to 11.7 ± 0.88 mm while only 4 (25.0%) showed inhibitory effect against *Salmonella* sp. with zones of inhibition ranging from 5.3 ± 0.88 to 11.0 ± 1.15 mm.

Table 4.4: Antibacterial Activity (Zone of Inhibition) of Yeast Isolates

Isolate code	Zones of Inhibition (mm)		
	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Salmonella</i> sp.
LB4	18.7 ± 0.88^c	0.0 ± 0.00^a	11.0 ± 1.15^b
Br1	11.3 ± 0.33^b	0.0 ± 0.00^a	0.0 ± 0.00^a
F2	16.3 ± 0.67^b	0.0 ± 0.00^a	0.0 ± 0.00^a
F1	10.0 ± 0.58^b	9.7 ± 0.67^b	0.0 ± 0.00^a
Br4	21.3 ± 0.67^b	0.0 ± 0.00^a	0.0 ± 0.00^a
P4	11.3 ± 0.88^b	0.0 ± 0.00^a	0.0 ± 0.00^a
P1	17.3 ± 0.33^c	8.7 ± 0.33^b	7.7 ± 0.67^a
Pw1	20.0 ± 0.58^b	0.0 ± 0.00^a	0.0 ± 0.00^a
P5	17.7 ± 0.67^b	0.0 ± 0.00^a	0.0 ± 0.00^a
Br2	0.0 ± 0.00^a	0.0 ± 0.00^a	0.0 ± 0.00^a
P2	14.0 ± 0.58^b	0.0 ± 0.00^a	0.0 ± 0.00^a
Pw2	18.0 ± 0.58^c	0.0 ± 0.00^a	5.3 ± 0.88^b
F3	14.7 ± 3.18^b	0.0 ± 0.00^a	0.0 ± 0.00^a
P3	11.3 ± 1.20^b	11.7 ± 0.88^b	0.0 ± 0.00^a
Pw5	16.3 ± 1.45^c	0.0 ± 0.00^a	9.3 ± 1.20^b
Pw3	20.7 ± 0.88^b	0.0 ± 0.00^a	0.0 ± 0.00^a

Values are presented as mean $\pm$ standard error of mean (SEM) of two replicates

Values with different superscripts in a column are significantly different at $p < 0.05$

Acid and Bile Tolerance of Selected Yeast Isolates: Figure 2 and 3 shows the %survival of each yeast isolates after 3 hours in acidic conditions and bile salts. The acid tolerance test showed considerable variation in the ability of the yeast isolates to survive acidic conditions over a 3-hour period. Initial viable counts at 0 hours ranged from 3

to 63 CFU, while survival after 3 hours (0-13 CFU) differed widely among the isolates. Whereas initial viable counts in bile salts at 0 hours ranged from 5 to 57 CFU/mL, while viable counts after 3 hours decreased to values between 3 and 47 CFU/mL, depending on the isolate

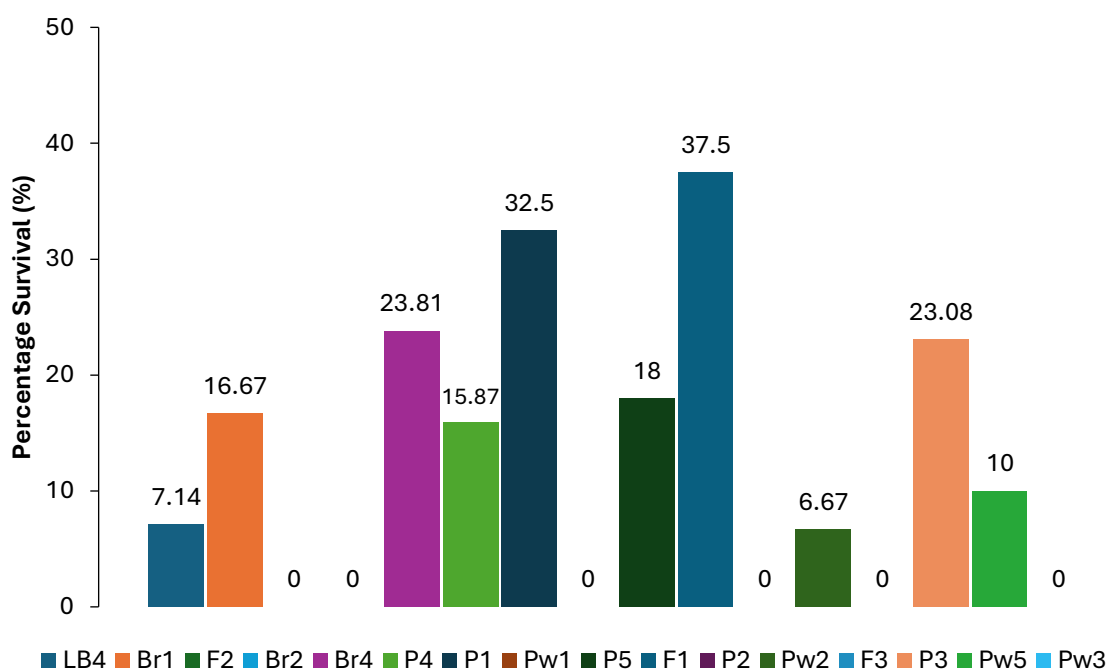


Figure 2: Acid tolerance of yeast isolates (%Survival after 3h)

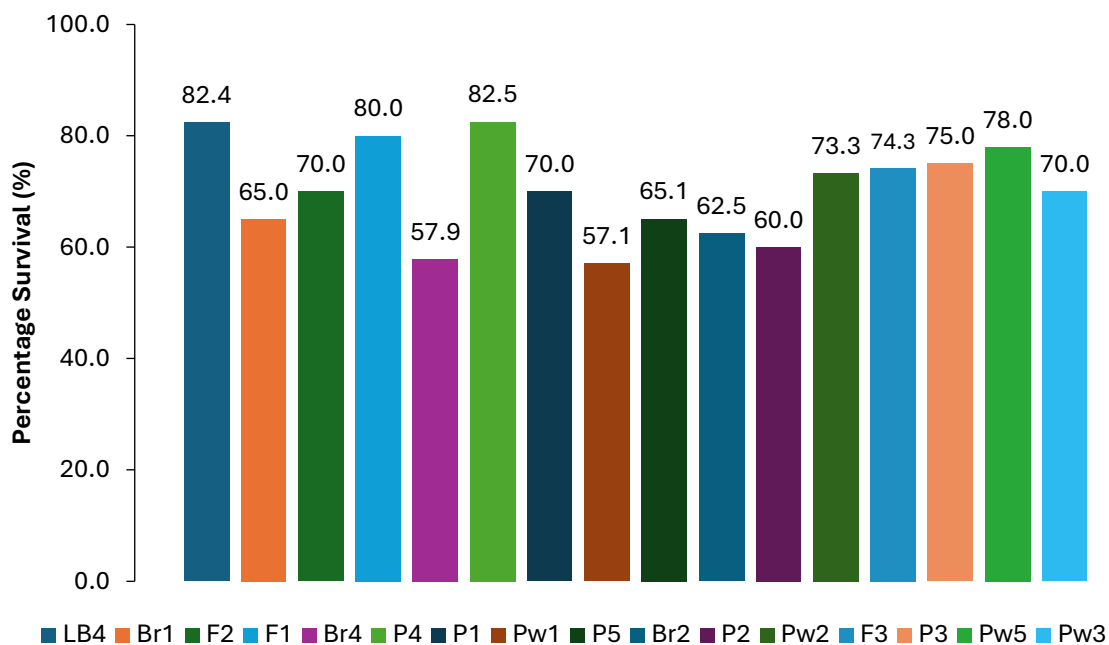


Figure 3: Bile tolerance of yeast isolates (%Survival after 3h)

Hydrophobicity, co-aggregation, and auto-aggregation abilities of Yeast Isolates: The heatmap (Figure 4) illustrates these trends by showing individual isolate performance. For hydrophobicity, top isolates included F1 (75.0%), Br4 (75.0%), P3 (62.5%), and Br2 (40.0%), while most isolates displayed negligible hydrophobicity. In terms of co-aggregation, F2 (70.0%), Br4 (65.0%), P1 (63.75%), and Br2 (61.25%) were the strongest performers, whereas P4 (2.5%) had the lowest co-aggregation capacity. Auto-aggregation was high for most isolates, with LB4 (96.25%), F1 (93.75%), Pw5 (91.25%), and Pw2 (90.0%) showing the highest self-aggregation ability, while Pw1 and Br2 exhibited minimal auto-aggregation (0.0%).

Antioxidant properties of Yeast isolates:

The DPPH radical scavenging activity of the best yeast isolates F1 and P3 based on their overall performance across the preliminary probiotic screening assays is presented in Figure 5. *Saccharomyces cerevisiae* (F1) exhibited the highest antioxidant activity among the isolates, with $60.0 \pm 0.13\%$ inhibition, followed by *Pichia kudriavzevii* (P3), which recorded $53.0 \pm 0.14\%$ inhibition. The standard reference, ascorbic acid, showed the highest activity overall, with $97.5 \pm 0.65\%$ inhibition.

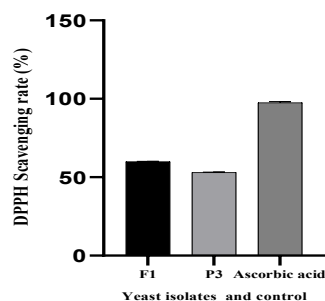


Figure 4: DPPH Radical Scavenging Activity of Selected Yeast Isolates

Values are presented as mean $\pm$ standard error of mean (SEM) of three replicates

DISCUSSION

This study isolated yeast strains from locally fermented foods, palm wine recorded the highest yeast count of 3.0×10^7 CFU/mL, followed by locust beans (2.4×10^7 CFU/g), burukutu (2.3×10^7 CFU/mL), while Fufu and ogi recorded lower counts of 1.7×10^7 CFU/g and 1.3×10^7 CFU/g, respectively. The observed variation in yeast counts was statistically significant ($p < 0.05$), showing differences in fermentative dynamics and substrate suitability among the food types. The high yeast count observed in palm wine is consistent with its naturally fermentative nature and the abundance of simple sugars in the sap, which provides a conducive environment for rapid yeast growth [28]. The yeast isolates obtained in this study displayed distinct macroscopic and microscopic characteristics typical of fermentative yeasts. Their morphology and sugar fermentation profiles suggest that many belong to genera such as *Saccharomyces*, *Pichia*, *Candida* and *Hanseniaspora*, which are commonly associated with spontaneous fermentations of traditional foods [29].

Safety assessment is a fundamental requirement when evaluating microorganisms intended for use in fermented foods, biotechnology, or potential probiotic applications [30]. In the present study, most of the isolates exhibited gamma (γ) haemolysis, showing absence of haemolytic activity. The lack of haemolysis suggests that the isolates do not produce haemolysins

and are unlikely to possess haemolysis-associated virulence traits. This finding aligns with safety criteria expected of microorganisms intended for food or probiotic use. Similar observations were reported by [31], who found no haemolytic activity among yeasts isolated from agro-food sources. The isolates also showed variable susceptibility to the tested antifungal agents while some were resistant. The general susceptibility pattern observed suggests limited antifungal resistance risk. Resistance detected in single agents may reflect intrinsic or strain-specific variability rather than multidrug resistance.

The ability to inhibit pathogenic microorganisms is a key selection criterion for probiotic candidates, as it contributes to microbial balance and host protection [32]. In the present study, the yeast isolates exhibited inhibitory activity against all tested bacterial pathogens. A high proportion of the isolates (93.8%) exhibited inhibitory activity against *Pseudomonas aeruginosa*. In contrast, antimicrobial activity against *Escherichia coli* was limited, with only 18.8% of the isolates showing measurable inhibition. Similarly, only 25.0% of the yeast isolates inhibited *Salmonella* spp., indicating moderate antagonistic potential. These antimicrobial effects may be attributed to the production of organic acids, ethanol, antimicrobial peptides, killer toxins, and other bioactive metabolites.

Survival under acidic and bile conditions is essential for probiotic functionality, as these conditions simulate passage through the gastrointestinal tract [33]. *Saccharomyces cerevisiae* (F1)

recorded higher tolerance to both acid (37.5%) and bile (80.0%). Acid exposure resulted in substantial reductions in viable counts, indicating moderate acid resistance. Bile tolerance was comparatively stronger, suggesting intrinsic resilience to bile salt stress. The difference between acid and bile responses confirms that these traits are independent and strain-specific.

Cell surface hydrophobicity, auto-aggregation, and co-aggregation are important indicators of adhesion and colonization potential [34]. These values show strong adhesion-related properties. High auto-aggregation suggests enhanced self-association and potential biofilm formation, which may support gut persistence. Reported hydrophobicity ranges in literature vary widely (6-87%) [34]. Co-aggregation values are also comparable to those reported by [35]. The exceptionally high auto-aggregation values observed, particularly for F1, indicate strong self-association capacity, which may enhance competitive exclusion of pathogens and stable colonization.

The DPPH assay revealed moderate antioxidant activity, with F1 (60.0%) outperforming P3 (53.0%), though both were lower than ascorbic acid (97.5%). These values fall within reported ranges (30-70%) for probiotic yeasts reported by [36]. The antioxidant potential may be attributed to production of phenolic compounds, vitamins, antioxidant enzymes, or metal-chelating metabolites [37]. Although not comparable to pure ascorbic acid, the activity observed suggests functional antioxidant capability that may contribute to health-promoting effects.

CONCLUSION

None

This study showed that locally fermented foods serve as valuable reservoirs of yeast strains with promising probiotic potential. Most isolates were non-haemolytic and largely susceptible to commonly used antifungal agents, supporting their safety for potential probiotic application.

The yeasts exhibited significant antibacterial activity, appreciable tolerance to acidic and bile conditions, strong adhesion-related properties (hydrophobicity, auto-aggregation, and co-aggregation), and significant antioxidant activity.

DECLARATION SECTION**Ethics Approval and Informed Consent.**

This study did not use human or animal subjects. Therefore, ethical consideration was not applicable.

Authors' Contribution

Conceptualization: Bala Jeremiah David and Madaki Fatima Muhammad; **Supervision:** Bala Jeremiah David and Akande Kemi Eunice; **Investigation:** Bala Jeremiah David and Jibrin Ramatu; **Writing the first draft of the article:** Jibrin Ramatu; **Editing:** Ummulkhair Salamah Ilyasu, Jibrin Ramatu and Enagi Fatima Muhammad. All authors contributed to the development of the final manuscript and approved its submission.

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Disclosure of Conflict of interest.**REFERENCES**

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