



Microbial Characterization and Probiotic Profiling of Starter Cultures in Palm Wine Fermentation: A Biotechnological Assessment

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Abstract: Palm wine, a traditional fermented beverage vital to West and Central Africa's socio-cultural fabric, faces challenges in quality and safety due to spontaneous fermentation. This study aimed to develop standardized starter cultures by isolating and characterizing dominant fermentative microorganisms from Nigerian *Elaeis guineensis* (oil palm) and *Raphia hookeri* (raphia palm) sap. Microbial dynamics were monitored over 36 hours, revealing high initial aerobic flora (10^8 CFU/mL) and yeast populations (10^6 CFU/mL). Biochemical and molecular analyses identified *Saccharomyces cerevisiae* (yeast) and *Leuconostoc mesenteroides* (LAB) as dominant species, exhibiting robust stress tolerance (15% ethanol, 45°C) and probiotic potential, with 89–90% survival under simulated gastrointestinal conditions. LAB isolates demonstrated significant acidification ($\Delta pH \geq 1.5$) and bile salt tolerance (88–90% survival), while both yeast and LAB showed non-hemolytic activity and antibiotic susceptibility, confirming safety. Starter cultures of these strains enabled consistent fermentation, enhancing ethanol yield (5.2–7.0% v/v) and flavor compound production. The study highlights the potential of *S. cerevisiae* and *L. mesenteroides* as multifunctional starter cultures to standardize palm wine production, ensuring reproducibility, safety, and probiotic functionality. These findings bridge traditional practices with biotechnological innovation, offering scalable solutions to improve quality, extend shelf-life, and position palm wine as a functional health beverage in global markets. Collaborative efforts to integrate these cultures into artisanal production could empower rural economies while preserving cultural heritage.

Keywords: *Saccharomyces cerevisiae*, *Leuconostoc mesenteroides*, fermentation, probiotics, palm wine, gastrointestinal survival.

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Introduction

Palm wine, a traditional alcoholic beverage deeply rooted in the cultural and socio-economic fabric of West and Central Africa, is produced through the spontaneous fermentation of sap extracted from palm species such as *Elaeis guineensis* (oil palm) and *Raphia hookeri* (raphia palm) [38-42]. For centuries, it has served as a staple in rituals, ceremonies, and daily nutrition, while also functioning as a critical income source for rural communities for instance, in Nigeria, palm wine tapping employs over 75% of males in palm-rich regions, producing up to 10,000 liters daily [1, 2]. Despite its cultural reverence and nutritional richness marked by B vitamins, potassium, and antioxidants like gallic acid palm wine production remains largely artisanal, relying on wild

microbial consortia that introduce significant variability in quality, safety, and sensory profiles [3]. This unpredictability, compounded by risks of contamination with pathogens such as *Klebsiella pneumoniae* and rapid spoilage (≤ 48 hours), limits its commercial potential and poses public health concerns [4, 5].

Nigeria is endowed with a wide range of fermentable indigenous staple foods (e.g., cassava, sorghum, millet, maize, plantains, and legumes), which serve as dietary foundations and hold significant potential for probiotic and functional food development [36]. Recent advances in food microbiology have highlighted the transformative potential of controlled fermentation using defined starter cultures [33-35]. Such cultures not only standardize production but also enhance functional properties, such as



probiotic activity and bioactive compound synthesis, positioning palm wine as a functional health beverage [6, 7]. Pioneering studies by Faparusi [8] laid the groundwork for this approach, isolating key fermentative microbes like *Saccharomyces cerevisiae* and *Leuconostoc mesenteroides* and linking their metabolic activities to flavor development and preservation. However, a comprehensive evaluation of these strains' stress tolerance, safety, and probiotic efficacy remains underexplored, particularly in the context of artisanal production systems.

This study aimed to bridge this gap by systematically isolating, characterizing, and evaluating dominant fermentative microorganisms from palm wine for their suitability as starter cultures. Isolates were subjected to rigorous morphological and biochemical profiling to confirm taxonomic identity, followed by assessments of their fermentation kinetics under stress conditions (e.g., pH 3–9, temperatures 25–45°C, and ethanol concentrations up to 10% v/v). Probiotic potential was evaluated through *in vitro* assays for acid and bile tolerance, adhesion to intestinal epithelial cells, and antimicrobial activity against enteric pathogens. Safety was verified via hemolytic activity screening and antibiotic resistance profiling.

By identifying robust, multifunctional strains capable of standardizing fermentation while enhancing health-promoting attributes, this work seeks to redefine palm wine as a reproducible, safe, and functionally enriched beverage. The findings hold promise for empowering rural producers through scalable biotechnological solutions, aligning traditional practices with modern food safety standards and global functional food trends.

2. Materials and Methods

2.1 Sample Collection

Oil palm (*Elaeis guineensis*) sap was obtained from Udi community, Udi LGA, Enugu State, Nigeria (Latitude: 6.33330 N, Longitude: 7.43330 E), and Raffia palm (*Raffia hookeri*) sap was sourced from Umuawulu community, Awka South LGA, Anambra State (Latitude: 6° 8' 60.00" N, Longitude: 7° 05' 60.00" E). In both cases, traditional tappers aseptically collected the fresh sap into separate 500ml capped Erlenmeyer flasks within 30-60 mins of tapping. Before tapping, tappers were thoroughly instructed on aseptic palm sap collection protocols, emphasizing the use of sterilized materials. The collected samples were subsequently transported under cold chain management to the Department of Applied Microbiology and Brewing Laboratory, Nnamdi Azikiwe University, Nigeria for further physicochemical and biochemical analyses. The fermentation was monitored from 0 to 36 hours.

2.2 Enumeration of Microorganisms in Palm Sap (Oil palm and Raffia palm)

One mL of each of the samples was mixed with 9 mL of physiological saline (0.85% NaCl) and homogenised thoroughly for 2 min. Homogenised samples were diluted serially in the same diluent; 1 mL of diluent from the particular dilution was plated on plate count agar (HiMedia, Mumbai, India) and Sabouraud Dextrose Agar (HiMedia, Mumbai, India) for the enumeration of bacteria and yeasts respectively. Bacterial plates were incubated at 37 °C for 24 h and yeast plates were incubated at 28 °C for 48 h. After incubation, the number of colonies from the respective plates was counted and expressed as colony-forming units (cfu)/mL.

Lactic acid bacteria (LAB) from samples were enumerated using MRS agar medium (GM641, HiMedia, Mumbai, India) as per the method described by Shangpliang and Tamang [9]. Homogenised samples were inoculated into MRS agar plates supplemented with 1% CaCO₃ by pour plating method. The plates were incubated anaerobically at 30 °C for 48 h. After incubation, isolates were selected primarily and re-inoculated into the MRS broth (M1926, HiMedia, Mumbai, India) and incubated for 24 h at 30 °C and their growth was checked at 600 nm using a spectrophotometer.

2.3 Preliminary Identification of Yeasts and LAB

2.3.1 Microscopic examination

Macroscopically, isolates were observed for the following features: colony elevation, colour and other distinct and unique features. For microscopy, thin smear was prepared in accordance to Zongo *et al.* [10], by emulsifying a loopful of an isolate under test on a clean slide with a drop of water. The film was spread to make a thin film and then air dried after which it was stained with a methylene blue dye and observed with a light microscope under X10 and X40 objective lenses.

2.3.2 Biochemical tests for the identification of yeasts

2.3.2.1. Carbon assimilation test

Yeast Nitrogen Base (Difco) (YNB) broth, which includes all important nutrients and vitamins required for yeast development except for a carbon source, was made by dissolving 6.7 g of the base and 5 g of carbohydrate in 100 ml of warmed distilled water. The preparation was meticulously combined, filter sterilised, and kept in a refrigerator at 2-8°C for a maximum duration of one week prior to use. An inoculum culture of the test organism was cultivated on PDA plates for two days at 25°C and subsequently used to inoculate the YNB carbohydrate broth medium, which was incubated at 25°C for 6-7 days. Following incubation, the tubes were agitated and examined for growth. The carbohydrates examined with YNB included glucose, sucrose, maltose, xylose, galactose, lactose, raffinose, melibiose, mannitol, and trehalose [11].

2.3.2.2. Nitrogen assimilation

Yeast Carbon Base broth (Difco) was utilised. The base includes all vital nutrients and vitamins required for yeast cultivation, excluding a nitrogen source. To prepare the broth, 11.7 g of the base and 2 g of nitrogen source were well mixed in 100 ml of distilled water; filter sterilised, and kept at 2-8°C. The prepared medium was aseptically distributed into tubes and injected with the test organism as previously outlined for the YNB assimilative test. Following incubation, the tubes were agitated to evaluate growth. The nitrogen sources utilised in the experiment were lysine and nitrate [11].

2.3.2.3. Urea hydrolysis

Urea broth was formulated by combining 9.68 g of Urea R broth (Difco) with 250 ml of distilled water, which was subsequently filter-sterilized. A volume of 0.5 ml was aseptically dispensed into sterile, empty test tubes. Each experimental organism was inoculated into its corresponding labelled tube using a flame-sterilized inoculation loop and incubated for 24-48 h at 37°C. The incubated broths were monitored for a colour change from yellow to bright pink [11].

2.3.3 Biochemical characterization of LAB strains

A total of thirty LAB were isolated from the fresh and fermented sap of oil palm and raffia palm. Purified microorganisms were grown on MRSa at 30°C for 48 hrs and then characterized.

The biochemical characterization includes cell morphology, bacteria Gram stain, catalase and oxidase test, cell motility and sugars fermentation profile was carried out using standard methods in microbiology.

The study of the fermentation profile of sugars of lactic acid bacteria strains was carried out using a liquid base medium containing only the sugar to be tested as a carbon source. The liquid medium contained distilled water, 5 g/L of yeast extract, 1 g/L of KH_2PO_4 , 1 g/L of NH_4Cl , 0.5 g/L of $\text{FeSO}_4 \cdot (6\text{H}_2\text{O})$ and 3 g/L of sugar. The sugars that were tested include glucose, fructose, sucrose, galactose, lactose, maltose, xylose, arabinose, ribose and mannose. Lactic acid bacteria in the exponential growth phase (18-24 h) were used to inoculate the liquid medium containing Bromothymol Blue as indicator. The pH of the basal medium (green color) was adjusted to 7.2. The change in color from green to yellow after 24 to 48 hrs of incubation reflects the ability of the strain to ferment the sugar in question. The grouping of pure strains was based on their morphological, cultural, biochemical and physiological characters. Microorganisms with the same characteristics were placed in the same group [10].

2.4 Selection of Starter Cultures (Yeast and Lactic Acid Bacteria)

2.4.1. Yeast Isolates

2.4.1.1 Flocculation Test

In this test, isolates (yeast) were inoculated in 10 ml of Sabouraud Dextrose broth and incubated at 30°C for three days. After incubation, tubes were agitated to observe the flocculation formed. The sediment was suspended and the degree of flocculation assessed visually [11].

2.4.1.2. Determination of Ethanol Tolerance

The ability of the isolated yeast strains to grow in higher ethanol concentrations medium was tested in relation to varying sample locations by growing them in potato dextrose broth containing 3 different concentrations of ethanol, 5%, 10%, 15% and 20% (v/v), respectively and was incubated at 30°C for 72 h [11].

2.4.1.3. Temperature Tolerance Test

The ability of the yeast to grow at higher temperatures was verified by plating the yeast isolates onto Sabouraud Dextrose Agar medium and incubated at 3 different temperatures i.e. 30, 37 and 45°C for 72 h [10]. The resulting growth was observed and recorded.

2.4.2. Lactic Acid Bacteria Isolates

2.4.2.1. Selection of LAB starter cultures with acidifying activity

Sterilized palm sap (50ml) (70°C; 25 min) was inoculated with 2% of culture at early stationary growth phase on MRS broth of the lactic acid bacteria strain. The palm sap was incubated at 37°C. The pH was measured on aliquots after 6 h. The decrease in pH (ΔpH) was calculated as the difference between the values

immediately after inoculation ($t=0\text{h}$) and the values at 6 h ($\Delta\text{pH} = \text{pH at } 6\text{h} - \text{pH zero time}$). Three trials of each isolates were carried out [12].

2.4.3. Functional characterization of the probiotic potentials of pre-selected isolates

2.4.3.1. Screening and Selection of Acid and Bile Salt Tolerant LAB as the Potent Probiotic.

All LAB isolates were tested for their tolerance to acidic conditions and bile salts. Tolerance to acidic conditions was determined using the method of Argyri, *et al.* [13], with some modification. Briefly, an overnight culture of LAB strains in MRS broth at 37°C was harvested by centrifugation at $8000 \times g$ for 10 min at 4 °C, and then washed twice with phosphate-buffered saline (PBS), pH 7.2. The washed cell pellets were resuspended in PBS to approximately 10^8 CFU/mL. A total 0.1 mL of LAB cell suspension was transferred into 10 mL of PBS pH 7.2 adjusted with 1.0 M hydrochloric acid, and cells suspended with PBS (pH 7.2) were used as control. All mixtures were incubated at 37°C for 3 h. A viable cell count was determined by plating on MRS agar and incubated at 37°C for 12h. The viable cell count was expressed as the log value of colony-forming units per mL ($\log\text{CFU/mL}$).

The bile salts tolerance of LAB isolates was determined according to the method of García-Hernández, *et al.* [14]. Briefly, 0.1 mL of LAB cell suspension was inoculated into 10 mL of PBS supplemented with 0.3% (w/v) bile salts (Oxgall, Merck, Germany) and PBS without bile salts served as the control; all cultures were incubated at 37 °C for 3h. The viable cell count was determined, and the survival rate was calculated as follows:

$$\text{Survival (\%)} = \frac{[\text{final } (\log\text{CFU/mL}) / \text{control } (\log\text{CFU/mL})]}{(1)} \times 100$$

2.4.3.2. Assessment of the ability of isolates to survive in simulated gastrointestinal juices.

Two LAB and two yeast isolates were selected due to their better pH activities as compared to the other seven of LAB and eight of yeast isolates, respectively. These four selected isolates were tested for their *in vitro* gastrointestinal tract tolerance following the modified method of Casarotti and Penna [15]. Simulated gastric juice (pH 2.5) (6.2 g/1 NaCl, 2.2 g/1 KCl, 0.22 g/1 CaCl_2 , 1.2 g/1 NaHCO_3 , 0.3% pepsin, pH 2.5), duodenal juice (pH 5.0), and intestinal juice (6.4 g/1 NaHCO_3 , 0.239 g/1 KCl, 1.28 g/1 NaCl, 0.1% pancreatin) (pH 8.0) were prepared as described in Muganga *et al.* [16]. The cells were harvested from overnight cultures by centrifugation (804 g, 4°C, 15 min). The cells were washed twice with an equal volume of sterile PBS (pH 7.4), and resuspended in simulated gastric juice (pH 2.5).

Determination of total viable cell counts was performed after 0 and 3 h of incubation in simulated gastric juice at 37°C, after which 1 mL cell suspension was transferred into 9 mL simulated duodenal juice and incubated (37°C, 2 h). After duodenal juice digestion, the cell suspensions (1 mL) were transferred into 9 mL simulated intestinal juice and incubated at 37°C for 8 h. After the intestinal juice digestion, cell viability was determined. One mL aliquot was serially diluted in a sterile saline solution (0.85% NaCl) and plated on the MRS agar for LAB and SDA for the yeast isolates and incubated at the respective temperature (37°C for LAB and 30°C for yeast) for 72 h. The microbial tolerance in simulated gastric,

duodenal, and intestinal juice were expressed as survival (%) and calculated as $(\beta/\alpha) \times 100$, where α was the log₁₀ colony forming units (CFU)/mL of the assayed strain before treatment, and β was the log₁₀ CFU/mL of the same strain after incubation.

2.4.4. Safety Evaluation of Selected Isolates.

2.4.4.1. Hemolytic Activity

The non-virulent nature of the isolates toward blood cells was assessed on SDA and MRS agar supplemented with 5% sheep blood. On this medium, 24 hr old cultures were deposited as spots and incubated at 37°C for 48 h. The development of clear (β -haemolysis), and greenish (α -haemolysis) areas was looked for around the colonies [17].

2.4.4.2. Antimicrobial Susceptibility Assay

This was performed by the disc diffusion method according to the CLSI protocol [18]. Fresh yeast and lactic acid bacteria cultures were used respectively to prepare the McFarland 0.5 densities (1.5×10^8 CFU/ml). This was grown in Mueller-Hinton agar plates supplemented with 2% glucose and 0.5mg/l Methylene Blue. The antimicrobial discs were placed on the surface of the medium and incubated for 24 hr. Blue areas around the discs were measured and the isolates were classified as susceptible (S), intermediate (I), or resistant (R).

2.5. Molecular characterization of selected starter cultures (Lactic Acid Bacteria and Yeast)

2.5.1. Genomic DNA Isolation

Genomic DNA of LAB isolates was extracted as per the method of Shangpliang and Tamang [9]. The method of Lama and Tamang [19] was followed for the isolation of DNA from yeast isolates.

2.5.2. Repetitive Sequence-Based PCR

LAB and yeasts isolated were grouped through the repetitive sequence-based PCR using GTG₅ primer (5'-GTGGTGGTGGTGGTG-3') as described by Cissé *et al.* [20].

2.5.3. Amplification and DNA Sequencing

The 16S rRNA gene from the representative bacterial isolates was amplified using 27F (27F 5'-AGAGTTTGATCATGGCTCAG-3') and 1492R (5'-GTTACCTTGTACGACTT-3') primers by following the method of Shangpliang and Tamang (2021). For representative yeast isolates, the D1–D2 region of the 26S rRNA gene was amplified for the final identification using NL1 (5'-GCA TAT CAA TAA GCG GAG GAA AAG-3') and NL4 (5'-GGT CCG TGTTC AAG ACG G-3') primers as described by Palla *et al.* [21].

3. Results and Discussions

3.1 Microbial Load and Fermentation Kinetics A time-dependent increase in microbial populations was observed within the first 24 hours, followed by a slight decline at 36 hours. Oil palm wine showed higher initial aerobic flora (1.02×10^8 CFU/mL) compared to raffia palm wine (6.7×10^7 CFU/mL). Similar trends were observed for fungi, LAB, and yeasts, consistent with observations by Karamoko *et al.* [22] and Zongo *et al.* [23].

Table 3.1: Microbiological Enumeration of Fresh Sap from Oil Palm and Raffia Palm Fermented from 0h – 36h

	0h	12h	24h	36h
Total Aerobic Flora Count				
Oil palm wine	1.02×10^8	1.34×10^8	1.41×10^8	1.22×10^7
Raffia palm wine	6.7×10^7	1.0×10^8	1.2×10^8	1.6×10^7
Total Fungal Count				
Oil palm wine	1.83×10^5	1.67×10^6	1.8×10^6	1.5×10^6
Raffia palm wine	1.66×10^5	1.55×10^6	1.72×10^6	1.45×10^6
Total Lactic Acid Bacterial Count				
Oil palm wine	1.80×10^6	1.43×10^7	1.79×10^6	1.52×10^6
Raffia palm wine	1.88×10^6	2.56×10^6	1.99×10^6	1.43×10^6
Total Yeast Count				
Oil palm wine	1.35×10^6	1.68×10^6	1.98×10^6	2.3×10^6
Raffia palm wine	1.44×10^6	1.62×10^6	1.90×10^6	1.92×10^6

3.2 Biochemical Characterization of Yeasts and LAB Yeast isolates (YP1–YP4, RY1–RY4) fermented glucose and fructose but not lactose or galactose, identifying them as *Saccharomyces cerevisiae*, in line with Kurtzman *et al.* [24]. LAB isolates (OP1–OP4, RP1–RP3) showed characteristics consistent with *Leuconostoc mesenteroides*, including fermentation of glucose, maltose, sucrose, and fructose, with negative catalase and oxidase tests, as reported by Amoa-Awua *et al.* [2] and Ouoba *et al.* [25].

Table 3.2: Sugars fermentation profile of yeasts isolated from oil palm wine and raffia palm wine

Isolates	Man	Mal	Lac	Glu	Gal	Rib	Suc	Xylo	Fru	Arab
YP1	+	-	-	+	-	-	-	-	+	-
YP2	+	-	-	+	-	-	-	-	+	-
YP3	+	-	-	+	-	-	-	-	+	-
YP4	+	-	-	+	-	-	-	-	+	-
RY1	+	-	-	+	-	-	-	-	+	-
RY2	+	-	-	+	-	-	-	-	+	-
RY3	+	-	-	+	-	-	-	-	+	-
RY4	+	-	-	+	-	-	-	-	+	-

Legend: (+): Positive reaction, (-): Negative reaction,

Mann- Mannose, Mal – Maltose, Lac- Lactose, Glu – Glucose, Gal- Galactose, Rib- Ribose, Suc- Sucrose, Xylose, Fruct- Fructose, Arabi- Arabinose. YP1, YP2, YP3 and YP4 are all yeast isolates from oil palm wine and RY1, RY2, RY3 and RY4 are yeast isolates from Raffia palm wine.

Table 3.3: Morphology and Biochemical characteristics of lactic acid bacteria isolated from oil palm wine and raffia palm wine

Isolates	Cell Morphology	Gram	Catalase	Motility	Oxidase
OP1	Milky white colony, small cocci alone	+	-	-	-
OP2	Milky white colony, cocci, alone	+	-	-	-
OP3	Milky white colony, cocci in chains	+	-	-	-
OP4	Milky white colony, cocci alone	+	-	-	-
RP1	Milky white colony, small cocci in pairs	+	-	-	-
RP2	Milky white colony, cocci in pairs	+	-	-	-
RP3	Milky white colony, small cocci in chains	+	-	-	-

Legend: (+): Positive reaction, (-): Negative reaction, OP1, OP2, OP3, and OP4 are LAB isolates from oil palm wine and RP1, RP2, RP3 AND RP4 are LAB isolates from Raffia palm wine.

Table 3.4: Sugars fermentation profile of lactic acid bacteria isolated from oil palm wine and raffia palm wine

Isolates	Mann	Malt	Lac	Glu	Galac	Ribose	Sucrose	Xylose	Fruct	Arabi
OP1	-	+	-	+	-	-	+	-	+	-
OP2	-	+	-	+	-	-	+	-	+	-
OP3	-	+	-	+	-	-	+	-	+	-
OP4	-	+	-	+	-	-	+	-	+	-
RP1	-	+	-	+	-	-	+	-	+	-
RP2	-	+	-	+	-	-	+	-	+	-
RP3	-	+	-	+	-	-	+	-	+	-

Legend: (+): Positive reaction, (-): Negative reaction,

Mann- Mannose, Mal – Maltose, Lac- Lactose, Glu – Glucose, Galac- Galactose, Rib- Ribose, Suc- Sucrose, Xylose, Fruct- Fructose, Arabi- Arabinose; OP1, OP2, OP3, and OP4 are LAB isolates from oil palm wine and RP1, RP2, RP3 and RP4 are LAB isolates from Raffia palm wine.

3.3 Stress Response and Flocculation At 5% ethanol, all yeast isolates showed high tolerance (+++), while tolerance declined at 15% and ceased at 20% ethanol, affirming reports by Olowonibi [11]. Yeast flocculation, important for downstream processing, was strongest in YP2 and RY3 (+++). Temperature tolerance peaked at 30°C, with inhibition at 45°C, confirming findings by Willaert and Nedović [26].

Table 3.5: Stress Exclusion Tests Results Showing the Ethanol at different Concentrations, Temperature tolerance and Level of Flocculation of the Different Yeast Isolates.

Isolates	Ethanol at different Concentrations				Temperature tolerance			Level of Flocculation
	5%	10%	15%	20%	30°C	37°C	45°C	
YP1	+++	++	+	-	+++	++	-	+
YP2	+++	++	++	-	+++	+++	+	+++
YP3	+++	++	+	-	+++	++	-	++
YP4	+++	+++	+	-	+++	++	-	++
RY1	+++	++	+	-	+++	++	-	++
RY2	+++	++	-	-	+++	++	-	-
RY3	+++	++	++	-	+++	+++	-	+++
RY4	+++	++	-	-	+++	++	-	-

+++=Intensive response, ++=Moderate response, +=Low response, -=No response

3.4 Acid and Bile Salt Tolerance OP1 and RP2 demonstrated exceptional survival at pH 2.0 (89.11% and 90.21%, respectively) and at 0.3% bile salts (90.53% and 88.60%, respectively). These traits align with requirements for effective gastrointestinal probiotics [27, 28].

Table 3.6: Viability and survival percentages of seven LAB isolates incubated at 0.3% (w/v) Bile Salt at 37°C for 3h.

Isolates	Viable Cell. LogCFU/ml		Survival Rate %
	Without bile	0.3% bile	
OP1	8.45±0.04	7.65±1.72	90.53
OP2	8.40±0.02	6.15±0.04	73.21
OP3	8.46±0.04	5.26±1.17	62.17
OP4	8.38±0.01	5.30±1.08	63.25
RP1	8.32±0.00	6.20±0.02	74.52
RP2	8.42±0.03	7.46±1.25	88.60
RP3	7.38±0.77	6.34±0.00	85.90

Values are means of three measurements for each sample ± standard deviation

Table 3.7: Viability and survival percentages of Seven LAB isolates incubated at pH 2.0 at 37°C for 3h.

Isolates	Viable Cell. LogCFU/ml		Survival Rate %
	pH 7.0	pH 2.0	
OP1	8.36±0.00	7.45±1.00	89.11
OP2	8.30±0.00	6.15±0.09	74.10
OP3	8.32±0.00	6.11±0.12	73.44
OP4	8.26±0.00	5.30±1.32	64.16
RP1	8.28±0.00	6.41±0.00	77.46
RP2	8.38±0.00	7.56±1.23	90.21
RP3	8.28±0.00	6.20±0.06	74.88

Values are means of three measurements for each sample

3.5 Assessment of the ability of isolates to survive in simulated gastrointestinal juices.

An *in vitro* model was employed to assess survival under simulated gastrointestinal circumstances for two LAB and two yeast isolates. Figure 3.1 illustrates the impact of gastric and duodenal fluids on the viability of LAB and yeast isolates.

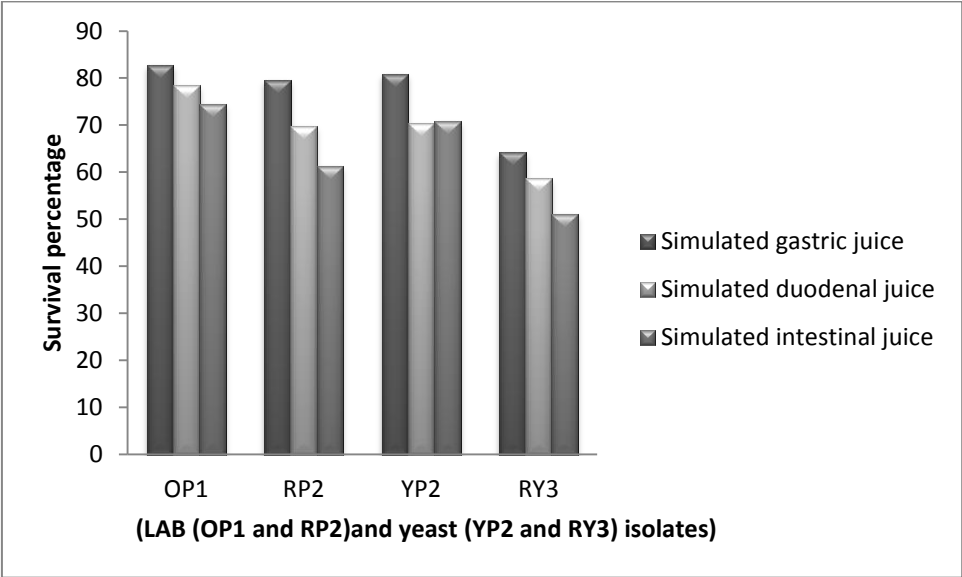


Figure 3.1: Survival of LAB (OP1 and RP2) and Yeast (YP2 and RY3) isolates under simulated gastrointestinal conditions.

3.6 Safety Evaluation and Antibiotic Susceptibility

In table 3.8 no hemolytic activity was observed for any isolates (γ -hemolysis), indicating non-pathogenicity, as corroborated by Fadda *et al.* [17]. LAB isolates were sensitive to all tested antibiotics in table 3.9 but resistant to antifungals, whereas yeast isolates were resistant to antibiotics and sensitive to antifungals, supporting findings by Syal and Vohra [30].

Table 3.8: Haemolytic Activity Assay of LAB and Yeast Isolates on Blood Agar

Isolates	Haemolytic Activity
OP1	Non- haemolytic
RP2	Non- haemolytic
YP2	Non- haemolytic
RY3	Non- haemolytic

OP1 and RP2 represents LAB isolates, while YP2 and RY3 isolates represents yeast isolates

Table 3.9. Antimicrobial Susceptibility Assay

Isolates	Antibacterials						Antifungals		
	Amox	Eryh	Cipro	Genta	Tetra	Levo	Nysta	Fluco	Itra
OP2	S	S	S	S	S	S	R	R	R
RP2	S	S	S	S	S	S	R	R	R
YP2	R	R	R	R	R	R	S	S	S
RY3	R	R	R	R	R	R	S	S	S

OP2 and RP2 are LAB isolates while YP2 and RY3 are yeasts isolate; Amox: amoxicillin, Ery: erythromycin, Cipro: ciprofloxacin, Genta: gentamicin, Tetra: tetracycline, Amp: ampicillin, Levo: levofloxacin, Fluco: fluconazole, Itra: itraconazole, Nyst: nystatin.

3.6 Molecular Identification 16S rRNA and 26S rRNA sequence analysis confirmed OP2 and RP2 as *Leuconostoc mesenteroides* (96% and 98% similarity) and YP2 and RY3 as *Saccharomyces*

cerevisiae (98%–99%). These taxa are widely documented in traditional and industrial fermentations [30, 31].

The study validates the biotechnological viability of *S. cerevisiae* and *L. mesenteroides* as robust starter cultures for palm wine fermentation. Their stress tolerance, acidification potential, flocculation capacity, and probiotic viability position them as candidates for commercial applications.

In contrast to spontaneous fermentation, the use of starter cultures ensures controlled microbial succession, consistent ethanol

production, and enhanced functional benefits [2, 22]. The compatibility of yeasts and LAB further enhances co-fermentation, improving flavour, shelf life, and consumer safety [27, 32].

Conclusion

The strategic application of *S. cerevisiae* and *L. mesenteroides* as starter cultures transforms palm wine from a variable traditional beverage into a standardized, health-promoting product. By harnessing their fermentative efficiency, probiotic potential, and bioactive output, palm wine can emerge as a functional beverage competitive in global markets. Collaborative efforts between microbiologists, food technologists, and indigenous communities will ensure these innovations respect cultural heritage while delivering measurable public health benefits. Future research should prioritize clinical trials to validate cholesterol-lowering and glycemic control claims, solidifying palm wine's place in the functional food revolution.

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