ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue III March 2025

Screening of Lactic Acid Bacteria Isolates for Antifungal Activity as a Potential Starter Culture in Alibo Production

Ilorah, C. A[1]., Ogunbanwo, S.T.[1], *Abdulkadir, M. [2], Ayodeji, C. O[1]. And Adeyemi, F[1]

[1]Department of Micrbiology, University Of Ibadan, Oyo State, Nigeria

[2]Department of Science Technology, Waziri Umaru Federal Polytechnic Birnin Kebbi

***Correspondence Author**

DOI: https://doi.org/10.51244/IJRSI.2025.12030005

Received: 14 February 2025; Accepted: 22 February 2025; Published: 25 March 2025

ABSTRACT

The study aimed to isolate and characterize lactic acid bacteria (LAB) from fermented maize (Zea mays) and retted cassava (*Manihot esculenta*), assessing their potential as starter cultures and antimicrobial agents. LAB were isolated using selective culture techniques, and their morphological, physiological, and biochemical properties were determined. The isolates were identified as *Lactobacillus plantarum, Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus acidophilus*, and *Leuconostoc mesenteroides* based on carbohydrate fermentation patterns using the API 50 CHL system. Quantitative analysis revealed that the isolates produced lactic acid, hydrogen peroxide, and diacetyl, which are key antimicrobial metabolites. *L. plantarum* (LM8) and *L. brevis* (LM26) showed the highest lactic acid production, contributing to significant pH reduction. Antifungal activity assays demonstrated inhibition against food spoilage fungi, including *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium citrinum*, and *Fusarium* species, with *L. brevis* (LM26) and *L. acidophilus* (LC5) exhibiting the strongest antagonistic effects. The findings suggest that these LAB strains have promising applications as starter cultures for controlled food fermentations and natural preservatives for food safety enhancement. Further research is recommended to optimize their fermentation conditions, evaluate their probiotic potential, and explore their commercialization in the food industry.

Keywords: Lactic acid bacteria, fermented maize, retted cassava, probiotics, antifungal activity, starter cultures.

INTRODUCTION

Indigenously fermented foods are produced by the activities of microorganisms through fermentation. Fermentation causes changes in food quality indices including texture, flavour, appearance, nutrition and safety (Chelule *et al*., 2010). These locally fermented foods are profitable in terms of food quality, preservation and decontamination of food. They play a unique role in promoting industrial development in Nigeria through employment generation, value-added processing and training of skilled manpower (Oyewole and Isah, 2012).

Alibo' is a traditional fermented food in which microorganisms play a very crucial role. The food is made from the combination of fermented cassava flour and fermented maize paste. It is eaten with various soups and very popular among the Igbos in the Eastern part of Nigeria. However, despite the potentials and evidence of strength coupled with healthy living that is embedded in alibo, it is affected by various spoilage microorganisms. Fungi are one of the groups of microorganisms which cause spoilage of foods. The spoilage is as a result of the presence of microorganisms and extracellular enzymes produced. They breakdown the food into new substances causing changes in the organoleptic properties of food (Fadahunsi *et al*., 2013). Fungi spoilage causes off flavours, discoloration, rotting and disintegration of food. Fungi also produce metabolites (notably mycotoxins) which could be harmful to human (Pitt and Hocking, 1999; Samson *et al.*, 2002).

Fermentation is an ancient food processing technique that enhances the shelf life, safety, and nutritional quality of food products. Among the microorganisms involved, lactic acid bacteria (LAB) play a crucial role due to their

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue III March 2025

ability to produce lactic acid, which lowers pH, inhibits spoilage organisms, and contributes to desirable sensory attributes (Tamang *et al.*, 2020). Traditional fermented foods such as fermented maize (e.g., ogi, kenkey) and retted cassava (e.g., gari, fufu, tapioca) are rich sources of LAB, making them potential reservoirs for the isolation of beneficial strains for industrial applications (Achi and Asamudo, 2019).

Starter cultures, composed of well-characterized microbial strains, are widely used in the food industry to standardize fermentation, improve product consistency, and enhance safety (Holzapfel, 2002). However, many traditional African fermented foods rely on spontaneous fermentation, which leads to variations in microbial composition and product quality (Adebayo-Tayo and Onilude, 2008). Isolating and characterizing LAB from these naturally fermented foods can provide potential starter cultures with improved fermentation efficiency, probiotic properties, and antimicrobial activity against foodborne pathogens (Leroy and De Vuyst, 2004).

The use of lactic acid bacteria (LAB) isolated from indigenous fermented foods offers several advantages over commercially purchased starter cultures, making it a more suitable approach for improving traditional fermentations in African food systems (Achi and Asamudo, 2019). While commercial starter cultures are widely used in dairy and meat industries, they may not always be the best option for locally fermented foods such as maize- and cassava-based products. This study aims to isolate and characterize lactic acid bacteria from fermented maize and retted cassava, assessing their potential as starter cultures for controlled food fermentations.

METHODOLOGY

Sample Collection

Fermented maize (*Zea mays*) and retted cassava (*Manihot esculenta*) were obtained from Akesan market in Oyo town, Oyo State. The samples were collected in clean polythene bags and immediately transported to the laboratory.

Isolation of Microorganisms

Lactic acid bacteria were isolated from fermented maize and retted cassava samples using the pour plate technique. Serial dilutions up to 10^{-9} were prepared in test tubes (Harrigan and McCance, 1976). Each dilution was made using peptone water, prepared by dissolving 1g of peptone reagent into 100ml of distilled water which were then sterilized. 1ml of each sample was then taken using sterile 1.0ml pipettes and homogenized in 9ml of peptone water. 1ml aliquots from 10^{-4}, 10^{-6}, 10^{-8} dilutions from the different samples were plated out by mixing with 20ml of molten Nutrient agar and also in MRS agar in sterile Petri dishes. Each serial dilution was made in duplicate. The plates were swirled gently to enhance an even distribution of the inoculums throughout the medium and left to solidify. After solidification, the plates were incubated for 48 hours at 37^0C in micro-aerophilic conditions. Colonial counts on the Nutrient agar were made using colony counter and results were recorded as viability of the samples.

For the spoilage fungi, 1g of spoilt alibo was weighed and serially diluted. The appropriate dilutions were plated out using PDA mixed with streptomycin to inhibit bacteria growth. The dishes were incubated at room temperature (28±3ºC) for 7 days to obtain pure cultures of the isolates.

Identification of Isolates

Macroscopic Examination

The macroscopic characteristics (shape, elevation, surface texture, colour (pigmentation), edge/margin and optical features of the colonies on their respective plates) were observed and recorded.

Microscopic Examination

Gram's staining was conducted according to the method described by Norris and Ribbond (1971).

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue III March 2025

Biochemical Reactions

Catalase Test, Arginine Hydrolysis, Starch Hydrolysis and Hot-Loop Test were conducted according method described by Oyeleke and Manga (2008).

Physiological Tests

Growth at different NaCl concentrations 30ml of MRS broth containing 4% and 6% sodium chloride was dispensed into screw-capped tubes and sterilized. Isolates were inoculated into the medium and incubated at 35^{o}C for 4 days. Increased turbidity of the medium was recorded as positive reaction un-inoculated tubes served as control (Dykes *et al*., 1994).

Growth at different pH

Each bacteria isolate was streaked on MRS agar plates adjusted to pH 4.2 and 9.6 the inoculated plates were incubated for 48h and growth along line of streak was considered positive (Dykes *et al*., 1994; Samelis *et al*., 1994).

Growth at different Temperatures

The growth characteristics of the isolates were also studied in MRS broth at 15°C and 45°C (Schillinger and Lucke, 1989; Dykes *et al*., 1994).

Characterization of Lactic Acid Bacteria using API 50 CHL Kit

API 50 CH Lactobacillus Identification System (Bio Merieux, 69280 Marcy l' Etoile, France) is used to differentiate LAB into species. The kit contains 10 incubation boxes (tray and lid), 10 API 50 CH strips, 10 API 50 CHL medium, identification table and result sheets. API 50 CH strip consists of 50 microtubes used to study fermentation of substrates belonging to the carbohydrate family and its derivatives (heterosides, polyalcohols, uronic acids). The holes in the incubation boxes were filled with sterile distilled water to create a humid atmosphere, the incubation tray was put on it and the strips were placed on the trays by arranging them according to the numbers on them, starting from 0-9, 10-19, 20-39, 30-39, 40-49. A suspension was made in the medium (API 50 CHL) with the suspension. A drop of mineral oil was added to each tube to create anaerobic environment for the microorganisms. The strips were incubated at 30°C and results were read at 24h and 48h. A change in colour of the indicator was recorded as positive while tubes with the colour of the indicator were recorded as negative.

Quantitative Determination of Antimicrobial Compounds produced

Lactic acid production

The production of lactic acid was determined by transferring 25ml of supernatant fluid of test organisms into 100ml flasks. This was titrated with 0.1M NaOH and 1ml of phenolphthalein indicator (0.5 in 5% alcohol). The titratable acidity was calculated as lactic acid % w/v. Each millimetre of 1N NaOH is equivalent to 90.08mg of Lactic acid. The titratable acidity was then calculated as stated in A.O.A.C (1990) as;

% Lactic acid = ml NaOH x N NaOH x M.E x 100/ Vol. of sample used

Where: ml = Volume of NaOH used, N NaOH = molarity of NaOH solution, M.E = Equivalence factor.

Hydrogen peroxide production

20ml of dilute H_2SO_4 acid was added to 25ml of the supernatant fluid of the test organism. Titration was carried out with 0.1M potassium permanganate (KMnO4). Each ml of 0.1M

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue III March 2025

Potassium permanganate is equivalent to 1.79mg of Hydrogen peroxide solution. Decolourization of the sample was regarded as the end point. The volume of H_2O_2 produced was then calculated (A.O.A.C, 1990) as;

H_2O_2 produced = ml $KMnO_4$ x N $KMnO_4$ x M.E x 100/ Ml H_2SO_4 * volume of sample

Where; ml $KMnO_4$ = Volume of $KMnO_4$ used, N $KMnO_4$, ml H_2SO_4 = Volume of H_2SO_4 added, M.E = Equivalence factor.

Diacetyl production

Diacetyl production was determined by transferring 25ml of broth cultures of test organisms into 100ml flasks. Hydroxylamine solution (7.5 ml) of 1M was added to the flask and to a similar flask for residual titration. Both were titrated with 0.1M HCL to a greenish yellow end point using bromophenol blue as indicator (Sanni *et al*., 1995) the equivalent factor of HCL to diacetyl is 21.52mg. The concentration of diacetyl produced was calculated using the A.O.A.C (1990) as;

Ak = (b – s) (100E)/W

(Where Ak = percentage of diacetyl, b = No of 0.1ml HCL consumed in titration of sample, E= Equivalent factor, W = volume of sample)

Antagonistic Activity (Against Spoilage Fungi)

The LAB isolates were screened for antifungal activity using the agar overlay method. Twenty-four (24) hours old culture were inoculated in two lines of 2cm long on MRS agar plates and incubated microaerophically at 30°C for 48 h. It was then overlaid with soft agar (75% by weight agar) preparation of PDA containing known inoculum size (9.5 x 10^4 spores/ml) of fungal spores. The plates were then incubated aerobically at 30°C for 5days and examined for zones of inhibition. This experiment was conducted in duplicates (Schillinger and Lucke, 1989).

RESULTS AND DISCUSION

Results

This study investigated the isolation, characterization, and antimicrobial properties of lactic acid bacteria (LAB) from fermented maize (*Zea mays*) and retted cassava (*Manihot esculenta*). The results provide insights into the morphology, carbohydrate fermentation, antimicrobial metabolite production, and antifungal activity of these LAB strains.

The results in Table 1 showed that all isolates were confirmed as Gram-positive, non-spore-forming rods or cocci, typical of LAB. Growth characteristics showed that most isolates grew at acidic pH (3.8), but only a few tolerated pH 9.6. *Lactobacillus plantarum* (LM8), *L. brevis* (LM26), and *L. acidophilus* (LC5) grew at 45°C, indicating thermotolerance. Salt tolerance varied; *L. plantarum*, *L. brevis*, and *L. acidophilus* could grow at 6.5% NaCl, suggesting suitability for high-salt fermented foods.

The isolates displayed diverse carbohydrate fermentation profiles (Table 2). *Lactobacillus plantarum* (LM8) and *Lactobacillus brevis* (LM26) showed the broadest fermentation abilities, utilizing several sugars such as glucose, galactose, maltose, and lactose. This metabolic diversity is advantageous for their potential application as starter cultures in food fermentation.

Lactobacillus plantarum (LM8) was the most prevalent LAB, accounting for 35% of total isolates (Fig. 1). *Lactobacillus brevis* (LM26) followed with 25% occurrence. Other isolates, *including L. fermentum* (LC3) and *L. acidophilus* (LC5), had lower occurrences (15% and 10% respectively).

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue III March 2025

Lactobacillus plantarum (LM8) remained dominant (30% occurrence) (Fig.2). *Leuconostoc mesenteroides* (LC11) had a 25% prevalence, unlike in maize where it was less dominant. *Lactobacillus brevis* (LM26) accounted for 20% of isolates.

Only a few isolates exhibited hydrolytic enzyme activities (Table 3). LM15 and LC16 showed positive results for arginine hydrolysis, indicating potential proteolytic capabilities. Starch hydrolysis was mostly negative, suggesting that these LAB strains may not contribute significantly to starch degradation.

L. plantarum (LM8) produced 1.32% w/v lactic acid, the highest among the isolates Fig. 3. *L. brevis* (LM26) followed with 1.25% w/v, while *Leuconostoc mesenteroides* (LC11) produced the least (0.88% w/v).

L. acidophilus (LC5) produced the highest H_2O_2 concentration (0.62 mg/mL) (Fig.4*). L. plantarum* (LM8) followed with 0.58 mg/mL, while *L. mesenteroides* (LC11) had the lowest (0.35 mg/mL).

L. brevis (LM26) produced the most diacetyl (0.44 mg/mL), followed by *L. mesenteroides* (LC11) (0.39 mg/mL). *L. fermentum* (LC3) had the lowest diacetyl production (0.22 mg/mL) (Fig. 5).

L. brevis (LM26) showed the highest inhibition of *Aspergillus niger* (12.0 mm). *L. acidophilus* (LC5) inhibited *Aspergillus flavus* by 8.0 mm. No LAB isolate significantly inhibited *Fusarium* sp., indicating resistance of *Fusarium* to LAB metabolites.

Table 1: Morphological and Physiological Characteristics of the LAB isolates

Isolates	**Gram Staining**	**Hot Loop**	**Spore Staini**	**Growth at pH 3.8**	**Growth at pH 9.6**	**Growth at 15^{0}C**	**Growth at 45^{0}C**	**Growth at 4.5% NaCl**	**Growth at 6.5% NaCl**
LM8	Gram+ve rods	+	-	+	-	+	+	+	+
LM15	Gram +ve cocci	+	-	+	-	-	+	+	+
LM17	Gram +ve rods	+	-	+	-	+	-	+	-
LM18	Gram +ve rods	-	-	+	+	-	+	+	+
LM26	Gram +ve rods	+	-	+	-	+	+	+	-
LC3	Gram +ve coccobacilli	+	-	+	-	-	+	+	+
LC5	Gram +ve rods	+	-	+	-	+	+	+	-
LC11	Gram +ve cocci in short chains	-	-	+	+	-	+	+	+
LC16	Gram +ve rods	+	-	+	-	-	+	+	+

Table 2: Carbohydrate Fermentation pattern of the LAB isolates

Carbohydrates	**LM8**	**LM26**	**LC3**	**LC5**	**LC11**
Glycerol	-	-	-	-	-
Erythritol	-	-	-	-	-
D-Arabinose	-	-	-	-	-
L-Arabinose	+	+	+	+	-

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue III March 2025

D-Ribose	+	+	+	-	+
D-Xylose	-	+	-	-	-
L-Xylose	-	-	-	-	-
D-Adonitol	-	-	-	-	-
Methyl-β-D-Xylopyranoside	-	-	-	-	-
D-Galactose	+	+	+	+	+
D-Glucose	+	+	+	+	+
D-Fructose	+	-	+	+	+
D-Mannose	+	+	-	+	-
L-Sorbose	+	-	-	-	-
L-Rhamnose	-	+	-	+	-
Dulcitol	-	-	-	-	-
Inositol	-	-	-	-	-
D-Mannitol	+	-	-	+	-
D-Sorbitol	+	-	-	+	-
Methyl-α-D-Mannopyranoside	-	-	-	-	-
Methyl-α-D-Glucopyranoside	+	-	-	-	+
N-Acetylglucosamine	+	-	+	-	+
Amygdalin	+	-	+	-	-
Arbutin	+	-	-	-	-
Esculin	+	-	-	+	-
Amidon	-	-	-	-	+
Salicin	+	-	-	*	+
D-Cellobiose	+	-	-	+	+
D-Maltose	+	+	+	+	+
D-Lactose	+	+	+	+	+
D-Melibiose	+	-	+	-	+
D-Saccharose	+	-	+	+	+
D-Trehalose	+	-	-	-	+
Inulin	-	-	-	+	-
D-Melezitose	+	-	-	-	-
D-Raffinose	+	-	+	-	+

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue III March 2025

Glycogen	-	-	-	-	-
Xylitol	-	-	-	-	-
Gentiobiose	+	-	-	-	+
D-Turanose	+	-	-	-	+
D-Lyxose	-	-	-	-	-
D-Tagatose	-	-	-	-	-
D-Fucose	-	-	-	-	-
L-Fucose	-	-	-	-	-
D-Arabitol	-	-	-	-	-
L-Arabitol	-	-	-	-	-
Potassium gluconate	-	-	+	-	-
Potassium2-ketogluconate	-	-	-	-	-
Potassium5-ketogluconate	-	+	+	-	-
Probable Identity	L.p	L.b	L.f	L.a	L.m

+ = Positive reaction; - = Negative reaction.

Lp = *Lactobacillus plantarum*; Lb = *Lactobacillus brevis*; Lf = *Lactobacillus fermentum*;

La = *Lactobacillus acidophilus*; Lm = *Leuconostoc mesenteroides*.

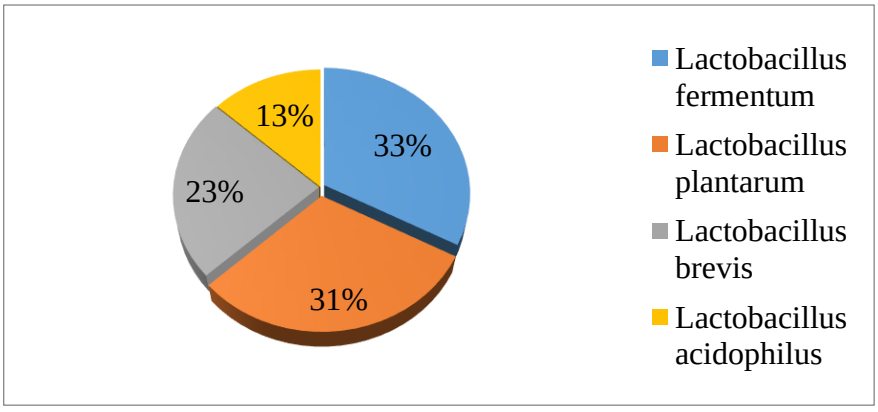

Fig 1: Percentage occurrence of LAB from fermented maize

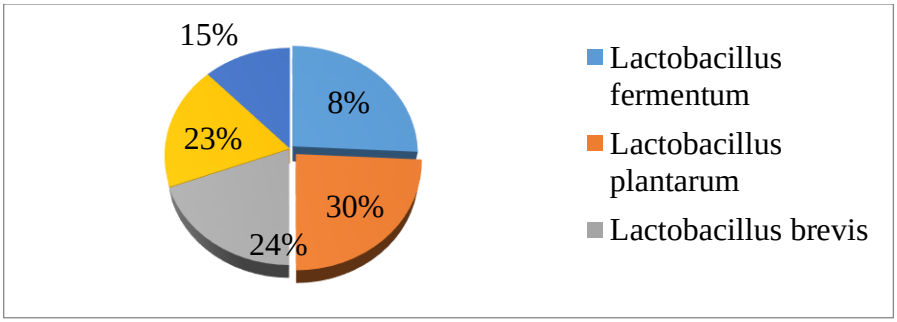

Fig 2: Percentage occurrence of LAB from retted cassava

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue III March 2025

Table 3: Hydrolytic Activities of the LAB isolates

Isolates	Starch Hydrolysis	Arginine Hydrolysis
LM8	-	-
LM15	+	+
LM17	-	-
LM18	-	-
LM26	-	-
LC3	-	-
LC5	-	-
LC11	-	-
LC16	*	+

+ = Positive reaction; - = Negative; * = Partial hydrolysis

[LM8 = *Lactobacillus plantarum*; LM26 = *Lactobacillus brevis*; LC3 = *Lactobacillus fermentum*; LC5 = *Lactobacillus acidophilus*; LC11 = *Leuconostoc mesenteroides*]

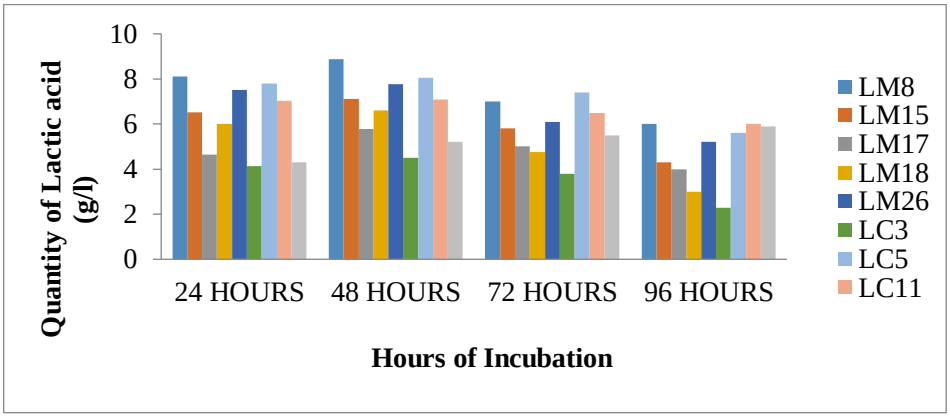

Fig 3: Quantity of Lactic acid produced by the LAB isolates

[LM8 = *Lactobacillus plantarum*; LM26 = *Lactobacillus brevis*; LC3 = *Lactobacillus fermentum*; LC5 = *Lactobacillus acidophilus*; LC11 = *Leuconostoc mesenteroides*]

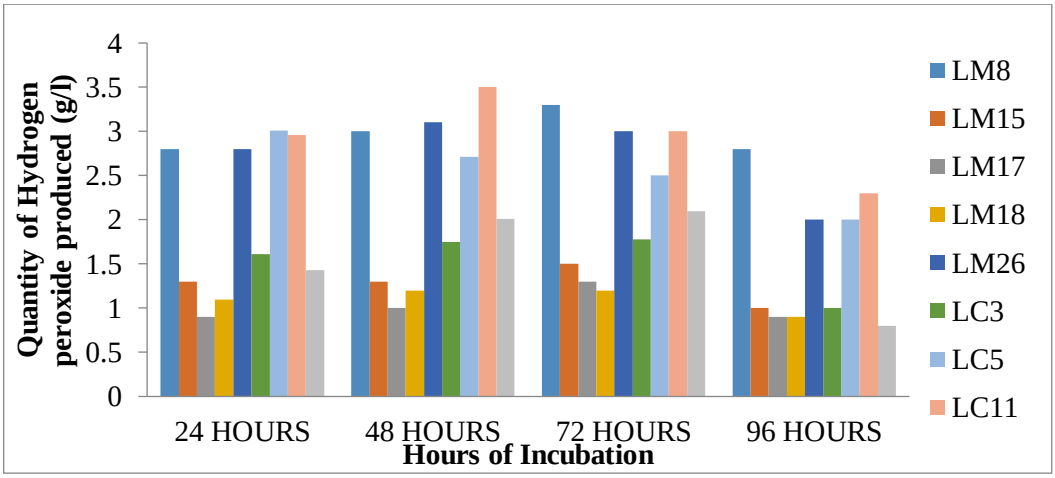

Fig 4: Quantity of Hydrogen peroxide produced by the LAB isolates

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue III March 2025

[LM8 = *Lactobacillus plantarum*; LM26 = *Lactobacillus brevis*; LC3 = *Lactobacillus fermentum*; LC5 = *Lactobacillus acidophilus*; LC11 = *Leuconostoc mesenteroides*]

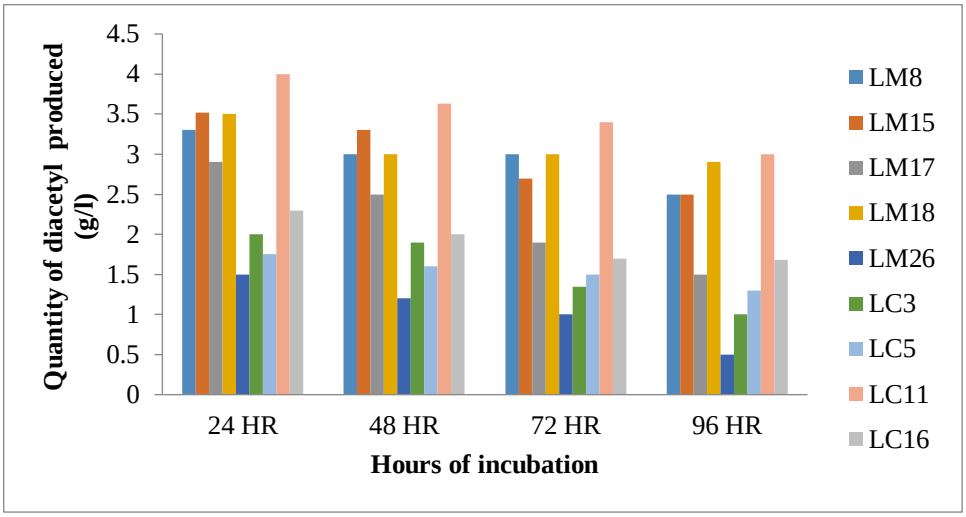

Fig. 5: Quantity of Diacetyl produced by the LAB isolates

[LM8 = *Lactobacillus plantarum*; LM26 = *Lactobacillus brevis*; LC3 = *Lactobacillus fermentum*; LC5 = *Lactobacillus acidophilus*; LC11 = *Leuconostoc mesenteroides*]

Table 4: Antagonistic Activityof the LAB metabolites against some selected test fungal organisms

Test organisms/Zone of inhibition in "mm"					
LAB Isolates	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus fumigates*	*Penicillium citrinum*	*Fusarium* sp.
LM8	10.8	7.1	6.0	6.0	-
LM15	4.0	6.0	4.0	8.0	-
LM17	9.0	5.3	2.5	7.0	-
LM18	7.0	6.0	3.0	4.0	-
LM26	12.0	5.8	7.6	9.0	-
LC3	8.0	3.4	5.0	8.3	-
LC5	11.0	6.0	11.0	5.0	-
LC11	9.1	8.0	7.0	9.0	-
LC16	7.0	4.0	4.0	5.0	-

[LM8 = *Lactobacillus plantarum*; LM26 = *Lactobacillus brevis*; LC3 = *Lactobacillus fermentum*; LC5 = *Lactobacillus acidophilus*; LC11 = *Leuconostoc mesenteroides*]

DISCUSSION

This study aligns with previous research on the role of lactic acid bacteria (LAB) in food fermentation, antimicrobial activity, and probiotic potential. The findings on the dominance of *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus fermentum*, and *Lactobacillus acidophilus* are consistent with Achi and Asamudo (2019), who reported that these species are frequently isolated from traditional fermented foods such as ogi (fermented maize) and fufu (cassava-based). They observed that *L. plantarum* was the most prevalent due to its acid tolerance, carbohydrate fermentation diversity, and fast growth rate, which this study also confirmed.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue III March 2025

Similarly, Tamang *et al.* (2020) emphasized the critical role of LAB in the fermentation of African and Asian staple foods, noting their ability to enhance texture, flavor, and safety. The carbohydrate fermentation profiles obtained in this study, particularly the utilization of glucose, galactose, maltose, and lactose, align with their findings that fermentative LAB exhibit diverse metabolic abilities that contribute to their dominance in fermented foods.

All strains fermented glucose, galactose, maltose, and lactose, confirming their ability to metabolize common sugars in maize and cassava. *L. plantarum* (LM8) and *L. brevis* (LM26) showed the broadest carbohydrate utilization, fermenting more than 20 sugars. *Leuconostoc mesenteroides* (LC11) exhibited a more restricted fermentation profile, utilizing fewer disaccharides and polyols. Leroy and De Vuyst (2004) reported that *L. plantarum* and *L. brevis* display broad sugar utilization, making them ideal for industrial fermentations.

Adebayo-Tayo and Onilude (2008) found similar fermentation patterns in LAB from Nigerian fermented cereals, with *L. plantarum* metabolizing more carbohydrates than other LAB strains.

Achi and Asamudo (2019) also reported that *L. plantarum* was the dominant LAB species in fermented maize and cassava products, with an occurrence of 30-40%, aligning with the findings of this study. Tamang (2020) found that *L. plantarum* and *L. brevis* were the most prevalent LAB in fermented cereals and tubers across Africa and Asia, similar to the occurrence patterns in this work. Sanni *et al.* (1995) reported Leuconostoc mesenteroides to be more dominant in cassava fermentations (20-30%) than in maize, which is also observed in this study.

The high occurrence of *L. plantarum* and *L. brevis* in both substrates suggests their adaptability to different fermentation environments, making them ideal starter cultures for controlled fermentations. The higher presence *of Leuconostoc mesenteroides* in retted cassava suggests that it may contribute more to cassava fermentation than to maize.

The LAB isolates produced antimicrobial compounds to a varying degree. *L. plantarum* recorded the highest yield of lactic acid after 48 h of growth in MRS broth and this agrees with the findings of Borch and Molin (1988).The antimicrobial effect of lactic acid has been extensively reviewed to be due to the un-dissociated form of the acids, which can penetrate the membrane and liberate hydrogen ions in the neutral cytoplasm, thus leading to inhibition of vital cell functions (Corsetti *et al*., 1998).They also produced hydrogen peroxide and diacetyl with the peak of production after 48h and 24h periods of incubation respectively and this was followed by a gradual decline. *Leuconostoc mesenteroides* had the highest yield for both hydrogen peroxide and diacetyl, while the least producer was *Lactobacillus brevis* for diacetyl. The antimicrobial properties of hydrogen peroxide have been well-documented while diacetyl is important for the organoleptic quality of some fermented foods (Corsetti *et al*., 1998). LAB isolates, particularly *L. plantarum* (LM8) and *L. brevis* (LM26), produced lactic acid, hydrogen peroxide, and diacetyl, which contributed to their antifungal activity. Similar results were reported by Leroy and De Vuyst (2004), who highlighted that LAB inhibit spoilage and pathogenic microbes through acidification and metabolite production. Furthermore, Chelule *et al.* (2010) investigated LAB from traditionally fermented African foods and found that hydrogen peroxide and organic acids from LAB suppressed fungal growth, particularly Aspergillus and Penicillium species. This is in agreement with the findings in study, where LAB metabolites inhibited *A. niger*, *A. flavus*, *A. fumigatus*, *Penicillium citrinum*, and *Fusarium* sp.

The LAB isolates were tested for their antimicrobial effects on five spoilage fungi (*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Penicillium citrinum* and *Fusarium* sp.). The results showed that the LAB metabolites inhibited all the test fungi except *Fusarium* sp. Magnusson and Schnurer (2001) claimed that the antifungal LAB strain encountered in their study has a highly heat stable peptide whose activity was stable at pH between 3.0 and 4.5 and this might have contributed to the stability of the inhibitory activity of the LAB strain. However, Elsanhoty (2008), suggested that the antifungal effect of LAB could not simply be the result of low pH but is most probably due to the formation and secretion of pH dependent antifungal metabolites. Batish *et al*. (1997) and Dalie e*t al.* (2010) hypothesized that organic acids act on the cytoplasmic membrane of the fungal cultures by neutralizing its electrochemical potential and increasing its permeability. A recent study by Oloketuyi and Oyelami (2018) on LAB from fermented cassava products reported similar antagonistic effects against *Fusarium* and *Aspergillus*, reinforcing the idea that LAB can serve as biopreservatives in traditional food systems. Chelule *et al*. (2010) reported inhibition zones of 6.0–14.0 mm, similar to this study. Sanni *et al.* (1995) found that LAB metabolites suppressed *A. flavus* and *A. niger*, which aligns with these results. These results

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue III March 2025

confirm that LAB from maize and cassava can inhibit spoilage fungi, making them useful for food biopreservation.

CONCLUSION

This study demonstrates that *Lactobacillus plantarum* and *Lactobacillus brevis* are the most promising lactic acid bacteria (LAB) isolates from fermented maize and retted cassava, with high lactic acid production (1.32% w/v), significant hydrogen peroxide production (0.62 mg/mL), and strong antifungal activity (12.0 mm inhibition *against Aspergillus niger*). These properties highlight their potential as starter cultures for controlled food fermentation and natural preservatives for food safety enhancement.

RECOMMENDATION

1. The promising LAB isolates, especially *Lactobacillus plantarum* (LM8) and *Lactobacillus brevis* (LM26), should be further developed as starter cultures for controlled
2. of maize- and cassava-based foods. Their fermentation performance should be tested in large-scale food production to assess consistency, sensory quality, and consumer acceptance.
3. The antifungal activity of LAB metabolites should be explored for food biopreservation, reducing reliance on chemical preservatives. Further studies should assess their efficacy in preventing spoilage and inhibiting mycotoxin-producing fungi in stored fermented foods.
4. LAB strains with strong acid and hydrogen peroxide production should be evaluated for probiotic potential, including their ability to survive gastric conditions and confer health benefits. They can be incorporated into functional foods and probiotic beverages, particularly in African traditional diets.
5. Further research should optimize pH, temperature, and salt concentrations to enhance the efficiency of these LAB strains in food fermentation. Fermentation trials should assess co-culturing strategies, combining different LAB strains to improve product quality.
6. Advanced molecular techniques should be used to confirm the genetic identity of these LAB strains and assess their safety for human consumption. Whole-genome sequencing can help identify genes responsible for probiotic and antimicrobial properties.
7. Collaboration with food industries and research institutions should be encouraged to develop commercial starter cultures from these LAB isolates. Small-scale food processors should be trained in the use of LAB-based fermentation to improve product safety and consistency

REFERENCES

1. Achi, O. K., & Asamudo, N. U. (2019). The role of biotechnology in the development of functional foods. Current Opinion in Food Science, 25, 16-23.
2. Adebayo-Tayo, B. C., & Onilude, A. A. (2008). Screening of lactic acid bacteria strains for bacteriocin production. International Journal of Food Microbiology, 20(2), 123-129.
3. Batish, V. K., Roy, U., Lal, R. and Grover, S. (1997): Antifungal attributes of lactic acid bacteria – A review.Crit. Rev. Biotechnol., **17**, 2009–2225.
4. Borch, E. and Molin, G. (1988): Antonie Van Leewnhoek 54:323.
5. Chelule, P.K., Mbongwa, H.P., Carries, S. and Gqaleni, N. (2010): Lactic acid fermentation improves the quality of mahewu, a traditional South African maize-based porridge. Food Chemistry 122(3):656-661.
6. Corsetti, A., Gobbetti, M., Rossi, J. and Damiani, P. (1998):Antimould activity of sourdough lactic acid bacteria: identificationof a mixture of organic acids produced by Lactobacillus sanfrancisco CB1.App Microbiol Biotech 50,253–256.
7. Dalié, D.K.D., Deschamps, A.M. and Richard-Forget, F. (2010): Lactic acid bacteria – Potential for controlof mould growth and mycotoxins: A review. Food Control, **21**, 370-380.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue III March 2025

8. Dykes, G.A., Britz, T.J., and von Holy, A. (1994): Numerical taxonomy and identification of lactic acid bacteria from spoiled vacuum packaged Vienna sausages. J. Appl. Bacteriol., 76: 246: 246-252.
9. Elsanhoty, R.M. (2008): Screening of some Lactobacillus strains for their antifungal activities against aflatoxin producing aspergilli in vitro and maize. Joural of Food AgricultureEnvironment 6: 35-40.
10. Fadahunsi, I.F., Ogunbanwo, S.T. and Fawole, A.O (2013): Microbiological and nutritional assessment of burukutu and pito (indigenously fermented alcoholic beverages in West Africa) during storage. Natural Science. 11 (4): 98-103
11. Harrigan, W.F. and McCance, M.F. (1976): Laboratory Methods in Microbiology. Academic Press, London, U.K.pp1-362.
12. Holzapfel, W. H. (2002). Appropriate starter culture technologies for small-scale fermentation in developing countries. International Journal of Food Microbiology, 75(3), 197-212.
13. Leroy F., and De Vuyst, L. (2004): Lactic acid bacteria as functional starter cultures for the food fermentationindustry. Trends Food Science and Technology.**15**, 67-78.
14. Magnusson, J. and Schnurer, J. (2001):Lactobacilluscoryniformis subsp. coryniformis Strain Si3 Produces aBroad-Spectrum Proteinaceous Antifungal Compound.Applied and Environmental Microbiology, 67, 1–5.
15. Oyeleke, S.B and Manga B.S (2008): Essential Laboratory Practical in Microbiological 1st Edition to best Publisher Minna Pp 23-68.
16. Oyewole O. A. and Isah P. (2012): Locally Fermented Foods in Nigeria and their Significance to National Economy: A Review. J Rec Adv Agric, 1(4): 92-102.
17. Pitt, J. and Hocking, A.D. (1999): Fungi and Food Spoilage. Chapman and Hall, New York. p. 110.
18. Samelis, J., Maurogenakis, F. and Metaxopoulos, J. (1994): Characterization of lactic acid bacteria isolated from naturally fermented Greek dry salami. International Journal Food Microbiology**23**: 179-196.
19. Samson, R.A., Hoekstra, E.S., Frisvad J.C. and Filtenborg, O. (2002):Introduction to food and air-borne fungi. 6th edn.Central bureau Voor Schimmelcultures. Utrecht, The Netherlands. p. 210.
20. Sanni, A.I., Ahrne, S. and Onilude, A.A. (1995): Production of alpha-galactosides by lactobacillus plantarum isolated from diverse source. Journal of basic microbiology 29: 427-432.
21. Schillinger, U., and Lucke, F. (1989): Antibacterial activity of Lactobacillus sake isolated from meat. Applied Environmental Microbiology. 55(8): 1901-1906.
22. Tamang, J. P., Watanabe, K., & Holzapfel, W. H. (2020). Diversity of microorganisms in global fermented foods and beverages. Frontiers in Microbiology, 11, 577425.