

Original Research Article

Isolation, identification and characterization of potential probiotics from fermented food products

Abstract

Potential probiotics were isolated, identified and characterised from ogi, fufu, nunu, palmwine and fermented tigernut milk. Pour plate method was used for the isolation of lactic acid bacteria (LAB) on De Man Rogosa Sharpe (MRS) media. Three (3) pure colonies were distinctly obtained from each of the fermented food sources with microbial mean counts ranging from 8.13 - 8.25 Log CFU. Isolated strains were identified and characterized using morphological, API-50 CHL (Bio-merieux, France) and molecular PCR analysis. Ten (10) of the isolated microorganisms were identified as Lactobacilli, two (2) Micrococci and a Lactococcus. The Lactobacilli were catalase negative and oxidase negative rod-shaped bacteria. The identification accuracy of the isolates based on similarities from the computer-aided API and PCR GenBank databases ranged from 49.00 - 99.90% and 79.00 - 99.00% respectively. The amplification pattern of the 16S regions of the sequenced isolates showed DNA fragments with 500 - 1000 base pairs. The LAB strains identified are *Lactobacillus fermentum* NBRC 15885, *Leuconostoc mesenteroides* LM, *Lactobacillus plantarum* CIP 10315.1, *Lactobacillus plantarum* NBRC 15891, *Lactobacillus parabuchneri* LMG 11457, *Lactobacillus pentosus* 124-3 and *Lactobacillus brevis* ATCC 14869. These strains had high correlation in both the API and PCR identification techniques that was used in this study. Potential probiotic lactic acid bacteria can be isolated and identified from ogi, fufu, nunu, palmwine and fermented tigernut milk.

Keywords: potential probiotics, LAB, fermented food, isolates, Lactobacillus, API, PCR

1.0 Introduction

Probiotics are live microbial cultures which when consumed by humans can beneficially affect health by improving the original intestinal microbiota (Sathyabama *et al.*, 2014; Aslam and Qazi 2010). Some lactic acid bacteria (LAB) are probiotics while others may be potential probiotics or just fermentation cultures that are widely distributed in nature and can be used in the food industry (Ricci *et al.*, 2019). LAB are group of microorganisms consisting of gram-positive, aerotolerant, acid-tolerant, usually non-sporulating and non-respiring rods or cocci bacteria. Some play important roles in the fermentation of foods and have earned the GRAS status (generally regarded as safe). Several in vitro studies have shown that the growth of food-borne

pathogenic microbes was inhibited by probiotic LAB (Dowarah *et al.*, 2018; Tesfaye *et al.*, 2011; Tadesse *et al.*, 2005).

Moreover, LAB can metabolize host prebiotics, elicit immunomodulatory activities and possess cholesterol-reducing abilities (Mokoena *et al.*, 2016; Di Cagno *et al.*, 2013; Vitalli *et al.*, 2012). The therapeutic evidence of probiotics in the prevention and treatment of health problems have also been demonstrated. These include, alleviation of lactose intolerance, protection against gastrointestinal infection, stimulation of immune system, lowering of serum cholesterol and anti-allergic qualities, and prevention of urogenital diseases (Parmjit, 2011; Carlos *et al.*, 2010).

LAB can be isolated from different food sources such as fruit, vegetables, juices, grain products and fermented foods. Recent studies in Nigeria have shown that some strains of LAB isolated from fermented foods display attributes desirable for probiotic culture. Ngene *et al.*, (2019) isolated *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus casei* and *Lactococcus lactis* from ogiri, yoghurt, and ugba. David *et al.*, (2019) isolated *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus fabifermentan* and *Bacillus species* from fermented corn gruel (ogi) and fermented milk (nono). Berebon *et al.*, (2018) reported that eighteen (18) potential probiotics were isolated from locally fermented food products (akamu, Aqua Rafa® yoghurt, ogiri, okpeye, and kunu) in Enugu state, Nigeria. Olokun *et al.*, (2018) in a study carried out to produce fermented drink from milk extract of tigernut, isolated *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus lactis* and *Streptococcus thermophilus* from locally fermented milk (nono). Obinna-Echem *et al.*, (2014) isolated *Lactobacillus plantarum* strains from fermented maize (ogi).

However, it is very important to carefully select potential probiotic strains that could be used as starter cultures in food processing. This study was therefore aimed at isolation, identification and characterization of potential probiotics from fermented food sources.

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The need to develop alternatives to lactic acid fermented dairy products necessitated this study

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2.0 Materials and Methods

2.1 Materials

Fresh cow milk was sourced from Fulani settlements at Aluu in Obio-Akpor Local Government Area, Rivers State, Nigeria. Fresh palmwine was sourced from tappers at Mgbueto, Emohua Local Government Area, Rivers State. The yellow variety of maize seed, fresh and dried yellow varieties of tigernuts, and fresh cassava roots were purchased from hawkers at Aluu market in Ikwerre Local Government Area, Rivers State. All samples were collected in sterilized containers/polyethene bags and transported to the Food Science Laboratory in the Department of Food Science and Technology, Rivers State University, Port-Harcourt. Soybean (Tax 1448-2E) were obtained from IITA (International Institute of Tropical Agriculture), Ibadan, Oyo State, Nigeria. These samples were collected in sterilized polythene bags and transported to the Food Processing Laboratory in Federal Institute of Industrial Research Oshodi (FIIRO), Lagos for further processing. Analytical grade chemicals were procured for this study.

2.2.1 Preparation of fermented maize gruel (ogi)

Fermented maize (ogi) was prepared according to the method described by Akin-Osanaiye and Kamalu, (2019). One kilogram of the yellow variety of maize grain was used for this study. The grains were sorted and washed with portable water. The clean grains were soaked in portable water for 48 h, followed by wet-milling and sieving to remove bran, hulls and germ. The filtrate was fermented for 24 h at ambient temperature to yield ogi. This was decanted and stored in a covered container, and used for isolation of LAB.

2.2.2 Preparation of fermented cassava (fufu)

Fermented cassava (fufu) was prepared using the method described by Ayodeji *et al.*, (2017). Two (2) kilograms of the white variety of fresh cassava tubers were sorted, peeled and properly washed with portable water. The clean tubers were cut into smaller sizes and fermented for 24 h at ambient temperature. The resulting soft fermented cassava roots were hand pulverized and sieved using sieve of about 1-mm aperture. The sieved mash was allowed to sediment for 24 h

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and decanted. The resulting wet mash (fufu) was stored and used for the isolation of lactic acid bacteria.

2.2.3 Preparation of fermented tigernut milk

Fermented tigernut milk was produced using the method described by Belewu and Abodunrin (2008) and Wakil *et al.* (2014). About 1 kg of fresh yellow variety of tigernut tubers were sorted and washed with portable water. The clean grains were soaked in 2 litres of portable water for 24 h. Thereafter, they were washed, wet-milled and filtered with double-lined cheese cloth to separate the milk from the insoluble chaff. Spontaneous fermentation was carried out for 18 h by the natural flora of the milk at ambient temperature ($25 \pm 2^{\circ}\text{C}$). The fermented milk was stored and used for isolation of LAB.

2.3 Total counts of LAB from the fermented foods

Isolation of lactic acid bacteria was carried out using the method described by Vantsawa *et al.*, (2017) with slight modification. 10 ml of each fermented samples was introduced into 90 mls of sterilised peptone water and homogenised. Serial dilutions (10^{-1} - 10^{-6}) were performed and 0.1 ml aliquot of the appropriate dilution was directly inoculated in duplicate on solidified MRS agar plates mixed with Nystatein to inhibit yeast growth. The plates were incubated for 48 h at 45°C under aseptic anaerobic conditions using anaerobic jars. Thereafter, the numbers of microbial colonies were counted and the total viable count was calculated using:

$$\frac{\text{Average counts of microbial colonies}}{(\text{Dilution plated}) \times (\text{ml plated})} = \text{Log CFU/ml}$$

2.4 Isolation and purification of LAB

Colonies with distinct morphological characteristics such as colour, size, and shape were isolated from the MRS agar plates as presumptive lactic acid bacteria isolates. These isolates were purified by repeated streaking on solidified MRS agar plates according to the method described by Mahantesh *et al.*, (2010). The purified isolates were streaked on MRS agar slants and stored at 4°C for further analyses.

2.5 Preliminary Identification of LAB

Preliminary identification of strains obtained in pure cultures were based on gram reaction, catalase production, and oxidase reaction. Macroscopic appearance of all the colonies was examined for cultural and morphological characteristics. Their sizes, shapes, colours, and texture were examined and recorded.

2.5 Carbohydrate fermentation profile of LAB isolates

The result obtained in the preliminary method above was subjected to biochemical test using the API 50 CHL system (Biomérieux, France) biochemical test kit for *Lactobacillus* (Biomérieux, 2005). The API 50 CHL carbohydrate identification kit contain 50 biochemical tests. The kit is made up of 5 small strips, containing 10 wells with different carbohydrate substrates. The inoculum was prepared according to the manufacturer's instruction. Pure LAB culture was aseptically transferred from the MRS agar into the API Suspension Medium ampoule (2.0 mL) using sterile swab. The suspension was mixed and 350 µL was transferred to a second API Suspension Medium ampoule (5.0 mL) to reach turbidity equivalent to McFarland standard # 2. The final inoculum was prepared by transferring 700 µL from the initial bacterial suspension (API Suspension Medium ampoule, 2.0 mL) into an API 50 CHL Medium (10.0 mL). The suspension was mixed and 150 µL (inoculated API 50 CHL medium suspensions) was measured into the well using sterilized micropipette and covered with 50 µL mineral oil. The strips were incubated (Mettler, Germany) at 37°C for 48 h. After the incubation, each well was observed for colour changes. The positive result was confirmed by the change of colour of bromocresol purple indicator from purple to yellow. The first well on the strip was used as a control. No change in the colour indicated negative result. The result was analyzed using api-web™ identification software database (Biomérieux, France, V 5.1) to identify *Lactobacillus* species.

2.6 Molecular identification of isolated LAB strains using 16S rRNA gene sequencing

2.6.1 Genomic DNA extraction

Five ~~millilitres~~milliliter of an overnight broth culture of the bacterial isolates in Luria Bertani (LB) broth were spun at 14000 rpm for 3 min. The cells were re-suspended in 500 µl of normal saline and heated at 95°C for 20 mins. The heated bacterial suspension were cooled in ice and spun for 3 mins at 14000 rpm. The supernatant containing the DNA was transferred to 1.5 ml microcentrifuge tube and stored at -20°C for other downstream reactions.

2.6.2 DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was ~~la~~unched by double clicking on the Nanodrop icon. The equipment was initialized with 2 µl of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the “measure” button.

2.6.3 PCR amplification of 16S rDNA

PCR was carried out to identify LAB using the primer pair BSF-8 (AGAGTTTGATCCTGGCTCAG) and BSR - 534 (ATTACCGCGGCTGCTGGC). The PCR reaction was carried out using the Solis Biodyne 5X HOT FIREPol Blend Master mix. The PCR was performed in 25 µL of a reaction mixture with concentration reduced from 5X to 1X (this contains 1X Blend Master mix buffer (Solis Biodyne). The reaction mixture was done according to standards (1.5 mMol MgCl₂, 200 µM of each deoxynucleoside triphosphates (dNTP) (Solis Biodyne), 25pMol of each primers (BIOMERS, Germany), 2 units of Hot FIREPol DNA polymerase (Solis Biodyne), Proof~~_~~reading Enzyme, 5 µL of the extracted DNA, and sterile distilled water). Thermal cycling was conducted in an Eppendorf Vapo protect thermal cycler (Nexus Series) for an initial denaturation of 95°C for 15 min. This was followed by 35 amplification cycles of 30 s at 95°C, 1 min at 58°C and 1.5 min at 72°C. Thereafter, a final

extension step of 10 min at 72°C was done. The amplification product was separated on 1.5% agarose gel and electrophoresis was carried out at 80 V for 1.5 h. After the electrophoresis, DNA bands were visualized by ethidium bromide staining and 100 bp DNA ladder was used as DNA molecular weight standard.

2.6.4 Sequencing of the PCR products

The PCR products were subjected to Sanger sequencing at Epoch Life science (USA). The sequencing was done at a final volume of 10 µl, the components included 0.25µl Big_Dye® terminator v1.1/v3.1, 2.25 ul of 5X Big_Dye sequencing buffer, 10µM PCR primer, and 2-10ng PCR template per 100bp. The sequencing condition were as followed: 32 cycles of 96°C for 10 secs, 55°C for 5 secs and 60°C for 4 mins.

2.7 Statistical analysis

All experiments were done in three replicates and data obtained from analysis were computed in Microsoft excel spreadsheet and used to express data as Mean \pm SD. The data obtained was analyzed using Minitab Release 18.1 statistical software to compare means. The significant difference between the means was analyzed using Fisher pairwise Test. All statistical tests were performed at 5% significant level.

3.0 Results and Discussions

3.1 LAB counts in selected fermented foods

In this study, pure cultures of microorganisms were isolated from ogi, fufu, nunu, palmwine and fermented tigernut milk. Three (3) isolates were obtained from each of these food sources with microbial counts ranging from 8.13^b Log CFU/ml in fufu to 8.25^a Log CFU/ml in nunu (Table 1). There were no significant ($P>0.05$) differences in the total LAB counts of the isolates obtained from ogi, nunu and fermented tigernut milk. However, there was significant ($P<0.05$) differences in the total counts obtained from fufu and palmwine. This study revealed high total viable counts which may be attributed to the availability of some easily metabolizable substances in the fermented food products that enhanced microbial growth. The ability of LAB

to generate high levels of lactic acids, increasing the anaerobic condition of the fermenting medium and favouring the growth of only facultative anaerobes can also increase the counts. It may also have been as a result of the antifungal activity of the nystatein to inhibit the growth of yeast in the medium, encouraging LAB growth.

Table 1: LAB counts (cfu/ml) in selected fermented foods

S/No	Food source	Number of isolates	LAB counts (Log CFU/ml)
1.	Fermented maize (ogi)	3	8.23 \pm 0.03 ^a
2.	Fermented cassava (fufu)	3	8.13 \pm 0.07 ^b
3.	Fermented tigernut	3	8.23 \pm 0.04 ^a
4.	Palm wine	3	8.19 \pm 0.03 ^{ab}
5.	Fermented cow milk (nono)	3	8.25 \pm 0.04 ^a

Values are means of triplicate LAB counts \pm standard deviation

Means with the same superscript in the same column do not differ significantly (P > 0.05).

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3.2 Morphological and physiological characteristics of isolated microorganisms

The microorganisms isolated in this study had various distinct colonial morphologies ranging from cream to white colonies. The colonies are small, smooth and grainy-looking with flat or raised elevation. They appear clear or opaque in the medium as presented in Table 2. Based on these morphological characteristics, 10 *Lactobacilli* species, 2 *Micrococci* and a *Lactococcus* species were isolated, as well as, 2 *Micrococci* and a *Lactococcus* species. This finding agrees with Arimah *et al.*, (2014) who isolated *Lactobacillus*, *Leuconostoc* and *Lactococcus* species from fura, wara and nono. Similarly, Nkemnaso, (2018) isolated *Lactobacillus*, *Lactococcus* and *Leuconostoc* species from palmwine. —David *et al.*, (2019) isolated both *Lactobacilli* and *Bacillus* species from ogi and nono (David *et al.*, 2019). —Obinna-Echem *et al.*, (2014) isolated *Lactobacillus* strains from fermented maize (ogi) (Obinna-Echem *et al.*, 2014). The ten

presumptive *Lactobacilli* species isolated in this study were gram positive, catalase negative, and oxidase negative rod-shaped bacteria. The higher prevalence of this rod-shaped LABs in this study is similar to the work of Nwokoro and Chukwu, (2012) who reported that the genus *Lactobacilli* commonly predominates during fermentation of plant-based foods (Nsofor *et al.*, 2014; Nwokoro and Chukwu, 2012). Lactobacilli have great economic importance due to their status as generally recognized as safe (GRAS) bacteria (Pyar and Peh, 2013; Widyastuti *et al.*, 2014). They have been used as starter cultures in food processing and as probiotics, health-promoting bacteria (Solieri *et al.*, 2014).

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Table 2: Morphological and physiological characteristics of isolated Microorganisms

Isolate Code	Form	Elevation	Margin	Opacity	Colour	Gram Reaction	Catalase	Oxidase	Shape	Probable ID
FM1	Small Circular	Flat	Entire	Opaque	White	+ve	-ve	-ve	Discrete tiny rods	<i>Lactobacillus spp.</i>
FM2	Small Circular	Raised	Entire	Clear	Cream	+ve	-ve	-ve	Discrete tiny rods	<i>Lactobacillus spp.</i>
FM3	Medium Circular	Raised	Entire	Clear	Cream	+ve	+ve	+ve	Clustered cocci	<i>Micrococcus spp.</i>
FC1	Small Circular	Flat	Entire	Opaque	White	+ve	-ve	-ve	Tiny rods	<i>Lactobacillus spp.</i>
FC2	Big Circular	Convex	Undulated	Opaque	White	+ve	-ve	-ve	Long slender rods	<i>Lactobacillus spp.</i>
FT1	Small Circular	Flat	Entire	Opaque	White	+ve	-ve	-ve	Tiny rods	<i>Lactobacillus spp.</i>
FT2	Medium Circular	Raised	Entire	Clear	Cream	+ve	-ve	-ve	Discrete rods	<i>Lactobacillus spp.</i>
FP1	Small Circular	Raised	Entire	Opaque	White	+ve	-ve	-ve	Short tiny rods	<i>Lactobacillus spp.</i>
FP2	Medium Circular	Raised	Entire	Clear	Cream	+ve	-ve	-ve	Discrete tiny rods	<i>Lactobacillus spp.</i>
FP3	Small Circular	Raised	Entire	Clear	Cream	+ve	-ve	-ve	Cocci	<i>Lactococcus spp.</i>
FCm1	Medium Circular	Raised	Entire	Clear	Cream	+ve	-ve	-ve	Tiny rods	<i>Lactobacillus spp.</i>
FCm2	Small Circular	Raised	Entire	Opaque	White	+ve	-ve	-ve	Tiny rods	<i>Lactobacillus spp.</i>
FCm3	Big Circular	Flat	Entire	Opaque	Yellowish	+ve	+ve	+ve	Cocci	<i>Micrococcus spp.</i>

FM = Fermented maize (ogi); FC = Fermented cassava (fufu); FT = Fermented tigernut; FP = Fermented palmwine; FCm = Fermented cowmilk (nunu)

3.3 Carbohydrate fermentation profile of LAB isolates using API 50 CHL

The result of the carbohydrate fermentation profiles of the isolated LAB using the API 50 CHL tests are presented in Table 3. All the isolates showed negative results for erythritol, D-arabinose, L-xylose, adonitol, β -metil-D-xiloside, dulcitol, inositol, amidon, glycogen, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose and 2-keto-gluconate. However, they fermented glycerol, L-arabinose, ribose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, L-sorbose, rhamnose, mannitol, sorbitol, α -methyl-D-mannoside, α -methyl-D-glucoside, N-acetyl-

glucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, lactose, melibiose, saccharose, trehalose, inulin, melezitose, D-raffinose, δ -gentiobiose, D-turanose, D-arabitol, L-arabitol, gluconate and 5keto-gluconate. This shows that the LAB can grow in different habitats utilizing different type of carbohydrates. The result corresponds with the report given by Manas Ranjan et al., (2014) that *Lactobacillus fermentum* uses several carbohydrates such as arabinose, cellobiose, galactose, maltose, mannose, melibiose, raffinose, ribose, sucrose, trehalose, and xylose. This result also confirms the report presented by Hedberg *et al.*, (2008) that *Lactobacillus plantarum* can ferment a wide range of carbohydrates, including L-Arabinose, rhamnose, mannitol, cellobiose, arbutin, esculin, salicin, lactose, melezitose, turanose, galactose, maltose, mannose, melibiose, raffinose, ribose, sucrose, sorbitol, trehalose, and gluconate. Studies have shown that most *Lactobacillus* are able to use a wide range of simple and complex carbohydrates due to the availability of sugar-utilizing cassettes in some LAB species (O'Donnell *et al.*, 2013; Campanaro *et al.*, 2014). The results from this study also confirms the predominance of LAB in Nigeria fermented foods as reported by other researchers (Ngene *et al.*, 2019; David *et al.*, 2019; Olatunde *et al.*, 2018; Osita *et al.*, 2018; Obinna-Echem *et al.*, 2014; Sanni *et al.*, 2013). The API 50 CHL identification kit had been reported as an important tool for Lactobacilli identification (Coeuret *et al.*, 2003; Dimitonova *et al.*, 2008; Herbel *et al.*, 2013). However, the biochemical-based methodologies for identification might not be conclusive in many cases since various LABs have similar nutritional and growth requirements (Fguiri *et al.*, 2015).

Table 3a: Carbohydrate fermentation profile of LAB isolates using API 50 CHL

Isolate Code	Carbohydrates																														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
FM1	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	
FM2	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
FC1	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-
FC2	-	-	-	-	+	+	-	-	-	-	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
FT1	-	-	-	-	-	+	-	-	-	-	+	+	+	+	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+	-
FT2	-	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+

FC1	+	+	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	<i>L. acidophilus</i> 1	49.00
																				<i>L. delbrueckii ssp. lactis</i> 2	36.80
																				<i>Pediococcus spp.</i>	13.20
																				<i>L. paracasei ssp. paracasei</i> 3	0.60
FC2	+	+	-	+	+	-	-	-	+	+	-	-	-	-	+	-	+	-	-	<i>L. plantarum</i> 1	99.90
																				<i>L. pentosus</i>	0.10
FT1	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	<i>L. plantarum</i> 2	96.20
																				<i>Lactococcus lactis ssp. Lactis</i> 1	2.10
FT2	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	<i>L. buchneri</i>	97.30
																				<i>L. brevis</i> 2	2.60
FP1	+	+	-	-	+	-	-	-	+	+	-	-	-	-	-	-	+	-	-	<i>L. pentosus</i>	99.90
																				<i>L. plantarum</i> 1	0.10
FP2	+	+	+	+	-	-	-	-	+	+	-	+	-	-	-	+	+	-	-	<i>L. paracasei ssp. paracasei</i> 1	99.90
																				<i>L. rhamnosus (L. casei ssp. rhamnosus)</i>	0.10
FCm1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	<i>L. brevis</i> 3	99.60
																				<i>L. collinoides</i>	0.20
FCm2	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<i>L. helveticus</i>	71.30
																				<i>L. delbrueckii ssp. lactis</i> 1	26.50
																				<i>Leuconostoc lactis</i>	1.00

- = Absence of carbohydrate; + = Presence of carbohydrate

FM = Fermented maize (ogi); FC = Fermented cassava (fufu); FT = Fermented tigernut;

FP = Fermented palmwine; FCm = Fermented cow milk (nunu); D: Identity (%), the percentages

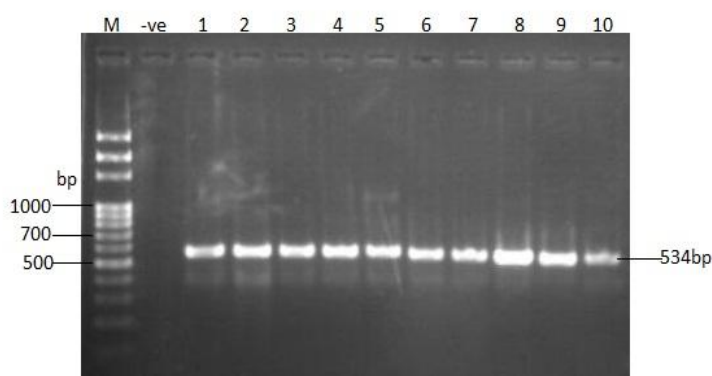
following the scientific names of species represent the similarities from the computer-aided database of the API-webTM API 50 CHL V5.1 software

31. Saccharose
32. Trehalose
33. Inulin
34. Melezitose
35. D-Raffino

36. Amidon
37. Glycogen
38. Xylitol
39. δ -Gentiobiose
40. D-Turanose

41. D-Lyxose
42. D-Tagatose
43. D-Fucose
44. L-Fucose
45. D-Arabitol

46. L-Arabitol
47. Gluconate
48. 2 keto - gluconate
49. 5 keto - gluconate



M - DNA marker, bp – base pairs between 500 - 1000
1 (FM1); 2 (FM2); 3 (FC2); 4 (FT1); 5 (FT2); 6 (FP1); 7 (FP2); 8 (FCm1); 9 (FCm2)
FM = Fermented maize (ogi); FC = Fermented cassava (fufu); FT = Fermented tigernut;
FP = Fermented palmwine; FCm = Fermented cow milk (nunu)

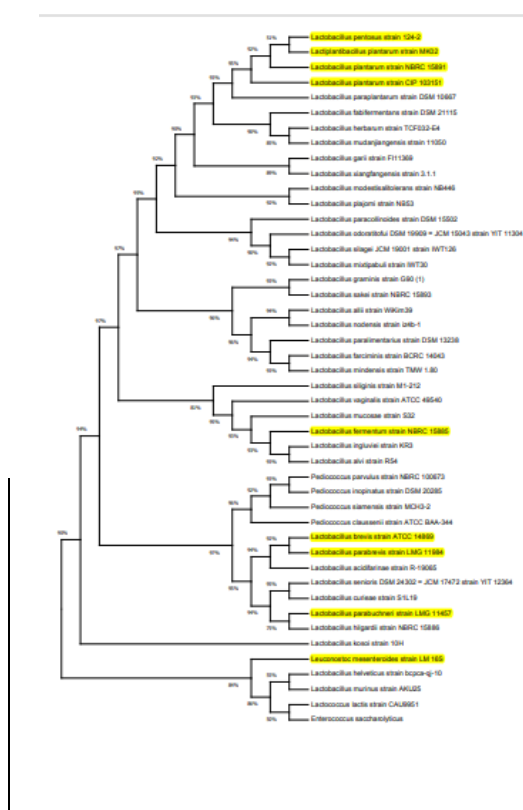


Table 4: Molecular identification of selected isolates using PCR

Table 4: Molecular identification of selected isolates using PCR

Food source	Matches to 16S rRNA Sequences from GenBank Database	Sequence ID	Identity with GenBank Database (%)
FM1	<i>L. fermentum</i> NBRC 15885	NR 113335.1	87
FM2	<i>Leuconostoc mesenteroides</i> LM	MF 927885.1	79
FC2	<i>L. plantarum</i> CIP 10315.1	NR 104573.1	99
FT1	<i>L. plantarum</i> NBRC 15891	NR 113338.1	99
FT2	<i>L. parabuchneri</i> LMG 11457	NR 115116.1	89
FP1	<i>L. pentosus</i> 124-3	NR 029133.1	99
FP2	<i>L. parabrevis</i> LMG 11984	NR 042456.1	90
FCm1	<i>L. brevis</i> ATCC 14869	NR 044704.2	89
FCm2	<i>Lactiplantibacillus plantarum</i> MK 02	MW 309840.1	85

FM = Fermented maize (ogi); FC = Fermented cassava (fufu); FT = Fermented tigernut; FP = Fermented palmwine; FCm = Fermented cow milk (nunu)

3.5 Correlation between the LAB identified with API and PCR technique

The two techniques used in this study provided different patterns of genera and species identification for the LAB isolates. The result in Table 5 shows that seven (7) out of the nine (9) LAB isolates identified using both techniques had high correlation and similarities. There was no correlation between the results obtained from the biochemical and genotypic identification for the FP2 and FCm2. This result is similar to the result obtained by Gutiérrez-Cortés *et al.*, (2017) who showed that three (3) out of the nineteen (19) isolates identified by API did not correspond to the results obtained through molecular identification. However, the study conducted by Moraes *et al.*, (2013) did not agree with this current result. Results from this study shows that both the API and PCR method can be used to identify potential LAB isolates from fermented food. API identification technique could be used as trial test while PCR can be used as confirmatory since they are more accurate. The API test could only detect to the species level while the PCR analysis identified the strains of each LABs. However, considering the difficulties in differentiating some LAB species with 16S rDNA sequencing and phenotypic

tests, the application of specific molecular techniques such as species-specific PCR can be employed (Fguiri *et al.*, 2015).

Table 5: Comparison between LABs identified with the API and PCR method

LAB source	LABs identified using API	LABs identified using PCR	Observation
FM1	<i>L. fermentum</i>	<i>L. fermentum</i> NBRC 15885	High correlation
FM2	<i>Leuconostoc mesentroides</i> ssp. <i>mesenteroides</i> / <i>dextranicum</i> 1	<i>Leuconostoc mesenteroides</i> LM	High correlation
FC2	<i>L. plantarum</i> 1	<i>L. plantarum</i> CIP 10315.1	High correlation
FT1	<i>L. plantarum</i> 2	<i>L. plantarum</i> NBRC 15891	High correlation
FT2	<i>L. buchneri</i>	<i>L. parabuchneri</i> LMG 11457	High correlation
FP1	<i>L. pentosus</i>	<i>L. pentosus</i> 124-3	High correlation
FP2	<i>L. paracasei</i> ssp <i>paracasei</i> 1	<i>L. parabrevis</i> LMG 11984	No correlation
FCm1	<i>L. brevis</i> 3	<i>L. brevis</i> ATCC 14869	High correlation
FCm2	<i>L. helveticus</i>	<i>Lactiplantibacillus plantarum</i> MK 02	No correlation

FM = Fermented maize (ogi); FC = Fermented cassava (fufu); FT = Fermented tigernut; FP = Fermented palmwine; FCm = Fermented cow milk (nunu)

4.0 Conclusion and Recommendation

This study has shown that potential probiotics can be isolated, identified and characterised from fermented food products. From this study, *L. fermentum* NBRC 15885, *Leuconostoc mesenteroides* LM, *L. plantarum* CIP 10315.1, *L. plantarum* NBRC 15891, *L. parabuchneri* LMG 11457, *L. pentosus* 124-3 and *L. brevis* ATCC 14869 were identified in ogi, fufu, nunu, Palmwine and fermented tigernut milk using API 50 CHL (Biomérieux, France) and PCR techniques. It is recommended that these strains can be useful in food production as potential probiotic cultures. However, further research work is needed to evaluate the probiotic characteristics/potentials of these LAB.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

Comment [8]: Brief Conflict of Interest

Comment [U9]: I've stated this in the revised file

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