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 Polymerase chain reaction (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, paimwine 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).

The need to develop alternatives to lactic acid fermented dairy products necessitated this study. However, it is very important to carefully select potential probiotic strains from different indigenous fermented foods to enhance their utilization as starter cultures in fermented foods. This study was therefore aimed at isolation, identification and characterization of potential probiotics.

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 (*Zea mays*) 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) kg of the white variety of fresh cassava (*Manihot esculenta*) 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 and decanted. The resulting wet mash (fufu) was stored and used for the isolation LAB.

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 (*Cyperus esculentus*) tubers were sorted and washed with portable water. The clean grains were soaked in 2 litre 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 ± 2^0C). 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 nystatin to inhibit yeast growth. The plates were incubated for 48 h at 45^0C under aseptic anaerobic conditions using anaerobic jars. Thereafter, the numbers of microbial colonies were counted and the total viable count was calculated using:

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

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 (Biomerieux, France) biochemical test kit for *Lactobacillus* (Biomerieux, 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 (Memmert, 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-webTM 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 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 95^0C for 20 min. The heated bacterial suspension was cooled in ice and spun for 3 min at 14000 rpm. The supernatant containing the DNA was transferred to 1.5 ml microcentrifuge tube and stored at -20^oC 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 launched by double clicking on the Nanodrop icon. The equipment was initialized with 2 µl of sterile distilled water and blanked using normal saline. Two microliter 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

Polymerase chain reaction (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 $MgCl_2$, 200 µM of each deoxynucleoside triphosphates (dNTP) (Solis Biodyne), 25pMol of each primer (BIOMERS, GERMANY), 2 units of Hot FIREPol DNA polymerase (Solis Biodyne), Proofreading 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 BigDye® terminator v1.1/v3.1, 2.25 ul of 5X BigDye 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 sec and 60°C for 4 min. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree is shown in figure 2. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in the units of the number of base substitutions per site. This analysis involved 46 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1615 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018).

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 ± 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 Log cfu/ml in fufu to 8.25 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 of lactic acid bacteria. Makarova, *et al*., (2006) reported that LAB species can metabolise hexose sugars into lactic acid producing an acid environment which inhibits the growth of several species of harmful and spoilage microorganisms. This high viable LAB counts may also have been as a result of the antifungal activity of nystatin used in the media to inhibit the yeast growth while encouraging LAB growth (No-Hee *et al*., 2017).

Table 1: LAB counts (Log 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 ± 0.03^{a}
2.	Fermented cassava (fufu)	3	8.13 ± 0.07^{b}
3.	Fermented tigernut	3	8.23 ± 0.04^{a}
4.	Palm wine	3	8.19 ± 0.03^{ab}
5.	Fermented cowmilk (nono)	3	8.25 ± 0.04^{a}

Values are means of triplicate LAB counts ± standard deviation

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

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. 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. *Lactobacilli* and *Bacillus* species were isolated from ogi and nono (David *et al*., 2019). *Lactobacillus* strains were isolated 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).

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	*Lactobacillus spp.*
FM2	Small Circular	Raised	Entire	Clear	Cream	+ve	-ve	-ve	Discrete tiny rods	*Lactobacillus spp.*
FM3	Medium Circular	Raised	Entire	Clear	Cream	+ve	+ve	+ve	Clustered cocci	*Micrococcus spp.*
FC1	Small Circular	Flat	Entire	Opaque	White	+ve	-ve	-ve	Tiny rods	*Lactobacillus spp.*
FC2	Big Circular	Convex	Undulated	Opaque	White	+ve	-ve	-ve	Long slender rods	*Lactobacillus spp.*
FT1	Small Circular	Flat	Entire	Opaque	White	+ve	-ve	-ve	Tiny rods	*Lactobacillus spp.*
FT2	Medium Circular	Raised	Entire	Clear	Cream	+ve	-ve	-ve	Discrete rods	*Lactobacillus spp.*
FP1	Small Circular	Raised	Entire	Opaque	White	+ve	-ve	-ve	Short tiny rods	*Lactobacillus spp.*
FP2	Medium Circular	Raised	Entire	Clear	Cream	+ve	-ve	-ve	Discrete tiny rods	*Lactobacillus spp.*
FP3	Small Circular	Raised	Entire	Clear	Cream	+ve	-ve	-ve	Cocci	*Lactococcus spp.*

FCm1	Medium Circular	Raised	Entire	Clear	Cream	+ve	-ve	-ve	Tiny rods	*Lactobacillus spp.*
FCm2	Small Circular	Raised	Entire	Opaque	White	+ve	-ve	-ve	Tiny rods	*Lactobacillus spp.*
FCm3	Big Circular	Flat	Entire	Opaque	Yellowish	+ve	+ve	+ve	Cocci	*Micrococcus spp.*

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-frutose, 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 ManasRanjan *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	-	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	+
FP1	-	+	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
FP2	-	-	-	-	-	+	-	-	-	-	+	+	+	+	+	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	-
FCm1	-	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	+
FCm2	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-

- = Absence of carbohydrate; + = Presence of carbohydrate; FM = ogi; FC = fufu; FT = Fermented tigernut; FP = palmwine; FCm = nunu

0. Control
1. Glycerol
2. Erythritol
3. D - Arabinose
4. L - Arabinose
5. Ribose
6. D - Xylose
7. L - Xylose
8. Adonitol
9. β-Metil-D-xiloside
10.Galactose
11. D - Glucose
12. D - Fructose
13. D - Mannose
14. L - Sorbose
15. Rhamnose
16.Dulcitol
17.Inositol
18. Mannitol
19. Sorbitol
20.α-Methyl-D-mannoside
21. α-Methyl-D-glucoside
22. N-Acetyl-glucosamine
23. Amygdalin
24.Arbutin
25.Esculin
26. Salicin
27. Cellobiose
28. Maltose
29. Lactose
30.Melibiose

3.4 Molecular identification of selected isolates using PCR-based methods

Lactobacillus plantarum CIP 10315.1, *L. plantarum NBRC 15891* and *Lactiplantibacillus plantarum MK 02* were identified from fufu, fermented tigernut milk and nunu respectively

(Table 4). Research have shown that *L. plantarum* is one of the most predominant species of LAB with high occurrence rate isolated from plant sources through fermentation (David *et al*., 2019). *L. pentosus 124-3* and *L. parabrevis LMG 11984* were identified in palmwine. *L. brevis ATCC 14869* was identified in nunu, *L. parabuchneri LMG 11457* in fermented tigernut milk and both *L. fermentum NBRC 15885* and *Leuconostoc mesenteroides LM* were identified in ogi.

The 16S rRNA gene sequence (figure 1) of the LAB strains identified in this study gave the basis for the construction of the phylogenetic tree (figure 2). The 16S rRNA sequence is used for various phylogenetic studies because it is highly conserved between different species of bacteria (Bushell and Burns, 2012).

Table 3b: Carbohydrate fermentation profile of LAB isolates Continued

Isolate Codes	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	LABs Identified	ID%
FM1	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	*L. fermentum* *L. brevis*	98.50 0.90
FM2	+	+	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	*Leuconostoc mesentroides ssp. mesenteroides/dextranicum 1* *L. brevis* 1	99.80 0.10
FC1	+	+	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	*L. acidophilus* 1 *L. delbrueckii ssp. lactis* 2 *Pediococcus spp.* *L. paracasei ssp. paracasei* 3	49.00 36.80 13.20 0.60
FC2	+	+	-	+	+	-	-	-	+	+	-	-	-	-	+	-	+	-	-	*L. plantarum* 1 *L. pentosus*	99.90 0.10
FT1	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	*L. plantarum* 2 *Lactococcus lactis ssp. Lactis 1*	96.20 2.10
FT2	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	*L. buchneri* *L. brevis* 2	97.30 2.60
FP1	+	+	-	-	+	-	-	-	+	+	-	-	-	-	-	-	+	-	-	*L. pentosus* *L. plantarum* 1	99.90 0.10
FP2	+	+	+	+	-	-	-	-	+	+	-	+	-	-	-	+	+	-	-	*L. paracasei ssp. paracasei* 1 *L. rhamnosus* (*L. casei ssp. rhamnosus*)	99.90 0.10
FCm1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	*L. brevis* 3 *L. collinoides*	99.60 0.20
FCm2	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*L. helveticus* *L. delbrueckii ssp. lactis* 1 *Leuconostoc lactis*	71.30 26.50 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

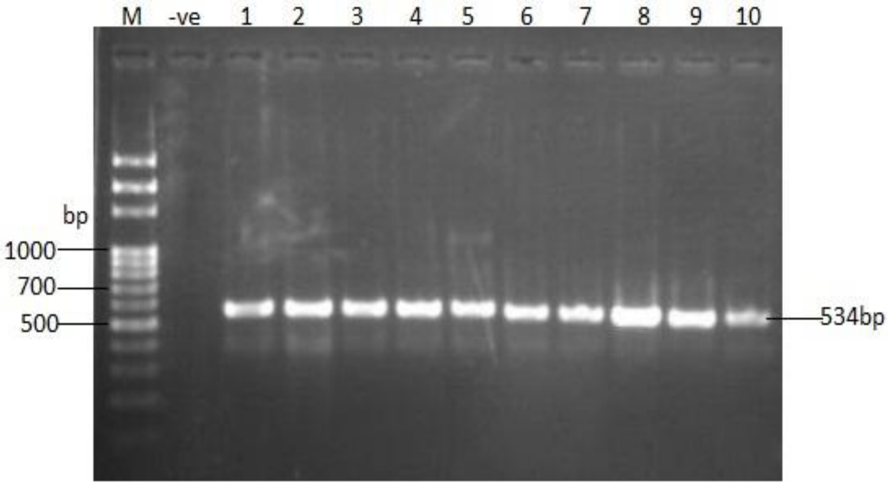

Figure 1: PCR amplification patterns of the *Lactobacillus species*

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

Food source	Matches to 16S rRNA Sequences from GenBank Database	Sequence ID	Identity with GenBank Database (%)
FM1	*L. fermentum NBRC 15885*	NR 113335.1	87
FM2	*Leuconostoc mesenteroides LM*	MF 927885.1	79
FC2	*L. plantarum CIP 10315.1*	NR 104573.1	99
FT1	*L. plantarum NBRC 15891*	NR 113338.1	99
FT2	*L. parabuchneri LMG 11457*	NR 115116.1	89
FP1	*L. pentosus 124-3*	NR 029133.1	99
FP2	*L. parabrevis LMG 11984*	NR 042456.1	90
FCm1	*L. brevis ATCC 14869*	NR 044704.2	89
FCm2	*Lactiplantibacillus plantarum 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	*L. fermentum*	*L. fermentum* NBRC 15885	High correlation
FM2	*Leuconostoc mesentroides ssp. mesenteroides/ dextranicum 1*	*Leuconostoc mesenteroides* LM	High correlation
FC2	*L. plantarum 1*	*L. plantarum* CIP 10315.1	High correlation
FT1	*L. plantarum 2*	*L. plantarum* NBRC 15891	High correlation
FT2	*L. buchneri*	*L. parabuchneri* LMG 11457	High correlation
FP1	*L. pentosus*	*L. pentosus* 124-3	High correlation
FP2	*L. paracasei ssp paracasei 1*	*L. parabrevis* LMG 11984	No correlation
FCm1	*L. brevis 3*	*L. brevis* ATCC 14869	High correlation
FCm2	*L. helveticus*	*Lactiplantibacillus plantarum* 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 (Biomerieux, 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 potentials of these LAB.

Authors' contributions

This work was carried out in collaboration among all authors. Author SYG designed the study. Author LIB managed the analysis, performed the statistical analysis, performed the literature search and wrote the manuscript. Authors SYG, LIB and PCO supervised the analysis and were part of the initial draft of the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist.

Figure 2 The phylogenetic tree analysis

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