Genotyping of Probiotic Lactobacilli in Nigerian Fermented Condiments for Improved Food Safety

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Abstract

BACKGROUND: Plant-based naturally fermented condiments usually result in poor quality products with various bacteria and fungi contaminants. Previous reports suggested the use of starter cultures from previously fermented condiments in fermentation processes to ensure health-promoting benefits, improved quality, shelf life, and organoleptic properties for the achievement of healthy nutrition, safe, and quality food.

AIM: This study aimed to genotype potential lactobacilli from locally fermented condiments for improved food safety.

METHODS: The lactobacilli colonies isolated from fermented condiments purchased from food markets in Southwest Nigeria were profiled for probiotic activities, hemolytic activities, antibiotics susceptibility, and inhibitory activities against food pathogens. Interesting probiotic lactobacilli were identified using 16S rRNA gene sequencing and evaluated for phylogenetic relatedness with other globally reported probiotic lactobacilli.

RESULTS: Lactobacillus species which expressed significant probiotics, γ-hemolysis, anti-spoilage, and anti-listerial activities (P < 0.05) with tolerable safety profiles were identified as Lactiplantibacillus plantarum YD001 (MW280136), Lactiplantibacillus plantarum YD002 (MW280139), L. plantarum YD003 (MW280137), and Lactocaseibacillus paracasei YD004 (MW280138) possessed 50.75, 50.61, 50.75, and 52.54 mol% DNA G+C contents, respectively. The species clustered into different phylogroups with high clonal relatedness with other potential lactobacilli meta-data (≥96.80%) obtained from the public repository.

CONCLUSION: Obtained genotyped Lactobacillus species are potential starter cultures for improved fermentation processes, control of food pathogens, and spoilage organisms.

Significance of the Study

- Probiotic Lactobacillus species were isolated from Nigerian fermented condiments
- New Lactobacillus species were suggested as starter cultures for food fermentation industries
- Biotechnological approach toward real-life sustainable development applications.

Introduction

Fermented condiments are rich sources of legume-based proteins that are usually consumed in Nigeria to obtain affordable dietary protein needed to ease malnutrition [1]. Fermented condiments [locust beans “Iru” or “Dawadawa” (Parkia biglobosa); oil beans “Ugba” (Pentaclethra macrophylla); and castor seeds “Ogiri” (Ricinus communis)] are usually added to improve sensory properties of food and soups in many Nigerian homes [2]. Other Leguminosae used to produce fermented condiments included African Yam beans “Owoh” (Sphenostylis stenocardia), cotton plant seeds (Gossypium hirsutum), melon seeds “Ogiri” (Citrullus vulgaris), fluted pumpkin seeds “Ogiri” (Telfairia occidentalis), and Prosopis Africana seeds “Okpiye” (P. africana) [3], [4], [5]. These seeds are naturally fermented to detoxify, improve digestibility, and improve nutritional components before human and animal consumption. However, improved nutritional, safety, and shelf-life quality would be achieved with the application of starter cultures in the production process. The presence of potentially probiotic Lactobacillus species has been reported perspective as their application as starter cultures in fermentation enhanced nutritional benefits, digestibility, accessibility, safety, and quality of fermented foods [6], [7], [8], [9], [10]. At present, fermented condiments have only achieved local commercial acceptance to due poor hygiene during processing, transportation, and storage [11], [12], [13]. Prolonged fermentation of protein components in these fermented condiments by fermenting microorganisms during the spontaneous fermentation produces protease enzyme
which breaks down the proteins resulting in pungent odor, sticky texture, and characteristic taste due to release of $\lambda$-polyglutamic acid metabolite [14], [15]. This study aims to substantiate the benefits of probiotic lactobacilli in Nigerian fermented condiments as potential starter cultures that would ensure health-promoting benefits, organoleptic properties, and improve shelf-life qualities of these condiments.

Materials and Methods

Sampling and proximate analyses of fermented condiments

Freshly fermented condiments [(African locust beans “Iru” (P. biglobosa), castor oil seed “Ogiri” (R. communis), and African oil beans “Ugba” (P. macrophylla)] were randomly purchased in triplicates from major local food markets in Southwest Nigeria between August 2019 and January 2020. The coordinates of the points of purchase in the markets were recorded using a global positioning system device as described [16] to substantiate the geographical area where samples were obtained in the Nigerian most popular food markets. All the samples were transported in the cold chain to the Microbiology Laboratory, Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria, for further analysis.

The fermented samples were evaluated for proximate composition including the moisture content, ash content, crude fiber, fat, protein, and carbohydrate to quantify the nutritional components in samples as described [17].

Identification and probiotic activities of Lactobacillus species

One gram from each of the fermented condiment samples was serially diluted and inoculated into enriched de Mann Rogosa and Sharpe (MRS) agar (BD BBL, Franklin Lakes, NJ, USA) and incubated anaerobically at 37°C for 48 h. Growth colonies were identified for the sugar fermentation profile as described [3]. Pure cultures of selected Lactobacillus isolates were slanted on MRS agar with 20% (v/v) glycerol to await further analysis.

Acid tolerance assay

Each Lactobacillus culture (10^8 CFU/ml) was inoculated into sterile MRS broth 1% (v/v) at pH 2, 3, 4, and 5.5 and the control had inoculum at pH 7. Each culture was incubated at 37°C for 6 h and absorbance was analyzed using a microtiter plate reader (Labtech Auto Elisa P, Italy) at 630 nm wavelength. Then, 100 µL of 24 h cultures at different pH were plated by dropping in sterile MRS agar. The plates were incubated at 37°C for 48 h and the colonies were enumerated as described [18]. The survival rate was calculated using Equation 1 below.

Bile tolerance assay

The tolerance of Lactobacillus species to bile salt at different concentrations was evaluated using 96-well microtiter plates as described [19]. The MRS broth was supplemented with sodium thioglycolate (Bio-basic, Canada) at different concentrations of 0.1%, 0.3%, 0.7%, and 1.0%. The 96 wells were inoculated with Lactobacillus cultures and incubated anaerobically at 37°C for 48 h. After incubation, the absorbance was measured using a microtiter plate reader (Labtech Auto Elisa P, Italy) at 630 nm wavelength. The control setup was without bile salts. Then, 100 µL of 24-h MRS broth at different bile concentrations were plated by dropping in sterile MRS agar. The plates were incubated at 37°C for 48 h and the colonies were enumerated using Equation 1 below.

Phenol tolerance assay

Phenol tolerance was performed using overnight cultures of the bacterial isolates inoculated 1% (v/v) into MRS broth with 0.1, 0.3, 0.5, and 1.0% (v/v) phenol for without phenol. After 24 h of incubation at 37°C, viable counts on the MRS agar plates were observed and counted. The absorbance of the culture at 630 nm was determined using a spectrophotometer (Thermo Fisher Scientific 5225, USA) [20].

Cholesterol reduction assay

Cholesterol assimilation of Lactobacillus species was evaluated using the quantitative determination of total cholesterol reagent 200 g/ml (Biolabo SAS, France) using 96-well microtiter plates as described [21]. The medium was then inoculated with each tested Lactobacillus culture and incubated anaerobically at 37°C for 24 h. After incubation, absorbance measuring the optical density of cell culture using a microtiter plate reader (Labtech Auto Elisa P, Italy) at 630 nm wavelength was compared with control (without cholesterol reagent). Then, 100 µL of the 24 h culture was inoculated on MRS agar. The plates were incubated anaerobically at 37°C for 48 h and the colonies were enumerated using the Equation 1 below:

\[
\text{Survival Rate} (\%) = \left( \frac{\text{Number of viable cells survived} (\text{CFU}/\text{ml})}{\text{Number of Initially viable cells inoculated} (\text{CFU}/\text{ml})} \right) \times 100
\]
Hemolytic and antibiotic susceptibility assay

The hemolytic activities of each of the selected Lactobacillus species was investigated. Fresh Lactobacillus cultures was inoculated on blood agar and incubated at 37°C for 48 hours. The agar plates were examined for hemolysis around the colonies as described by Clinical and Laboratory Standards Institute [22]. Lactobacillus isolates were profiled for antibiotics susceptibility using the Kirby–Bauer disk diffusion method. Briefly, each isolate culture of 0.5 McFarland turbidity was spread on Mueller-Hilton agar. The antibiotic disk of ceftazidime (30 µg), cefuroxime (30 µg), cefixime (30 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), gentamicin (10 µg), nitrofurantoin (30 µg), and augmentin (30 µg) was added and incubated at 37°C for 24 h. The zone of inhibition obtained was measured and evaluated [23].

Antibacterial activities of Lactobacillus isolates against food pathogens

The antibacterial activity of Lactobacillus isolates was determined by the spot overlay method [24], [25]. Lactobacillus isolates were inoculated in MRS broth anaerobically at 37°C for 24 h. The 24 h culture of each isolate was spotted sterile MRS agar and incubated anaerobically at 37°C for 24 h. Foodborne pathogens (indicator organisms) including Listeria monocytogenes (ATCC13337), Pseudomonas aeruginosa (DPC6504), Staphylococcus aureus (DPC7016), Hafnia alvei ATCC13337, and Escherichia coli ATCC20133 were cultured in Trypticase soy broth (10² CFU/ml) and incubated at 37°C for 24 h. About 100 µL of indicator cell culture were mixed with 10 ml of soft top agar (0.8% w/v sloppy agar) and used to overlay the spotted Lactobacillus isolates. The top agar was allowed to solidify and then incubated at 37°C for 24 h. The zones of inhibition around the spotted Lactobacillus isolates were recorded.

Genotyping and phylogenetic analysis of Lactobacillus species

Genomic DNA from Lactobacillus isolates was purified using a GenElute DNA extraction kit (Sigma-Aldrich, USA) following manufacturers’ instructions. PCR amplification of 16S rRNA gene of selected Lactobacillus isolates was performed using the primers: 16SF: 5’-AGTTTGATCCTGGTCAG-3’ and 16SR: 5’-TACCTTGTTACGACTT-3’ and carried out in a Hybrid PCR express unit (Hybrid Ltd., Middlesex, UK) at an initial denaturation temperature of 94°C for 5 min for one cycle which was followed by 35 cycles of denaturation at 95°C for 1 min. Annealing was at 55°C for 45 s and extension was at 72°C for 1 min. The final extension was carried out at 72°C for 7 min [26], [27]. DNA Amplicons were resolved on 1.0% agarose gel and electrophoresis was carried out at 120 V for 40 min.

The DNA bands were visualized on agarose gel stained with SYBR Green with 1.0 kbp DNA weight marker (Solis BioDyne, USA) on photo-documented UV light. Amplicons were purified with a QIAquick PCR purification kit (Qiagen, USA) and sequenced using the ABI Prism Big Dye Terminator version 3.0 sequencer [28], [29]. Partial 16S rRNA gene sequences were blasted with Basic Local Alignment Search Tool concerning Bacillus, Lactobacillus, and Lactococcus genomes in NCBI GenBank, and DNA G+C contents were calculated [30], [31]. The 16S rRNA sequences obtained from probiotic lactobacilli were evaluated for phylogenetic relatedness with globally reported probiotics Lactobacillus species from fermented foods in the GenBank database. Closely related strains were decided using manual alignment with homologous sequences curated into the multiple sequence alignment in MEGA X software version 7 (http://www.megasoftware.net) to construct the phylogenetic trees using the neighbor-joining method considering the maximum parsimony and maximum-likelihood analysis with bootstrap consensus tree deduced from 1000 replicates [32], [33].

Statistical analysis

All experiments and measurements were done in triplicate. The results are expressed on a dry matter basis as mean ± standard deviation. The significance of the probiotic potential isolates was analyzed using SPSS Software version 20.0 using Chi-square at P < 0.05 to evaluate survival rates at different conditions while the antibiogram profile data were analyzed with descriptive statistical methods.

Results and Discussion

Geospatial distribution and nutritional composition of fermented condiments

This study aimed to genotype potential lactobacilli strains from the Nigerian fermented condiments and substantiates their potential use as starter cultures in the fermentation of Nigerian indigenous plant-based condiments. Fermented condiments (Figure 1) were purchased from eighteen major Nigerian food markets located on lat. 7.2571 ± 1.500° and long. 5.2058 ± 1.5500° (Figure 2). The markets served as a place where the farm produce and other locally produced food materials were displayed by consumers.

Proximate analysis of fermented condiments

The nutritional compositions in sampled fermented condiments are shown in Table 1. Percentage
moisture content in the fermented condiments ranged 39.60 ± 0.42% – 52.82 ± 0.01% with fermented African locust beans (Iru) having significantly high moisture content (52.82 ± 0.01%). There was also moderate to low moisture content in fermented castor oil seed (Ogiri) and fermented oil beans (Ugba) as 39.60 ± 0.42% - 49.83 ± 0.42%, respectively. The percentage fat content in the samples ranged between 8.84 ± 0.02% and 16.51 ± 0.01%. The result revealed African locust beans (Iru) had high-fat content (16.51 ± 0.01%), fermented oil beans (Ugba) and castor oil seeds (Ogiri) had low-fat content 9.81 ± 0.01% and 8.84 ± 0.02%, respectively.

Table 1: Nutrient composition in selected fermented condiment samples retailed in Southwest Nigeria

<table>
<thead>
<tr>
<th>Macromolecules</th>
<th>Percentage composition (%)</th>
<th>Mean ± S. D</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>Iru</td>
<td>52.82 ± 0.01$^a$</td>
<td>49.83 ± 0.21$^b$</td>
</tr>
<tr>
<td>Fat content</td>
<td>Iru</td>
<td>16.51 ± 0.01$^a$</td>
<td>8.84 ± 0.02$^b$</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>Iru</td>
<td>4.12 ± 0.02$^a$</td>
<td>1.45 ± 0.04$^b$</td>
</tr>
<tr>
<td>Crude protein</td>
<td>Iru</td>
<td>22.58 ± 0.01$^a$</td>
<td>22.02 ± 0.02$^b$</td>
</tr>
<tr>
<td>Ash content</td>
<td>Iru</td>
<td>3.98 ± 0.01$^a$</td>
<td>1.65 ± 0.02$^b$</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Iru</td>
<td>16.05 ± 0.01$^a$</td>
<td>26.46 ± 0.02$^b$</td>
</tr>
</tbody>
</table>

Iru: Parkia biglobosa (African locust beans), Ogiri: Ricinus communis (castor oil seed), Ugba: Pentaclethra macrophylla (African oil beans), S.D: Standard deviation. Values with superscript $^a,b,c$ within the same row show significant differences.

The fermented condiment samples had very low crude fiber content with Iru, Ogiri, and Ugba containing 4.12 ± 0.02%, 1.45 ± 0.04%, and 2.21 ± 0.01%, respectively. Furthermore, fermented condiment the samples had significantly moderate crude protein content with Iru, Ogiri, and Ugba containing 22.58 ± 0.01%, 22.02 ± 0.02%, and 36.28 ± 0.02%, respectively. The fermented condiment samples used in this study had significantly low ash content with Iru, Ogiri, and Ugba containing 3.98 ± 0.01%, 1.65 ± 0.02%, and 2.32 ± 0.02%,
respectively. There was significantly moderate carbohydrate content in the condiments with Iru, Ogiri, and Ugba containing 16.05 ± 0.01%, 26.46 ± 0.02%, and 15.55 ± 0.07%, respectively. Significantly high moisture content in fermented condiments in this study is in agreement with previously reported studies [34], [35] resulting from absorption of moisture during boiling, fermentation, and hydrolytic breakdown of the seed component. Observed crude protein content in this study was in agreement with the previous reports indicating proteolysis of the legume seeds amino by secretion of protease enzyme by fermenting microorganisms during the fermentation of these condiments [36]. Variations in carbohydrate contents in sampled fermented condiments resulted in conversion of complex oligosaccharides into simple utilisable sugars by biochemical activities of microbiota in the samples [37]. Similar carbohydrate contents in fermented locust beans, soybeans, and other fermented condiments containing carbohydrates classes (including stachyose, melibiose, raffinose, starch) was reported in Nigeria [38]. In addition, similar low ash content in fermented locust beans and soybean condiments was reported [37], [38]. Varied ash content is an indication of mineral abundance in the samples food was attributed to addition of local ash powder to raw seeds during processing of condiments in some local communities. Crude fiber content in analysed samples was lower than values previously reported for fermented locust bean, mung beans and soybean condiments [37], [38]. Observed low ash contents observed in the samples similarly reported in fermented locust beans, soybean, and “Soumbala” (commonly consumed fermented locust beans in Burkina Faso) due to the level of mineral abundance in the food products could be attributed to addition of local ash powder during processing of raw seeds [39]. High-fat content in Soumbala <40.47% was attributed to hydrolysis of fat contents in the seeds as reported to improve the organoleptic properties of food products [40]. Observed low crude fiber contents was, however, lower than previously reported values in fermented condiments as a result of processing methods of the legume-based seeds [41], [42].

**Identification of Lactobacillus isolates**

The isolated colonies appeared creamy in color with smooth edges on Difco™ Lactobacilli MRS agar (BD Biosciences, Ireland). Selected colonies were circular, with approximately 0.4–2.0 mm in diameter, 1.0–3.0 mm in length, and grew at pH 2.0–8.0 and optimum growth temperature of 30–37°C in anaerobic conditions for 24–48 h. Isolates were Gram-positive rod-shaped, catalase-negative, oxidase-negative, non-motile, facultative anaerobes, and non-spore-forming. Gas was not produced during the fermentation of glucose, lactose, and sucrose; however, gas production was observed with fermentation D-glucose, D-galactose, D-fructose, D-mannitol, and D-sorbitol (Table 2). Four Lactobacillus species found to be anaerobic, Gram positive, bacilli, catalase and oxidase negative, non-spore forming, and non-motile were isolated from Nigerian fermented condiments derived from leguminous seeds. The isolates exhibited morphologically, and biochemical resemblance with other previously reported probiotic strains of Lactobacillus plajomi, Lactobacillus plantarum, and Lactobacillus paracasei which were isolated from a wide variety of fermented foods [29], [43], [44].

**Probiotic activities of Lactobacillus isolates**

Selected Lactobacillus isolates from fermented condiments significantly exhibited above 89.0% survival rate in acidic media ranging from pH =2.0 and pH =5.5 for at least 6 h. The isolates survived the extremely acidic environment (pH =2.0) and had more survival rates in other acidic environments (Table 3). The isolates exhibited potential to survive in the GI tract at pH =3.0 with food passage for about 3 h. The result revealed these Lactobacillus isolates exhibited significantly above 98% survival rate in 0.3% bile and 100% survival rate in 1.0% bile after 6 h of incubation. Reduction of the fatty acids and lipid content in the cell membrane which eventually decreases the survival rate of bacteria in extreme bile environments (Bile =1.0%) was not observed in the studied isolates (Table 3). Selected Lactobacillus isolates exhibited ≥97.0% survival rate at P < 0.05 in 0.1% phenol concentration and ≥96% survival rate in 1.0% phenol concentration after 24 h. The presence of toxic metabolites in the cell membrane which may significantly decrease bacteria survival rate in extreme phenol environments (Phenol =1%) was not observed in the selected isolates (Table 3). The selected Lactobacillus isolates showed a good ability

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**Table 2: Phenotypic identification of Lactobacillus species in fermented condiments**

<table>
<thead>
<tr>
<th>Isolate code</th>
<th>Morphology</th>
<th>Gram's reaction</th>
<th>Catalase</th>
<th>Oxidase</th>
<th>Coagulase</th>
<th>Citrate</th>
<th>Urease</th>
<th>MR/VP</th>
<th>Motility</th>
<th>Spore presence</th>
<th>Lactose</th>
<th>Maltose</th>
<th>Glucose</th>
<th>Sucrose</th>
<th>Mannitol</th>
<th>Identity of LAB isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB004</td>
<td>Milk</td>
<td>irregular, flat</td>
<td>Gram-positive</td>
<td>rod diplobacilli</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Ab</td>
<td>AG</td>
<td>AN</td>
<td>AN</td>
<td>AN</td>
<td>AN</td>
<td>NR</td>
<td>Lactobacillus sp.</td>
</tr>
<tr>
<td>LB005</td>
<td>Milk</td>
<td>irregular, flat</td>
<td>Gram-positive</td>
<td>rod diplobacilli</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Ab</td>
<td>AG</td>
<td>AN</td>
<td>AN</td>
<td>AN</td>
<td>AN</td>
<td>NR</td>
<td>Lactobacillus sp.</td>
</tr>
<tr>
<td>LB006</td>
<td>Milk</td>
<td>irregular, flat</td>
<td>Gram-positive</td>
<td>rod diplobacilli</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Ab</td>
<td>AG</td>
<td>AN</td>
<td>AN</td>
<td>AN</td>
<td>AN</td>
<td>NR</td>
<td>Lactobacillus sp.</td>
</tr>
<tr>
<td>LB010</td>
<td>Milk</td>
<td>irregular, flat</td>
<td>Gram-positive</td>
<td>rod diplobacilli</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Ab</td>
<td>AG</td>
<td>AN</td>
<td>AN</td>
<td>AN</td>
<td>AN</td>
<td>NR</td>
<td>Lactobacillus sp.</td>
</tr>
</tbody>
</table>

to assimilate cholesterol after 24 h of incubation. The isolates significantly assimilated the cholesterol at $P < 0.05$ with a survival rate $\geq 90.0\%$ (Table 3). The initial study for screening of Lactobacillus isolates for probiotics activities to mimic extreme environments found in the human gut system. These conditions may inhibit the growth of probiotic bacteria, including low pH, bile salts tolerance, phenol tolerance, and good cholesterol assimilation capabilities. Lactobacillus isolates were tolerant to pH $\geq 2.0$, 1.0% bile salts, 1.0% phenol, and 200 mg/dl cholesterol with over 80% survival rate in agreement with studies previously reported [45, 46, 47, 48].

**Table 3: Probiotic activities of Lactobacillus species**

<table>
<thead>
<tr>
<th>Probiotic properties</th>
<th>Parameter</th>
<th>Survival rate of Lactobacillus isolates (%) ± standard deviation (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LB004</td>
</tr>
<tr>
<td>Acid tolerance</td>
<td>pH=2</td>
<td>103.98 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>pH=3</td>
<td>77.45 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>pH=4</td>
<td>77.45 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>pH=5.5</td>
<td>87.17 ± 0.03</td>
</tr>
<tr>
<td>Bile tolerance</td>
<td>Bile0.1%</td>
<td>129.05 ± 0.007</td>
</tr>
<tr>
<td></td>
<td>Bile0.3%</td>
<td>135.64 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Bile0.7%</td>
<td>137.95 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Bile1.0%</td>
<td>137.96 ± 0.01</td>
</tr>
<tr>
<td>Phenol tolerance</td>
<td>Phenol0.1%</td>
<td>122.81 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Phenol0.3%</td>
<td>112.28 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>Phenol0.4%</td>
<td>118.48 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>Phenol1.0%</td>
<td>104.10 ± 0.07</td>
</tr>
<tr>
<td>Cholesterol assimilation</td>
<td>200mg/dl</td>
<td>90.29 ± 0.01</td>
</tr>
</tbody>
</table>

Table 3: Probiotic activities of Lactobacillus species

**Figure 3:** Gel electrophoresis of 16S rRNA genes obtained from polymerase chain reaction amplicons of Lactobacillus species.

**Hemolytic and antibiostics susceptibility assay**

The selected Lactobacillus isolates exhibited γ-hemolytic activities (no zones of inhibition) on blood agar. This signifies an absence of virulence activities in the isolates which may be a significant criterion for potential probiotic strains selection (plates not shown). The result also revealed generally regarded as safe status of isolated Lactobacillus isolates as implicated by γ-hemolysis (no hemolysis) on blood agar. The negative hemolytic result signifies an absence of virulence factors in the isolated Lactobacillus species which may be a crucial benchmark for potential probiotic strains selection. Our result supported other previous reports showing negative hemolysis in many Lactobacillus species [49, 50, 51]. Lactobacillus isolates were sensitive (with the zone of inhibition ≥20 mm in diameter) to erythromycin 5 μg, ceftriaxone 30 μg, cefuroxime 30 μg, gentamicin 10 μg, and ofloxacin 5 μg. Moderate susceptibility to potentially acquired resistance to other antibiotics such as ceftazidime 30 μg, cefoxitin 5 μg, and amoxicillin clavulanic acid 30 μg which protect activities of β-lactamase enzymes was also observed (Table 4). Susceptibility to inhibitors of cell wall synthesis and protein synthesis by these bacteria served as important selection criteria for the safety evaluation of potential probiotic bacteria for biotechnological applications. Our result was in agreement with the previous reports revealing the antibiotic sensitivity of selected Lactobacillus isolates to antibiotics such as erythromycin 5 μg, ceftriaxone 30 μg, cefuroxime 30 μg, gentamicin 10 μg, and ofloxacin 5 μg [52]. Moderate susceptibility observed to other antibiotics such as ceftazidime 30 μg, cefoxitin 5 μg, and amoxicillin clavulanic acid 30 μg which protected activities of β-lactamase enzymes observed among selected Lactobacillus species was also observed in the previous reports [53, 54].

**Table 4: Antibiotic susceptibility profile of Lactobacillus isolates**

**Antimicrobial activities of Lactobacillus species**

Lactobacillus isolates were selected based on their ability to produce a maximum radial zone of inhibition (≥20 mm) when overlaid with sloppy agar seeded with indicator strain such as L. monocytogenes DPC6579, P. aeruginosa DPC6054, S. aureus DPC7016, H. alvei ATCC 13337, and E. coli ATCC2013 (Table 5). Lactobacillus isolates produced antimicrobial substances that inhibited the growth of food pathogens during the fermentation of food products. Our result revealed the inhibitory activities of the identified probiotic lactobacilli strains on the growth of common food pathogens including L. monocytogenes DPC6579, [45], [46], [47], [48].
Obafemi et al. Probiotic Lactobacilli in Nigerian Fermented Condiments


Table 5: Antimicrobial activities of selected Lactobacillus isolates against food pathogens

<table>
<thead>
<tr>
<th>Lactobacillus species</th>
<th>Mean zone of Inhibition (mm) ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Listeria monocytogenes DPC6579</td>
<td>25.0 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa DPC6054</td>
<td>27.0 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus DPC7016</td>
<td>24.0 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Haemophilus influenzae ATCC13337</td>
<td>13.0 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli ATCC20133</td>
<td>15.0 ± 0.9</td>
<td>0.001</td>
</tr>
</tbody>
</table>

P. aeruginosa DPC6054, S. aureus DPC7016, H. alvei ATCC 13337, and E. coli ATCC20133 by the production of acidic organic metabolites into the surrounding medium; however, there was no inhibition against closely related Lactobacillus strains including L. plantarum DPC6730. Our result is related to some previous reports showing the antimicrobial activities of some Lactobacillus species such as L. plantarum against food pathogens.

Figure 4: Phylogenetic tree constructed using neighbor-joining algorithm in MEGA X with 1000 bootstrap replicates to compare isolated with related Lactobacillus species from based on 16S rRNA gene sequences. G1–G2 represents the diversity of groups while taxa in red represent Lactobacillus species isolated from Nigerian fermented condiments.
GCC_19M1 and L. plantarum isolated from fermented milk and vegetable, respectively [28], [55], [56], [57].

Genotyping of Lactobacillus species

Purified DNA concentrations of >50 ng/µL and A280 / 260 ratio >1.60 were used for the PCR amplification. High-molecular-weight bands observed on agarose gel. A negative control (molecular grade water) revealed no band (-ve) while bands in lane 1 are specific for the positive control (L. plantarum DPC 6682) and lanes 2–5 confirmed that the other four isolates belong to Lactobacillus genus (Figure 3). Analysis of partial 16S rRNA gene sequence identified isolates LB004, LB005, LB006, and LB010 as Lactiplantibacillus plajomi YD001 (MW280136), Lactiplantibacillus plantarum YD002 (MW280139), L. plantarum YD003 (MW280137), and Lacticaseibacillus paracasei YD004 (MW280138), respectively (Table 6).

<table>
<thead>
<tr>
<th>Isolate ID</th>
<th>Food Source</th>
<th>NCBI GenBank closest match</th>
<th>Sequence similarity index (%)</th>
<th>Identity G+C (%)</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB004</td>
<td>Iru</td>
<td>Lactobacillus plajomi NB53</td>
<td>99.80</td>
<td>Lactiplantibacillus plajomi YD001</td>
<td>50.75</td>
</tr>
<tr>
<td>LB005</td>
<td>Ugbia</td>
<td>Lactobacillus plantarum R12</td>
<td>96.68</td>
<td>Lactiplantibacillus plajomi YD001</td>
<td>50.61</td>
</tr>
<tr>
<td>LB006</td>
<td>Iru</td>
<td>Lactobacillus plantarum</td>
<td>99.80</td>
<td>Lactiplantibacillus plantarum YD002</td>
<td>50.75</td>
</tr>
<tr>
<td>LB010</td>
<td>Ogun</td>
<td>Lactobacillus paracasei</td>
<td>99.18</td>
<td>Lacticaseibacillus paracasei YD004</td>
<td>52.54</td>
</tr>
</tbody>
</table>

Species identity based on new Lactobacillus species reclassification 2020.

Conclusion

The results presented in this study revealed that genotyped L. plajomi YD001 (MW280136), L. plantarum YD002 (MW280139), L. plantarum YD003 (MW280137), and L. paracasei YD004 (MW280138) isolated from Nigerian fermented condiments could be probiotic safe species that can be used as potential starter cultures for fermentation processes and biological preservatives in foods. This study affirms our positive contribution toward the enhancement of real-life sustainable development in Nigeria through the provision of healthy nutrition and empowerment initiatives for the low-skilled women who are major producers of these condiments.

Authors’ Contributions

Background concept: YDO and SUO; sample collection and experimental design: YDO and PAA; manuscript preparation and editing: YDO and PAA; manuscript reviewing: PAA, SUO, and KOA; and supervision: SUO and KOA. All authors read and approved the final manuscript.

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