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Research article

Probiotic potential of *Lactobacillus* species isolated from fermented cassava and corn meal

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Abstract
Probiotics, particularly *Lactobacillus* species, are beneficial microorganisms known for enhancing gut health, boosting immunity, and inhibiting pathogens. Fermented cassava and corn products, widely consumed in Nigeria, serve as rich sources of these probiotics due to traditional fermentation. This study aimed to evaluate the probiotic properties of bacteria isolated from fermented cassava and corn in Benin, Nigeria. A total of 100 samples were collected from various areas in Benin Metropolis and analyzed in the laboratory. Microbial assays and 16S rRNA gene sequence analysis identified bacterial strains, including *Lactococcus lactis* (AE89, HBUAS53615), *Lactobacillus reuteri* (CI), *Lactobacillus plantarum* (NRIC0383), *Lysinibacillus sphaericus* (Z15), *Bacillus cereus* (ABRIFBI-64), and *Bacillus pacificus* (MP6). Antibiotic resistance among *Lactobacillus* species ranged from 71.4% to 100%. The strains exhibited acid tolerance, with survival rates of 77.98 to 97.11% at pH 2.5 and 65.58 to 90.49% at pH 3 over three and six hours. Biofilm formation rates varied, with *Lactobacillus fermentum* (92.3%), *Lactobacillus plantarum* (92.9%), and *Bacillus pacificus* (81.9%) showing significant formation. Survival rate in 2.0% bile salt exceeded 70%, but declined to 60.00 to 71.43% at 2.5% and 53.85 to 64.29% at 3.0% respectively. Antimicrobial resistance showed inhibition zones ranging from 8.6±1.50mm to 18.1±0.10mm. These findings highlight the high probiotic potential of *Lactobacillus* species from fermented cassava and corn, supporting their role in promoting gastrointestinal health and encouraging their continued consumption.

Keywords: Antibiotic resistance, Biofilm formation, Fermentation, Gene sequencing, *Lactobacillus* species, Probiotic potential.

Introduction

Probiotics, integral to digestive health, are living organisms incorporated into food to maintain microbial balance within the gastrointestinal tract (Goel *et al.,* 2020). Among these, Lactobacillus, the largest genus within lactic acid bacteria, plays a pivotal role. Phylogenetically, *Lactobacilli* are distributed into seven groups based on the 16S rRNA sequence (Nkhata *et al.,* 2022). These bacteria are key players in fermentation pathways, exhibiting diverse characteristics essential for their probiotic functions (Hill *et al.,* 2009). The principal genera of lactic acid bacteria, including *Lactobacillus*, *Leuconostoc*, and *Bifidobacterium*, contribute significantly to gastrointestinal health by fostering the growth of beneficial microorganisms and reducing the incidence of gastrointestinal disorders (Marco *et al.,* 2021). *Lactobacilli*, abundant in the environment, possess the capability to inhibit the growth of harmful bacteria through the production of specific chemicals, thus serving as natural biopreservatives (Oguntoyinbo & Narbad, 2015). Moreover, they are resistant to gastric secretions and bile production, making them effective probiotic agents (Pasolli *et al.,* 2020).

Lactobacillus species are recognized for their probiotic potential owing to their ability to halt the growth of pathogenic bacteria and regulate undesirable microflora in the gut (Oyedeji *et al.,* 2013). Their antimicrobial activities, attributed to the production of organic acids, hydrogen peroxide, bacteriocins, and antifungal substances during fermentation, contribute to inhibiting the growth of sensitive pathogenic bacteria (Pisoschi *et al.,* 2018). Unlike antibiotics, bacteriocins

possess a distinct digestive system breakdown mechanism, further enhancing their efficacy against harmful microbes (Pfeiler & Klaenhammer, 2017).

Lactobacillus strains exhibit resilience to adverse conditions within the gastrointestinal tract, making them ideal candidates for probiotic formulations (Oguntoyinbo *et al.,* 2023). Their ability to generate antimicrobial chemicals alters the metabolism or toxicity of pathogenic bacteria, establishing a barrier between beneficial and harmful bacteria in fermented food products (Pisoschi *et al.,* 2018). Consequently, *Lactobacilli* play a crucial role in preserving food quality and enhancing its safety. In Nigeria, cassava and corn are staple crops utilized in the production of various fermented food products, including fufu and garri (Diaz *et al.,* 2019). Fermentation not only enhances the nutrient content of cassava but also reduces its antinutrient levels, thereby improving its nutritional quality (Freire *et al.,* 2021).

Traditional fermentation techniques have been employed for centuries, resulting in a rich diversity of fermented foods integral to Nigerian cuisine. Fufu, a fermented cassava mash consumed across Nigeria, particularly in the eastern and southern regions, undergoes a meticulous fermentation process involving the disintegration of tissue structures and enzymatic breakdown of linamarin (Olopade *et al.,* 2022). This process softens the root, rendering it suitable for further processing into fufu. Additionally, corn fermentation, often involving pretreatments like nixtamalization, contributes to the development of unique flavors and textures in fermented maize products (Karaca *et al.,* 2017). By elucidating the probiotic potential of these strains, researchers aim to develop locally sourced probiotic products tailored to the nutritional needs of the Nigerian population (Olasupo *et al.,* 2021). Understanding the microbial diversity and dynamics during the fermentation of cassava and corn provides valuable insights into traditional food processing techniques and their impact on microbial communities (Rozos *et al.,* 2018; Sanni & Adesulu, 2023). Moreover, by harnessing the probiotic potential of indigenous *Lactobacillus* strains, researchers can contribute to the development of sustainable solutions for improving dietary quality and enhancing food security in Nigeria. This study therefore focused on the probiotic potential of *Lactobacillus* species isolated from fermented cassava and corn meal in Benin City, Edo State, Nigeria.

Materials and Methods

Study Area

The study area was Benin City metropolis, Edo State, South- South Nigeria. Edo State is located between 6°- 20°N and latitude 8°- 60°S. The State has a bimodal pattern of rainfall from April-July and September-November every year.

Sample Collection

Fermented cassava and maize were collected from ten different locations which include: Ikpoba hill area, Ekiosa market, New Benin market, Okha area, Uselu area, Oliha area, Oka market, Isihor area, Ugbighoko market, and Aduwawa market. A total of 100 samples were collected from ten (10) different location with fifty (50) samples each for ground fermented cassava and fermented maize for a period of one month between August and September, 2023. The samples were obtained in foil paper, maintained in their original packaging, tagged, and delivered right away to the microbiology lab at Benson Idahosa University for analysis.

Fermentation conditions

Fermentation of the slurry was performed in a batch by using 500ml fermenting pot filled to 450mL of distilled water. Cassava tubers were processed which include washing, peeling, chopping, and re-washing with water. The chopped or sliced cassava tubers were allowed to ferment for four days at room temperature in fermenting pots filled with sterile distilled water. Additionally, some maize grains (corn) were fermented for four days at room temperature in a fermenting pot filled with sterile distilled water. To a fineness of 0.05um, the fermented maize grains (corn) were ground. The cassava and corn flour slurry were rinse and sample are obtained in sterile wide-mouthed jars, maintained in their original pack tagged and delivered right away to the microbiology lab at Benson Idahosa University for analysis.

Sterilization of Materials

Before and after use, materials underwent a variety of sterilization processes. Test tubes, conical flasks, and pipettes were among the glassware that was carefully cleaned with detergents, rinsed with

water, and drained. Before being used, these materials were wrapped in foil paper and sanitized at 170^0C in a hot air oven. The manufacturers' instructions were followed when preparing the media used in this investigation. The prepared media and distilled water were autoclaved at 121°C for fifteen minutes. Hardware such as the inoculating loop was burned until red. Prior to analysis, the work bench was swabbed with 70% alcohol to disinfect it.

Isolation of *Lactobacillus* sp. from Fermented Cassava and Corn/Maize

The isolation of the *Lactobacillus* spp. on de ManRogosa-Sharpe Agar (MRS; Oxoid, Cambridge, UK), was performed according to the method described by Nkhata *et al.,* (2022) and (Nkhata *et al.,* (2022). 45 mL of sterile peptone water were used to homogenize 5 g of each sample. 1 ml of the homogenized sample was serially diluted in 9 ml of peptone water and 1ml of the solution was plated in MRS Agar. Triplicate plates were inoculated using the pour plate method and incubated at 37 °C for 48 hours in anaerobic jar 5–10% CO2. A morphologically unique, well-isolated colony was included in each sample, and it was chosen and streaked onto fresh MRS plates until a pure culture was obtained. The bacterial cells were suspended in MRS Agar in order to maintain pure cultures for use in subsequent studies (Oguntoyinbo & Narbad, 2015).

Identification of *Lactobacillus* sp.

The bacterial isolates were identified through cultural, morphological, and biochemical characterizations from fresh cultures of *Lactobacillus* spp. (grown on MRS Agar for 24 hours). The identification procedures followed modified techniques from Nkhata *et al.,* (2022) and Marco *et al.,* (2021). The following tests were performed: Gram staining, motility (Nkhata *et al.,* 2022), catalase, oxidase, coagulation (Freire *et al.,* 2021); citrate, indole (Marco *et al.,* 2021), endospore formation (Marco *et al.,* 2021), hydrogen sulfide production, gas production from glucose fermentation, and sugar fermentation (Freire *et al.,* 2021).

***In Vitro* Characterization of Probiotic Properties**

Tolerance to low pH, tolerance to bile salt, antibiotic susceptibility, and antimicrobial activity are among the frequently used techniques for *in vitro* analysis of probiotic characteristics.

Tolerance to Low pH

The isolates were cultured independently for an entire night at +37°C in 5 mL of MRS broth in anaerobic conditions. To get an initial inoculum level of log 3 CFU/ml, a volume of 1 ml of log 4 CFU/ml of each overnight-grown culture was inoculated into 10 ml of MRS broth. After that, the culture was centrifuged for ten minutes at 5000 rpm. Two washes of the pellets in phosphate buffer (pH 7.2) were performed. In order to replicate the gastric environment, the pellets were again suspended in 5 ml of sterile MRS broth that had been pH-corrected to 2.0, 2.5, and 3.0 using 1% HCl. At 37°C, the test tubes were incubated for three and six hours. 1 ml of the culture was diluted in sterile 9-ml phosphate buffer (0.1 mM, pH 6.2) prepared in accordance with the manufacturer's instructions to counteract the acidity of the medium. Then, the culture was plated on MRS agar medium using a 100-µl aliquot and its 10-fold serial dilutions. The inoculated plates were placed in an anaerobic jar (BBL, Gas Pack System) and incubated for 48 hours at 37°C without oxygen. The colonies formed by the grown *Lactobacillus* were expressed as colony-forming units per mililiter (CFU/ml). The percentage of *Lactobacillus* colonies grown on MRS agar relative to the starting bacterial concentration was used to compute the survival rate (Equation 1).

$$\text{survival rate}(\%) = \frac{LogCFUN_1}{LogCFUN_o} x100\% \qquad (1)$$

Where LogCFUN_1 is the viable count of isolates after incubation and LogCFUN_0 is the initial viable count.

Bile salt tolerance

The isolates were separately grown overnight in MRS broth at 37°C under anaerobic conditions to estimate the bile salt tolerance of *Lactobacillus* (those only were grown at pH 2.0, 2.5, and/or 3.0). After that, each culture was centrifuged for 10 minutes at 5000 rpm. Two rounds of washing in pH 7.2 phosphate-saline buffer (PBS) were performed on the pellets. In sterile MRS broth supplemented with 0.3% (w/v) bile salt (Oxgall,

USA), cell pellets were resuspended. As previously mentioned, samples were collected 24 hours after the start of incubation to assess the viability of the cells. Simultaneously, a positive control was established using MRS broth without bile salts that had been inoculated with each distinct culture. In order to neutralize the medium, 1 ml of each distinct culture was separately diluted in sterile 9-ml phosphate buffer (Sigma, St. Louis, USA) prepared in accordance with the manufacturer's instructions (0.1 mM, pH 6.2). This was done after 3 hour and 6 hour of incubation. Briefly put, MRS agar medium was plated with a 100-µl aliquot of the culture and its 10-fold serial dilutions. Anaerobic conditions were maintained for 24 to 48 hours at 37°C on plates using an anaerobic jar (BBL, Gas Pack System). The colony-forming units per milliliter (CFU/ml) used to express *Lactobacillus* counts were the survival rate was calculated as the percentage of *Lactobacillus* colonies grown on MRS agar compared to the initial bacterial concentration.

$$\text{survival rate}(\%) = \frac{LogCFUN_1}{LogCFUN_o} x100\% \qquad (2)$$

Where LogCFUN_1 is the viable count of isolates after incubation and LogCFUN_0 is the initial viable count.

Antibiotics Susceptibility Testing

The Kirby-Bauer dics diffusion method was used to determine antibiotic resistance profiles. In a nutshell, sterile swabs were used to select and streak the corresponding colonies on Mueller-Hinton Agar (Oxoid). Using sterile forceps, the antibiotic discs (Oxoid) were placed onto the agar plate surface using a multi-disc. The plates were incubated for the entire night at 30°C for 48 hours. The different zones of inhibition were identified and documented, and the diameters of the inhibition zones were measured (Bax, 2001). The antibiotics used in this study were Cilastatin 10µg, Gentamicin 10µg, Oflaxacin 5µg, Azithromycin 15µg, Amoxicillin 30µg, Cefotaxime 25µg, Ciprofloxacin 5 µg, Cefexime 5µg, Levofloxacin 5µg, Erythromycin 15µg, Ceftriaxone 45µg, and Cefuroxime 30µg (Ce Tech. Diagnostic. www.celtechproduct.com Belgium Inc.)

Determination of antimicrobial properties of the isolates

The antimicrobial qualities of the isolates were ascertained in duplicate using the agar-well diffusion experiment. First, seven pathogenic species were grown in MHA (Mueller-Hinton Agar) as a growth medium and incubated for 24 hours at 37 °C to create McFarland 0.5 standard solutions. These species included *Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactococcus lactis, Lactobacillus reuteri, Lactobacillus plantarum, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*Two milliliters of each pathogen culture (*Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, Citrobacter spp, Klebsiella pneumonia, and Escherichia coli*) made using McFarland 0.5 standard solutions were pipetted out, flood inoculated onto MHA plates (90 mm in diameter), and then the plate was rotated to distribute the inoculum. After that, the wells on the MHA plate were made using sterile, 9-mm cork borers. Following that, 180 µL of pH-adjusted cell-free isolate supernatants were added to each well. This was accomplished by centrifuging the MRS broth, which contained bacteria that had been cultured for a full day, for ten minutes at 10,000 g. Finally, the plates were incubated at 37 °C for 24 hours, and the zone of inhibition in each well was assessed. Inhibitions with diameter zones larger than 1 mm were considered to have strong antibacterial activity.

Detection of biofilm production by microtiter plate assay

A microtiter plate (MtP) assay method was used to gauge the amount of biofilm that is forming. The bacterial suspension is prepared by adding 1% glucose supplement to Mueller-Hinton Broth (MHB), and it is subsequently adjusted to 0.5 McFarland. This bacterial culture was diluted 20 times (1/20) to yield 5×10^5 cfu/ml. 180 µl of this bacterial culture supplemented with 1% glucose were added to a 96-well flat-bottomed sterile polystyrene microplate to create an infection that will eventually reach a final concentration of 5×10^4 using a micropipette. The microplates were incubated at 37^0C for 24 hours after washing twice with phosphate-buffered saline (PBS) (pH 7.2) and drying the wells at 60°C for an hour. The

Lactobacillus isolates whose biofilms formed on the walls of the microplate wells are stained for 15 minutes with only 50 µl 0.3% w/y crystal violet stain and ethanol (95% w/y); a decolorizer was added and left for 10 seconds. After that, PBS was used twice to wash the crystal violet stained. A microplate reader then measures the microplate spectrophotometrically at 570 nm. Three duplicates of each study are conducted. Blanks inoculated with sterile MHB supplemented with 1% glucose, are regarded as the negative controls. The blank absorbance readings are utilized to determine whether or not isolates develop biofilms. Biofilm-producing isolates are those whose wells have optical density (OD) values greater than those of a blank well. It is possible to classify isolates as strong, moderate, or weak biofilm using the cut-off value (ODc).

Molecular Identification

DNA Extraction Protocol

Genomic DNA was extracted from the cultures using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005). The 16S target region was amplified using OneTaq® Quick-Load® 2X Master Mix (NEB, Catalogue No. M0486) with the primers presents in table 3.1. The PCR products were run on a gel and cleaned up enzymatically using the EXOSAP method. The extracted fragments were sequenced in the forward and reverse direction (Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3 -100/1000) and purified (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit™, Catalogue No. D4050). The purified fragments were analysed on the ABI 3500xl Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific) for each reaction for every sample, as listed in Section 1. BioEdit Sequence Alignment Editor version 7.2.5 was used for the analysis.

Results and Discussion

The result in Table 1 shows the total anaerobic Count of *Lactobacillus* species recorded for fermented Cassava for Ten (10) Selected locations in Benin Metropolis with Aduwawa market having the highest *Lactobacillus* Count while New Benin aera having the Lowest *Lactobacillus* Count. The total *Lactobacillus* count for fermented cassava samples ranged from 7.17×10^3cfu/g recorded for fermented cassava obtained from New Benin aera to 9.30×10^3cfu/g recorded from Aduwawa market in Benin Metropolis. Also, the total anaerobic Count of *Lactobacillus* species recorded for fermented maize (corn) for ten (10) selected location in Benin Metropolis shows that a total *Lactobacillus* count for fermented corn/maize ranged from 6.20×10^3cfu/g recorded for fermented corn/maize obtained from Ikpobahill area market to 8.35×10^3cfu/g obtained from Aduwawa market in Benin Metropolis.

Table 1: Total anaerobic count of *Lactobacillus* species recorded for fermented cassava and corn obtained from different location in Benin metropolis.

Sample Location	Fermented Cassava 10^3 ± SD (cfu/g)	Fermented Corn 10^3 ± SD (cfu/g)
Ikpobahill area	7.20± 0.25	6.20± 0.26
Ekiosa market	7.93± 0.50	6.93± 0.06
New Benin area	7.17± 0.35	7.00± 0.01
Okha area	7.83± 0.40	6.83± 0.04
Uselu area	8.00± 0.20	8.00± 0.03
Oliha area	8.00± 0.05	7.00± 0.16
Oka market	7.27± 0.22	6.22± 0.26
Isihor area	7.20± 0.30	6.25± 0.05
Ugbighoko market	8.43± 0.10	7.13± 0.08
Aduwawa market	9.30± 0.20	8.35± 0.12
Average count	7.83± 0.25	6.99± 0.10

Identification of the Isolates at the Genus Level

Table 2 shows the organisms isolated from fermented cassava samples from the various locations in Benin metropolis, based on biochemical test. A total of ninety (90) isolates were recorded. These isolates were obtained from hundred (100) samples collected with ten samples each from each location. The microorganisms

isolated from each location were recorded according to the location of the samples (that is Ikpobahill area, Ekiosa market, New Benin area, Okha area, Uselu area, Oliha area, Oka market, Isihor area, Ugbighoko market, Aduwawa market) and the percentage of samples with growth of organisms was also recorded.

Table 2: Organisms Isolated from Fermented Cassava and Corn Samples from the Various Locations in Benin City, Nigeria.

Location	Number of Samples	Number with Isolated Organisms	Isolates
Ikpobahill area	10	9(90%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactococcus lactis, Lactobacillus reuteri, Lactobacillus plantarum,, Bacillus cereus, Bacillus pacificus*
Ekiosa market	10	10(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactococcus lactis, Lactobacillus reuteri, Lactobacillus plantarum, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus*
New Benin area	10	10(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactococcus lactis, Lactobacillus reuteri, Lactobacillus plantarum, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus*
Okha area	10	10(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactococcus lactis, Lactobacillus reuteri, Lactobacillus plantarum, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus*
Uselu area	10	10(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactococcus lactis, Lactobacillus reuteri, Lactobacillus plantarum, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus*
Oliha area	10	10(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus, Lactococcus lactis, Lactobacillus reuteri, Lactobacillus plantarum, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus*
Oka market	10	10(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactococcus lactis, Lactobacillus reuteri, Lactobacillus plantarum, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus*
Isihor area	10	10(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactococcus lactis, Lactobacillus reuteri, Lactobacillus plantarum, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus*
Ugbighoko market	10	10(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactococcus lactis, Lactobacillus reuteri, Lactobacillus plantarum, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus*
Aduwawa market	10	10(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactococcus lactis, Lactobacillus reuteri, Lactobacillus plantarum, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus*
Total	100	99(99%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactococcus lactis, Lactobacillus reuteri, Lactobacillus plantarum, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*

Table 3: Percentage Distribution of Bacterial Species Isolated from Fermented Cassava and Corn Sample from Benin City, Based on Biochemical Test.

Location	*LACTOBACILLUS* ISOLATES									
	Lactobacillus fermentum	*Lactobacillus ghanensis*	*Lactobacillus delbrueckii*	*Lactobacillus plantarum*	*Lactococcus lactis*	*Lactobacillus reuteri*	*Lysinibacillus sphaericus*	*Bacillus cereus*	*Bacillus pacificus*	Total Organism
Ikpobahill area	1	1	-	2	-	1	-	1	2	8
Ekiosa market	1	1	1	2	1	1	-	-	1	8
New Benin area	1	-	1	1	1	1	1	1	-	7
Okha area	1	1	2	1	1	-	-	-	1	7
Uselu area	2	1	1	1	1	1	-	1	2	10
Oliha area	1	2	1	1	1	1	1	1	1	10
Oka market	2	-	2	1	1	1	1	1	1	10
Isihor area	1	1	-	2	1	-	2	2	1	10
Ugbighoko market	2	1	1	2	1	2	1	1	-	11
Aduwawa market	1	1	1	1	1	-	1	1	2	9
	13(14.4%)	9(10%)	10(11.1%)	14(15.6%)	9(10%)	8(8.9%)	7(7.8%)	9(10%)	11(12.2%)	90(100%)

Table 3 shows the percentage distribution of *Lactobacillus* species isolated based on biochemical test. A total of nine (9) *Lactobacillus* species (*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactococcus lactis, Lactobacillus reuteri*, *Lactobacillus plantarum, Lysinibacillus sphaericus*, *Bacillus cereus*, *Bacillus pacificus*) were isolated. The total number of organisms based on different location was also recorded. The total percentage of each organism was recorded based on biochemical test of the percentage distribution of the lactic acid bacterial isolates. A total of 90 isolates were identified, with *Lactobacillus plantarum* recording the most isolates 14 (15.6%), followed by *Lactobacillus fermentum* with isolates 13 (14.4%). *Lactobacillus ghanensis, Lactococcus lactis* and *Bacillus cereus*, had 9 (10%) each. Moreover, 10 (11.1%) isolates were found in *Lactobacillus delbrueckii*, 8 (8.9%) in *Lactobacillus reuteri*, 7 (7.8%) in *Lysinibacillus sphaericus*, and 11 (12.2%) in *Bacillus pacificus* respectively. Table 4 shows the antibiotics resistance profile of bacterials isolated from fermented cassava samples. Isolates showed 87.0% resistance towards Cilastatin and 93.9% resistance towards Gentamicin while Oflaxacin show 78.8% resistance and Azithromycin shows 80.1% resistance towards isolates. Also, isolates showed 94.2% resistance towards Amoxicillin, 81.9% resistance towards Cefotaxime, 73.8% resistance towards Ciprofloxacin, 88.3% resistance towards Cefexime, 72.8% resistance towards Levofloxacin, 96.3% resistance towards Erythromycin, 74.6% resistance towards Ceftriaxone and 81.5% resistance towards Cefuroxime. Isolates were more sensitive to Ciprofloxacin and Levofloxacin. *Lactobacillus fermentum* recorded 92.9%, *Lactobacillus ghanensis* recorded 87.0%, *Lactobacillus delbrueckii* recorded 86.7%, *Lactobacillus plantarum* recorded 86.3%, *Lactococcus lactis* recorded 84.3%, *Lactobacillus reuteri* recorded 72.9%, *Lysinibacillus sphaericus* recorded 84.5%, *Bacillus cereus* recorded 73.2% and *Bacillus pacificus* recorded 84.9%.

Table 4: Antibiotics Resistance Profile of Bacterials Isolated from Fermented Cassava and corn Samples: Resistant Isolates, N (%)

Antibiotics	*Lactobacillus fermentum (n=13)*	*Lactobacillus ghanensis (n=9)*	*Lactobacillus delbrueckii (n=10)*	*Lactobacillus plantarum (n=14)*	*Lactococcus lactis (n=9)*	*Lactobacillus reuteri, (n=8)*	*Lysinibacillus sphaericus, (n=7)*	*Bacillus cereus (n=9)*	*Bacillus pacificus (n=11)*
Cilastatin (10µg)	12(92.3%)	8(88.9%)	10(100%)	12(85.7%)	7(77.8%)	7(87.5%)	5(71.4%)	8(88.9%)	10(90.9%)
Gentamycin (10µg)	13(100%)	8(88.9%)	10(100%)	14(100%)	9(100%)	6(75.0%)	7(100%)	9(100%)	9(81.8%)
Oflaxacin (5µg)	11(84.6%)	7(77.8%)	8(80%)	12(85.7%)	9(100%)	6(75.0%)	5(71.4%)	7(77.8%)	10(90.9%)
Azithromycin (15µg)	10(76.9%)	8(88.9%)	7(70%)	11(78.6%)	9(100%)	6(75.0%)	6(85.7%)	8(88.9%)	10(90.9%)
Amoxicillin (30µg)	13(100%)	9(100%)	9(90%)	13(92.9%)	9(100%)	7(87.5%)	7(100%)	9(100%)	11(100%)
Cefotaxime (25µg)	13(100%)	7(77.8%)	10(100%)	12(85.7%)	9(100%)	8(100%)	5(71.4%)	8(97.7%)	8(72.7%)
Ciprofloxacin (5µg)	11(84.6%)	9(100%)	8(80%)	11(78.6%)	8(88.9%)	5(62.5%)	6(85.7%)	9(.100%)	8(72.7%)
Cefexime (5µg)	12(92.3%)	9(100%)	9(90%)	13(92.9%)	7(77.8%)	6(75.0%)	7(100%)	9(100%)	11(100%)
Levofloxacin (5µg)	12(92.3%)	8(88.9%)	7(70%)	11(78.6%)	8(88.9%)	5(82.5%)	4(57.1%)	9(100%)	8(72.9%)
Erythromycin (15µg)	13(100%)	9(100%)	10(100%)	14(100%)	9(100%)	7(87.5%)	7(100%)	8(88.9%)	10(90.9%)
Ceftriaxone (45µg)	12(92.3%)	7(77.8%)	7(70%)	10(71.4%)	7(77.8%)	5(82.5%)	5(71.4%)	8(88.9%)	9(81.8%)
Cefuroxime (30µg)	13(100%)	8(88.9%)	9(90%)	12(85.7%)	8(88.9%)	5(92.5%)	7(100%)	9(100%)	8(72.7%)

Table 5: Antimicrobial Resistance Profile of *Lactobacillus* sp from Fermented Cassava and Corn, Isolated Pathogenic Bacterial Isolates (Growth Inhibition Zone Diameter in Milliliter **(mm) of Pathogens with Nine *Lactobacillus* spp.)**

	Clinical Pathogenic Bacterial isolates from Central Hospital, Benin City (Mean±SD), Zone of inhibition (mm)							
S/N	*Lactobacillus* sp.	*Candida* sp.	*Staphylococcus* sp.	*Pseudomonas* sp.	*Klebsiella* sp.	*Escherichia coli*	*Salmonella* sp.	*Streptococcus* sp.
1	*Lactobacillus fermentum*	14.2±1.01	13.5±2.50	12.4±2.50	15.2±0.16	13.1±1.20	13.5±0.50	11.0±0.02
2	*Lactobacillus ghanensis*	11.5±1.50	11.6±0.80	12.0±0.40	11.5±0.50	10.0±0.20	9.0±0.70	10.0±0.30
3	*Lactobacillus delbrueckii*	9.0±0.60	9.0±0.50	10.0±0.80	9.0±1.40	11.0±1.30	9.5±2.10	7.0±0.60
4	*Lactobacillus plantarum*	15.0±0.16	16.5±0.25	17.8±0.50	18.1±0.10	14.8±0.50	17.5±0.80	8.0±0.90
5	*Lactococcus lactis*	9.0±1.30	10.4±0.30	8.0±1.60	8.0±0.15	9.0±0.16	9.0±0.16	7.0±0.90
6	*Lactobacillus reuteri*	11.0±0.80	10±1.20	10.5±0.12	12.4±0.22	9.0±1.80	11.2±0.50	9.0±0.12
7	*Lysinibacillus sphaericus*	10.5±0.40	8.5±1.50	9.0±1.10	9.0±0.15	12.0±0.25	11.2±0.80	9.0±0.10
8	*Bacillus cereus*	8.6±1.50	9.0±0.50	8.0±0.13	7.0±0.85	6.0±0.50	9.5.0±0.88	9.0±0.11
9	*Bacillus pacificus*	10.0±0.24	12.0±1.02	14.0±1.10	13.5±0.90	11.2±2.10	12.8.0±1.54	7.0±0.35

Table 5 illustrates the antimicrobial resistance profile of *Lactobacillus* spp. isolated from fermented cassava and corn against various clinical pathogenic bacteria. Nine *Lactobacillus* isolates displayed inhibition zones against *Candida* sp., *Salmonella* sp., *Staphylococcus* sp., *Streptococcus* sp., *Pseudomonas* sp., *Klebsiella* sp., and *Escherichia coli*. Notably, *Lactobacillus fermentum* and *Lactobacillus plantarum* exhibited the highest growth inhibition, while *Bacillus cereus* showed the lowest. The inhibition zones ranged from 7.0 to 18.1 mm, indicating varying degrees of effectiveness against the pathogens. All isolates displayed some level of inhibition against the tested pathogens, suggesting their potential as probiotics to combat common human pathogens. The results highlight the inhibitory nature of *Lactobacillus* sp. against pathogenic bacteria, supporting their use as a potential treatment for various human infections. These findings underscore the importance of probiotic bacteria in addressing microbial infections and promoting human health.

Tolerance to Low pH

In Table 6, the survival rates of the isolates varied greatly over the course of three and six hours of incubation, ranging from 38.40 to 98.50% at different pH values (pH 2, pH 2.5 and pH 3 respectively). Highly tolerant strains of bacteria were found to persist above 50% for three and six hours, including "*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactococcus lactis, Lactobacillus reuteri, Lactobacillus plantarum, Lysinibacillus sphaericus*, *Bacillus cereus*, and *Bacillus pacificus*." On the other hand, more isolates failed to grow above 55% at pH 2.0 after three and six hours of exposure (Table 7). Every isolate proceeded to the next experiments even though, after six hours at pH 2.0, their survival rate had significantly decreased.

Table 6: Percentage survival of probiotic *Lactobacillus* sp. at different pH levels

Isolates	pH tolerance (%)					
	3 h			6 h		
	pH 2	pH 2.5	pH 3	pH 2	pH 2.5	pH 3
Lactobacillus fermentum	47.35 ± 1.07	80.39 ± 1.59	91.00 ± 1.60	39.40 ± 1.24	70.50 ± 1.11	83.29 ± 1.97
Lactobacillus ghanensis	52.29 ± 1.40	91.13 ± 1.11	96.11 ± 2.19	50.34 ± 1.17	71.52 ± 1.23	90.19 ± 1.76
Lactobacillus delbrueckii	47.35 ± 1.07	78.39 ± 1.50	93.00 ± 1.60	45.40 ± 1.34	78.50 ± 1.45	88.39 ± 1.67
Lactococcus lactis	48.36 ± 1.30	81.31 ± 1.98	90.02 ± 1.42	44.68 ± 1.23	67.58 ± 1.78	90.58 ± 1.08
Lactobacillus reuteri	45.35 ± 1.22	75.39 ± 1.50	95.00 ± 1.66	40.40 ± 1.54	70.50 ± 1.18	87.49 ± 1.77
Lactobacillus plantarum	55.00 ± 0.40	90.13 ± 1.17	96.11 ± 2.19	49.34 ± 1.27	69.52 ± 1.90	90.89 ± 1.26
Lysinibacillus sphaericus	50.15 ± 0.70	79.98 ± 1.10	92.28 ± 2.88	48.41 ± 0.77	71.11 ± 1.01	84.19 ± 0.99
Bacillus cereus	45.35 ± 1.34	76.39 ± 1.50	93.00 ± 1.66	47.40 ± 1.25	75.50 ± 1.18	86.36 ± 1.25
Bacillus pacificus	54.36 ± 1.56	78.31 ± 1.79	97.02 ± 1.41	51.68 ± 1.53	63.58 ± 1.48	89.98 ± 1.80

Table 7: Percentage survival of probiotic *Lactobacillus* sp. at 2%, 2.5% and 3.0% of bile salt after 24 hours

Number of *Lactobacillus* Isolates	Bile Salt tolerance at 2%	Bile Salt tolerance at 2.5%	Bile Salt tolerance at 3.0%
Lactobacillus fermentum, n=13	11(84.62%)	9(69.23%)	7(53.85%)
Lactobacillus ghanensis, n=9	7(77.78%)	6(66.67%)	5(55.56%)
Lactobacillus delbrueckii, n=10	7(70.00%)	6(60.00%)	5(50.00%)
Lactococcus lactis, n=14	11(78.57%)	9(64.29%)	9(64.29%)
Lactobacillus reuteri, n=9	8(88.89%)	6(66.67%)	5(55.56%)
Lactobacillus plantarum, n=8	6(75.00%)	5(62.50%)	5(62.50%)
Lysinibacillus sphaericus, n=7	5(71.43%)	5(71.43%)	4(57.14%)
Bacillus cereus, n=9	7(77.78%)	5(55.56%)	5(55.56%)
Bacillus pacificus, n=11	8(72.72%)	7(63.63%)	6(54.54%)

Tolerance to Bile Salts

In the presence of 2.0% bile salt, all nine the *Lactobacillus* isolates were able to survive at a rate greater than 70% (Table 8). The most tolerant isolate was *Lactobacillus reuteri*, which had a survival rate of 88.8%. Other isolates with survival rates of 84.62%, 77.78%, 70.00%, 78.57%, 75.00%, 71.43%, 77.78%, and 72.72% were "*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactococcus lactis, Lactobacillus plantarum, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus* isolates respectively" (Table 4.10). Moreover, in the presence of 2.5% bile salt, there was percentage decrease in the survival rate of the *Lactobacillus* spp which ranged from 60.00%, to 71.43%. Furthermore, in the presence of 3.0% bile salt, there was further decrease in the percentage of survival rate which also ranged from 53.85%, to 64.29%. The results obtained show that the higher the percentage of bile salt the lower the survival rate of the *Lactobacillus* species.

Table 8: Biofilm Formation in *Lactobacillus species* Isolated from Fermented Cassava and Corn Samples

Biofilm Results				
Isolates	Biofilm formation strength			
	No biofilm	Weak	Moderate	Strong
Lactobacillus fermentum (n=13)	1(7.7%)	2(15.4%)	4(30.8%)	6(46.1%)
Lactobacillus ghanensis (n=9)	2(22.2%)	3(33.3%)	2(22.2%)	2(22.2%)
Lactobacillus delbrueckii (n=10)	2(20.0%)	2(20.0%)	4(40.0%)	2(20.0%)
Lactobacillus plantarum (n=14)	1(7.1%)	3(21.4%)	4(28.6%)	6(42.9%)
Lactococcus lactis (n=9)	2(22.2%)	4(44.4%)	2(22.2%)	1(11.1%)
Lactobacillus reuteri (n=8)	2(25.0%)	2(25.0%)	3(37.5%)	1(12.5%)
Lysinibacillus sphaericus (n=7)	3(42.9%)	2(28.6%)	2(28.6%)	0(00.0%)
Bacillus cereus (n=9)	2(22.2%)	2(22.2%)	4(44.4%)	1(11.1%)
Bacillus pacificus (n=11)	2(18.2%)	4(36.4%)	3(27.3%)	2(18.2%)

Table 8 shows the biofilm formation in *Lactobacillus* sp. Isolated from fermented Cassava and corn samples was obtained using microtiter plate assay method. It was observed that high percentages (92.3%) of *Lactobacillus fermentum* isolates were able to form biofilms, and 46.1% of them formed biofilm strongly. The percentages of biofilm formation in "*Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus"* were 77.7%, 80.0%, 92.9%, 77.7%, 75.0%, 57.2%, 77.7% and 81.9% respectively. The results indicate that *Lactobacillus ghanensis* had weak biofilm formation strength 3(33.3%), *Lactobacillus delbrueckii* had moderate biofilm formation strength 4(40.0%), *Lactobacillus plantarum* had strong biofilm formation strength 6(42.9%), *Lactococcus lactis* had weak biofilm formation strength 4(44.4%), *Lactobacillus reuteri* had moderate biofilm formation strength 3(37.5%), *Lysinibacillus sphaericus* had weak biofilm formation strength 3(42.9%), *Bacillus cereus* had no biofilm formation strength 4(44.4%), and *Bacillus pacificus* had weak biofilm formation strength 4(36.4%), The strong biofilm formation in some strain indicates the sensitivity of the *Lactobacillus* sp.

Table 9: BLAST Prediction/Analysis

S/N	Sample ID	Organism	Strain	Sequence length (bp)	Identity/ Similarity (%)	Accession no of BLAST hit	P value	Alignment score	Highest query coverage (%)
1	N2	*Lactococcus lactis*	AE89	1,510	93.15%	OQ652962.1	0.010	≥200	98%
2	N3	Lactobacillus reuteri	CI	1,538	99.93%	EF412975	0.011	≥200	99%
3	S1	*Lactobacillus plantarum*	NRIC0383	1,554	98.92%	AB362652.1	0.002	≥200	98%
4	S2	*Lysinibacillus sphaericus*	Z15	887	98.99%	MH921646.1	0.003	≥200	99%
5	S3	*Bacillus cereus*	ABRIFBI-64	1,364	88.06%	ON387635.1	0.005	≥200	91%
6	S5	*Bacillus pacificus*	MP6	1470	99.73%	CP093424.1	0.00	≥200	99%
7	S6	*Lactococcus lactis*	HBUAS53615	1,470	94.79%	MZ787686.1	0.00	≥200	100%

Table 10: Molecular Identification of Isolates Using 16SrRNA (Fermented Cassava and Corn)

Isolate Similarity in Gene Bank	Similarity (%)	p-value
Lactococcus lactis	93.15%	0.010
Lactobacillus reuteri	99.93%	0.011
Lactobacillus plantarum	98.92%	0.002
Lysinibacillus sphaericus	98.99%	0.003
Bacillus cereus	88.06%	0.005
Bacillus pacificus	99.73%	0.00
Lactococcus lactis	94.79%	0.00

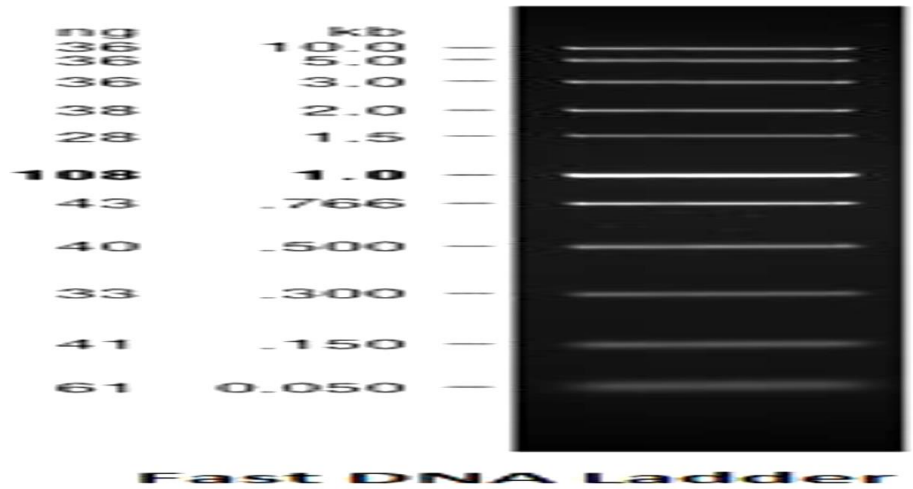

Figure 1: Annotation of ladder used in gel

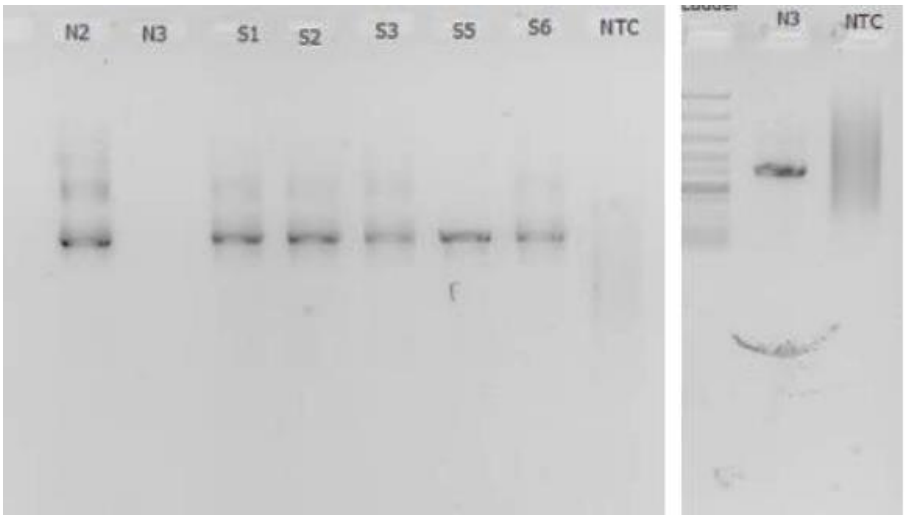

Figure 2: A photographic image of an agarose gel indicting the amplification of the 16S target region of *Lactobacillus* Sp.

Isolate N2: *Lactococcus lactis*, strain AE89, accession number OQ652962.1
Isolate N3: *Lactobacillus reuteri*, strain CI, accession number EF412975
Isolate S1: *Lactobacillus plantarum*, strain NRIC0383, accession number AB362652.1
Isolate S2: *Lysinibacillus sphaericus*, strain Z15, accession number MH921646.1
Isolate S3: *Bacillus cereus*, strain ABRIFBI-64, accession number ON387635.1
Isolate S4: *Bacillus pacificus*, strain MP6, accession number CP093424.1
Isolate S5: *Lactococcus lactis*, strain HBUAS53615, accession number MZ787686.1

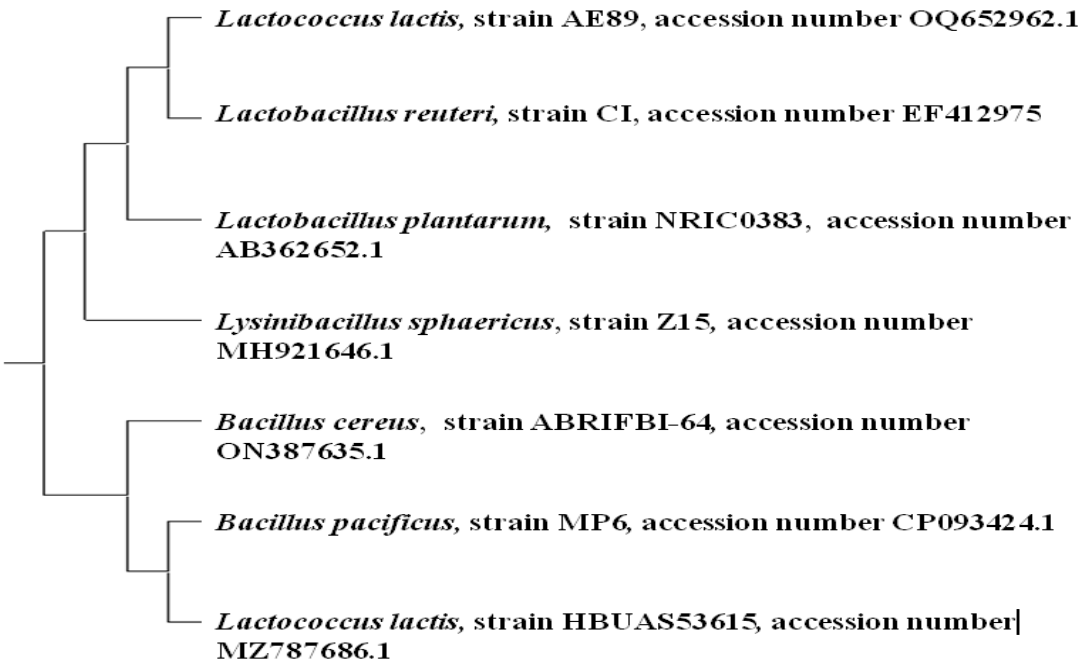

Figure 3: Phylogenetic Tree of Isolates

Discussion

Table 1 presents the total number of microbial isolates obtained from fermented cassava and corn samples. A total of ninety isolates were identified, with *Lactobacillus plantarum* being the most prevalent (15.6%), followed by *Lactobacillus fermentum* (14.4%). Other identified isolates include *Lactobacillus ghanensis, Lactobacillus reuteri, Lysinibacillus sphaericus, Lactobacillus delbrueckii, Bacillus cereus, Lactococcus lactis*, and *Bacillus pacificus*. Table 5 displays the antibiotic resistance profile of bacterial isolates from fermented cassava and corn samples. The isolates exhibited high resistance percentages towards antibiotics such as Cilastatin (87.0%), Gentamicin (93.9%), Oflaxacin (78.8%), and Azithromycin (80.1%). However, they showed relatively lower resistance to Ciprofloxacin and Levofloxacin. Notably, all tested *Lactobacillus* spp. displayed resistance to multiple antibiotics, consistent with previous research.

Table 7 presents the antimicrobial activity of *Lactobacillus* isolates against various pathogens. Lactobacillus fermentum and *Lactobacillus plantarum* exhibited the highest inhibition against pathogens such as *Candida* sp., *Staphylococcus* sp., and *Escherichia coli*. The inhibition zones ranged from 8.0 to 18.1 mm in diameter, indicating strong antagonistic activity against food-borne pathogens. According to Handa's (2012) research, isolates with clearance zones against the test pathogens that were less than 9 mm and more than 12 mm in diameter, respectively, indicated weak and strong antimicrobial activity. In this study, the survival rate of the nine *Lactobacillus* species exposed to pH 2.0 for six hours ranged from 70.00 to 88.89% (Table 7). According to a related study by Mourad and Nour-Eddine (2022), *Lactobacillus plantarum* OL12, OL9, OL15, and OL33, which were isolated from fermented olives, had survival percentages of 55%, 49%, 65%, and 57%, respectively, after being exposed to pH 2.0 for two hours. The results of Rajoka *et al*., (2021) and Akalu *et al.*, (2017), who reported that most *L. plantarum* strains isolated from different sources showed a survival rate above 80% at pH 2 for 3 hours, their investigation contradicts the result obtained by Mourad and Nour-Eddine.

In this study, in the presence of 2.0 to 3.0% bile salt, all the nine *Lactobacillus* isolates were able to survive at a rate ranging from 53.85% to 88.89% (Table 8). Bile salt tolerance is thought to be a critical selection criterion for probiotic isolates in order for them to survive in the small intestine. Additionally, as previously demonstrated, different

Lactobacillus species isolated from Omegisool, a traditionally fermented millet alcoholic beverage popular in Korea, also exhibit strain-specific tolerance to high bile salt conditions. Significant bile salt tolerance was shown by these species (Oh and Jung, 2020). In agreement with the present findings, earlier studies' findings have shown that each isolated strain showed a high level of tolerance to bile salt conditions, with survival rates for *Lactobacillus* strains ranging from 88% to 92% (Haghshenas *et al.*, 2019).

The characterization of the bacteria isolates using 16SrRNA gene sequence analysis shows the isolates to include the following; *Lactococcus lactis,* strain AE89 with accension number , *Lactobacillus reuteri,* strain CI with accension number EF412975, *Lactobacillus plantarum,* strain NRIC0383 with accension number AB362652.1, *Lysinibacillus sphaericus*, strain Z15 with accension number MH921646.1, *Bacillus cereus*, strain ABRIFBI-64 with accension number ON387635.1, *Bacillus pacificus*, strain MP6 with accension number CP093424, *Lactococcus lactis,* strain AE89, accession number OQ652962.1, *Lactococcus lactis,* strain HBUAS53615, accession number MZ787686.1 respectively (Table 9). According to Shokryazdan *et al.* (2020), a comparative 16S rRNA gene analysis showed that the *Lactobacillus* species isolates belonged to *L. acidophilus, L. fermentum, L. buchneri,* and *L. casei*. Additionally, Rajoka *et al*. (2021) identified Lactobacillus strains from breast-feeding piglets' faeces that might have probiotic properties using 16S rRNA gene analysis. Similar to this, other recent studies (Dowarah *et al*., 2018) have demonstrated the strain-level identification of various *Lactobacillus* sp. with strong probiotic qualities isolated from various substrates using phylogenetic estimation of 16S rDNA genes. Their findings were in agreement with this study.

Conclusion

In conclusion, the study on the probiotic potential of *Lactobacillus* species isolated from fermented cassava and corn meal highlights promising findings. Throughs rigorous analysis, it was evident that these *Lactobacillus* strains exhibit traits indicative of probiotic activity, such as acid and bile tolerance, antimicrobial properties, and adhesion capabilities. Furthermore, their ability to survive and proliferate in the gastrointestinal tract suggests their potential for exerting beneficial effects on human health. The findings underscore the significance of fermented cassava and corn meal as potential sources of probiotic strains, offering a natural and culturally relevant means of enhancing gut health. However, further research is warranted to explore the specific mechanisms underlying their probiotic effects, optimize their production, and evaluate their efficacy in human trials. Such endeavors hold promise for the development of novel probiotic products that could contribute to improved digestive health and overall well-being.

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