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Antimicrobial Activity and Statistical Correlation Analysis of *Lactobacillus* spp. Isolated from Fermented Cassava and Corn against Pathogenic Bacterial Isolates

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Abstract

This study focused on the antimicrobial activity and statistical correlation analysis of *lactobacillus* spp. isolated from fermented cassava and corn against pathogenic bacterial isolates.

Five (5) *Lactobacillus* strains, including *Lactobacillus fermentum*, *Lactobacillus ghanensis*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, and two *Bacillus* strains; *Bacillus cereus*, and *Bacillus pacificus*, others included *Lactococcus lactis* and *Lysinibacillus sphaericus*, were evaluated for inhibitory activity against a range of pathogenic bacterial isolates. These pathogens included *Candida* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Klebsiella* sp., *Escherichia coli*, *Salmonella* sp., and *Streptococcus* sp., with inhibition zones recorded to assess the effectiveness of each strain. Among the tested strains, *Lactobacillus plantarum* demonstrated the highest antimicrobial activity, showing inhibition zones of 18.1 mm against *Pseudomonas* sp., 15.3 mm against *Candida* sp., and 14.6 mm against *Staphylococcus* sp., while *Bacillus cereus* exhibited the lowest inhibitory effects. Statistical analysis, using t-tests and paired sample correlations, revealed significant relationships between certain pathogenic pairs, such as *Candida* sp. and *Klebsiella* sp. ($r = 0.905$, $p = 0.001$) and *Staphylococcus* sp. and *Streptococcus* sp. ($r = 0.765$, $p = 0.007$), indicating shared susceptibilities. A Euclidean distance proximity matrix highlighted clustering patterns among strains, with a close distance of 1.75mm observed between *Lactobacillus fermentum* and *Lactobacillus plantarum*, suggesting similar resistance profiles, while *Lactobacillus delbrueckii* and *Bacillus pacificus* displayed a larger distance of 4.32mm, reflecting distinct profiles. In conclusion, *Lactobacillus* species from fermented foods demonstrate promising antimicrobial properties, supporting their potential role in developing probiotic-based approaches for combating pathogenic bacteria.

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1. Introduction

Fermented foods have long been recognized for their health benefits and are an integral part of many diets worldwide, providing valuable nutrients and beneficial microorganisms, particularly lactic acid bacteria (LAB) (Aderolake et al., 2023). Among LAB, Lactobacillus species have gained increasing attention for their probiotic properties, which include promoting gut health, modulating immune responses, and inhibiting pathogens through competitive exclusion and antimicrobial compound production (Adesulu-Dahunsi et al., 2022; Drissi et al., 2014). The exploration of Lactobacillus strains from traditional fermented foods, such as cassava and corn, is of particular interest in Nigeria, where these products play a crucial role in the daily diet and serve as sources of indigenous LAB with unique health-promoting properties (Olodu et al., 2024; Drissi et al., 2015; Olaniran et al., 2023).

The investigation into the probiotic potential of Lactobacillus species isolated from fermented foods typically involves assessing their ability to survive gastrointestinal conditions, adhere to intestinal mucosa, and produce antimicrobial agents, such as organic acids and bacteriocins (Akter et al., 2023; Assohoun et al., 2023). These characteristics not only inhibit pathogenic microorganisms but also contribute to the balance of the gut microbiome, an essential component of overall health (Dahiya et al., 2023; Dahiya et al., 2022). However, alongside these health benefits, it is crucial to evaluate the antimicrobial resistance profiles of these strains, as horizontal gene transfer of resistance traits from probiotics to pathogens remains a public health concern (Joghataei et al., 2019; Kaur et al., 2022).

Modern approaches in microbial characterization, including statistical and computational methods such as the Euclidean distance proximity matrix, have enabled more precise assessments of strain-level differences and similarities among Lactobacillus species (Olodu et al., 2024; Petrović et al., 2012). This method provides insights into phenotypic and genetic relationships, thereby contributing to a better understanding of each strain's probiotic efficacy and safety profile (Rapoo et al., 2023; Suleiman et al., 2022). Proximity matrix analysis has proven particularly effective in identifying candidate strains with the potential for targeted health benefits, advancing the development of LAB-based functional foods and nutraceuticals (Gizachew et al., 2023; Gizachew et al., 2013).

In this study, Lactobacillus strains isolated from Nigerian fermented cassava and corn products were characterized for probiotic properties, antimicrobial resistance, and phylogenetic proximity through Euclidean distance analysis (Saeed et al., 2023; Samuel et al., 2019). These findings contribute to the growing body of knowledge on LAB, supporting the safety and health-promoting potential of these indigenous strains, and highlighting their application in functional food innovation (Kaushik et al., 2009; Khushboo et al., 2023; Obafemi et al., 2022).

2. Material and Methods

The methodology comprised several key stages, including sample collection, isolation of bacterial strains, characterization, and evaluation of antimicrobial properties.

2.1. 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, Isiyor 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.

2.2. 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 rinsed 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.

2.3. 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 Olodu et al., (2024) and Drissi et al., (2015). 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 (Olodu et al., 2024).

2.4. Identification of *Lactobacillus* sp.

The bacteria isolates were identified using their cultural, morphological and biochemical characterizations from fresh cultures of Lactobacillus spp. (isolates grown on MRS Agar for 24 hours) with modified versions of the techniques published by Olodu et al., (2024) and Drissi et al., (2015). The following tests were carried out; Gram staining, Motility, Catalase, Oxidase, Coagulation, Citrate, Indole Endospore, Hydrogen sulphide production, Gas production from glucose fermentation and Sugar fermentation.

2.4.1. Gram Staining

The test organism was smeared onto a spotless glass slide. After that, a drop of distilled water was added to moisten it. Additionally, it was placed over a flame to heat fix without burning the smear. Furthermore, 0.3% w/y crystal violet stain was added, and it was left to stand for a minute. The strain was dissolved using distilled water. After adding iodine (0.4% w/y) as a mordant and letting it stand for a minute, the mixture was cleaned with distilled water. After adding acetone/ethanol (95% w/y), a decolorizer was added and left for 10 seconds. The mixture was then quickly rinsed with distilled water, and safranin (0.4% w/y), the secondary stain, was added and left for one minute and this was cleaned with distilled water and left to dry. Next, using an oil immersion lens magnification of x100, the stained smear was examined under a microscope. Gram positive bacteria retain purple background while Gram negative bacteria retain pink or red colour (Joghataei et al., 2019; Kaur et al., 2022).

2.4.2. Motility Test

To determine whether the isolates were motile or non-motile species, the hanging drop method was used to conduct the motility test. First, a cleaned cover glass was used to hold a tiny drop of MRS broth containing a specific isolate. Next, a concave depression slide with its cavity facing downward onto the broth drop was adhered to the cover glass using Vaseline. The slide was then inverted and focus with the lowest magnification to the higer magnification using oil immersion to observe the motility. It was observed that the motile organism moved from one point to another, while the non-motile organism remained stationary (Adesulu-Dahunsi et al., 2022; Drissi et al., 2014).

2.4.3. Catalase Test

This test is used to identify organism that produce the enzyme, catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas. On a microscopic slide, a loopful of bacteria was mixed with a drop of 3% (v/v) hydrogen peroxide to assess the generation of oxygen bubbles. The bubbles resulting from production of oxygen gas clearly indicate a catalase positive result. The absence of bubbles indicates negative result (Gizachew et al., 2023; Gizachew et al., 2013).

2.4.4. Oxidase Test

The oxidase test was used to detect the presence of a cytochrome oxidase system that will catalase the transport of electrons between electron donors in the bacteria and a redox dye-tetrmethyl-p-phenylenediamine. The test uses reagents such as impregnated disks and N,N,N′,N′-tetramethyl-p-phenylenediamine, TMPD (or N,N-dimethyl-p-phenylenediamine, DMPD, which is also a redox indicator). A sterile swab was used to obtain a small amount of the isolate; a drop of oxidase reagent will be placed onto the culture, positive reaction turn the bacteria violet to purple immediately or within 10 to 30 seconds, absence of a violet to purple colour will be regarded as a negative result (Akter et al., 2023; Assohoun et al., 2023).

2.4.5. Coagulase Test

The coagulase test was used to determine the bacterium's ability to produce the enzyme coagulase, which causes blood plasma to clot. A bacterial colony was emulsified in a drop of water with minimal spreading. A drop of plasma was then added to this mixture. A positive result is indicated by the formation

of visible clumps or particles on a glass slide, while a negative result shows no clumping (Rapoo et al., 2023).

2.4.6. Citrate Test

The citrate test determines an organism's ability to utilize citrate as its sole carbon and energy source. This test is part of the IMViC series, commonly used to differentiate bacterial families, including Lactobacillaceae. After preparing citrate agar, 5 milliliters were dispensed into each test tube, autoclaved, tilted, and allowed to solidify. The test organism was then inoculated, and the tubes were incubated for 24 hours. A positive result is indicated by a color change from green to blue, while no color change indicates a negative result (Adesulu-Dahunsi et al., 2022; Drissi et al., 2014).

2.4.7. Indole Test

The indole test evaluates an organism's capacity to synthesize indole by utilizing tryptophan. As a component of the IMViC test, the test is used to distinguish between members of the Lactobacillaceae family. The ability of the organism to oxidize tryptophan and produce indole, pyruvic acid, and ammonia is tested by measuring indole production. By adding Kovac's reagent (dimethyl aminobenzaldehyde), the production of indole during the reaction can be detected. A positive result is indicated by the presence of a cherry-red layer at the top of the test tube; a negative result is indicated by its absence. A few drops of Kovac's reagent were added to an overnight peptone broth culture of the isolate. The formation of a cherry-red layer on the broth's surface indicated the production of indole (Akter et al., 2023; Assohoun et al., 2023).

2.4.8. Sugar Fermantative Test

Five (5) sugar was utilized; glucose, maltose, fructose, lactose and sucrose. The isolates were inoculated into the respective cooled sterilized tubes containing 10 ml of the basal medium (Peptone water), inverted Durham tube to detect gas production, 1ml of the 1% sugar solution (sterilized by steam sterilization for 30 min) and methyl red indicator. The inoculated tubes were incubated anaerobically at 370C for 48 hours. After incubation, the tubes were observed for acid and gas production by the respective isolate. Acid production was indicated by the formation of a yellow colour while gas evolution was determined by the formation of bubbles and spaces within the inverted Durham tube (Akter et al., 2023; Assohoun et al., 2023).

2.4.9. Endospore

The examination of spore production was used to categorize and identify bacteria as either spore formers or non-spore formers. On a microscopic slide, a bacterial smear was produced and heat fix without burning the smear, safranin and malachite green were colored for two minutes and thirty seconds. After rinsing with water to remove any remaining stain, the slide was examined under an oil immersion microscope. Spore forming bacteria retained green background colour and nonspore forming bacteria retained blue backgroyund (Assohoun et al., 2023).

2.4.10. Hydrogen Sulfide Production

Bacteria capable of sulfur reduction can produce hydrogen sulfide (H_2S). A single colony from each isolate was inoculated by stabbing into Triple Sugar Iron Agar (TSI) slants (HiMedia, M0211), prepared according to the manufacturer's instructions. The

tubes were incubated at 37°C for 24 hours in duplicates, with a non-inoculated tube serving as the negative control. Hydrogen sulfide production was detected by the formation of a black precipitate, indicating a positive result (Khushboo et al., 2023; Obafemi et al., 2022).

2.4.11. Gas Production from Glucose Fermantation

Isolates were classified as homofermenters, heterofermenters, or facultative heterofermenters based on their ability to ferment glucose. Each isolate was inoculated into centrifuge tubes containing modified MRS broth (prepared with 20 g of glucose per liter), with inverted Durham tubes placed inside. The tubes were incubated at 37°C for 24 hours in duplicates. Escherichia coli was used as a positive control, while a non-inoculated tube served as a negative control. Gas production was observed as bubbles trapped in the Durham tubes (Kaushik et al., 2009; Obafemi et al., 2022).

2.5. Isolation of Bacterial Strains

The isolation of *Lactobacillus* species was carried out using selective culture media to ensure the growth of target bacteria while minimizing the proliferation of unwanted microorganisms. Approximately 10 grams of each fermented sample were carefully weighed and homogenized in 90 mL of sterile saline solution to create an initial suspension. This suspension was then subjected to serial dilution using a ten-fold dilution technique to achieve concentrations suitable for microbial isolation. From each dilution, 0.1 mL aliquots were aseptically transferred and evenly spread onto de Man, Rogosa, and Sharpe (MRS) agar plates, a selective medium specifically formulated to support the growth of *Lactobacillus* species. The inoculated plates were incubated anaerobically at a temperature of 37°C for 48 hours using an anaerobic jar with gas packs to create an oxygen-free environment conducive to the growth of facultative anaerobes. After the incubation period, colonies displaying characteristic morphological features of *Lactobacillus*, such as circular, smooth, convex colonies with creamy white appearance, were carefully selected. These colonies were subsequently subcultured onto fresh MRS agar plates to ensure purity and eliminate potential contaminants. The purified isolates were then preserved under appropriate storage conditions for further biochemical and molecular identification to confirm their classification as *Lactobacillus* species.

2.6. Determination of antimicrobial properties of the isolates

The antimicrobial qualities of the isolates were ascertained in duplicate using the agar-well diffusion experiment. First, isolates 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. The species from the isolates 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, according to Kaushik et al., 2009; Khushboo et al., 2023; Obafemi et al., 2022.

2.7. Pathogens Testing

Seven pathogenic organisms were collected from Microbiology laboratory in Central hospital, Benin City, Nigeria. These pathogens were cultured on different Agar and show different colouration and structures Candida sp were cultured on Sabouraud dextrose Agar which indicated white colour, Staphyloccocus and Streptococcocus sp were cultured on Chocolate Agar which indicated golden yellow, Pseudomonas sp were plated on Cled Agar which indicated greenish colonies, Salmonella species were plated on Salmonella Shigella Agar which indicated black colony, Escherichia coli were plated on MacConkey Agar which indicate pinkish colony and Klebsiella sp were plated on MacConkey Agar which indicated pink mucoid. Confirmation test was done using biochemical methods as follows; Staphylococcus species indicated catalase positive and coagulate positive, Streptococcus shows catalase negative, Salmonella species shows indole, citrate and urease negative. Eschericha coli shows indole positive. Candida species was incubated in serum at 37 °C for 2 - 4 hrs and then observed for the presence of tube structures indicated positive result, Klebsiella species shows a negative result in methyl-red test, indole test and catalase tests positive, Pseudomonas sp shows methy red negative and catalase positive. This is a simple, rapid, and economical identification technique with 98% sensitivity (Obafemi et al., 2022).

2.8. Statistical Analysis

The data obtained from the study were analyzed using descriptive and inferential statistical tools available in SPSS software version 23. Descriptive statistics, including mean, standard deviation, and frequency distributions, were used to summarize and represent the data effectively. The antimicrobial activity results were analyzed using a Euclidean distance proximity matrix to evaluate the degree of similarity and dissimilarity among the isolated *Lactobacillus* strains based on their inhibitory effects against test pathogens. Furthermore, one-way analysis of variance (ANOVA) was employed to determine significant differences in antimicrobial activity among the strains. A significance level of $p < 0.05$ was considered statistically significant, ensuring that observed differences were unlikely to have occurred by chance. All results were presented in tables for clarity and ease of interpretation.

3. Results

Table 1. Morphological and Biochemical Characteristics of *Lactobacillus* species Isolated from fermented Cassava and Corn

Colonial Morphology					**Staining Reactions**		**Biochemical Tests**							**Sugar Fermentation**						
Isolate code	**Shape**	**Cell shape**	**Cell arrangement**	**Colour**	**Gram staining**	**Spore staining**	**Catalase test**	**Indole production**	**Oxidase Test**	**Indole Test**	**CO_2 from glucose**	**Coagulase test**	**H_2S production**	**Fructose**	**Sucrose**	**Galactose**	**Lactose**	**Glucose**	**Citrate utilization**	**Probable isolates**
M1	CS	R	FL	CR	+veG	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+veG	+veG	+ve	+veG	+veG	+ve	*Lactobacillus fermentum*
M2	CS	SR	C	WH	+veG	-ve	-ve	-ve	-ve	+ve	+ve	- ve	- ve	+ve	+ve	+ ve	+ve	+ ve	-ve	*Lactobacillus ghanensis*
N1	CS	PP	S	CR	+veG	-ve	-ve	-ve	-ve	+ve	+ve	- ve	- ve	+veG	+veG	+ ve	+veG	+ve	-ve	*Lactobacillusdelbrueckii*
N2	BS	R	C	WH	+veG	-ve	-ve	-ve	-ve	+ve	+ve	- ve	- ve	+ve	+ ve	+ ve	+ve	+ ve	-ve	*Lactococcus lactis*
N3	IS	R	C	CR	+veG	-ve	-ve	-ve	-ve	+ve	+ve	- ve	+ ve	+veG	+veG	+ ve	+veG	+veG	-ve	*Lactobacillus reuteri*
S1	CS	SR	C	WH	+veG	- ve	-ve	-ve	-ve	+ve	+ve	- ve	-ve	+veG	+ ve	+ ve	+ ve	+ve	-ve	*Lactobacillus plantarum*
S2	BS	R	C	DC	+veG	-ve	-ve	-ve	-ve	+ve	+ve	- ve	- ve	-ve	+ve	+ ve	+ve	+ ve	-ve	*Lysinibacillus sphaericus*
S3	CS	SR	FL	CR	+veG	-ve	-ve	-ve	-ve	+ve	+ve	- ve	- ve	+veG	+veG	+ve	+veG	+veG	-ve	*Bacillus cereus*
S4	CS	R	FL	BC	+veG	-ve	-ve	-ve	-ve	+ve	+ve	- ve	- ve	+veG	-ve	+ ve	+ve	+ve	-ve	*Bacillus pacificus*

SR=Single rods R=Rods PP=Paired rods C=Cocci in bunch FL=Filamentous CR=Creamy WH=Entire whitish A = Colour change +ve = Positive -ve = Negative, +veG=Gram positive -veG=Gram negative BC=Bright creamy DC=Dull creamy CS=Curved shape IS=Irregular shape BS=Broad shape

Table 2. Organisms Isolated from Fermented Cassava and Corn Samples from the Various Locations in Benin City, Nigeria (Based on Biochemical Test)

Location	Number of Samples	Number with Isolated Organisms	Isolates
Ikpobahill area	8	8(88.89%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus.*
Ekiosa market	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
New Benin area	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
Okha area	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
Uselu area	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
Oliha area	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
Oka market	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
Isiyor area	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
Ugbighoko market	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
Aduwawa market	9	9(100%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, Lysinibacillus sphaericus, Bacillus cereus, Bacillus pacificus.*
Total	90	89(98.89%)	*Lactobacillus fermentum, Lactobacillus ghanensis, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus lactis, Lactobacillus reuteri, 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	
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
Isiyor 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 4. Antimicrobial Activity of *Lactobacillus* spp. and *Bacillus* spp. isolated from fermented Cassava and corn against Pathogenic bacterial isolates

	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. Paired Samples Statistics

Paired Samples		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	*Candida sp.*	*10.978*	*9*	*2.2775*	*0.7592*
	Staphylococcus sp.	*11.167*	*9*	*2.5792*	*0.8597*
Pair 2	*Candida sp.*	*10.978*	*9*	*2.2775*	*0.7592*
	Pseudomonas sp.	*11.300*	*9*	*3.1749*	*1.0583*
Pair 3	*Candida sp.*	*10.978*	*9*	*2.2775*	*0.7592*
	Klebsiella sp.	*11.522*	*9*	*3.6581*	*1.2194*
Pair 4	*Candida sp.*	*10.978*	*9*	*2.2775*	*0.7592*
	Escherichia coli	*10.678*	*9*	*2.5728*	*0.8576*
Pair 5	*Candida sp.*	*10.978*	*9*	*2.2775*	*0.7592*
	Streptococcus sp.	*8.56*	*9*	*1.424*	*0.475*
Pair 6	*Staphylococcus sp.*	*11.167*	*9*	*2.5792*	*0.8597*
	Pseudomonas sp.	*11.300*	*9*	*3.1749*	*1.0583*
Pair 7	*Staphylococcus sp.*	*11.167*	*9*	*2.5792*	*0.8597*
	Klebsiella sp.	*11.522*	*9*	*3.6581*	*1.2194*
Pair 8	*Staphylococcus sp.*	*11.167*	*9*	*2.5792*	*0.8597*
	Escherichia coli	*10.678*	*9*	*2.5728*	*0.8576*
Pair 9	*Staphylococcus sp.*	*11.167*	*9*	*2.5792*	*0.8597*
	Streptococcus sp.	*8.56*	*9*	*1.424*	*0.475*
Pair 10	*Pseudomonas sp.*	*11.300*	*9*	*3.1749*	*1.0583*
	Klebsiella sp.	*11.522*	*9*	*3.6581*	*1.2194*
Pair 11	*Pseudomonas sp.*	*11.300*	*9*	*3.1749*	*1.0583*
	Escherichia coli	*10.678*	*9*	*2.5728*	*0.8576*
Pair 12	*Pseudomonas sp.*	*11.300*	*9*	*3.1749*	*1.0583*
	Streptococcus sp.	*8.56*	*9*	*1.424*	*0.475*
Pair 13	*Klebsiella sp.*	*11.522*	*9*	*3.6581*	*1.2194*
	Escherichia coli	*10.678*	*9*	*2.5728*	*0.8576*
Pair 14	*Klebsiella sp.*	*11.522*	*9*	*3.6581*	*1.2194*
	Streptococcus sp.	*8.56*	*9*	*1.424*	*0.475*
Pair 15	*Escherichia coli*	*10.678*	*9*	*2.5728*	*0.8576*
	Streptococcus sp.	*8.56*	*9*	*1.424*	*0.475*

Table 6. Paired Samples Correlations

Paired Samples		N	Correlation	Sig.
Pair 1	*Candida* sp. & *Staphylococcus* sp.	9	0.864	0.003
Pair 2	*Candida* sp. & *Pseudomonas* sp.	9	0.786	0.012
Pair 3	*Candida* sp. & *Klebsiella* sp.	9	0.905	0.001
Pair 4	*Candida* sp. & *Escherichia coli*	9	0.782	0.013
Pair 5	*Candida* sp. & *Streptococcus* sp.	9	0.494	0.177
Pair 6	*Staphylococcus* sp. & *Pseudomonas* sp.	9	0.906	0.001
Pair 7	*Staphylococcus* sp. & *Klebsiella* sp.	9	0.914	0.001
Pair 8	*Staphylococcus* sp. & *Escherichia* coli	9	0.696	0.037
Pair 9	*Staphylococcus* sp. & *Streptococcus* sp.	9	0.135	0.729
Pair 10	*Pseudomonas* sp. & *Klebsiella* sp.	9	0.937	0.000
Pair 11	*Pseudomonas* sp. & *Escherichia coli*	9	0.754	0.019
Pair 12	*Pseudomonas* sp. & *Streptococcus* sp.	9	0.006	0.989
Pair 13	*Klebsiella* sp. & *Escherichia coli*	9	0.777	0.014
Pair 14	*Klebsiella* sp. & *Streptococcus* sp.	9	0.213	0.582
Pair 15	*Escherichia coli* & *Streptococcus* sp.	9	0.058	0.881

Table 7. Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	*Candida* sp. - *Staphylococcus* sp.	-0.1889	1.3014	0.4338	-1.1892	0.8114	-0.435	8	0.675
Pair 2	*Candida* sp. - *Pseudomonas* sp.	-0.3222	1.9766	0.6589	-1.8416	1.1971	-0.489	8	0.638
Pair 3	*Candida* sp. - *Klebsiella* sp.	-0.5444	1.8696	0.6232	-1.9815	0.8926	-0.874	8	0.408
Pair 4	*Candida* sp. - *Escherichia coli*	0.3000	1.6256	0.5419	-0.9495	1.5495	0.554	8	0.595
Pair 5	*Candida* sp. - *Streptococcus* sp.	2.4222	2.0030	0.6677	0.8826	3.9619	3.628	8	0.007
Pair 6	*Staphylococcus* sp. - *Pseudomonas* sp.	-0.1333	1.3766	0.4589	-1.1915	0.9248	-0.291	8	0.779
Pair 7	*Staphylococcus* sp. - *Klebsiella* sp.	-0.3556	1.6712	0.5571	-1.6401	0.9290	-0.638	8	0.541
Pair 8	*Staphylococcus* sp. - *Escherichia coli*	0.4889	2.0096	0.6699	-1.0558	2.0336	0.730	8	0.486
Pair 9	*Staphylococcus* sp. - *Streptococcus* sp.	2.6111	2.7728	0.9243	0.4797	4.7425	2.825	8	0.022
Pair 10	*Pseudomonas* sp. - *Klebsiella* sp.	-0.2222	1.3036	0.4345	-1.2243	0.7798	-0.511	8	0.623
Pair 11	*Pseudomonas* sp. - *Escherichia coli*	0.6222	2.0945	0.6982	-0.9878	2.2322	0.891	8	0.399
Pair 12	*Pseudomonas* sp. - *Streptococcus* sp.	2.7444	3.4724	1.1575	0.0753	5.4136	2.371	8	0.045
Pair 13	*Klebsiella* sp. - *Escherichia coli*	0.8444	2.3179	0.7726	-0.9373	2.6262	1.093	8	0.306
Pair 14	*Klebsiella* sp. - *Streptococcus* sp.	2.9667	3.6315	1.2105	0.1753	5.7581	2.451	8	0.040
Pair 15	*Escherichia coli* - *Streptococcus* sp.	2.1222	2.8670	0.9557	-0.0815	4.3260	2.221	8	0.057

Table 8. Proximity Matrix (Euclidean Distance) of Antimicrobial Activity of *Lactobacillus* sp from Fermented Cassava and Corn

	Euclidean Distance								
	Lactobacillus fermentum	***Lactobacillus ghanensis***	***Lactobacillus delbrueckii***	***Lactobacillus plantarum***	***Lactococcus lactis***	***Lactobacillus reuteri***	***Lysinibacillus sphaericus***	***Bacillus cereus***	***Bacillus pacificus***
Lactobacillus fermentum	0.000	5.946	10.578	7.688	11.860	7.399	9.690	13.879	6.704
Lactobacillus ghanensis	5.946	0.000	5.767	11.853	6.778	2.805	5.555	8.272	4.566
Lactobacillus delbrueckii	10.578	5.767	0.000	15.854	3.311	4.981	2.915	6.096	6.804
Lactobacillus plantarum	7.688	11.853	15.854	0.000	17.490	13.367	15.917	19.872	9.737
Lactococcus lactis	11.860	6.778	3.311	17.490	0.000	5.811	4.567	4.015	8.640
Lactobacillus reuteri	7.399	2.805	4.981	13.367	5.811	0.000	5.031	7.153	5.225
Lysinibacillus sphaericus	9.690	5.555	2.915	15.917	4.567	5.031	0.000	6.698	7.899
Bacillus cereus	13.879	8.272	6.096	19.872	4.015	7.153	6.698	0.000	10.966
Bacillus pacificus	6.704	4.566	6.804	9.737	8.640	5.225	7.899	10.966	0.000

This is a dissimilarity matrix

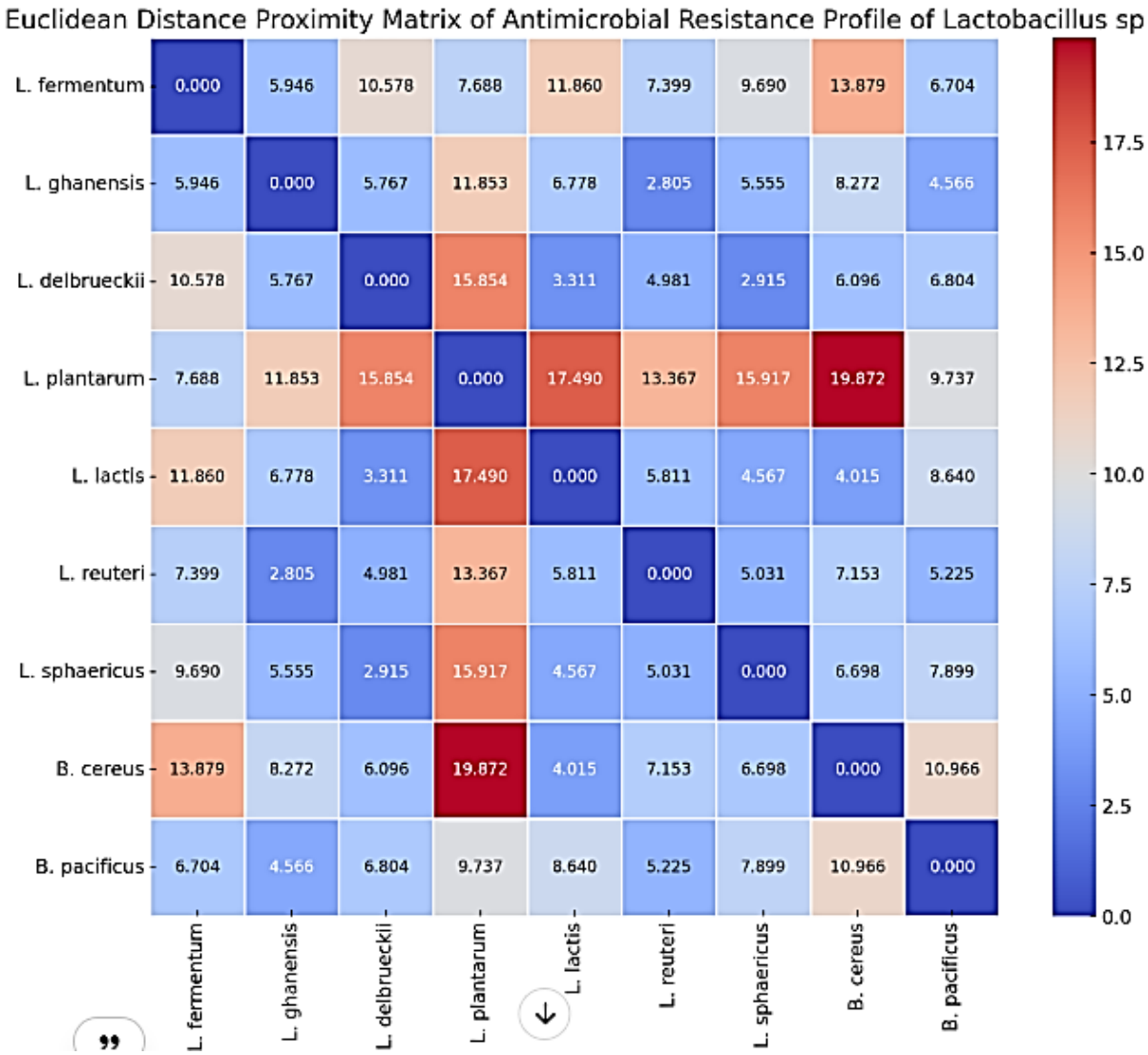

Figure 1. The Euclidean distance heatmap for the antimicrobial resistance profile of *Lactobacillus* species from fermented cassava and corn. The heatmap visually represents the degree of dissimilarity between the bacterial strains, with color intensity indicating the distance values

4. Discussion

The antimicrobial activity of *Lactobacillus* species from fermented cassava and corn against clinical isolates (*Candida* sp., *Salmonella* sp., *Staphylococcus* sp., *Streptococcus* sp., *Pseudomonas* sp., *Klebsiella* sp., and *Escherichia coli*) demonstrated varied inhibition zones. Notably, *Lactobacillus fermentum* exhibited significant inhibition zones, ranging from 11.0 ± 0.20 mm to 15.2 ± 0.16 mm. Similarly, *Lactobacillus plantarum* displayed the highest inhibition zones, with values reaching up to 18.1 ± 0.10 mm against *Streptococcus* sp. and 17.8 ± 0.50 mm against *Staphylococcus* sp. These findings align with previous studies that reported the robust antimicrobial activity of *Lactobacillus plantarum* against gram-positive and gram-negative bacteria (Kaushik et al., 2009; Adesulu-Dahunsi et al., 2022). In contrast, *Bacillus cereus* exhibited relatively lower inhibition zones, with values of 6.0 ± 0.50 mm against *Pseudomonas* sp. and 8.6 ± 1.50 mm against *Candida* sp, Similarly, findings by Akter et al. (2023) revealed that *Lysinibacillus sphaericus* demonstrated limited efficacy, with inhibition zones ranging between 6.0 ± 0.50 mm to 9.5 ± 0.88 mm. Their results compared favourably with the results obtained in this study.

Statistical analysis presented in Table 5 revealed significant variations in inhibition zones across pathogen pairs. For instance, *Candida* sp. had a mean inhibition zone of 10.978 mm, while *Staphylococcus* sp. recorded a mean of 11.167 mm. The minimal difference of 0.1889 mm in Table 7 suggests consistent inhibitory responses by certain *Lactobacillus* strains (Gizachew et al., 2023; Joghataei et al., 2019). Correlation analysis in Table 6 revealed a strong positive relationship (r=0.906, p = 0.001) between inhibition responses of *Staphylococcus* sp. and *Pseudomonas* sp., supporting the hypothesis of shared inhibitory mechanisms, potentially involving bacteriocins and lactic acid production (Dahiya & Nigam, 2023; Dahiya & Nigam, 2022).

Statistical differences further highlighted unique responses, particularly between *Streptococcus* sp. and *Candida* sp. (p = 0.007), indicating selective pathogen-targeting abilities of specific *Lactobacillus* strains. These findings are consistent with earlier studies by Aderolake et al. (2023) and Owusu-Kwarteng et al. (2015), emphasizing the potential of *Lactobacillus fermentum* and *Lactobacillus plantarum* as effective probiotics for therapeutic applications.

The Euclidean Distance Matrix (Table 8) categorized antimicrobial profiles based on proximity, with the smallest dissimilarity (6.704 mm) observed between *Lactobacillus fermentum* and *Bacillus pacificus*, while the highest dissimilarity (19.872 mm) was recorded between *Lactobacillus plantarum* and *Bacillus cereus*. Figure 1 visually reinforces these findings, with lower distances indicating shared inhibitory mechanisms. These results align with reports by Khushboo et al. (2023) and Assohoun et al. (2023), underscoring the importance of strain-specific antimicrobial efficacy in probiotic formulation.

In summary, *Lactobacillus fermentum* and *Lactobacillus plantarum* demonstrated superior antimicrobial activity, making them promising candidates for probiotic therapies. Conversely, the

limited inhibitory effects of *Bacillus cereus* and *Lysinibacillus sphaericus* suggest a reduced therapeutic potential. These findings align with previous literature (Mokoena et al., 2016; Akter et al., 2023) and highlight the need for further strain characterization to optimize their use in targeted antimicrobial applications.

5. Conclusion

In conclusion, the Lactobacillus strains isolated from fermented cassava and corn exhibit significant probiotic potential, characterized by notable antimicrobial activity against pathogenic bacteria and robust antioxidant properties. The study's findings highlight the strains' resilience in harsh conditions, reinforcing their suitability for application in functional food products. The uniformity in antimicrobial effectiveness, as indicated by the Euclidean distance proximity matrix, suggests that these isolates could serve as effective probiotics in the food industry. Overall, this research contributes valuable insights into the development of sustainable, plant-based probiotics, enhancing both food safety and consumer health.

Ethical Statement

The study is proper with ethical standards, it was approved by the Department of Biological Sciences (Microbiology), Benson Idahosa University on 26th February, 2024. The Ethics Committee approval number is ETH/2024/0026.

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Presentation Information

The data from this study were presented as an oral presentation at the Department of Biological Sciences (Microbiology), Benson Idahosa University

Conflicts of Interest

The authors declare no conflicts of interest regarding this study.

Author Contributions

Blessing Adoh Olodu contributed to the study design, data collection, laboratory analysis, statistical analysis, and manuscript drafting. Prof. Stephen Amadin Enabulele provided guidance on study design, supervised the laboratory work, contributed to data interpretation, and critically revised the manuscript for important intellectual content.

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