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EVALUATION OF INHIBITORY SUBSTANCE- PRODUCING BACTERIA ISOLATED FROM PALM WINE (*ELAEIS GUINEENIS)* SOLD IN ELELE MARKET, RIVERS STATE, NIGERIA.

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ABSTRACT: *The increase in the number of antimicrobial-resistant food-borne pathogens has become a cause for concern. There is a need to find novel strategies to combat these pathogens, in order to reduce food poisoning and even promote food security. Probiotic bacteria, including lactic acid bacteria, were isolated from palm wine (Elaeis guineensis) to evaluate antimicrobial effects on some selected spoilage and pathogenic microorganisms (Escherichia sp, Pseudomonas sp and Staphylococcus sp). They were identified by gram staining, catalase test, spore staining tests, sugar fermentation test and molecular analysis. These isolates were tested against pathogens to determine if they were capable of inhibiting their growth, and the isolates that exhibited antimicrobial properties were Bacillus cereus, Lactobacillus sp 1 and Lactobacillus sp 2. Bacillus cereus showed 18 mm against both Staphylococcus aureus and Pseudomonas aeruginosa, Lactobacillus sp 1 showed 20 mm against Pseudomonas aeruginosa, while Lactobacillus sp 2 showed 21 mm against Staphylococcus aureus. The quantitative analysis of the molecular results shows the absorbance ratio (A260/A280) for Lactobacillus sp 1 as 1.49, Bacillus cereus as 1.69 and Lactobacillus sp 2 as 1.29. The results indicated that Bacillus cereus, Lactobacillus sp 1 and Lactobacillus sp 2 exhibited probiotic potential.*

KEYWORDS: Antimicrobial, Probiotic; Inhibitory, Lactic Acid Bacteria, Food spoilage, Pathogens.

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INTRODUCTION

Probiotics are defined as microorganisms that live in symbiosis with the human host. When ingested in adequate quantities, probiotics may modulate biological functions, with health benefits. Different biological properties have been reported for probiotics, including antimicrobial activity (da Silva et al., 2023). Studies have shown that different probiotics, in addition to having beneficial effects on the host's health, have a very good ability to eliminate and neutralize pathogens and their toxins in foods, which leads to enhanced food safety (Ansari et al., 2023). The antimicrobial or antagonistic activity of probiotics is an important property that includes the production of antimicrobial compounds, such as bacteriocins, competitive exclusion of pathogens, enhancement of the intestinal barrier function in resisting pathogens, as well as enhancing the immune system of the host in order to successfully combat pathogens (Fijan, 2023).

Prolonged usage of probiotics in food ingredients for human as well as in animal feed has not exposed any side effects yet. Although antibiotics play a crucial role in the treatment of various diseases, there are adverse side effects associated with them, such as allergic reactions, gastrointestinal effects (e.g., nausea, loss of appetite, bloating, vomiting, abdominal pain, diarrhoea) resulting from the disturbance of gut flora, and so on (Acharjee et al., 2022). Hence, to formulate new drugs beside the available antibiotics, the natural components from plant origin should come into focus in terms of undermining the potential antibiotic resistant bacteria (Sharmin et al., 2014). Recently, innovative technologies have attracted more attention. Bio-preservation is an approach against food spoilage to produce more natural food that meets consumer demands, including preservative-free and less processed foods (Erfani et al., 2024).

The aim of the study was to carry out comprehensively the evaluation of inhibitory substance-producing bacteria from food source (palm wine) gotten from Elele Market, Rivers State, Nigeria.

MATERIALS AND METHOD

Study Area

Elele is a town in Rivers State, Nigeria. Madonna University is located in Elele. Elele has about 20,600 residents with a longitude of 6.81417°or 6°48' 51" east and latitude of 5.10167°or 5°6' 6" north. In Elele, the wet season is warm and overcast, the dry season is hot and mostly cloudy, and it is oppressive year round. Over the course of the year, the temperature typically varies from 69°F to 88°F and is rarely below 61°F or above 91°F.

Collection of Samples

The palm wine samples were collected in Elele Market in Rivers State, South region of Nigeria. Five samples were collected, and the samples were introduced into sterile bottles, placed in a cooler at temperature of 4°C and transported to the laboratory for the isolation of inhibitory substance-producing bacteria.

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Sample Processing

Each sample was cultured on de Man Rogosa and Sharpe Agar (MRS) and incubated at 37ºC for 24 hrs under aerobic and anaerobic conditions.

Isolation and Phenotypic Identification of Lactic Acid Bacteria

Lactic acid bacteria were isolated from a palm wine sample by pour plate method using de Man Rogosa and Sharpe Agar (MRS). For this purpose, ten-fold serial dilution was realized with saline solution (NaCl, 0.85% w/v). Then, 1 ml aliquot of the 10^{-2}, 10^{-4} and 10^{-6} dilutions were aseptically disposed on sterile plates and were done in duplicates. MRS agar was poured onto it and allowed to set. All plates were incubated at 37°C for 24 hrs under both aerobic and anaerobic conditions. Only counts of 30 and 300 cfu were considered. The counts were then multiplied by the dilution factor inverse for the respective plates, to give the total aerobic and anaerobic counts per ml of sample. Pure colonies cultures of the isolates were obtained by repeatedly streaking the lactic acid bacteria isolates on Nutrient agar. In order to obtain pure cultures, the colony was inoculated in MRS agar medium in a slant bijou bottle and incubated at 37ºC. Lactic acid bacteria were identified via their morphological and biochemical characteristics, and molecular methods (Schillinger et al., 1989).

Morphological and Cultural Examination

Cultural Characteristics

The cultural characteristics observed after incubating for 24 hrs at 37ºC included shape, color, texture, elevation, form, edge and size, which were all noted and recorded.

Morphological Characteristics

Gram Staining Technique

This was done by placing a small drop of isolate on a slide and adding a drop of normal saline which was allowed to dry. The primary stain (crystal violet) was applied using a dropper to the slide and was allowed it to sit for 1 minute. The slide was rinsed with water for 5 seconds to remove excess stain. A dropper was used to apply Gram's iodine (Lugol's iodine) to the slide to fix the crystal violet to the cell wall and let to sit for 1 minute. The slide was then decolorized with alcohol or acetone for about 3 seconds, and followed immediately with a gentle rinse using water. The secondary stain (safranin) was then applied, and allowed to sit for 1 minute. It was then rinsed with water for 5 seconds. The slide was viewed using a compound microscope. A magnification of 100x oil immersion was used. It was expected that the gram-negative cells will show a pink color, while the gram-positive cells will remain violet (Mannan et al., 2017).

Catalase Test

It is based on the ability of the isolates to produce the enzyme catalase which oxidizes hydrogen to water and oxygen. So, 2-3 of hydrogen peroxide solution was dropped on a clean slide. Using a sterile wire loop, a good proportion of the test organism was picked from the slant and placed on the slide containing the hydrogen peroxide. The presence of bubbles indicated a positive test while the absence of bubbles indicated a negative test (Dawodu et al., 2021).

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Spore Staining

A smear of bacterial isolate of not less than 48 hrs old was made on a clean slide and covered with a piece of bibulous paper. The slide was then held over a steam bath while applying malachite green. The paper was kept moist over the smear by reapplying the malachite green over and over again for 3 minutes. After 3 minutes, the stain was rinsed off (both sides). Safranin was then applied for 30 seconds and rinsed off from both sides. The slide was then allowed to air dry. Bacterial cells were stained pink while spores appeared green under the microscope (Prescott et al., 2002).

Sugar Fermentation Test

Several carbohydrate sugars were used during this test; 4 grams of the particular sugar was dissolved in distilled water inside a bijou. Durham's tube was put inside as an indicator for the presence of gas production, phenol red (a drop) was introduced into the solution to indicate change in pH, and the broth was autoclaved at 121ºC. Then 1 ml of lactic acid bacteria broth (normal saline and lactic acid bacteria) was introduced into the solution and incubated for 24 hrs at 37ºC. A change of color of the solution from red to yellow indicated that fermentation had taken place. Then the presence of bubbles in the Durham's tube, was an indication of gas production (Mannan et al., 2017).

Preparation of Bacteria Culture Supernatants

Bacteria were propagated aerobically and anaerobically in MRS broth for two nights. The isolated LAB strains were inoculated in 9 ml of MRS broth and incubated under aerobic condition at 37°C for 18-24 hrs. For the extraction of cell-free solution, the culture was spun in a centrifuge at 10,000xrpm for 20 minutes (Boris et al., 2000). To clarify whether the antimicrobial activity detected was derived from an organic acid or hydrogen peroxide (H2O2), the CSF was adjusted to pH 7.0 by adding 1N NaOH to eliminate the inhibitory effect of organic acids and 3000 u/ml of catalase was added to eliminate the potential inhibitory effect of hydrogen peroxide produced by the isolates. The treated CFS was then filtered through 0.45cμm filter and used as crude bacteriocin solution (Bonadè et al., 2001).

Antimicrobial Screening

Lactic acid bacteria produce substances generally called inhibitory substances and these substances tend to inhibit the growth of pathogenic and spoilage organisms. The three different organisms that were used were *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. If the inhibitory substances were effective against these pathogenic bacteria, no colonies would grow. This is called the zone of inhibition. The size of the zone of inhibition was then measured to determine the effectiveness of the bacteria. The method of Antimicrobial Susceptibility Testing (AST) that was used was the Agar Well Diffusion method (Boris et al., 2000). Each test organism was standardized with a spectrophotometer using 0.08-0.13 absorbance reading before carrying out the antimicrobial susceptibility testing with the test organisms.

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Agar Well Diffusion Method

Agar well diffusion was employed. The already standardized test organisms were streaked individually on separate plates of Mueller Hinton Agar using sterile swab sticks. The plates were then allowed to dry for about 10 minutes. Then a sterile cork-borer was used to bore four wells each in the already inoculated agar plates, and about 100 ul (0.1 ml) of each isolate was inoculated in the well. Diffusion of the isolates was allowed for about 15 minutes before incubating at 37ºC, and observations for clear zones of inhibition were taken at the 10^{th}, 12^{th}, 14^{th}, 16^{th} and 18^{th} hours.

Molecular Characterization of Isolates

Materials used included Primers, Quick Load Taq 2x Master Mix (containing Taq DNA Polymerase, dNTPs, MgCl2, KCl, tracking dyes and stabilizers), Quick-DNA Fungal/Bacterial Miniprep Kit, Microcentrifuge, Vortex, Bead Beating, Spin Column Purification System, Genomic Lysis Buffer, Bashing Bead Buffer, DNA Pre-wash Buffer, g-DNA Wash Buffer, DNA Elution Buffer, ZR Bashing Bead Lysis Tubes (0.1 & 0.5 mm), Zymo-Spin III-F Filters, Nuclease-free Water, Zymo-Spin IICR Columns and Collection Tubes.

DNA Extraction

Quick-DNA Fungal/Bacterial Miniprep kit was used. It is a simple, efficient isolation of DNA (up to 25 µg/prep) from all types of tough-to-lyse fungi (e.g., yeast) and bacteria in as little as 15 minutes. The state-of-the-art, ultra-high density Bashing Beads are fracture resistant and chemically inert, which omits the use of organic denaturants as well as proteinases. The Quick-DNA Fungal/Bacterial Miniprep kit is designed for the simple, rapid isolation of DNA from tough-to-lyse fungi, including *A. fumigatus, C. albicans, N. crassa, S. cerevisiae, S. pombe*, as well as from mycelium and gram positive and gram negative bacteria. The procedure is easy and can be completed in as little as 15 minutes: fungal and/or bacterial samples were added directly to a ZR Bashing Bead Lysis Tube (0.1 & 0.5 mm), and rapidly and efficiently lysed by bead beating without using organic denaturants or proteinases. The DNA was then isolated and purified using the Zymo-Spin Technology and is ideal for downstream molecular-based applications, including PCR, array, etc.

Sample Sources

About 50-100 mg (wet weight) fungi or bacteria; this equated to approximately 109 bacterial cells and 108 yeast cells.

DNA Purity

High quality DNA was eluted with DNA Elution Buffer, making it perfect for PCR.

DNA Size Limits

This is capable of recovering genomic DNA up to and above 40 kb. In most instances, mitochondrial DNA and viral DNA (if present) will also be recovered.

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DNA Recovery

Up to 25 µg total DNA was eluted into 100 µl (35 µl minimum) DNA Elution Buffer per sample.

Procedures

For optimal performance, beta-mercaptoethanol was added to the Genomic Lysis Buffer to a final dilution of 0.5% (v/v), i.e., 500 µl per 100 ml. Briefly, 50-100 mg (wet weight) fungal or bacterial cells that had been resuspended in up to 200 µl of water or isotonic buffer (e.g., PBS) was transferred to a ZR Bashing Bead Lysis Tube (0.1 & 0.5 mm). Also, 750 µl of Bashing Bead Buffer was added to the tube and vortexed for 20 minutes. Afterwards, the ZR Bashing bead Lysis Tube (0.1 & 0.5 mm) was centrifuged in a microcentrifuge at 10,000 xg for 1 minute. Consequently, 400 µl of the supernatant was transferred to a Zymo-Spin III-F Filter in a Collection Tube and centrifuged at 8,000 xg for 1 minute. Afterwards, 1,200 µl of Genomic Lysis Buffer was added to the filtrate in the Collection Tube from the above step. Then, 800 µl of the mixture was transferred to the Zymo-Spin IICR Column in a Collection Tube. This was centrifuged at 8,000cxg for 1 minute. The flow through was discarded from the Collection Tube and the remaining 800 µl of the mixture above was transferred to the Zymo-Spin IICR Column in a Collection Tube and centrifuged at 8,000 xg for 1 minute. Furthermore, 200 µl of DNA Pre-Wash Buffer was added to the Zymo-Spin IICR Column in a new Collection Tube and centrifuged at 10,000 xg for 1 minute. Afterwards, 500 ul of g-DNA Wash Buffer was added to the Zymo-Spin IICR Column and centrifuged at 10,000 xg for 1 minute. Then, the Zymo-Spin IICR Column was transferred to a clean 1.5 ml microcentrifuge tube and 100 µl (35 µl minimum) of DNA Elution Buffer was decanted directly to the column matrix. This was centrifuged at 10,000cxg for 30 seconds to elute the DNA. The ready-to-use ultra-pure DNA was stored at the refrigerator until use.

DNA Amplification

Genomic DNA of bacterial isolates was purified using the Quick-DNA Fungal/Bacterial Miniprep Kit. The 16S rRNA genes were amplified by PCR using a set of 0.5 ul (16 s forward and 16 s reverse) each of universal primers. The reaction mixture contained One Taq Quickload 2x Master mix (12.5 ul), 1 ul of the DNA and 10.5 ul of nuclease-free water in a total volume of 25 µl. The mixture was heated at an initial denaturation of 95°C for 5 mins and subjected to 30 rounds of thermal cycling at 95°C for 15 secs, 54°C for 30 secs for annealing, 72°C for 1 min for elongation and 72°C for 5 mins for final extension; it was then was held at 10°C. The amplicon was purified prior to gel electrophoresis (2.0% [w/v]).

Gel Electrophoresis

Gel electrophoresis was used as the standard lab procedure for separating mixtures of the amplified DNA with the expected size of 1,550 sbp for visualization and purification. Electrophoresis uses an electrical field to move the negatively charged DNA through an agarose gel matrix toward a positive electrode. In gel electrophoresis, the molecules to be separated are pushed by an electrical field through a gel that contains small pores.

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Preparation of Gel

Gel solution was prepared using a 250 mL flask. To the flask, 2.0 g of agarose and 100 mL of TBE diluted (1x) buffer solution was added. The mixture was swirled to disperse any clump of agarose powder. The flask was covered with plastic wrap to minimize evaporation. The flask was placed in a microwave oven and heated with high temperature for 2-3 minutes to allow the dissolution of the agarose solution. The agarose solution was cooled to 50°C-55ºC with careful swirling to promote even dissipation of heat and then poured into the casting trays fitted with a five toothed comb. The gel was allowed to stand for 20 minutes to completely solidify.

Preparation of Samples

The PCR product was mixed in a ratio of 1:1 with the loading buffer. A 5 µl of PCR amplicons underwent electrophoresis on a 2.0% agarose gel stained with Safe View^{TM} Classics in the presence of the running buffer. Also, 10 ul of the DNA ladder control was included in one of the wells.

Running the Gel

The loading tip was inserted to a few mm from the well bottom and the samples were delivered to the well. The power supply was attached by putting the lid on. (The connection was ensured to be in the correct way, that is, black-black and red-red.) The electrophoresis was carried out at 150 volts for 40 minutes.

DNA Sequencing and BLAST Search

The PCR products generated were shipped to Inqaba Biotech, South Africa for sequencing. All raw sequence generated were cleaned using Molecular Evolutionary Genetics Analysis (MEGA) 6. Version 6.06 prior to blast search using the NCBI Nucleotide database “16s rRNA sequence (Bacteria and Archaea)” with the program selection optimized for Somewhat Similar Sequence (blastn).

Sequence Alignment of 16S rRNA Genes & Phylogenetic Analysis

Following the alignment of the clean reads against 16s rRNA gene sequences in the NCBI nucleotide database, the percentage similarities were noted and the phylogenetic trees were produced using the BLAST pairwise alignment.

RESULTS

Out of 16 isolates of lactic acid, four showed inhibitory effect against the test organism and three were further characterized by molecular analysis with one definite result.

Table 1 shows the average count of lactic acid bacteria/probiotic from Elele Market, Rivers State Nigeria. It indicates the count for aerobically- and anaerobically-grown isolates. Table 2 shows the zones of inhibition of these lactic acid/probiotic bacteria against the test organisms. Table 3 shows the quantitative analysis of molecular results. The results for catalase, gram

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stain, pore stain and sugar fermentation test are presented in Tables 4, 5, 6 and 7 respectively. The quantitative analysis for the molecular results is shown in Table 8.

Figure 1 shows the gel electrophoresis results of the DNA bands. Figure 2 shows the phylogenetic tree using blast pairwise alignments. Plate 1 shows *Bacillus cereus* against *Staphylococcus aureus* while Plate 2 shows the zone of inhibition of Lactic Acid Bacteria against *Pseudomonas aeruginosa.*

Bacteriological Analysis of Lactic Acid Bacteria Isolated from Palm Wine

Table 1 shows the average count of lactic acid/probiotic bacteria isolated from the stock sample of palm wine. Here, the culture of Sample 1 gave a colony count of 1.05 x 10^6 for aerobic and 1.63 x 10^6 for anaerobic incubation, Sample 2 gave a colony count of 6.7 x 10^5 for aerobic and 1.38 x 10^6 for anaerobic, Sample 3 gave a colony count of 1.65 x 10^6 for aerobic and 2.01x 10^6 for anaerobic, Sample 4 gave a colony count of 2.0 x 10^5 for aerobic and 1.0 x 10^5 for anaerobic and Sample 5 gave a colony count of 1.63 x 10^6 for aerobic and 5.0 x 10^5 for anaerobic.

Table 1: Average count of lactic acid bacteria isolated from palm wine sold in Elele market

Sample	Lactic Acid Bacteria count (Aerobic) Cfu/ml	Lactic Acid Bacteria count (Anaerobic) Cfu/ml
Sample1	1.05 x 10^6	1.63 x 10^6
Sample 2	6.7 x 10^5	1.38 x 10^6
Sample 3	1.65 x 10^6	2.01 x 10^6
Sample 4	2.0 x 10^5	1.0 x 10^5
Sample 5	1.63 x 10^6	5.0 x 10^5

Zone of Inhibition of the Inhibitory Substance-producing Lactic Acid

Table 2 shows the measurement of the zones of inhibition exacted by the lactic acid/probiotic bacteria isolated against the test organisms, with *Lactobacillus sp* 1 showing an inhibition of 20 mm against *Pseudomonas aeruginosa*, *Bacillus cereus* showing an inhibition of 18 mm against *Staphylococcus aureus* and 18 mm against *Pseudomonas aeruginosa*, and *Lactobacillus sp* 2 showing an inhibition of 21 mm against *Staphylococcus aureus*.

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Table 2: Zone of Inhibition (mm) of the inhibitory substance-producing lactic acid

Test Organism	*Lactobacillus sp* 1	*Bacillus cereus*	*Lactobacillus sp* 2
Escherichia coli	__	__	__
Staphylococcus aureus	__	18·00	21·00
Pseudomonas aeruginosa	20·00	18·00	__

Table 3: Cultural, morphological and biochemical characteristics of lactic acid

Characteristics	*Lactobacillus sp* 1 Isolate 3 (C)	*Bacillus cereus* Isolate 5 (A)	*Lactobacillus sp* 2 Isolate 11 (N)
Cultural characteristics			
Medium	MRS	MRS	MRS
Form	Puntiform	Round	Spindle
Elevation	Convex	Pulvinate	Raised
Color	Cream	Cream	Cream
Texture	Glistering	Glistering	Glistering
Size	Small	Small	Small
Edge	Entire	Entire	Entire
Gram Stain reaction	Positive	Positive	Positive
Catalase test reaction	Negative	Negative	Negative
Cell shape	Cocci	Cocci	Rod

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Table 4: Catalase test reaction of lactic acid

Isolate	Reaction
Lactobacillus sp 1	Negative
Bacillus cereus	Positive
Lactobacillus sp 2	Negative

Table 5: Gram stain reaction of lactic acid

Isolate	Reaction
Lactobacillus sp 1	Positive
Bacillus cereus	Positive
Lactobacillus sp 2	Positive

Table 6: Spore stain reaction of lactic acid

Isolate	Reaction
Lactobacillus sp 1	Negative
Bacillus cereus	Positive
Lactobacillus sp 2	Negative

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Table 7: Sugar fermentation test

Sugars	*Lactobacillus sp* 1	*Bacillus cereus*	*Lactobacillus sp* 2
Glucose	+	+	+
Sucrose	+	+	+
Lactose	+	-	+
Mannitol	+	-	+
Maltose	+	+	+
Fructose	+	+	+
Xylose	+	-	+

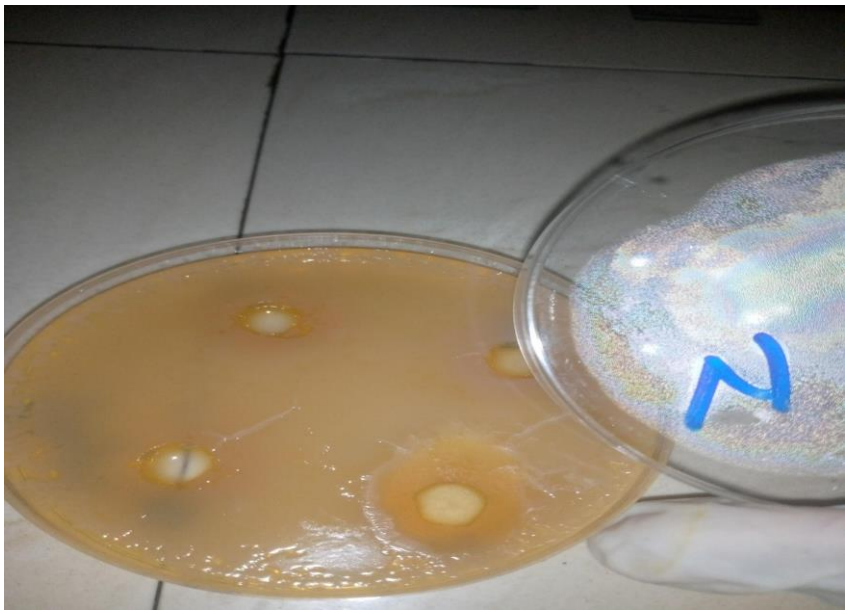

Plate 1: Zone of inhibition of *Bacillus cereus* against *Staphylococcus aureus*

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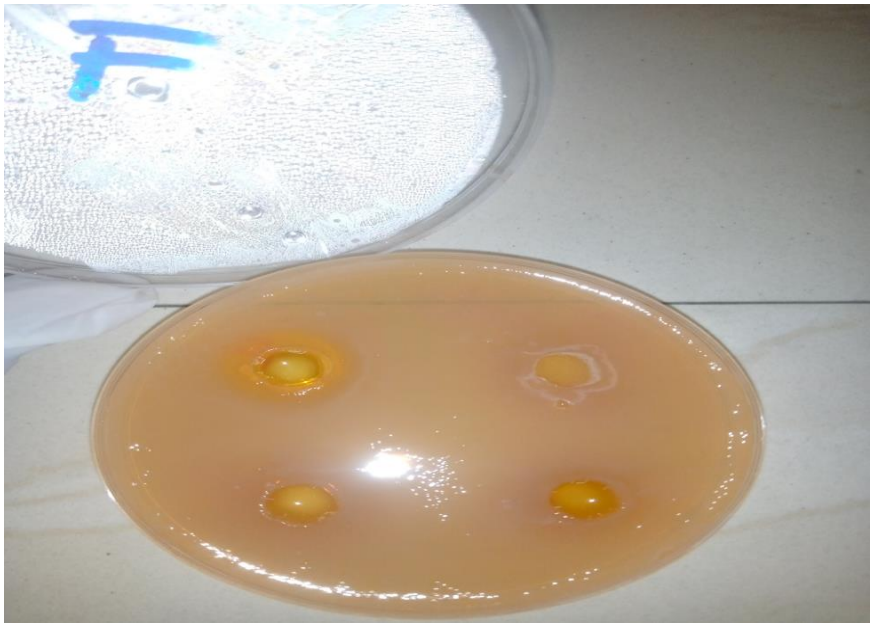

Plate 2: Zone of inhibition of lactic acid bacteria against *Pseudomonas aeruginosa*

Quantitative Analysis of Molecular Results

Table 8 shows the quantitative analysis for the DNA extracts concentration and purity. The ratio of absorbance 260 nm and 280 nm is used to assess the purity of the DNA, where a ratio between 1.60 and 2.0 indicates the accepted purity ratio.

Table 8: Quantitative analysis of molecular results

LABs	Concentration	Absorbance (Ratio A260/A280)	A260	A280
Bacillus cereus	57.4	1.69	1.15	0.28
Lactobacillus sp 2	12.8	1.29	0.26	0.20
Lactobacillus sp 1	16.8	1.49	0.34	0.23

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Gel Electrophoresis Showing the DNA Bands

Figure 1 shows the DNA bands following gel electrophoresis with a picture of the amplified DNA of expected size 1550 bp and the percentage similarities, and also the alignment of the clean reads against 16s rRNA gene sequences in the NCBI nucleotide database.

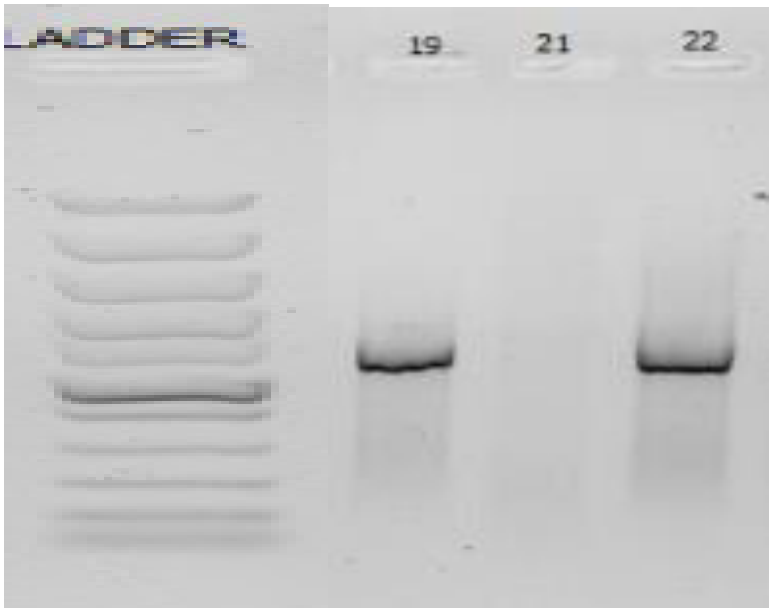
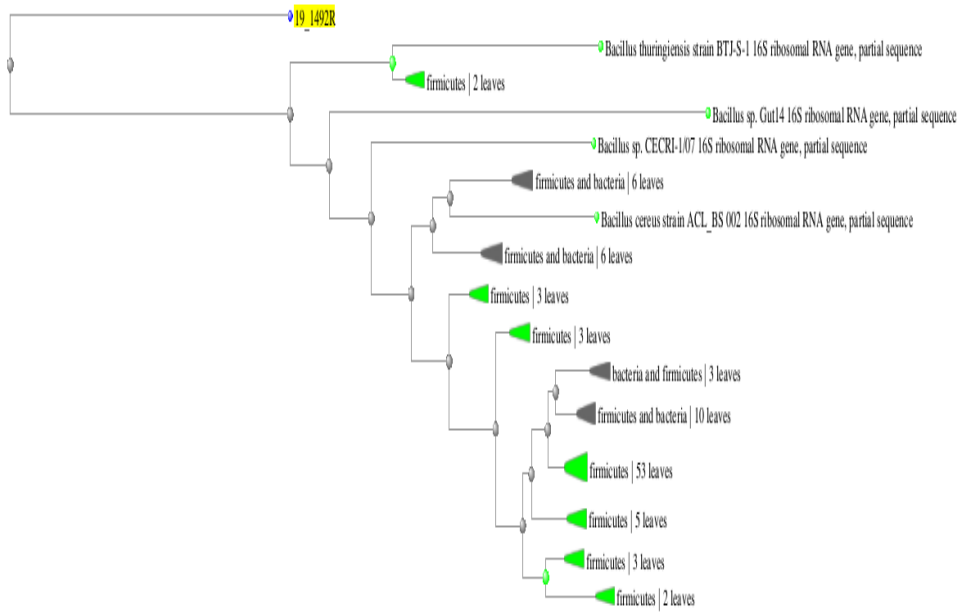


Figure 1: Gel electrophoresis showing the DNA bands

Phylogenetic Tree Using Blast Pairwise Alignments

Figure 2 shows the phylogenetic tree of bacteria strain using blast pairwise alignments, showing the relationship among taxa (or sequences) and their hypothetical common ancestors.



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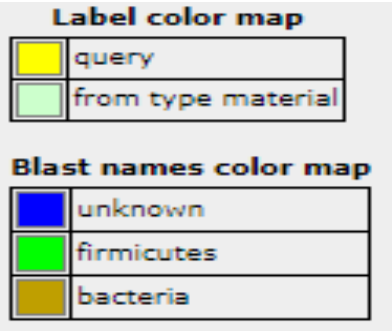

Figure 2: Phylogenetic tree using blast pairwise alignments

DISCUSSION

This research work was undertaken to evaluate inhibitory substance-producing lactic acid bacteria in palm wine sold in Elele Market, Rivers State. Some lactic acid/probiotic bacteria selected showed significant inhibitory activity against the selected test organisms: *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. This study shows the strong inhibitory effect of *Bacillus cereus* against an important pathogenic bacteria like *Pseudomonas aeruginosa,* which sheds more light on the important probiotic activity of *Bacillus*.

As a fermentation strain, bacteria, such as lactic acid bacteria, have several important metabolism characteristics, such as the ability to produce acid and aroma, the ability to hydrolyze protein, the ability to produce viscous exopolysaccharides and the ability to inhibit bacteria. In fermented foods, lactic acid bacteria produce antioxidant substances that are highly safe, and that can have a variety of beneficial effects on the human body through food. In recent years, there have been many reports on the synthesis of antioxidant metabolites by lactic acid bacteria. Antioxidant substances have excellent ability to scavenge free radicals and are closely related to human health. (Wang et al., 2021). In a study reported by Sharpe (2009), when *Lactoccocus lactis* and *Enteroccocus faecium* were inoculated onto fresh-cut salads, the growth of *Pseudomonas* sp., yeasts and total coliform were remarkably reduced controlling the growth of undesirable bacteria.

Also, isolated *Bacillus cereus* cultures used in the fermentation of African locust bean seeds "Parkia biglobosa" into the food condiment "daddawa" were evaluated for probiotic attributes; *Bacillus cereus* strains (BC1 and BC2) were tested for tolerance to acid, common salt (NaCl), and bile salt. Auto-aggregation and adhesion to epithelial cells, antibiotic sensitivity profile, hemolytic pattern, and antibacterial activity were also evaluated. To demonstrate further health benefit, spores of strain BC1 were investigated for anti-inflammatory potential employing the rat paw edema technique. Both Bacillus cereus strains showed antagonistic activity against pathogenic *Escherichia coli* and *Staphylococcus aureus*. The edema inhibition study revealed that spores of *Bacillus cereus* strain BC1 had anti-inflammation potential and produced no physiological toxicity on the animals (Nwagu et al., 2020).

The infectious diseases caused by bacteria are contributing to the death toll around the world, especially in poverty-stricken countries, with the situation further aggravated by the

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participation of human fungal infections as hidden killers. The morbidity and mortality rates among the world population are alarmingly on the rise as consequences of the widespread bacterial and fungal infections due to the emergence of the multi-drug resistance pathogens and the declining efficacy of the available antibiotics against them. Hence, seeking alternative ways through the utilization of the antimicrobial agents from natural sources for the improvement of the potency and the effectiveness of the currently available antibiotics along with the eradication of the detrimental effects associated with them should attract urgent concern at this juncture (Acharjee et al., 2022).

CONCLUSION

This research shows that probiotic strains not only have the potential to confer a lot of health benefits when consumed but also produce antimicrobial substances capable of inhibiting growth of pathogenic strains. These results indicate that the *Bacillus cereus* cultures for palm wine (*Elaeis guineesis*) are good candidates for probiotics and have the potential for application in both animal and human formulations for increased health benefit to consumers. There is a constant trend in the development of new policies for both food and pharmaceutical safety applications, thus encouraging the research on new antimicrobial alternatives that can meet these new policies. Particularly, the investment in bacteriocin research and patent development shows a clear upward trend in response to the potential applications of these antimicrobial peptides in the field of both new pharmaceuticals and food products.

Therefore, further research and findings on palm wine, as one of the sources of lactic acid bacteria, should be carried out in order to boost the bacteriocin arsenal against pathogenic/food spoilage organisms.

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