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Bacterial Diversity and Antibiotic-Producing *Bacillus* spp. in Ogiri-egusi: Potential for Biocontrol and Industrial Applications

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Abstract:

Ogiri-egusi, a traditional alkaline-fermented condiment derived from *Citrullus vulgaris* seeds, is produced through spontaneous fermentation, resulting in diverse microbial communities that influence its quality and safety. This study investigated the microbial diversity, fermentation dynamics, and antimicrobial potential of *Bacillus* spp. isolated from Ogiri-egusi. Samples were collected from four major markets in Makurdi metropolis, and bacterial isolates were identified based on cultural, morphological, and biochemical characteristics. The microbial analysis revealed that *Bacillus* spp. were the predominant fermentative bacteria (62.5%), alongside *Leuconostoc* spp., *Micrococcus* spp., *Proteus* spp., and *Lactobacillus* spp., indicating a diverse microbial community. The pH of the fermenting substrate increased from 6.3 to 7.9 over four days, consistent with proteolytic ammonia production, a hallmark of alkaline fermentation. Antimicrobial screening of *Bacillus* spp. crude extracts against bacterial pathogens using the agar well diffusion method revealed significant inhibitory effects on *Staphylococcus* spp. (8.00 ± 0.58 mm) and moderate inhibition against *Pseudomonas* spp. (4.67 ± 2.40 mm) and *Salmonella* spp. (3.33 ± 1.76 mm). However, *Proteus* spp., *Klebsiella* spp., and *Escherichia coli* exhibited resistance to the crude extracts. Nutrient composition influenced antimicrobial activity, with sucrose-based extracts exhibiting higher inhibition against *Staphylococcus* spp. and *Pseudomonas* spp., while nitrogen-based extracts showed enhanced activity against *Salmonella* spp. The absence of inhibition against *Proteus* and *Bacillus* spp. suggests intrinsic resistance mechanisms. These findings highlight the role of *Bacillus* spp. in Ogiri-egusi fermentation and their potential as natural biocontrol agents. Optimizing fermentation conditions could enhance antibiotic yield and efficacy, while further purification and molecular characterization of bioactive compounds are necessary. Harnessing beneficial microbes from traditional fermented foods may offer sustainable solutions for antibiotic production, food preservation, and biopharmaceutical applications.

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INTRODUCTION

Fermented foods are an integral part of traditional diets worldwide, particularly in Africa, where they contribute significantly to food security, nutrition, and health. Many of these foods undergo natural fermentation facilitated by diverse microbial communities, which enhance their flavor, texture, safety, and shelf life. One such traditional fermented food is Ogiri Egusi, a pungent, protein-rich condiment produced from melon seeds (*Citrullus colocynthis* or *Citrullus lanatus*). It is widely consumed in Nigeria and other West African countries as a seasoning for soups and stews (Azi *et al*., 2017). The fermentation of Ogiri Egusi is primarily driven by microorganisms such as *Bacillus* spp., *Lactobacillus* spp., *Saccharomyces* spp., (Adesemoye *et al*., 2025), and filamentous fungi, which contribute to the breakdown of complex seed components, releasing bioactive compounds in the process.

The microbial diversity in fermented foods has garnered interest due to their potential as reservoirs of antibiotic-producing microorganisms (Yunus *et al.,* 2017a). Antibiotics are essential bioactive compounds used in medicine and industry to combat pathogenic bacteria and fungi (Yunus *et al.,* 2016a). However, the rising incidence of antibiotic resistance has intensified the search for new antimicrobial compounds from natural sources (Ashraf and Iqbal, 2022; Iqbal and Ashraf, 2018, 2019; Saleem *et al.,* 2018; Shahzad *et al.,* 2017). Traditional fermented foods, which harbor complex microbial ecosystems, offer a promising yet underexplored avenue for discovering novel antibiotics (Cuamatzin-García *et al*., 2022; Valentino *et al*., 2024; Yunus *et al.,* 2017a). Many species of *Bacillus* and *Lactobacillus*, commonly found in Ogiri Egusi, produce antimicrobial peptides such as bacteriocins, which inhibit the growth of foodborne and clinical pathogens (Bamgbose *et al*., 2021; Darbandi *et al*., 2022). Similarly, certain filamentous fungi, including *Penicillium* and *Aspergillus* species, are known for producing secondary metabolites with potent antimicrobial properties (Zhgun, 2023).

Among the microorganisms involved in Ogiri Egusi fermentation, *Bacillus* spp. play a dominant role due to their ability to produce extracellular enzymes such as proteases, lipases, esterases, and amylases, which facilitate fermentation (Ogueke *et al*., 2013; Yunus *et al.,* 2017b). Beyond their fermentative functions, *Bacillus* spp. are also recognized for their ability to synthesize bioactive compounds, including bacteriocins, lipopeptides (surfactin, iturin, and fengycin), and peptide antibiotics (subtilin, bacitracin, and polymyxins) (Perez *et al*., 2017; Markelova and Chumak, 2025). These antimicrobial compounds contribute to the preservation and safety of fermented foods by suppressing the growth of undesirable microbes during fermentation. Studies have shown that *Bacillus* strains isolated from traditional African fermented foods exhibit antimicrobial properties against foodborne pathogens such as *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, and *Listeria monocytogenes* (Perez *et al*., 2017; Raphel and Halami, 2024), suggesting that Ogiri Egusi may possess inherent antimicrobial properties that enhance its safety and shelf stability.

Despite its cultural and economic importance, Ogiri Egusi remains under-researched compared to other fermented condiments such as Iru (fermented locust beans) and Dawadawa (fermented African locust beans). A deeper understanding of its microbiology, biochemical changes, and fermentation factors is essential for optimizing production, improving shelf stability, and ensuring product safety (Ogueke *et al*., 2013; Perez *et al*., 2017). Furthermore, the antibiotic-producing potential of *Bacillus* spp. isolated from Ogiri Egusi presents an exciting opportunity for biotechnological applications, particularly in the search for alternative antimicrobial agents to address rising antibiotic resistance.

This study aimed to collect Ogiri Egusi samples from different markets in Makurdi, isolate and identify the microbial diversity, and evaluate the antibiotic-producing potential of *Bacillus* spp. The research also investigated the effect of carbon and nitrogen sources on antibiotic production and assessed the antibacterial

activity of the antibiotic extract against selected pathogenic bacteria. By examining these aspects, the study seeks to enhance understanding of the microbial ecology of Ogiri Egusi, improve traditional fermentation practices, and explore the potential of *Bacillus* spp. for antibiotic production and food biopreservation.

MATERIALS AND METHODS

Sample Collection

Samples of fermented Ogiri-egusi (*Citrullus vulgaris*) were collected from four different locations in the Makurdi metropolis: Modern Market (MM), Wurukum Market (WM), Wadata Market (WDM), and North Bank Market (NBM). The samples were collected in 500 mL sterile wide-mouth bottles with screw caps and transported on ice packs for microbiological isolation in the Microbiology Laboratory at Joseph Sarwuan Tarkaa University, Makurdi. Additionally, fresh samples of *C. vulgaris* seeds were sourced from Modern Market for fermentation studies conducted in the laboratory. Ten grams of soil samples were randomly collected at two different sites along the bank of River Benue in Makurdi, Benue state. The samples were collected at 15 cm depth from the top using sterile soil auger and immediately transported to the laboratory for microbiological analysis.

Isolation and Identification of Bacteria

The study utilized Tryptone Soy Agar (TSA), Nutrient Agar (NA), *Salmonella Shigella* Agar, de Man, Rogosa, and Sharpe Agar (MRSA), Mannitol Salt Agar (MSA), and MacConkey Agar for bacterial enumeration and isolation. The media were sterilized by autoclaving at 121°C for 15 minutes and cooled to 45°C before inoculation. To ensure comprehensive microbial isolation, samples were separately meshed in a sterile porcelain mortar, and 1 g of each was subjected to ten-fold serial dilution using peptone water. Appropriate dilutions were plated by the pour plate method and incubated at 37°C for 24 hours. Discrete colonies were subcultured onto fresh agar to obtain pure cultures, which were maintained on slants at 4°C for further studies.

Microbial identification was based on cultural, morphological, and biochemical characteristics (Ebah *et al.,* 2024a; Hussain *et al.,* 2016; Iqbal *et al.,* 2015; Saleem *et al.,* 2020; Yunus *et al.,* 2016b). Gram staining was performed on 24-hour-old pure cultures to determine cell morphology and Gram reaction. Gram staining differentiated bacteria as Gram-positive or Gram-negative based on their ability to retain crystal violet or take up safranin as a counter stain (Paray *et al*., 2023). Biochemical characterization included Indole production, Methyl Red, Voges-Proskauer, Citrate utilization, Motility/Hydrogen sulfide production, Catalase, Oxidase, and Urease tests.

For the Indole test, isolates were inoculated into tryptone broth and incubated at 37°C for 24 hours. Kovac's reagent was added, and the formation of a bright red color indicated a positive result (MacFaddin, 2000). The Methyl Red test assessed the ability of bacterial isolates to produce stable acid end-products from glucose fermentation. After 48 hours of incubation in MR-VP broth, the addition of Methyl Red reagent resulted in a red color for a positive reaction and yellow for a negative one (MacFaddin, 2000). The Voges-Proskauer test determined the production of acetoin from glucose fermentation. After 24 hours of incubation in MR-VP broth, alpha-naphthol and potassium hydroxide were added. The development of a pinkish-red color indicated a positive result, while a yellow color signified a negative reaction.

Citrate utilization was evaluated by inoculating isolates onto Simmon's Citrate Agar slants and incubating at 37°C for 48 hours. A color change from light green to blue confirmed a positive result, while no color change indicated a negative reaction (MacFaddin, 2000). The oxidase test was performed using an oxidase reagent on filter paper. A positive reaction was indicated by a deep purple color within 10–30 seconds, while the absence of color change denoted a negative result. The urease test assessed the ability of isolates to hydrolyze urea

into ammonia. Isolates were inoculated onto Christensen's Urea Agar slants and incubated at 37°C for 24–48 hours. A pink color change confirmed a positive urease test, while a yellow or no color change indicated a negative result (Vitolo, 2022).

Antibiotics Production in Flask Cultures

Antibiotic production by *Bacillus* spp. was carried out in 500 mL Erlenmeyer flasks containing 100 mL of a defined production medium. The flasks were inoculated with *Bacillus* spp. at an initial inoculum size of 1×10^6 CFU/mL and incubated at 37°C on a rotary shaker at 180 rpm for 48 hours.

To optimize antibiotic production, the effects of a carbon and nitrogen source were evaluated. For carbon source optimization, 3.5 g of sucrose was incorporated into 100 mL of the defined medium, which contained 21.8 g KH_2PO_4, 5.7 g $NaHPO_4$, 0.5 g $MgSO_4$, 0.05 g $ZnSO_4$, 0.5 g $FeSO_4 \cdot 7H_2O$, and 10 g monosodium glutamate, adjusted to pH 7.0.

For nitrogen source optimization, 0.2 g of KNO_3 was added to a medium containing 21.8 g KH_2PO_4, 5.7 g $NaHPO_4$, 0.5 g $MgSO_4$, 0.05 g $ZnSO_4$, 0.5 g $FeSO_4 \cdot 7H_2O$, 10 g monosodium glutamate, and 3.5 g of the selected nitrogen source. Cultures were incubated under the same conditions as described above. The study followed the methodology of El-Banna and Qaddoumi, (2016).

Extraction of Crude Antibiotic

To extract the antibiotic compounds, 100 mL of ethanol was added to each of the flasks containing the cultured *Bacillus* spp. grown with carbon and nitrogen sources. The flasks were left at room temperature for 24 hours to allow the extraction process. After incubation, the mixtures were filtered to remove cell debris and insoluble particles. The filtrates were then left to evaporate, allowing the ethanol to completely dissipate, leaving behind the crude antibiotic extract for further analysis (Ilica *et al*., 2007).

Antimicrobial Sensitivity Assay

Seven test tubes containing sterile peptone water were allowed to cool before being inoculated with 24-hour-old cultures of the test organisms. The test organisms were isolated from soil samples on the bank of the River Benue. These included *Salmonella* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Klebsiella* spp., *Bacillus* spp., and *Escherichia* spp. The inoculated tubes were then incubated at 37°C for 24 hours to facilitate microbial growth. Nutrient agar was prepared and poured into seven sterile Petri dishes. Using a sterile swab, 100 µL of each test organism suspension was swabbed evenly onto the respective plates and allowed to dry. A cork borer, 6 mm in diameter, was used to create uniform wells in the agar medium. Additionally, 20 µL of antibiotic extracts from sucrose and KNO_3 were introduced into separate wells. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition around each well were measured to assess antimicrobial activity. The experiment was carried out in duplicates, and the activity was reported as the diameter of the zone of inhibition and standard deviation (ZOI ± SD) (Aernan *et al*., 2023; Aernan *et al*., 2024; Mulaw *et al*., 2019).

Laboratory Fermentation of *Citrullus vulgaris* for *Ogiri Egusi*

Mature *Citrullus vulgaris* seeds were washed thoroughly with potable water and boiled for 1 hour and 30 minutes to soften. The boiling water was drained, and the cotyledons were rinsed with sterile distilled water. Two hundred grams (200 g) of the boiled seeds were weighed and placed in sterile plantain leave (*Musa sapientun* var. *paradisiac* Linn.). *Bacillus* spp., previously isolated from Ogiri egusi samples, was used as a starter culture for fermentation by inoculating 1.0 mL of cell suspension (2×10^{10} cfu/mL). The inoculated seeds were allowed to ferment at 37°C for four (4) days to produce *Ogiri-egusi* (Ogueke *et al*., 2013).

The pH and temperature of the fermenting mash were monitored at 24-hour intervals throughout

the fermentation period using a calibrated pH meter and a sterile thermometer, respectively.

Determination of pH

The pH of the fermenting *Ogiri-egusi* was determined by suspending 1 g of the sample in 9 mL of sterile distilled water. The suspension was shaken thoroughly, and the pH was measured using a standardized pH meter at 24-hour intervals for four days.

Determination of Temperature

The temperature of the fermenting mash was measured by inserting a sterile thermometer into the sample. The readings were recorded after two minutes at 24-hour intervals for four days.

Statistics Analysis

Data was analyzed using statistics package for social science version software (20). The statistical significance of means was measured by using the ANOVA. $p<0.05$ was considered statistically significant (Ebah *et al.,* 2024b).

RESULTS

Figure 1 illustrates the frequencies and percentages of bacterial isolates found in Ogiri-egusi samples. *Bacillus* spp. was the most predominant isolate, accounting for 62.5%, followed by *Leuconostoc* spp. at 18.8%. *Micrococcus* spp., *Proteus* spp., and *Lactobacillus* spp. each represented 6.3% of the isolates.

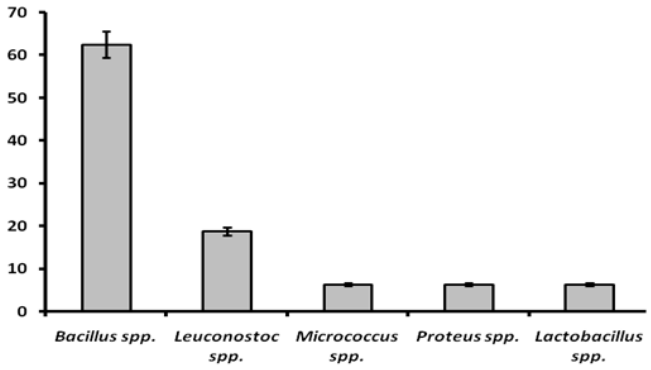

Fig. 1. Occurrence (%age) of bacterial isolate in Ogiri-egusi.

Table 1 shows the diversity and distribution of bacterial isolates in Ogiri-egusi samples according to their sample locations. *Bacillus* spp. was isolated from all four sample locations, while *Leuconostoc* spp. was found in three locations. *Micrococcus* spp. and *Proteus* spp. were isolated from the WDM location, and *Lactobacillus* spp. was isolated from the WKM location. The microbial diversity among the sampled locations was significantly different at $p<0.05$.

Tables 2 and 3 provide details about the cultural characteristics, morphological traits, and biochemical characteristics of the bacterial isolates from Ogiri-egusi samples across different markets. Tables 4 and 5 detail the cultural characteristics, morphological traits, and biochemical characteristics of bacteria isolated from the bank of River Benue in Makurdi.

Figure 2 shows the steady increase in pH 6.3 - 7.9 during laboratory fermentation of Ogiri Egusi for 4 days. Figure 3 presents the effects of sucrose and KNO_3 on the antimicrobial activity of the crude antibiotic against isolates from the bank of River Benue.

Table 1. Diversity and distribution of bacterial isolates in Ogiri Egusi samples from different markets.

Sr #	Code	Isolate
1	MM1	*Bacillus* spp.
2	MM2	*Leuconostoc* spp.
3	MM3	*Bacillus* spp.
4	MM4	*Leuconostoc* spp.
5	NBM1	*Bacillus* spp.
6	NBM2	*Bacillus* spp.
7	NBM3	*Bacillus* spp.
8	NBM4	*Bacillus* spp.
9	WDM1	*Bacillus* spp.
10	WDM2	*Bacillus* spp.
11	WDM3	*Micrococcus* spp.
12	WDM4	*Proteus* spp.
13	WKM1	*Bacillus* spp.
14	WKM2	*Leuconostoc* spp.
15	WKM3	*Lactobacillus spp.*
16	WKM4	*Bacillus* spp.

Key: MM=Modern Market, NBM=North Bank Market, WDM=Wadata Market, WKM= Wurukum Market.

Table 2. Cultural characteristics of bacterial isolates obtained from Ogiri-egusi samples at the different markets.

Sr #	Colony		Size	Shape	Elevation	Color	Margin	Surface appearance
	Code	Isolate						
1	MM1	*Bacillus* spp.	Large	Irregular	Raised	Creamy	Undulate	Smooth, Moist
2	MM2	*Leuconostoc* spp.	Small	Circular	Convex	White	Entire	Smooth, Mucoid
3	MM3	*Bacillus* spp.	Large	Irregular	Raised	White	Lobate	Rough, Moist
4	MM4	*Leuconostoc* spp.	Small	Circular	Convex	White	Entire	Mucoid, Shiny
5	NBM1	*Bacillus* spp.	Large	Irregular	Raised	Creamy	Undulate	Wrinkled, Moist
6	NBM2	*Bacillus* spp.	Large	Irregular	Flat	Creamy	Lobate	Smooth, Dry
7	NBM3	*Bacillus* spp.	Large	Irregular	Raised	White	Undulate	Moist, Rough
8	NBM4	*Bacillus* spp.	Large	Irregular	Flat	White	Lobate	Wrinkled, Moist
9	WDM1	*Bacillus* spp.	Large	Irregular	Raised	Creamy	Lobate	Smooth, Moist
10	WDM2	*Bacillus* spp.	Large	Irregular	Raised	White	Undulate	Moist, Rough
11	WDM3	*Micrococcus* spp.	Small	Circular	Convex	Yellow	Entire	Smooth, Shiny
12	WDM4	*Proteus* spp.	Medium	Irregular	Flat	Pale brown	Swarming	Moist, Shiny
13	WKM1	*Bacillus* spp.	Large	Irregular	Raised	White	Undulate	Rough, Moist
14	WKM2	*Leuconostoc* spp.	Small	Circular	Convex	White	Entire	Smooth, Mucoid
15	WKM3	*Lactobacillus spp.*	Small	Circular	Convex	Cream	Entire	Smooth, Mucoid
16	WKM4	*Bacillus* spp.	Large	Irregular	Raised	White	Lobate	Rough, Moist

Table 3. Morphological and biochemical characteristics of bacterial isolates obtained from Ogiri-egusi samples at the different markets.

Code	Isolate	Cell shape	Gram reaction	Urease	Citrate	Oxidase	Methyl red	Voges Proskauer	Catalase	H_2S	Motility	Indole
MM1	*Bacillus* ssp.	Rod	+	-	+	-	+	+	+	+	+	-
MM2	*Leuconostoc* spp.	Cocci	+	-	+	+	+	+	+	+	+	-
MM3	*Bacillus* ssp.	Rod	+	-	+	-	-	-	+	+	+	-
MM4	*Leuconostoc* spp.	Cocci	+	-	-	+	+	+	+	+	+	-
NBM1	*Bacillus* spp.	Rod	+	-	+	-	-	+	+	+	+	-
NBM2	*Bacillus* spp.	Rod	+	-	+	+	-	+	+	+	+	-
NBM3	*Bacillus* spp.	Rod	+	-	+	+	-	+	+	+	+	-
NBM4	*Bacillus* spp.	Rod	+	-	-	-	-	+	+	+	+	-
WDM1	*Bacillus* spp.	Rod	+	-	+	-	-	+	+	+	+	-
WDM2	*Bacillus* spp.	Rod	+	-	+	-	-	+	+	+	+	-
WDM3	*Micrococcus* spp.	Cocci	-	-	+	+	+	-	+	-	+	+
WDM4	*Proteus* spp.	Rod	-	-	-	-	-	-	+	+	+	-
WKM1	*Bacillus* spp.	Rod	+	-	+	-	-	+	+	+	+	-
WKM2	*Leuconostoc* spp.	Cocci	+	+	-	+	+	-	+	+	+	-
WKM3	*Lactobacillus* spp.	Rod	-	-	+	+	-	+	+	+	+	-
WKM4	*Bacillus* spp.	Rod	+	-	+	-	-	+	+	+	+	-

Key: Positive = +, Negative = -

Table 4. Morphological and biochemical characteristics of bacterial isolates from soil samples at the bank of River Benue.

Code	Isolate	Cell shape	Gram reaction	Urease	Citrate	Oxidase	Methyl red	Voges Proskauer	Catalase	H_2S	Motility	Indole
RB1	*Bacillus* spp.	Rod	+	-	+	-	-	+	+	+	+	-
RB2	*Staphylococcus* spp.	Cocci	+	-	+	-	+	+	+	-	+	-
RB3	*Pseudomonas* spp.	Rod	-	-	+	+	-	-	+	-	+	-
RB4	*Lactobacillus* spp.	Rod	+	-	+	-	+	+	+	-	+	-
RB5	*Bacillus* spp.	Rod	+	-	+	-	-	+	+	+	+	-
RB6	*Bacillus* spp.	Rod	+	-	+	-	-	+	+	+	+	-
RB7	*Lactobacillus* spp.	Rod	-	-	+	+	-	+	+	-	+	-
RB8	*Bacillus* spp.	Rod	+	-	+	-	-	+	+	+	+	-
RB9	*Escherichia coli*	Rod	-	-	-	-	+	-	+	-	+	+
RB10	*Salmonella* spp.	Rod	-	-	+	-	+	-	+	+	+	-
RB11	*Klebsiella* spp.	Rod	-	+	+	-	-	+	+	-	-	+

Table 5. Cultural characteristics of bacterial isolates obtained from soil samples at the bank of river Benue.

Sr #	Colony		Size	Shape	Elevation	Color	Margin	Surface appearance
	Code	Isolate						
1	RB1	*Bacillus* spp.	Large	Irregular	Raised	Creamy	Undulate	Smooth, Moist
2	RB2	*Staphylococcus* spp.	Medium	Circular	Convex	Pink	Entire	Smooth, Mucoid
3	RB3	*Pseudomonas* spp.	Large	Irregular	Raised	White	Undulate	Rough, Moist
4	RB4	*Lactobacillus* spp.	Small	Circular	Convex	Creamy	Entire	Smooth, Moist
5	RB5	*Bacillus* spp.	Large	Irregular	Raised	Creamy	Undulate	Wrinkled, Moist
6	RB6	*Bacillus* spp.	Large	Irregular	Flat	Creamy	Lobate	Smooth, Dry
7	RB7	*Lactobacillus* spp.	Small	Circular	Convex	Creamy	Entire	Smooth, Mucoid
8	RB8	*Bacillus* spp.	Large	Irregular	Raised	Creamy	Undulate	Smooth, Moist
9	RB9	*Escherichia coli*	Medium	Circular	Low	Pink/Red	Entire	Smooth, Moist
10	RB10	*Salmonella* spp.	Medium	Circular	Raised	Pale	Entire	Smooth, Moist
11	RB11	*Klebsiella* spp.	Large	Circular	Convex	Creamy/ White	Entire	Smooth, Shiny

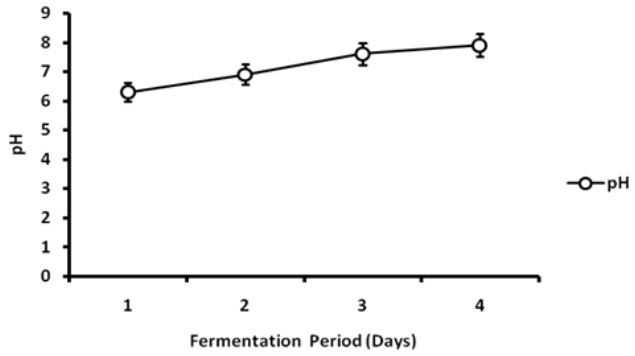

Fig. 2. pH variation during laboratory fermentation of Ogiri-egusi by *Bacillus* spp.

Staphylococcus spp. exhibited a higher zone of inhibition in response to the sucrose-based antibiotic extract (8.00 ± 0.58 mm) compared to the KNO_3-based extract (2.33 ± 2.33 mm). Similarly, *Pseudomonas* spp. demonstrated a comparable trend, with inhibition zones of 4.67 ± 2.40 mm for sucrose and 1.67 ± 1.67 mm for KNO_3. However, statistical analysis indicated no significant difference ($p > 0.05$) in the antimicrobial activity of antibiotics produced using sucrose and KNO_3.

Conversely, the crude KNO_3-based antibiotic extract showed a slightly greater inhibition zone (3.33 ± 1.76 mm) on *Salmonella* spp. than the sucrose-based extract (2.33 ± 2.33 mm). *Klebsiella* spp. exhibited a marginal increase in inhibition zones with carbon-based extracts (5.33 ± 2.91 mm) compared to nitrogen-based

extracts (4.67 ± 2.60 mm). *Escherichia coli* showed similar inhibition zones, with no significant difference observed between the two types of extracts ($p > 0.05$). For *Bacillus* spp., no inhibition zones were detected for either nitrogen (0.00 ± 0.00 mm) or carbon (0.00 ± 0.00 mm) sources.

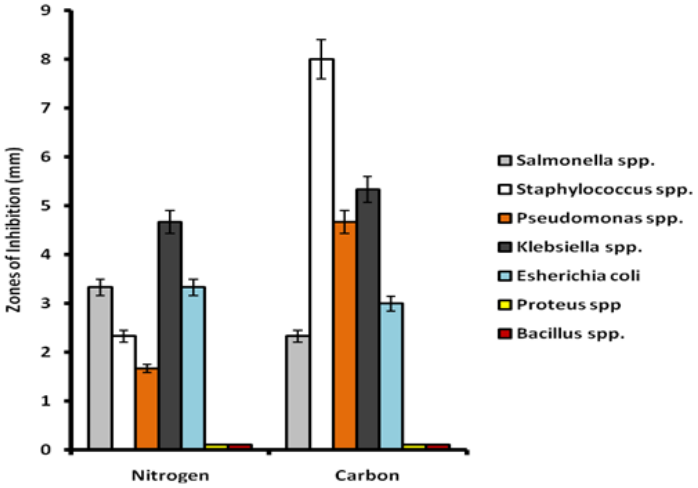

Fig. 3. Antimicrobial sensitivity tests of isolates on nitrogen and carbon sources showing the inhibition zones. The bar represents the standard error of duplicate determination.

DISCUSSION

The microbial diversity in Ogiri-egusi fermentation plays a critical role in shaping the biochemical and organoleptic properties of the final product. In this study, *Bacillus* spp. was the predominant isolate, accounting for 62.5% of the bacterial population. This finding is consistent with previous research, which has identified *Bacillus* spp. as the dominant microorganisms in alkaline-fermented foods due to their ability to thrive in high-pH environments. Similar observations have been made in the production of soumbala, a traditional alkaline-fermented condiment from *Parkia biglobosa* seeds in Burkina Faso, where *B. subtilis, B. amyloliquefaciens, B. licheniformis, B. pumilus, B. megaterium, B. sphaericus, B. cereus, B. badius,* and *B. fusiformis* were identified (Dabire *et al*., 2022).

The dominance of *Bacillus* spp. in alkaline fermentation is linked to their proteolytic activity, which contributes to enzymatic hydrolysis during fermentation, leading to the breakdown of proteins into amino acids (Ire *et al*., 2020). This metabolic process results in the release of ammonia and ammonium hydroxide, which elevate the pH and influence the characteristic aroma and texture of Ogiri-egusi (Owusu-Kwarteng *et al*., 2022).

Despite the prevalence of *Bacillus* spp., the presence of *Leuconostoc* spp., *Micrococcus* spp., *Proteus* spp., and *Lactobacillus* spp. suggests that Ogiri-egusi harbors a diverse microbial community that may contribute to both fermentation and spoilage (Obeta, 2008). The distribution of these bacteria across different sampling locations suggests that environmental and handling factors influence microbial composition. Significant differences in microbial counts across locations ($p = 0.008$) highlight the role of sanitation, fermentation conditions, and geographical variations in shaping microbial diversity. The absence of *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* previously reported as contaminants in unhygienic fermentations suggests improved

handling conditions in this study (Dike-Ndudim *et al*., 2021).

Traditionally, Ogiri-egusi fermentation takes three to five days (Ogueke *et al*., 2013). In this study, *Citrullus vulgaris* seeds were fermented for four days using *Bacillus* spp. as a starter culture. The pH increased from 6.3 to 7.9 as fermentation progressed, consistent with previous studies where pH shifts to alkalinity due to ammonia production from protein degradation (Ire *et al*., 2020). Similar trends were observed in Ogiri-egusi fermentation wrapped in different materials, where the pH ranged from 6.82 to 7.12 (Ire *et al*., 2020). The pH increase during fermentation is characteristic of alkaline-fermented foods, with peak proteolysis occurring at 96 hours in the study, leading to the highest production of amino acids (Ogueke *et al*., 2013).

The antimicrobial activity of *Bacillus* spp. was assessed through the inhibitory effects of crude extracts on selected bacterial pathogens. The results revealed no significant difference ($p > 0.05$) in antimicrobial activity between sucrose-based and KNO_3-based media. However, inhibition zones varied across bacterial isolates. *Staphylococcus* spp. exhibited the highest inhibition zone (8.00 ± 0.58 mm) in sucrose-based extracts. *Pseudomonas* spp. showed moderate inhibition (4.67 ± 2.40 mm). *Salmonella* spp. exhibited slightly higher inhibition in nitrogen-based extracts (3.33 ± 1.76 mm) than in sucrose-based extracts (2.33 ± 2.33 mm). No significant inhibition was observed against *Klebsiella* spp. and *Escherichia coli.*

The lack of inhibition against *Proteus* spp. and *Bacillus* spp. (0.00 mm inhibition zones) suggests the presence of intrinsic resistance mechanisms, possibly due to efflux pumps, biofilm formation, or the production of antagonistic metabolites (Cho and Chung, 2020; Wasfi *et al*., 2020). The resistance of *Bacillus* spp. to its own antimicrobial compounds could be attributed to endospore formation and the secretion of protective secondary metabolites.

Variations in inhibition zones between carbon- and nitrogen-based extracts suggest that nutrient composition affects antibiotic production. *Staphylococcus* spp. was significantly more inhibited by carbon-source extracts (8.00 mm) than nitrogen-source extracts (2.33 mm), suggesting enhanced antibiotic production under carbon-rich conditions. *Salmonella* spp. was more susceptible to nitrogen-source extracts (3.33 mm) than carbon-based extracts (2.33 mm), indicating potential nitrogen-enhanced antimicrobial synthesis. *E. coli* exhibited no significant difference ($p = 0.944$) in susceptibility, suggesting consistent resistance across nutrient conditions.

Studies have shown that glucose and ammonium ions can suppress antibiotic production due to catabolite and nitrogen repression (El-Banna and Qaddoumi, 2016). Secondary metabolite synthesis is often repressed under rapid growth conditions but is derepressed under nutrient-limiting conditions, allowing enhanced antibiotic production.

The variability in antimicrobial activity highlights the need for advanced purification and characterization of bioactive compounds to determine the precise chemical structures responsible for antibacterial activity. Optimization of fermentation conditions, including pH, temperature, and aeration, could enhance antibiotic yield and efficacy. Molecular studies to identify antimicrobial gene clusters and resistance mechanisms are essential for understanding how *Bacillus* spp. produce these compounds and why certain bacterial strains exhibit resistance. Exploring alternative carbon and nitrogen sources may improve antibiotic production, as nutrient availability influences secondary metabolite synthesis. Additionally, investigating probiotic applications of *Bacillus* spp. in food preservation could provide natural solutions to enhance the safety and shelf life of fermented products.

CONCLUSION

This study demonstrated that *Bacillus* spp. isolated from Ogiri-egusi produce antimicrobial compounds with varying efficacy against selected bacterial pathogens. Statistical analysis revealed no significant difference in antimicrobial

activity between sucrose- and KNO_3-based nutrient sources. However, the complete resistance of *Proteus* spp. and *Bacillus* spp. to the crude extracts suggests the presence of intrinsic resistance mechanisms that require further investigation.

Future research should focus on optimizing fermentation conditions to enhance antibiotic production, purifying and characterizing bioactive compounds for potential biopharmaceutical applications, and exploring the microbial ecology of Ogiri-egusi to understand the interactions between *Bacillus* spp. and other fermentative species. These findings highlight the potential of traditional fermented foods as sources of antimicrobial agents, contributing to food safety and sustainable antibiotic development.

REFERENCES

Adesemoye, E.T., Sanni, A.I., Spano, G., Capozzi, V., Fragasso, M., 2025. Lactic Acid Bacteria Diversity in Fermented Foods as Potential Bio-Resources Contributing to Alleviate Malnutrition in Developing Countries: Nigeria as a Case Study. Ferment., 11(2): 103.

Aernan, P.T., Odo, J.I., Omeji, J.M., Eunice, Y.M., Iqbal, M.N., 2023. Anti-bacterial Activity of *Moringa oleifera* Seeds against Selected Bacterial Pathogens. PSM Microbiol., 8(3): 82–90.

Aernan, P.T., Odo, J.I., Ado, B.V., Mende, I.U., Yaji, E.M., Iqbal, M.N., 2024. Phytochemical and Antibacterial Assessment of *Ageratum conyzoides* Cultivated in Benue State, Nigeria. PSM Biol. Res., 9(1): 41–50.

Ashraf, A., Iqbal, M.N., 2022. Antibacterial Compounds from Ethanolic Extract of *Scenedesmus obliquus* as Alternatives to Antibiotics. Int. J. Altern. Fuels. Energy., 6(1): 12-14.

Azi, F., Odo, M.O., Okorie, P.A., Njoku, H.A., Nwobasi, V.N., Nwankwegu, A.S., 2017. Fungi and aflatoxin analysis of processed ogiri-egusi and ogiri-ugba consumed in Abakaliki metropolis. Afr. J. Biotechnol., 16(42): 2024-2030.

Bamgbose, T., Atta, I.H., Anvikar, A.R., 2021. Bacteriocins of Lactic Acid Bacteria and Their Industrial Application. Curr. Top. Lact. Acid Bact. Probiotics., 7(1): 1-13.

Cho, W.I., Chung, M.S., 2020. *Bacillus* spores: a review of their properties and inactivation processing technologies. Food Sci. Biotechnol., 29: 1447–1461.

Cuamatzin-García, L., Rodríguez-Rugarcía, P., El-Kassis, E.G., Galicia, G., Meza-Jiménez, M. L., Baños-Lara, M.D.R., Zaragoza-Maldonado, D.S., Pérez-Armendáriz, B., 2022. Traditional Fermented Foods and Beverages from around the World and Their Health Benefits. Microorganisms., 10(6): 1151.

Dabire, Y., Somda, N.S., Somda, M.K., Compaoré, C.B., Mogmenga, I., Ezeogu, L.I., Traoré, A.S., Ugwuanyi, J.O., Dicko, M.H., 2022. Assessment of probiotic and technological properties of *Bacillus* spp. isolated from Burkinabe *Soumbala*. BMC Microbiol., 22(228).

Darbandi, A., Asadi, A., Ari, M.M., Ohadi, E., Talebi, M., Zadeh, H.M., Emamie, D.A., Ghanavati, R., Kakanj, M., 2022. Bacteriocins: Properties and potential use as antimicrobials. J. Clin. Lab. Anal., 36(1): e24093.

Dike-Ndudim, J.N., Ndubueze, C.W., Ezihe, J.C., Okechukwu, E., 2021. Isolation and identification of bacteria associated with fermentation of melon seed for Ogiri Sold in Owerri, Nigeria. Magna Sci. Adv. Res. Rev., 3(1): 57–61.

Ebah, E.E., Odo, J.I., Ogbada, I.E., Iqbal, M.N., 2024a. Isolation and Characterization of Bacteria Responsible for the Degradation

of Tributyltin (TBT) from Freshwater Sediment. PSM Biol. Res., 9(2), 66–71.

Ebah, E.E., Odo, J.I., Dickson, I.O.E., Iqbal, M.N., 2024b. Biodegradation of Combine Tributyltin and Diphenyltin by Bacteria in Freshwater Sediment. Int. J. Mol. Microbiol., 7(1): 29–38.

El-Banna, N., Qaddoumi, S.S., 2016. Antimicrobial activity of *Bacillus cereus*: Isolation, identification and the effect of carbon and nitrogen source on its antagonistic activity. J. Microbiol. Antimicr., 8(2): 7-13.

Hussain, F., Kalim, M., Ali, H., Ali, T., Khan, M., Xiao, S., Iqbal, M.N., Ashraf, A., 2016. Antibacterial Activities of Methanolic Extracts of *Datura inoxia*. PSM Microbiol., 01(1): 33-35.

Ilica, S.B., Konstantinovica, S.S., Todorovica, Z.B., Lazica, M.L., Veljkovica, V.B., Jokovica, N., Radovanovic, B.C., 2007. Characterization and Antimicrobial Activity of the Bioactive Metabolites in *Streptomycete* Isolates. Microbiol., 76(4): 421–428.

Iqbal, M.N., Anjum, A.A., Ali, M.A., Hussain, F., Ali, S., Muhammad, A., Irfan, M., Ahmad, A., Irfan, M., Shabbir, A., 2015. Assessment of microbial load of un-pasteurized fruit juices and *in vitro* antibacterial potential of honey against bacterial isolates. Open Microbiol. J., 9: 26-32.

Iqbal, M.N, Ashraf, A., 2018. Ceftazidime Resistant Bacteria in Clinical Samples: Do We Need New Antibiotics? Int. J. Mol. Microbiol., 1(2): 58-59.

Iqbal, M.N, Ashraf, A., 2019. *Withania somnifera*: Can it be a Therapeutic Alternative for Microbial Diseases in an Era of Progressive Antibiotic Resist-ance? Int. J. Nanotechnol. Allied Sci., 3(1): 16-18.

Ire, F., Eze, O., Maduka, N., 2020. A Influence of Different Wrapping Materials on Microbiological, Physicochemical and Sensory Properties of Condiment Product 'Ogiri-egusi'. J. Life. Bio. Sci. Res., 1(2), 34-43.

MacFaddin, J.F., 2000. Biochemical tests for identification of medical bacteria, (3rd ed.) Lippincott Williams & Wilkins, Philadelphia, PA.

Markelova, N., Chumak, A., 2025. Antimicrobial Activity of *Bacillus* Cyclic Lipopeptides and Their Role in the Host Adaptive Response to Changes in Environmental Conditions. Int. J. Mol. Sci., 26(1): 336.

Mulaw, G., Tessema, T.S., Muleta, D., Tesfaye, A., 2019. In Vitro Evaluation of Probiotic Properties of Lactic Acid Bacteria Isolated from Some Traditionally Fermented Ethiopian Food Products. Int. J. Microbiol., 2019: 7179514.

Obeta, J.A.N., 2008. A note on the microorganisms associated with the fermentation of seeds of the African oil bean tree (*Pentaclethra macrophylla* Benth). J. Appl. Microbiol., 54: 433 - 435.

Ogueke, C.C., Okoli, A.I., Owuamanam, C.I., Ahaotu, I., 2013. Fermentation of melon seeds for "Ogiri egusi" as affected by fermentation variables using *Bacillus subtilis*. Malaysian J. Microbiol., 9(4): 279 - 288.

Owusu-Kwarteng, J., Agyei, D., Akabanda, F., Atuna, R.A., Amagloh, F.K., 2022. Plant-Based Alkaline Fermented Foods as Sustainable Sources of Nutrients and Health-Promoting Bioactive Compounds. Front. Sustain. Food Syst., 6: 885328.

Paray, A.A., Singh, M., Mir, M.A., Kaur, A., 2023. Gram Staining: A Brief Review. Int. J. Res. Rev., 10: 336-341.

Perez, K.J., Viana, J.D., Lopes, F.C., et al., 2017. *Bacillus* spp. Isolated from Puba as a Source of Biosurfactants and

Antimicrobial Lipopeptides. Front Microbiol., 8: 61.

Raphel, S., Halami, P.M., 2024. Bioactive compounds from food-grade *Bacillus*. J. Sci. Food Agric., https://doi.org/10.1002/jsfa.13935

Saleem, M., Ain, N., Iqbal, M.N., 2020. Efficacy of Cleaning Agents against Bacterial Isolates from Raw Meat Sold in Market Places in Lahore. PSM Vet. Res., 5(1): 6-15.

Saleem, M., Batool, A., Iqbal, M.N, Ashraf, A., 2018. Characterization of Ceftazidime Resistance in Clinical Isolates of Bacteria in Lahore, Pakistan. Int. J. Mol. Microbiol., 1(2): 44-50.

Shahzad, M.I., Ashraf, H., Iqbal, M.N., Khanum, A., 2017. Medicinal Evaluation of Common Plants against Mouth Microflora. PSM Microbiol., 2(2): 34-40.

Valentino, V., Magliulo, R., Farsi, D., Cotter, P.D., O'Sullivan, O., Ercolini, D., De Filippis, F., 2024. Fermented foods, their microbiome and its potential in boosting human health. Microb. Biotechnol., 17(2): e14428.

Vitolo, M., 2022. Notes on Urea Hydrolysis by Urease. World J. Pharm. Pharm. Sci., 11. 96.

Wasfi, R., Hamed, S.M., Amer, M.A., Fahmy, L.I., 2020. *Proteus mirabilis* Biofilm: Development and Therapeutic Strategies. Front. Cell. Infect. Microbiol., doi: 10.3389/fcimb.2020.00414.

Yunus, F.N., Khalid, Z.Z., Rashid, F., Ashraf, A., Iqbal, M.N., Hussain, F., 2016a. Isolation and Screening of Antibiotic producing Bacteria from Soil in Lahore City. PSM Microbiol., 01(1): 01-04.

Yunus, F.N., Kanwal, F.,Rashid, F., Ashraf, A.,Iqbal, M.N., Xiao, S., 2016b. A Comparative Study on Isolation and Identification of *Bacillus thuringiensis* from Different Localities of GujranwalaCity. PSM Biol. Res., 01(1): 34-38.

Yunus, F.N., Riaz, A., Iqbal, M.N., Ashraf, A., 2017a. Isolation and Identification of Microflora from Some Bakery Products in Lahore. PSM Microbiol., 2(2): 29-33.

Yunus, F.N., Saeed, H., Rashid, F., Iqbal, M.N., Ashraf, A., 2017b. Isolation and Identification of Esterase Producing *Bacillus subtilis* from Soil. PSM Microbiol., 2(2): 24-28.

Zhgun, A.A., 2023. Fungal BGCs for Production of Secondary Metabolites: Main Types, Central Roles in Strain Improvement, and Regulation According to the Piano Principle. Int. J. Mol. Sci., 24(13): 11184.