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Bacteriological Quality, Virulence and Molecular Characteristics of Bacteria Isolated from Fermented African Locust Bean and Melon Seed Condiments Sold in Benin City

Kemini, B. C*., Ogundigie, O. P. and Akpoka, O. A.
Microbiology Unit, Department of Biological Sciences,
College of Natural and Applied Sciences, Igbinedion University,
Okada, Edo State, Nigeria. ***Corresponding Author:** *kemini.christianah@iuokada.edu.ng*

ABSTRACT

Iru (fermented African locust bean, *Parkia biglobosa*) and Ogiri/Evbarie (fermented melon seed, *Citrullus vulgaris*) are popular Nigerian condiments, but data on their bacteriological quality are scarce. This study is aimed at determining the bacteriological quality and molecular characterisation of bacteria isolated from fermented African locust bean and melon seed condiments sold in Benin City, Nigeria. One sample of Iru and two samples of Ogiri/Evbarie (A and B) were purchased from different vendors at Oba Market, Benin City, Nigeria. Total viable bacterial counts (TVBC) and total coliform counts (TCC) were determined in triplicate by pour plating. Thirty isolates (ten per sample) were characterised phenotypically and screened for biofilm formation, haemolysis and protease, lipase and cellulase production, and four were identified by 16S rRNA gene sequencing. Results revealed that mean TVBC was highest in Ogiri/Evbarie A (2.58×10^8 CFU/g), followed by Ogiri/Evbarie B (7.97×10^6 CFU/g) and Iru (4.00×10^6 CFU/g); mean TCC ranged from 2.43×10^3 CFU/g (Iru) to 7.75×10^6 CFU/g (Ogiri/Evbarie A), and both counts differed significantly among the samples ($p < 0.001$). Presumptive *Bacillus* (63.3%) and *Enterobacter* (30.0%) predominated. Biofilm formation and protease activity were each detected in ten isolates, lipase and cellulase activity in seven each, and β-haemolysis in seven presumptive *Bacillus* isolates, with no significant difference among samples ($p > 0.05$). Sequencing identified *Enterobacter hormaechei* and a *Priestia megaterium*-group isolate from Iru, *Staphylococcus kloosii* and *Micrococcus luteus* from Ogiri/Evbarie A and B, respectively (GenBank QB180977–QB180980). High coliform loads recorded in Ogiri/Evbarie and the recovery of *E. hormaechei* indicate a need for better hygiene in production and retail of these condiments, while the enzyme-producing isolates merit evaluation as starter cultures.

Keywords: Fermented condiments, Coliforms, 16S rRNA gene sequencing, *Enterobacter hormaechei*, Food safety.

Introduction

Fermented seed condiments are an integral part of West African cuisine, where small quantities are added to soups and stews for their characteristic flavour and as an inexpensive source of protein. Most are produced by spontaneous alkaline fermentation of cooked seeds, a process in which proteolytic *Bacillus* species usually dominate and in which protein breakdown releases peptides, amino acids and ammonia that raise the pH and produce the typical pungent aroma (Owusu-Kwarteng et al., 2022). In Nigeria, Iru is prepared from African locust bean (*Parkia biglobosa*) seeds and Ogiri/Evbarie from melon (*Citrullus vulgaris*) seeds, and both remain important household and commercial products (Obafemi et al., 2022; Oladeji et al., 2025).

Fermented melon seed is a single product, Ogiri/Evbarie, known as Ogiri in Yoruba and as Evbarie in Benin (Edo), and both names are used for it in Benin City markets. Ogiri/Evbarie from different processors and vendors is sold side by side, so comparing samples from different vendors indicates how much the microbiological quality of the same condiment varies within a single market.

Because these condiments are produced by small-scale processors under uncontrolled conditions and sold in open markets, they may carry undesirable microorganisms alongside the fermentative flora. *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* have been isolated from Ogiri sold in Owerri markets (Dike-

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Ndudim et al., 2021), and the safety limitations of Nigerian fermented seed condiments have recently been reviewed (Oladeji et al., 2025). In okpeye, another Nigerian alkaline-fermented condiment, enterotoxin-associated genes were detected in *Bacillus cereus* isolates (Agunwah et al., 2024). The microbial food-safety risks of African fermented foods have been reviewed by Anyogu et al. (2021).

Because fermented condiments are made in households and small enterprises and sold from open market stalls, often unpackaged or wrapped in leaves or polythene, hygiene during handling after fermentation matters as much to their safety as the fermentation itself (Anyogu et al., 2021; Oladeji et al., 2025).

In low- and middle-income settings, where routine microbiological surveillance of informally marketed foods is limited, market surveys provide some of the few available data on the quality of products as they reach consumers. Such data are lacking for Benin City, where fermented melon seed is sold under both its Yoruba and its Edo names.

Bacteria recovered from fermented condiments may also possess traits of technological value. Extracellular proteases, lipases and cellulases contribute to the breakdown of seed constituents and to flavour development, and protease-producing isolates are candidates for controlled starter cultures (Owusu-Kwarteng et al., 2022; Moyo et al., 2024).

Conversely, biofilm formation and haemolytic activity are commonly screened as virulence-associated phenotypes.

Molecular identification by 16S rRNA gene sequencing improves on phenotypic identification, although it cannot always discriminate closely related species (Church et al., 2020).

This study therefore determined the bacterial and coliform loads of Iru and of two vendor samples of fermented melon seed (Ogiri/Evbarie) in Benin City, characterised the culturable bacteria and screened them for selected technological and virulence-associated traits, and identified representative isolates by 16S rRNA gene sequencing.

Materials and Methods

Study area and sample collection

One sample of Iru and two samples of fermented melon seed (Ogiri/Evbarie), designated Ogiri/Evbarie A and B (approximately 50 g each), were purchased in July 2026 from three different vendors at Oba Market, one of the main traditional markets in Benin City, Edo State, Nigeria. Each sample was transferred aseptically into a sterile, labelled container, placed in an insulated cold box with ice packs, transported immediately to the Microbiology Laboratory, Department of Biological Sciences, Igbinedion University, Okada, and processed within 4 h of collection.

Enumeration of bacteria

Bacterial loads were determined by the pour-plate technique (Cappuccino and Welsh, 2022). Twenty-five grams of each homogenised sample was added to 225 mL of sterile 0.1% (w/v) peptone water, and tenfold serial dilutions were prepared to 10^{-6}. One-millilitre aliquots of appropriate dilutions were pour-plated with nutrient agar for the total viable bacterial count (TVBC) and with MacConkey agar (HiMedia, India) for the total coliform count (TCC); on MacConkey agar, lactose-fermenting Gram-negative bacteria form pink to red colonies, which were counted as presumptive coliforms. Plates were incubated at 35 °C for 24 h, after which plates bearing 30–300 colonies were counted with a colony counter. Counts were calculated as CFU/g = (number of colonies × dilution factor) ÷ volume plated (mL) and expressed as colony-forming units per gram (CFU/g). Each count was determined in triplicate from the same sample, and the mean of the three determinations was reported.

Isolation and presumptive identification

Colonies differing in size, shape, elevation, pigmentation, margin and surface appearance were picked from the enumeration plates, purified by repeated subculture and maintained on agar slants at refrigeration temperature. Purity was confirmed by uniform colony morphology and by Gram-stained smears examined under oil immersion, which were also used to record the Gram reaction, cell shape and arrangement.

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Ten isolates from each sample were characterised by colonial morphology, Gram reaction and cell shape, and by catalase, coagulase, oxidase, indole, citrate utilisation, methyl red, Voges–Proskauer and lactose fermentation tests selected according to the Gram reaction (Cappuccino and Welsh, 2022). The reactions of each isolate were interpreted collectively against standard identification criteria rather than on the basis of any single test.

Screening for virulence-associated traits

Haemolytic activity was determined on blood agar containing 5% (v/v) defibrinated sheep blood after aerobic incubation at 37 °C for 24–48 h; complete clearing was recorded as β-haemolysis, greenish partial clearing as α-haemolysis and no change as γ-haemolysis (Cappuccino and Welsh, 2022).

Biofilm formation was assessed on Congo red agar, with black, rough colonies after 24–48 h at 37 °C recorded as positive (Turu and Canlı, 2025).

Protease activity was detected as a clear zone around growth on casein agar, and lipase activity as clearing on tributyrin agar (Cappuccino and Welsh, 2022), after incubation at 35–37 °C for 24–48 h and 24–72 h, respectively.

Cellulase activity was detected on carboxymethyl cellulose agar flooded with 0.1% Congo red for 15 min and washed with 1 M sodium chloride, with a clear halo recorded as positive (Wang et al., 2024).

Molecular identification

Four isolates representing the three samples were selected for sequencing. Genomic DNA was extracted with a Zymo-Spin column kit and ZR BashingBead lysis tubes (Zymo Research, USA). Approximately 50–100 mg of cells were suspended in 200 µL of phosphate-buffered saline, disrupted by bead beating at maximum speed for 5 min and centrifuged at 10,000 × g for 1 min.

The supernatant was passed through a Zymo-Spin IV spin filter, mixed with DNA binding buffer and loaded onto a Zymo-Spin IIC column, which was washed with pre-wash and wash buffers before the DNA was eluted in 100 µL of elution buffer.

The 16S rRNA gene was amplified with primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) in 50 µL reactions containing 2 µL of template DNA, 2 mM $MgCl_2$, 200 µM of each dNTP, 0.5 µM of each primer and 2 U of GoTaq Hot Start polymerase (Promega, USA). Cycling comprised initial denaturation at 95 °C for 2 min; 40 cycles of 94 °C for 45 s, 55 °C for 60 s and 72 °C for 120 s; and a final extension at 72 °C for 5 min. Amplicons were checked on 2% agarose gels in Tris-borate-EDTA buffer containing 0.5 µg/mL ethidium bromide (100 V, 1 h) alongside a 100-bp ladder and a no-template control.

Amplicons were purified with ExoSAP-IT (Thermo Fisher Scientific, USA) and sequenced by the dideoxy chain-termination method (Crossley et al., 2020) using BigDye Terminator v3.1 chemistry on an ABI 3730xl DNA Analyzer (Applied Biosystems, USA). Sequences were edited in Sequencher 4.10.1 (Gene Codes Corporation, USA) and compared by megablast (BLAST+ 2.17.0) with the NCBI 16S ribosomal RNA (Bacteria and Archaea type strains) database.

An identity of at least 98.65% with the type strain of a species was taken to support species-level identification (Kim et al., 2014). The sequences were deposited in GenBank under accession numbers QB180977–QB180980.

Statistical analysis

Counts were $\log_{10}$-transformed, checked for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test), and compared among the three samples by one-way analysis of variance followed by Tukey's honestly significant difference test. Because each sample was a single purchase, the replicate counts reflect analytical rather than between-batch variation and the tests compare the samples examined rather than the product types in general.

The frequencies of presumptive genera and screened traits were compared among samples with the Fisher–Freeman–Halton exact test. Significance was set at $p < 0.05$. Analyses were performed in IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA); the Fisher–Freeman–Halton test was computed as the exact test in Crosstabs using the SPSS Exact Tests module.

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Results

Mean TVBC ranged from 4.00×10^6 CFU/g in Iru to 2.58×10^8 CFU/g in Ogiri/Evbarie A, and mean TCC from 2.43×10^3 CFU/g in Iru to 7.75×10^6 CFU/g in Ogiri/Evbarie A (Table 1; Figure 1). No departure from normality (Shapiro–Wilk, $p > 0.50$ for every group) or from equal variances was detected in the $\log_{10}$-transformed counts, although these tests have little power with three replicates (Levene's test, $p > 0.30$). The samples differed significantly in TVBC ($F(2, 6) = 1070.58$, $p < 0.001$) and TCC ($F(2, 6) = 9233.57$, $p < 0.001$), and Tukey's test separated all three samples for both counts. The TCC/TVBC ratio, calculated from counts on different media, was much higher in Ogiri/Evbarie B (69.04%) than in Ogiri/Evbarie A (3.00%) or Iru (0.06%).

Table 1: Total Viable and Total Coliform Counts of Iru and Two Ogiri/Evbarie Samples

Sample	Microbial Counts				
	Total Viable Bacteria (TVB)	$\log_{10}$ TVBC	Total Coliform (TC)	$\log_{10}$ TCC	TCC/TVBC (%)
Iru	$4.00 \times 10^6 \pm 2.78 \times 10^5$	6.60 ± 0.03^c	$2.43 \times 10^3 \pm 2.57 \times 10^2$	3.38 ± 0.05^c	0.06
Ogiri/Evbarie A	$2.58 \times 10^8 \pm 4.80 \times 10^7$	8.41 ± 0.08^a	$7.75 \times 10^6 \pm 2.29 \times 10^5$	6.89 ± 0.01^a	3.00
Ogiri/Evbarie B	$7.97 \times 10^6 \pm 4.31 \times 10^5$	6.90 ± 0.02^b	$5.50 \times 10^6 \pm 4.92 \times 10^5$	6.74 ± 0.04^b	69.04

Note: Values are means ± standard deviations of triplicate counts. Within a column, $\log_{10}$ values with different superscript letters differ significantly (one-way ANOVA with Tukey's HSD; TVBC, $F(2, 6) = 1070.58$; TCC, $F(2, 6) = 9233.57$; both $p < 0.001$). Ogiri/Evbarie (Yoruba/Benin names) is fermented melon seed; A and B are samples of the same product from different vendors.

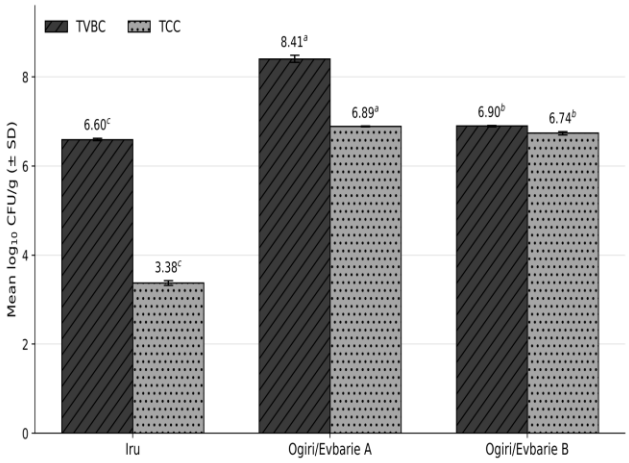


Figure 1: Mean (± SD) $\log_{10}$ total viable bacteria counts (TVBC) and total coliform counts (TCC) of Iru and two Ogiri/Evbarie samples ($n = 3$). Within each count type, bars with different letters differ significantly (Tukey's HSD, $p < 0.05$).

The 30 isolates were presumptively assigned to four genera on the basis of colonial, microscopic and biochemical characteristics (Table 2).

Presumptive *Bacillus* (19 isolates, 63.3%) predominated in all three samples, followed by *Enterobacter* (9 isolates, 30.0%), which also occurred in all three. Single isolates of *Staphylococcus* and *Micrococcus* were recovered from Ogiri/Evbarie A and B, respectively. The distribution of genera did not differ significantly among the samples (Fisher–Freeman–Halton exact test, p = 0.58; Table 4).

Table 2: Frequency of Distribution of Bacteria Isolated from Iru and Two Ogiri/Evbarie Samples

Bacteria Isolate	Condiment Sample		
	Iru	Ogiri/Evbarie A	Ogiri/Evbarie B
Bacillus	8 (80)	5 (50)	6 (60)
Enterobacter	2 (20)	4 (40)	3 (30)
Staphylococcus	-	1 (10)	-
Micrococcus	-	-	1 (10)

Note: Values are numbers (%) of isolates; ten isolates were characterised per sample. - = not isolated.

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The occurrence and distribution of virulence-associated traits among bacterial genera isolated from Iru and Two Ogiri/Evbarie Samples are presented in Table 3. Biofilm formation and protease activity were the most frequent traits (10 of 30 isolates each), followed by lipase and cellulase activity (7 isolates each) (Table 4). Cellulase activity was not detected among the Iru isolates, protease-positive isolates were most frequent in Ogiri/Evbarie B and lipase-positive isolates least frequent in that sample. Seven isolates (23.3%), all presumptive *Bacillus*, were β-haemolytic, the remaining 23 were γ-haemolytic, and no α-haemolysis was observed. None of the traits differed significantly in frequency among the samples.

The organisms expressing each trait are shown in Table 3. In Iru, biofilm formation was shown by two of the eight *Bacillus* isolates and one of the two *Enterobacter* isolates, whereas protease activity (2/8), lipase activity (3/8) and β-haemolysis (2/8) were confined to *Bacillus*. In Ogiri/Evbarie A, biofilm formation occurred in one of five *Bacillus*, two of four *Enterobacter* and the single *Staphylococcus* isolate; cellulase activity in two *Bacillus*, one *Enterobacter* and the *Staphylococcus* isolate; protease activity in two *Bacillus* and one *Enterobacter* isolate; lipase activity in one *Bacillus* and two *Enterobacter* isolates; and β-haemolysis in three *Bacillus* isolates.

Table 3: Occurrence and Distribution of Virulence-Associated Traits among Bacterial Genera Isolated from Iru and Two Ogiri/Evbarie Samples

Virulence Trait	Iru		Ogiri/Evbarie A			Ogiri/Evbarie B		
	Bacillus (n = 8)	*Enterobacter* (n = 2)	*Bacillus* (n = 5)	*Enterobacter* (n = 4)	*Staphylococcus* (n = 1)	*Bacillus* (n = 6)	*Enterobacter* (n = 3)	*Micrococcus* (n = 1)
Biofilm formation	2	1	1	2	1	1	1	1
Protease	2	0	2	1	0	2	3	0
Lipase	3	0	1	2	0	0	1	0
Cellulase	0	0	2	1	1	2	0	1
β-haemolysis	2	0	3	0	0	2	0	0
γ-haemolysis	6	2	2	4	1	4	3	1

Note: Values are numbers of isolates positive for each trait; n = number of isolates of that genus in the sample. No isolate showed α-haemolysis.

Table 4: Distribution of Presumptive Genera and Screened Traits among Isolates from Iru and Two Ogiri/Evbarie Samples

Presumptive genus	Iru (*n* = 10)	Ogiri/Evbarie A (*n* = 10)	Ogiri/Evbarie B (*n* = 10)	Total (*n* = 30)	*p*-value
Bacillus	8	5	6	19 (63.3)	
Enterobacter	2	4	3	9 (30.0)	0.580
Staphylococcus	0	1	0	1 (3.3)	
Micrococcus	0	0	1	1 (3.3)	
Biofilm formation	3	4	3	10 (33.3)	1.000
Protease	2	3	5	10 (33.3)	0.500
Lipase	3	3	1	7 (23.3)	0.642
Cellulase	0	4	3	7 (23.3)	0.151
β-haemolysis	2	3	2	7 (23.3)	1.000
γ-haemolysis	8	7	8	23 (76.7)	

Note: Values are numbers of isolates (percentage of 30 for the total). *p* values are from the Fisher–Freeman–Halton exact test comparing the three samples; for genus distribution, a 3 × 4 table was tested.

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In Ogiri/Evbarie B, biofilm formation was shown by one *Bacillus*, one *Enterobacter* and the single *Micrococcus* isolate; cellulase activity by two *Bacillus* isolates and the *Micrococcus* isolate; protease activity by two of six *Bacillus* and all three *Enterobacter* isolates; lipase activity by one *Enterobacter* isolate; and β-haemolysis by two *Bacillus* isolates.

Thus, β-haemolysis was restricted to *Bacillus* in all three samples, while biofilm formation and protease activity were expressed by both *Bacillus* and *Enterobacter*.

All four selected isolates for molecular identification yielded 16S rRNA gene amplicons of approximately 1,500 bp, with no band in the no-template control (Figure 2). Edited partial sequences of 923–1,009 bp were obtained (Table 5). BROG1, BROG3 and BROG4 shared 99.41%, 98.92% and 99.46% identity, respectively, with type strains of *E. hormaechei*, *S. kloosii* and *M. luteus* over 100% of the query length.

However, BROG1 showed equal identity (99.41%) to the type strain of the closely related *Enterobacter quasihormaechei* (WCHEs120003^T), and BROG3 equal identity (98.92%) to *Staphylococcus lloydii* 23_2_7_LY^T. BROG2 shared only 97.51% identity with the *Priestia megaterium* type strain (ATCC 14581^T), below the 98.65% species threshold (Kim et al., 2014).

Its highest identity (97.73%) was to *Priestia aryabhattai* B8W22^T and *Bacillus zanthoxyli* 1433^T, and *Priestia flexa* NBRC 15715^T also shared 97.51%. BROG2 is therefore reported as a *P. megaterium*-group isolate.

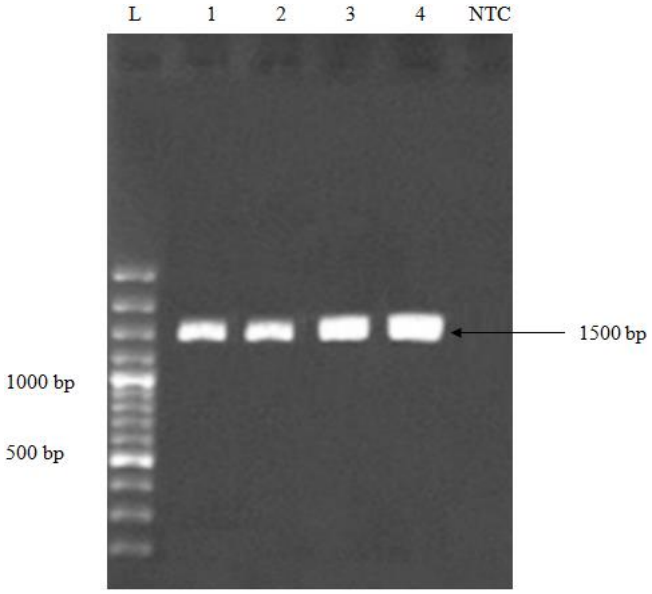


Figure 2: Agarose gel electrophoresis of PCR-amplified 16S rRNA gene fragments.
(L = DNA ladder; lanes 1 and 2 = Iru isolates (BROG1, BROG2); lane 3 = Ogiri/Evbarie A isolate (BROG3); lane 4 = Ogiri/Evbarie B isolate (BROG4); NTC = no-template control).

Table 5: 16S rRNA Gene-Based Identities of Selected Isolates

Sample	Strain	Assigned identity	Accession	Length (bp)	Closest type strain (NCBI accession)	Identity (%)	Coverage (%)
Iru	BROG1	*Enterobacter hormaechei*	QB180977	1,009	*E. hormaechei* subsp. *xiangfangensis* 10-17^T (NR_126208)	99.41	100
Iru	BROG2	*Priestia megaterium* group	QB180978	923	*P. megaterium* ATCC 14581^T (NR_116873)	97.51	100
Ogiri/Evbarie A	BROG3	*Staphylococcus kloosii*	QB180979	927	*S. kloosii* ATCC 43959^T (NR_024667)	98.92	100
Ogiri/Evbarie B	BROG4	*Micrococcus luteus*	QB180980	927	*M. luteus* NCTC 2665^T (NR_075062)	99.46	100

Note: Identity and coverage are from megablast searches against the NCBI 16S ribosomal RNA (Bacteria and Archaea type strains) database. ^{T} = type strain. Equal-identity matches and the basis for the *P. megaterium*-group assignment are given in the text.

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Discussion

The total viable counts of 10^6 to 10^8 CFU/g recorded in this study are consistent with the high microbial populations expected in finished alkaline-fermented condiments, in which proteolytic *Bacillus* species proliferate during fermentation (Owusu-Kwarteng et al., 2022). Ogiri/Evbarie A carried a load about 65 times that of Iru. Notably, the two Ogiri/Evbarie samples, the same melon-seed condiment obtained from different vendors, differed significantly in both TVBC and TCC, which suggests that processing and handling by individual producers and vendors, and not the substrate alone, influence the microbiological quality of the finished product, although single samples cannot establish this. Differences in seed substrate, moisture, fermentation duration and post-fermentation handling are plausible explanations, as such factors vary widely among traditional processors (Oladeji et al., 2025), but they were not measured here. Because the samples were finished products bought at the point of sale, the counts reflect both the fermentation and any growth or contamination during storage and display.

Of greater concern are the coliform loads. Coliforms were present in all three samples, at 10^6 to 10^7 CFU/g in both Ogiri/Evbarie samples, more than three orders of magnitude higher than in Iru. In Ogiri/Evbarie B, the coliform count was equivalent to about two-thirds of the total viable count. Coliforms are not part of the desirable fermentative flora of alkaline condiments, and such levels point to contamination during cooling, wrapping, storage or retail display rather than to the fermentation itself (Anyogu et al., 2021). Comparable post-processing contamination has been reported in other ready-to-eat staples sold in Edo State, including garri (Akpoka et al., 2026a) and fufu (Akpoka et al., 2026b). These findings agree with earlier reports of bacteria of public-health concern in Nigerian fermented seed condiments (Dike-Ndudim et al., 2021; Agunwah et al., 2024) and support calls for improved hygiene in the production and marketing of traditional condiments (Oladeji et al., 2025). The comparatively low coliform count in Iru suggests that cleaner products are achievable within the same market.

The predominance of presumptive *Bacillus* in all three samples agrees with the central role of *Bacillus* and related genera in alkaline seed fermentation (Owusu-Kwarteng et al., 2022) and with the recovery of novel *Bacillus*-related taxa from Iru (Akintayo et al., 2023). *Staphylococcus* was less frequent than in earlier Nigerian studies, which recovered *S. aureus* from Ogiri sold in Owerri (Dike-Ndudim et al., 2021) and reported *Staphylococcus* in 42.3% of 59 isolates from okpeye (Agunwah et al., 2024). Differences in the foods examined, sampling and identification methods, and the small number of isolates characterised here, probably account for this contrast.

Protease, lipase and cellulase activities were detected in isolates from all or most of the samples. Proteolysis drives flavour development and the rise in pH during alkaline fermentation (Owusu-Kwarteng et al., 2022) and may also release bioactive peptides (Moyo et al., 2024), while lipolysis is relevant to the oil-rich melon seeds used for Ogiri/Evbarie. The protease- and lipase-positive isolates are therefore candidates for further evaluation as starter cultures, subject to safety assessment. Because none of the traits differed significantly among the samples, these capacities appear to be broadly distributed among the culturable bacteria of both products.

One-third of the isolates formed biofilm on Congo red agar, and seven presumptive *Bacillus* isolates were β-haemolytic. Biofilm formation may favour persistence on processing surfaces and utensils, and β-haemolysis among *Bacillus* isolates raises the possibility of members of the *B. cereus* group, some of which produce enterotoxins; such genes were detected in *B. cereus* from okpeye (Agunwah et al., 2024). Both assays are qualitative screens, and toxin genes were not examined here, so these isolates should be prioritised for species-level identification and toxin-gene screening. Biofilms formed on fermentation vessels, utensils and wrapping materials may also act as persistent sources of contamination between production batches.

From a practical standpoint, the findings point to the post-fermentation stages as the main targets for intervention. Simple measures, including clean and covered containers, protection of the product from dust and hand contact at the point of sale, clean utensils for portioning and hygienic packaging, are within the means of small-scale producers and vendors and address the routes by which coliforms are most likely to enter the product (Anyogu et al., 2021).

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Periodic monitoring of total viable and coliform counts, combined with training of producers and vendors, would show whether such measures reduce contamination. For Ogiri/Evbarie, the contrast between the products of two vendors suggests that comparing samples from different sellers can help to identify good and poor practice within the same market.

Sequencing confirmed members of the dominant phenotypic groups and added species-level detail. The recovery of *E. hormaechei* from Iru is notable because this species belongs to the *Enterobacter cloacae* complex, which includes opportunistic pathogens and strains with emerging antimicrobial resistance (Davin-Regli et al., 2019); its presence in a ready-to-eat condiment warrants antimicrobial susceptibility testing.

BROG2 belonged to the *P. megaterium* group, spore-forming *Bacillus*-related bacteria consistent with the fermentative flora, while *S. kloosii*, a coagulase-negative *Staphylococcus*, and *M. luteus* are common environmental and skin-associated organisms whose presence is compatible with contamination during handling. The ties between BROG1 and *E. quasihormaechei* and between BROG3 and *S. lloydii*, and the sub-threshold identity of BROG2, illustrate the limited species-level resolution of partial 16S rRNA gene sequences (Church et al., 2020; Kim et al., 2014). Additional markers or genome sequencing would resolve these identities, as genome-based work on Iru has already revealed previously undescribed taxa (Akintayo et al., 2023). Presumptive identification remains useful for screening large numbers of isolates, but sequencing more of them would be needed to confirm the genus distribution reported here.

The study has limitations. Samples were obtained from a single market, and the triplicate counts were replicate determinations on the samples analysed rather than independent production batches, so the differences among samples describe the products examined and may not apply to all Iru and Ogiri/Evbarie in Benin City. Only ten isolates per sample were characterised, identification of most isolates was presumptive, only four isolates were sequenced. Future work should sample multiple markets and batches, measure pH and moisture, and screen isolates for toxin genes and antimicrobial resistance.

Conclusion

Iru and Ogiri/Evbarie sold in Benin City carried high and significantly different bacterial loads, with coliform levels in Ogiri/Evbarie that indicate poor hygiene during production or retail. Presumptive *Bacillus* predominated in all three samples, and protease-, lipase- and cellulase-producing isolates were common, but biofilm formation and β-haemolysis were also frequent. The recovery of *E. hormaechei*, an *E. cloacae* complex species, from Iru reinforces the need for hygienic processing, packaging and retail practices. The marked differences between the two Ogiri/Evbarie samples, from different vendors, suggest that microbiological quality depends on the producer and on handling, and the enzyme-producing isolates identified here merit evaluation as safe starter cultures.

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Declarations

Data availability: The 16S rRNA gene sequences are available in GenBank under accession numbers QB180977–QB180980. Other data are available from the corresponding author on reasonable request.

Conflict of interest: The authors declare no conflict of interest.

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