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Isolation and Molecular Identification of Bacteriocin-Associated Lactobacillus Strains from Indigenous Fermented Foods

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ABSTRACT

Bacteriocin-producing Lactobacillus species have been observed for their ability to inhibit foodborne pathogens and enhance food safety and serving a natural biopreservatives and probiotics. This study aimed to isolate and characterize bacteriocin-producing Lactobacillus strains from selected indigenous fermented foods for potential application in the food industry in Nigerian. The fermented food samples were serially diluted and were cultured on de Man, Rogosa and Sharpe (MRS) agar and used to isolate lactic acid bacteria (LAB). The isolates were characterized by using morphological, biochemical methods and molecular identification based on 16S rRNA gene amplification and sequencing. The LAB isolates that were predominant were subsequently screened for the presence of the gassericin bacteriocin gene using polymerase chain reaction (PCR) to screen for bacteriocin-producing potential. Phenotypic and molecular characterization identified the predominant isolates as Lacticaseibacillus paracasei, Lactiplantibacillus plantarum, Limosilactobacillus fermentum, and Limosilactobacillus reuteri, each were able to produce a distinct 16S rRNA gene amplicon of approximately 1,500 bp. The gassericin-associated gene, with an expected amplicon size of approximately 800 bp, was detected in all predominant isolates. Out of the 30 LAB isolates that were screened, 19 (63.33%) were positive for the targeted bacteriocin-associated genetic marker, with frequency of occurrence ranging from 50% in fermented melon seeds (Ogiri) to 70% in fermented milk. These findings in this study have shown that indigenous Nigerian fermented foods can constitute valuable reservoirs of lactobacilli that possess genes for bacteriocins, with potential applications as starter cultures, natural biopreservatives, and probiotic candidates that can be used to improve food safety and extend the shelf life of fermented foods and other food products.

INTRODUCTION

Lactic acid bacteria (LAB) are a diverse group of Gram-positive, non-spore-forming, catalase-negative, acid-tolerant microorganisms that can ferment food, act as preservatives, and play a vital role in human health. They are mostly used during production, as starter cultures of fermented dairy products, cereals, vegetables, meat products, and beverages, where they improve product safety, nutritional quality, flavor, and shelf life through the production of organic acids and antimicrobial metabolites (Liang *et al.*, 2025; Tian *et al.*, 2026). Members of the family Lactobacillaceae, are traditionally referred to as the genus Lactobacillus but recently reclassified into several genera, including Lactiplantibacillus, Lacticaseibacillus, and Limosilactobacillus, are recognized for their probiotic properties and remarkable ability to produce antimicrobial compounds such as organic acids, hydrogen peroxide, diacetyl, and bacteriocins (George-Okafor *et al.*, 2020).

Bacteriocins inhibit closely related bacteria as well as numerous foodborne pathogens and spoilage microorganisms with the aid of their ribosomally synthesized antimicrobial peptides. Bacteriocins are considered promising alternatives to conventional chemical preservatives and, in some cases, antibiotics because they are generally susceptible to gastrointestinal proteases and exhibit relatively targeted antimicrobial activity (Sugrue *et al.*, 2024; Tian *et al.*, 2026). Their antimicrobial activity is mediated through membrane permeabilization or interference with essential cellular processes, which leads to rapid cell death. Growing consumer demand for minimally processed, preservative-free, and "clean-label" foods has accelerated research into bacteriocin-producing LAB as natural bio-preservatives. Commercial bacteriocins such as nisin have demonstrated effectiveness against important foodborne pathogens including Listeria monocytogenes, Staphylococcus aureus, Clostridium botulinum, and several spoilage microorganisms, thereby improving food safety and extending product shelf life (Liang *et al.*, 2025; Sugrue *et al.*, 2024).

Bacteriocin-producing lactic acid bacteria (LAB) inhabit diverse environmental niches, including fermented foods, where spontaneous fermentation promotes the proliferation and dominance of microorganisms adapted to local substrates and processing conditions. Traditional fermented foods, including cereal, dairy, vegetable and tuber-based products, harbor diverse Lactobacillus species capable of producing bacteriocins and other antimicrobial metabolites and these bioactive compounds contribute to fermentation processes, inhibit undesirable and pathogenic microorganisms, and enhance the microbiological safety and overall quality of fermented products (Liang *et al.*, 2025).

The discovery and characterization of indigenous strains with broad antimicrobial spectra, desirable physicochemical

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stability, and probiotic potential could support the development of natural food preservatives, functional starter cultures, and alternative antimicrobial agents, while reducing dependence on imported microbial resources (Sugrue *et al.*, 2024). Despite the rich microbial diversity associated with fermented foods, bacteriocin-producing Lactobacillus strains remain insufficiently explored and characterized, particularly in developing countries where traditional fermentation practices are widely used. Therefore, this study aimed to isolate and characterize bacteriocin-producing Lactobacillus strains from selected indigenous fermented foods. The findings are expected to facilitate the identification of promising indigenous strains for applications in food preservation, food safety, and industrial biotechnology, particularly in low-resource settings where access to locally adapted microbial cultures remains limited.

LITERATURE REVIEW

Lactic Acid Bacteria in Fermented Foods

Lactic acid bacteria (LAB) are important in food fermentation through their carbohydrate-fermenting capacity and production of metabolites organic acids, bacteriocins, and other antimicrobial compounds that shape preservation, flavour, and microbial stability. The former broad genus Lactobacillus has been reorganized into several new genera, including Lactiplantibacillus, Lacticaseibacillus, and Limosilactobacillus, reflecting substantial genomic and functional diversity within the group (Mejía-Caballero and Marco, 2025), and LAB metabolites remain central to discussions of fermented-food quality and preservation (Doo *et al.*, 2024). Traditional fermented foods are a valuable reservoir of such organisms: Nigerian fermented foods, including ogi, fufu, and iru, have been shown to harbour bacteriocin-producing LAB with biopreservation potential (Nathaniel *et al.*, 2026). Because LAB composition varies with substrate, fermentation practice, and geography, further characterization across other indigenous Nigerian foods is still needed to identify strains with useful antimicrobial traits.

Bacteriocins and Molecular Detection

Bacteriocins are ribosomally synthesized antimicrobial peptides of growing interest as alternatives to conventional preservatives, with demonstrated activity against foodborne and spoilage organisms (Reuben and Torres, 2024), though practical use remains limited by production yield, antimicrobial spectrum, stability, and regulatory hurdles only a small number have reached commercial application (Liang *et al.*, 2025). Molecular screening is a key tool for identifying LAB carrying bacteriocin-associated genes, but detection of a single target gene is only preliminary evidence of genetic potential: biosynthesis typically involves structural, modification, transport, immunity, and regulatory genes, so gene detection alone cannot confirm active production (Sugrue *et al.*, 2024). Functional confirmation therefore requires pairing genetic screening with antimicrobial and biochemical assays before assigning confirmed bacteriocin-producing status (Bisht *et al.*, 2025). The importance of characterizing microorganisms associated with fermented foods is also evident in studies of Nigerian fermented products, where microbial profiling has been used to assess both beneficial and potentially undesirable microorganisms (Iember and Okonkwo, 2024).

Gassericin and the Present Study

Bacteriocins associated with gassericin are of particular interest given their antimicrobial relevance in lactobacilli, and genomic tools now make it easier to identify such determinants in food-associated strains (Sugrue *et al.*, 2024). Yet even with recent Nigerian studies on bacteriocin-producing LAB from ogi and iru, there is limited information on gassericin-associated genetic markers across multiple indigenous Nigerian food matrices. The present study addresses this gap by investigating LAB from corn pap (Akamu), fermented African oil bean seeds (Ugba), fermented melon seeds (Ogiri), and fermented locust beans (Iru), through enumeration, phenotypic and biochemical characterization, 16S rRNA gene sequencing, and PCR-based detection of the targeted gassericin-associated gene marker providing preliminary evidence of LAB diversity and bacteriocin-associated genetic potential across these food types.

Recent studies further demonstrate the value of molecular characterization of microorganisms associated with traditional fermented foods. For example, Fowotade *et al.* (2026) used full-length 16S rRNA sequencing to characterize microbial communities in selected traditional fermented Nigerian foods, including ogi, garri, fufu and iru, and reported the predominance of probiotic-associated taxa such as Lactobacillus delbrueckii and Limosilactobacillus fermentum. Such findings support the use of molecular approaches to complement conventional phenotypic characterization when investigating LAB diversity in indigenous Nigerian food matrices.

MATERIALS AND METHODS

Isolation of Lactic Acid Bacteria

Indigenous fermented food samples, namely corn pap (akamu), fermented milk, fermented melon seeds (ogiri), and fermented cabbage, were purchased from major markets in Enugu metropolis, Enugu State, Nigeria. Each food sample was aseptically packaged in labeled polyethylene bags and transported in an ice box to the laboratory for analysis. Thereafter, 1 g of each fermented food sample was aseptically homogenized using a sterile mortar and pestle and mixed with 9 mL of sterile physiological saline (0.85% NaCl) using a vortex mixer to obtain the initial suspension (10^{-1} dilution). Further ten-fold serial dilutions were prepared up to 10^{-6}. Subsequently, 0.1 mL aliquots of the appropriate dilutions of each food sample were inoculated onto de Man, Rogosa and Sharpe (MRS) agar plates by the pour-plate method and incubated anaerobically at 37°C for 24–48 h. Predominant, distinct colonies exhibiting characteristic morphological features suggestive of lactic acid bacteria (LAB) were selected and purified by repeated subculturing on fresh MRS agar plates.

Identification of Isolates

Selected isolates were subjected to Gram staining and biochemical tests including catalase, coagulase, oxidase, and sugar fermentation as described by Altheide (2019). Probable LAB isolates were further characterized via 16S rRNA sequencing for strain identification. The DNA of the isolates was extracted using the ZR Bacterial DNA Miniprep Kit (Zymo Research) as described by Shen (2025). Briefly, 2 mL of bacterial broth culture was transferred into a ZR BashingBead™ Lysis Tube, followed by the addition of 750 µL of Lysis Solution. The tube was secured in a bead-beating device and processed at maximum speed for more than 5 min. The lysate was centrifuged at >10,000 × g for 1 min, and up to 400 µL of the supernatant was transferred to a Zymo-Spin™ IV Spin Filter and centrifuged at 7,000 × g for 1 min. Thereafter, 1,200 µL of Bacterial DNA Binding Buffer was added to the filtrate, and 800 µL portions of the mixture were transferred to a Zymo-Spin™ IIC Column and centrifuged at 10,000 × g for 1 min, with the process repeated once. The column was subsequently washed with 200 µL DNA Pre-Wash Buffer and 500 µL Bacterial DNA Wash Buffer, each followed by centrifugation at 10,000 × g for 1 min. DNA was finally eluted by adding 100 µL of DNA Elution Buffer directly to the column matrix and centrifuging at 10,000 × g for 30 s.

The extracted DNA was used as template for amplification of the bacterial 16S rRNA gene according to the method described by Kargar *et al.* (2017), with slight modifications. The PCR reaction mixture consisted of 12.5 µL of Taq 2X Master Mix (New England Biolabs, M0270), 1 µL each of 10 µM forward primer 27F (AGAGTTTGATCMTGGCTCAG) and reverse primer 1525R (AAGGAGGTGWTCCARCCGCA), 2 µL of DNA template, and 8.5 µL of nuclease-free water, giving a final reaction volume of 25 µL. PCR amplification was performed with an initial denaturation at 94°C for 5 min, followed by 36 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 45 s, followed by a final extension at 72°C for 7 min and a hold at 10°C.

The amplified PCR products were detected by agarose gel electrophoresis. Briefly, 1 g of agarose was dissolved in 100 mL of 1× TAE buffer to prepare a 1% agarose gel. The solution was heated until completely dissolved, allowed to cool to approximately 50°C, and 10 µL of EZ Vision DNA stain was added. The gel was poured into a casting tray with a comb and allowed to solidify. Loading buffer was added to the DNA samples, and a molecular weight DNA ladder was loaded into the first well, followed by the PCR products in the subsequent wells. Electrophoresis was performed in 1× TAE buffer at 80–150 V for approximately 1–1.5 h. The amplified DNA fragments were subsequently visualized using a UV transilluminator. The confirmed 16S rRNA gene amplicons were sequenced using a Genetic Analyzer 3130xl sequencer (Applied Biosystems) according to the manufacturer's instructions, with the Big Dye Terminator v3.1 Cycle Sequencing Kit. The resulting sequences were analyzed using Bio-Edit software and MEGA X for genetic analysis and strain identification.

Bacteriocin Detection

Bacteriocin-associated genes were detected by polymerase chain reaction (PCR) using specific primers targeting the gassericin bacteriocin gene. The forward primer, designated BACTERIOCIN F, was 5'-GAACAGGTGCACTAATCGGT-3', while the reverse primer, BACTERIOCIN R, was 5'-CAGCTAAGTTAGAAGGGGCT-3'. PCR amplification was performed in a final reaction volume of 25 µL. The reaction mixture contained 12.5 µL of Taq 2X Master Mix (New England Biolabs, M0270), 1 µL each of 10 µM forward and reverse bacteriocin primers, 2 µL of extracted DNA template, and 8.5 µL of nuclease-free water.

PCR amplification was carried out using an initial denaturation at 94°C for 5 min, followed by 36 cycles consisting of denaturation at 94°C for 30 s, primer annealing at 55°C for 30 s, and extension at 72°C for 45 s. A final extension was performed at 72°C for 7 min, followed by a hold at 10°C. The PCR products were subsequently subjected to agarose gel electrophoresis for detection of the amplified bacteriocin gene. A 2% agarose gel was prepared by dissolving 2 g of agarose in 100 mL of 1× TAE buffer. After complete dissolution, the agarose solution was allowed to cool to approximately 50°C, after which 10 µL of EZ Vision DNA stain was added. The gel was cast in a tray with a comb and allowed to solidify. Loading buffer was added to the PCR products, and a molecular weight DNA ladder was loaded into the first well, followed by the PCR samples in the subsequent wells. Electrophoresis was performed in 1× TAE buffer at 80–150 V for approximately 1–1.5 h. The gel was then examined under a UV transilluminator. The presence of an appropriately sized DNA band was interpreted as a positive amplification of the targeted bacteriocin gene.

RESULTS AND DISCUSSION

Table 1: Enumeration of Lactic Acid Bacteria in Indigenous Fermented Foods

Fermented food	No. of samples (n)	(CFU/mL)
Corn pap (Akamu)	3	$7.23 \pm 1.076 \times 10^6$
Fermented Cabage	3	$6.673 \pm 1.120 \times 10^7$
Fermented melon seeds (Ogiri)	3	$6.127 \pm 0.006 \times 10^6$
Fermented Milk	3	$8.363 \pm 1.259 \times 10^7$

Table 2: Frequency of Occurrence of Bacteriocin-Producing LAB Isolates from Indigenous Fermented Foods

Fermented food	No. of LAB isolates screened	Bacteriocin-positive isolates	Frequency of occurrence (%)
Corn pap (Akamu)	6	4	66.67
Fermented Cabage	8	5	62.50
Fermented melon seeds (Ogiri)	6	3	50.00
Fermented Milk	10	7	70.00
Total	30	19	63.33

Table 3: Phenotypic Identification Scheme for the Selected Bacteriobiotic Isolates

S/N	Morphological Test		Biochemical tests								SuspectedOrganisms
	Growth on MRS Medium	Gram reaction	Cat	Oxi	Coa	Glu	Fru	Mal	Man	Lac Fm	
F	Small, round, white colonies	+ve rods	-v	-v	-v	Fm	Fm	Fm	Fm	AG	*Lactobacillus* sp.
G	White, raised,and moist colonies	+ve rods	-v	-v	-v	AG	AG	AG	AG	AG	*Lactobacillus* sp.
M	White, convex, smooth colonies	+ve rods	-v	-v	-v	AG	AG	AG	AG	AG	*Lactobacillus* sp.
C	White, flat, sticky colonies	+ve rods	-v	-v	-v	AG	AG	AG	AG	AG	*Lactobacillus* sp.
H	Small, round, white colonies	+ve rods	-v	-v	-v	AG	AG	AG	AG	AG	*Lactobacillus* sp.

KEY: Cat=Catalase test, Cit= Citrate test, Coa=Coagulase test, MR=Methyl red, Ind=Indole test, Oxi=Oxidase test, Gl=Glucose, F=D-Fructose, Ml=Maltose, Ma=Mannitol, La=Lactose, +=positive, - =negative, A=Acidic, AG=Acidic and Gas, G=Gas, +ve=positive, —ve = negative, Fm =Fermentation

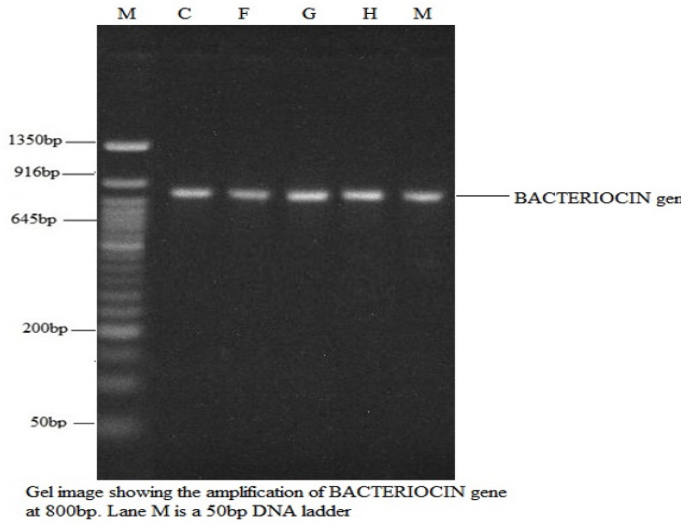


Figure 1: Gel image of Amplified DNA of LAB Isolates

Key:
C – *Lacticaseibacillus paracasei* strain IMUST00432 from Pap
F – *Limosilactobacillus fermentum* strain RM106 from fermented Milk
G – *Limosilactobacillus reuteri* strain EI1F-4 from fermMilk
H – *Lactiplantibacillus plantarum* strain 42D72 from fermented Castor oil seed (ogiri),
M – *Lactobacillus fermentum* strain hani.A ton 2 from fermented Cabage

Table 4: Phylogenetic affiliation of the Selected Bacteriocin-Producing Lactobacillus Isolates

Isolate	Closest NCBI reference strain	NCBI accession No.	Pairwise similarity (%)	Putative identification	Phylogenetic interpretation
C	Lacticaseibacillusparacasei strain IMUST00432	PV382596.1	95.26	*Lacticaseibacillusparacasei*	Clustered closely with L. paracasei, indicating close phylogenetic relatedness.
F	*Limosilactobacillus fermentum* strain RM106	MW658859.1	94.81	*Limosilactobacillus fermentum*	Closely related to L. fermentum, suggesting evolutionary affinity within the Limosilactobacillus lineage.
G	*Limosilactobacillusreuteri* strain EI1F-4	PQ008858.1	93.18	*Limosilactobacillus* reuteri	Clustered within the Limosilactobacillus clade but showed relatively low sequence similarity, suggesting strain divergence.
H	*Lactiplantibacillus plantarum* strain 42D72	OM760581.1	95.79	*Lactiplantibacillus plantarum*	Clustered closely with L. plantarum, indicating a close evolutionary relationship.
M	*Lactobacillus fermentum* strain hani.A ton 2	KM214424.1	89.07	*Lactobacillus fermentum*	Exhibited the lowest sequence similarity among the isolates, suggesting substantial genetic divergence from the reference strain.

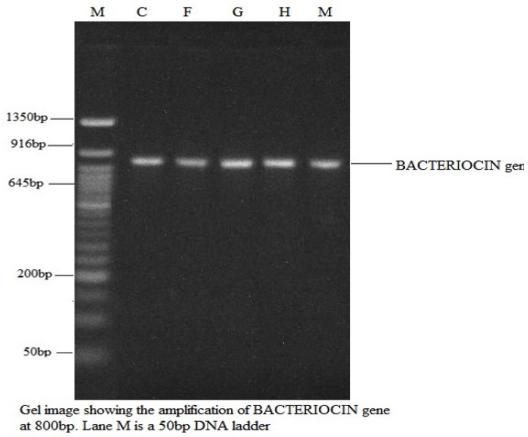


Figure 2: Gel Electrophoresis Showing PCR Amplification of the Bacteriocin Gene in Selected LAB Isolates

Discussion

The enumeration results showed that all the indigenous fermented foods examined harboured substantial populations of lactic acid bacteria (LAB), although the counts varied among the food types (Table 4.1). Fermented milk recorded the highest LAB count of $(8.363 \pm 1.259) \times 10^7$ CFU/mL, followed by fermented cabbage with $(6.673 \pm 1.120) \times 10^7$ CFU/mL. Corn pap (Akamu) and fermented melon seeds (Ogiri) recorded lower counts of $(7.23 \pm 1.076) \times 10^6$ and $(6.127 \pm 0.006) \times 10^6$ CFU/mL, respectively. The relatively high LAB populations observed indicate that these fermented foods provide favorable environments for LAB proliferation. This observation is consistent with the *established importance* of autochthonous microorganisms in

fermented-food ecosystems, where microbial growth and metabolism contribute to acidification, food safety, shelf-life extension, and sensory development (Mejía-Caballero and Marco, 2026; Auchtung *et al.*, 2025).

The selected isolates (F, G, M, C, and H) exhibited characteristics typical of *Lactobacillus*, including growth on MRS agar, Gram-positive rod morphology, and negative catalase, oxidase, and coagulase reactions (Table 4.3). These findings are consistent with the classical phenotypic characteristics of lactic acid bacteria reported by Mejía-Caballero and Marco (2026), who described lactobacilli as metabolically diverse, saccharolytic, fermentation-dependent bacteria that are widely associated with foods and other environments. All isolates fermented glucose, fructose, maltose, mannitol, and lactose with acid and gas production, demonstrating active carbohydrate metabolism. Such metabolic properties are important in fermented-food ecosystems because LAB metabolism contributes to acidification and the production of metabolites that influence microbial stability, flavour, and other food characteristics (Auchtung *et al.*, 2025; Mejía-Caballero and Marco, 2026). These properties are technologically important because rapid acidification can inhibit undesirable microorganisms and contribute to fermentation efficiency, microbial safety, and shelf-life extension. However, carbohydrate fermentation patterns alone are insufficient for definitive species identification because closely related bacterial species may exhibit overlapping phenotypic and biochemical characteristics (Martínez-Porchas and Vargas-Albores, 2017). The limitations of phenotypic identification therefore justify the use of molecular methods for more reliable taxonomic characterization.

The molecular results complemented the phenotypic characterization. PCR amplification of the 16S rRNA gene generated distinct amplicons of approximately 1,500 bp in all four isolates (Figure 4.1), confirming successful amplification of the bacterial marker gene. The subsequent sequencing and phylogenetic analysis provided additional evidence for the taxonomic affiliation of the isolates. The 16S rRNA gene remains an important molecular marker for bacterial identification and phylogenetic analysis; however, its discriminatory power can be insufficient for distinguishing some closely related species (Martínez-Porchas & Vargas-Albores, 2017). Previous methodological studies have similarly shown that 16S rRNA sequencing generally provides strong genus-level identification but may have limited species-level resolution where closely related taxa possess highly similar sequences. Thus, the identification obtained in the present study is appropriately interpreted in conjunction with the phenotypic characteristics and phylogenetic relationships rather than relying exclusively on 16S rRNA similarity.

The phylogenetic analysis based on the 16S rRNA gene sequences (Table 4.4) showed that the selected isolates belonged to diverse members of the family Lactobacillaceae. Isolate C was most closely related to Lacticaseibacillus paracasei, H to *Lactiplantibacillus plantarum*, F and M to *Limosilactobacillus fermentum*, and G to Limosilactobacillus reuteri. These findings are consistent with the recognized diversity of lactobacilli associated with food environments and their broad ecological and metabolic adaptability (Mejía-Caballero and Marco, 2026). The close relationship of isolate H to *Lactiplantibacillus plantarum* and isolate C to Lacticaseibacillus paracasei is particularly relevant because these taxa are associated with fermented-food environments and have been investigated for their technological and functional properties (Mejía-Caballero and Marco, 2026). *Lactiplantibacillus plantarum*, in particular, is widely distributed in fermented dairy, cereal, vegetable, meat, and other food systems and is recognized for its metabolic adaptability and potential contribution to fermentation, antimicrobial activity, and food quality (Mejía-Caballero and Marco, 2026). Likewise, members of *Limosilactobacillus fermentum* and *Limosilactobacillus reuteri* have been associated with antimicrobial and bacteriocin-related properties, supporting further investigation of these isolates for such functional traits.

The frequency of bacteriocin-positive LAB isolates further demonstrated the potential of the fermented foods as sources of LAB carrying the targeted bacteriocin-associated genetic marker (Table 4.2). Of the 30 LAB isolates screened, 19 (63.33%) were positive, indicating that more than half of the isolates examined yielded the expected PCR result. Fermented Milk recorded the highest proportion of positive isolates (7/10; 70.00%), followed by corn pap (Akamu) (4/6; 66.67%) and fermented cabage (5/8; 62.50%), while fermented melon seeds (Ogiri) recorded 3/6 (50.00%) positive isolates. The occurrence of bacteriocin-associated traits among isolates from all four fermented foods supports previous reports that traditional and ethnic fermented foods can serve as reservoirs of LAB with bacteriocin-related properties (Zheng *et al.*, 2020; Sugrue *et al.*, 2024). Reviews of fermented-food bacteriocins have similarly documented LAB-derived bacteriocins as important antimicrobial compounds with potential applications in food preservation and bioprotection.

PCR amplification further revealed an approximately 800 bp bacteriocin-associated gene amplicon in the selected isolates C, F, G, H, and M (Figure 4.2), indicating the presence of DNA sequences recognized by the bacteriocin primer pair. This finding provides molecular evidence that the selected isolates possess the targeted bacteriocin-associated genetic marker. Bacteriocins produced by LAB are ribosomally synthesized antimicrobial peptides whose biosynthesis is commonly associated with genetic clusters containing structural and accessory genes required for production, modification, transport, and regulation (Sugrue *et al.*, 2024). The detection of the targeted gene therefore provides a basis for further investigation of the bacteriocin-producing potential of the isolates.

The presence of bacteriocin-associated genetic markers in the selected isolates is also consistent with the recognized role of bacteriocins in microbial competition and food

biopreservation. LAB bacteriocins can inhibit susceptible microorganisms and have attracted considerable interest as natural antimicrobial agents for improving food safety and extending shelf life (Sugrue *et al.*, 2024). Reviews of bacteriocins from traditional fermented foods have specifically highlighted their potential as natural food preservatives and the possibility of using bacteriocin-producing LAB as protective or starter cultures (Zheng *et al.*, 2020; Sugrue *et al.*, 2024).

From an applied perspective, the substantial LAB populations recorded in Table 4.1, together with the 63.33% overall frequency of bacteriocin-associated PCR positivity recorded in Table 3.2, indicate that the indigenous fermented foods investigated are promising sources of LAB with potentially useful antimicrobial traits. The recovered isolates may therefore serve as candidates for further evaluation as protective starter cultures or sources of natural antimicrobial compounds. LAB and their bacteriocins have potential applications in food preservation, inhibition of foodborne and spoilage microorganisms, and hurdle-technology approaches to improve food safety and quality (Sugrue *et al.*, 2024). In particular, *Lactiplantibacillus plantarum* has been widely investigated for its technological and functional properties in fermented foods, including antimicrobial activity and potential application as a starter or protective culture (Mejía-Caballero and Marco, 2026).

However, the detection of a bacteriocin-associated gene does not by itself confirm bacteriocin production or antimicrobial activity. The presence of a structural or associated gene indicates genetic potential, but bacteriocin biosynthesis depends on appropriate expression, processing, transport, regulation, and production conditions. Bacteriocin gene clusters can contain multiple genes involved in precursor synthesis, modification, immunity, transport, and regulation, and the expression and activity of these systems may vary among strains (Sugrue *et al.*, 2024). Therefore, the PCR findings in the present study should be interpreted as evidence of the presence of the targeted bacteriocin-associated genetic marker, rather than definitive confirmation that the isolates produced active bacteriocins.

Consequently, further investigations involving antimicrobial activity assays, cell-free supernatant testing, protease sensitivity, bacteriocin purification, peptide characterization, and determination of the antimicrobial spectrum would be necessary to confirm functional bacteriocin production. This distinction is important because antimicrobial activity in LAB cultures may result from several compounds, including organic acids, hydrogen peroxide, and bacteriocins, and therefore activity alone may not always be attributable specifically to bacteriocins. Such additional characterization would also be necessary before the isolates could be recommended for commercial use as natural food preservatives, protective cultures, or probiotic candidates.

Overall, the findings demonstrate that the indigenous fermented foods examined contained appreciable populations of LAB, with counts ranging from 6.127×10^6 to 8.363×10^7 CFU/mL (Table 4.1). Furthermore, 19 of the 30 LAB isolates screened (63.33%) yielded positive amplification for the targeted bacteriocin-associated genetic marker (Table 4.2), while the selected isolates C, F, G, H, and M produced the expected approximately 800-bp PCR product (Figure 4.2). Collectively, these findings highlight the indigenous fermented foods as promising reservoirs of LAB carrying bacteriocin-associated genetic traits and provide a foundation for further functional characterization of the isolates for potential applications in food fermentation, microbial protection, and natural biopreservation.

CONCLUSION

The present study successfully isolated and detected bacteriocin-producing *Lactobacillus* strains from indigenous fermented foods and provides a scientific basis for harnessing indigenous Lactobacillus strains from local environment for sustainable food preservation and food biotechnology. However, further whole-genome sequencing and functional characterization of the expressed bacteriocins are recommended to facilitate their industrial exploitation.

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