A Study on the Screening for Lactic Acid Bacteria from Fura Da Nono with Antibacterial and Bio Preservative Properties

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Abstract. The study explores the isolation and screening of lactic acid bacteria (LAB) from the traditional Nigerian fermented beverage fura da nono with a focus on their antibacterial and biopreservative properties. A total of fifty LAB strains were isolated using selective MRS agar, and presumptive identification was achieved via phenotypic characterisation. Four isolates displaying prominent antibacterial activity were further identified through 16S rRNA gene sequencing, revealing *Lactobacillus plantarum* FJ390111, *Lactobacillus casei* CP14326, and *Enterococcus lactis* NR117562, with an additional isolate, *Lysinibacillus fusiformis*, identified as an environmental contaminant. Crude bacteriocin extracts, obtained via ammonium sulfate precipitation and dialysis, were evaluated against foodborne pathogens, including *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli* using agar diffusion assays. The LAB bacteriocins demonstrated optimal inhibitory activity at pH 6 and 35 °C, whereas significant declines in activity were noted at temperatures above 40 °C and during extended storage (96 hours at 37 °C). Graphical data and tables confirm that the bacteriocin extracts produced zones of inhibition ranging from approximately 11 to 15 mm under optimal conditions, with *Enterococcus lactis* showing particularly moderate to high activity. These results underscore the potential application of LAB-derived bacteriocins as natural bio-preservatives in the food industry. However, the heat sensitivity and degradation over time highlight the need for further formulation improvements. The study concludes that with enhanced stabilisation and rigorous quality control, bacteriocins from traditional LAB can serve as effective alternatives to chemical preservatives in ensuring food safety and extending product shelf life.

Keywords: Bacteriocin; Bio Preservative; Fura; da Nono; Lactic acid bacteria.

INTRODUCTION

Fermented dairy products play a crucial role in many cultures, providing not only nutrition but also numerous health benefits due to their microbial content [1]. Fura da nono, a traditional Nigerian beverage made by fermenting milk with cereal-based ingredients, is known for its unique texture, flavour, and its role in food preservation. However, the microbial load of fermented products may include spoilage organisms and pathogens [2]. In recent times, there has been increased research interest in lactic acid bacteria (LAB) because of their recognised ability to produce bacteriocins—antimicrobial proteins that naturally inhibit pathogenic bacteria and improve shelf life [3].

This study aims to isolate and screen LAB from fura da nono samples for their antibacterial and biopreservative properties. Additionally, the work seeks to characterise the optimum conditions (pH, temperature, and storage effects) for bacteriocin activity. Molecular identification via 16S rRNA sequence analysis further confirms the identity of the potent isolates. The findings are expected to contribute to safer food preservation strategies by providing naturally derived bio-preservatives as alternatives to chemical additives.

Fermented milk is created by using specific microorganisms to ferment milk, which may or may not have its composition altered [4]. This includes milk that has been reconstituted or recombined and then fermented by the appropriate microbes. The process might cause the milk to thicken, but not always. The cultures added must stay alive, active, and plentiful in the product until the expiration date listed on the packaging. However, if the product is heat-treated after fermentation, these standards don't apply. Certain fermented milk products are defined by the specific microbes used. For example, yoghurt requires fermentation through a cooperative interaction between *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus,* though it can also be made with *S. thermophilus* paired with any *Lactobacillus* species. Acidophilus milk, on the other hand, is made by fermenting milk with *Lactobacillus acidophilus* [5].

Lactobacilli are a type of lactic acid bacteria that are generally recognised as safe (GRAS) due to their natural presence in the normal microflora of the gastrointestinal (GI) tract. Certain members of lactic acid bacteria (LAB) are classified as probiotics. Among various strains such as Enterococcus, Bifidobacterium, Bacillus, and Saccharomyces, lactobacilli are commonly employed as probiotics. In modern times, probiotics hold significant medical importance for treating and controlling infections [6].

METHODS

Sample Collection and Isolation. Samples of locally produced fura da nono were aseptically collected from dairy markets in Abia State. The samples were stored at 4 °C and processed within 48 hours. LAB were isolated by homogenising the samples and plating on de Man, Rogosa, and Sharpe (MRS) agar. Incubation was carried out anaerobically at 37 °C for 24–48 hours. Isolates were identified as presumptive LAB based on being Gram-positive, catalase-negative, non–spore forming, and non-motile, as well as by sugar fermentation profiles [7].

Screening for Antibacterial Activity and Bacteriocin Production. A primary screening of the isolates for antibacterial activity was initially performed using the perpendicular streak plate and agar disk diffusion methods, as described by [8]. Test pathogens, including Staphylococcus aureus, Salmonella typhi, and Escherichia coli, were inoculated on nutrient and Mueller–Hinton agar and overlaid with the isolated LAB cultures. Isolates showing clear zones of inhibition were selected for further study.

Molecular Identification. Selected LAB isolates showing antibacterial potential were subjected to genomic DNA extraction using the boiling method. The universal primers 27F and 1492R were used for amplifying the 16S rRNA gene. PCR products were then sequenced using a cycle sequencing kit on an Applied Biosystems ABI 3500XL Genetic Analyser—sequence comparison using available databases allowed for species-level identification. The isolates were identified as primarily *Lactobacillus plantarum*, *Lactobacillus casei*, and *Enterococcus lactis*; an additional isolate, *Lysinibacillus fusiformis*, was also recovered and attributed to potential environmental contamination.

Bacteriocin Extraction, Purification, and Characterisation. Laboratory cultures of the selected isolates were grown in MRS broth until turbidity was achieved (approximately 18–24 hours). Cell-free supernatants (CFS) were obtained by centrifugation (6000 rpm for 10 minutes) at low temperature. To purify bacteriocins, the CFS were subjected to ammonium sulfate precipitation (using 70% and 40% saturation levels), and the resulting protein pellet was resuspended in phosphate-buffered saline (PBS, pH 7). Subsequent dialysis in PBS over 12 hours with successive buffer changes removed salts and ensured partial purification of the bioactive compounds [9].

Optimisation Studies. To understand the conditions affecting bacteriocin activity, the following tests were performed:

Effect of pH: Aliquots of bacteriocin extracts were adjusted to pH values ranging from 2 to 10 using diluted acids and bases. The activity against indicator organisms was measured by the agar well diffusion method. The indicator organisms utilised were isolates of *Escherichia coli, Staphylo-*

coccus aureus, and Salmonella typhi obtained from the National Root Crops Research Institute's general laboratory. The isolates were re-identified using standard biochemical tests. The plates were incubated at 30 °C for 24 hours under anaerobic conditions. After incubation, the respective plates were examined for the presence of clear zones of inhibition around the wells and the zones were measured in mm.

Effect of Temperature: The purified bacteriocins were exposed to different temperatures (40 °C, 60 °C, 80 °C, and 100 °C) for a fixed time period. Residual antibacterial activity was then evaluated using the well diffusion assay. The indicator organisms utilised were isolates of *Escherichia coli, Staphylococcus aureus, and Salmonella typhi obtained from the National Root Crops Research Institute's* general laboratory. The test isolates were re-identified using standard biochemical tests. The plates were incubated for 24h under anaerobic conditions. After incubation, the respective plates were examined for the presence of clear zones of inhibition around the wells and the zones were measured in mm.

Effect of Storage: Bacteriocin extracts were stored at 37 °C and sampled every 24 hours over 96 hours. The decline in antibacterial activity was monitored using the standard agar diffusion technique. The isolates were re-identified using standard biochemical tests. The indicator organisms utilised were clinical isolates of *Escherichia coli, Staphylococcus aureus, and Salmonella typhi obtained from the National Root Crops Research Institute's* general laboratory. The plates were incubated for 24h under anaerobic conditions. After incubation, the respective plates were examined for the presence of clear zones of inhibition around the wells and the zones were measured in mm.

RESULTS AND DISCUSSION

Isolation and Identification. Fifty LAB strains were isolated from fura da nono samples. The phenotypic characterisation confirmed typical LAB properties: Gram-positive rods or cocci, catalase-negative, and non-motile. From the pool, four isolates exhibiting the strongest antibacterial activity were selected. Molecular analysis of the 16S rRNA gene yielded sequences that, upon BLAST comparison, identified the isolates as *Lactobacillus plantarum* FJ390111, *Lactobacillus casei* CP14326, and *Enterococcus lactis* NR117562. An additional isolate, *Lysinibacillus fusiformis NBRIGS12*, was also detected, likely reflecting environmental contamination.

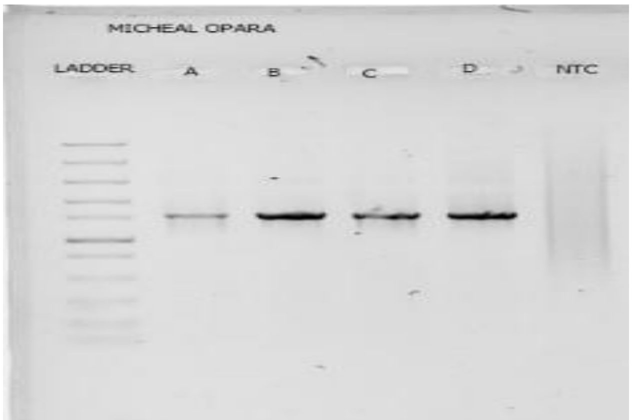

Figure 1 – Displays the PCR bands for the major isolates, confirming successful amplification of the 16S rRNA gene

Antibacterial and Bacteriocin Activity. Screening using the agar diffusion method showed that all selected LAB isolates produced bacteriocins with activity against the test pathogens. The bacteriocin extract from *Lactobacillus plantarum* demonstrated an inhibition zone of approximately 14 mm against *E. coli* and similar values against *S. typhi* and *S. aureus*. Meanwhile, the bacteriocin from *Enterococcus lactis* showed a slightly higher inhibition zone against *E. coli* (up to 14.67 mm).

Table 1 summarises the diameter of zones obtained, and Figure 2 includes a graph comparing the relative activity of bacteriocin extracts with gentamycin as a control.

Table 1 – Showing results for the screening of bacteriocin extract against some pathogenic organisms; antimicrobial activity (mm diameter)

ISOLATES	*Salmonella*	*Escherichia coli*	*Staphylococcus aureus*
Lactobacillus plantarum FJ390111	$14.33^{b} \pm 1.16$	$13.33^{bc} \pm 0.58$	$11.67^{b} \pm 1.16$
Lactobacillus casei CP14326	$13.33^{b} \pm 1.16$	$12.33^{c} \pm 1.16$	$8.33^{c} \pm 0.58$
Enterococcus lactis NR117562	$14.67^{b} \pm 0.58$	$14.33^{b} \pm 0.58$	$9.67^{c} \pm 0.58$
GENTAMYCIN	$23.33^{a} \pm 1.16$	$22.67^{a} \pm 1.16$	$19.33^{a} \pm 1.53$

CONTROL H_2O	$0.00^{c}\pm0.00$	$0.00^{d}\pm0.00$	$0.00^{d}\pm0.00$

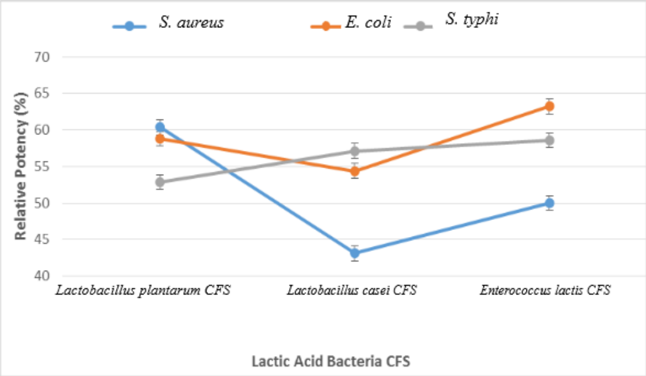

Figure 2 – Relative percentage potency of the Bacteriocin extract of the three Lactic acid bacteria to gentamycin

Optimisation of Bacteriocin Activity. Effect of pH: The bacteriocins from all three LAB isolates exhibited maximum antibacterial activity at pH 6. For example, the extract from *L. plantarum* inhibited *S. aureus*, *E. coli*, and *S. typhi*, with zones of inhibition of approximately 14 mm at this pH. Activity diminished remarkably below pH 4 and above pH 10 as shown in the graphs in Figures 3a–c.

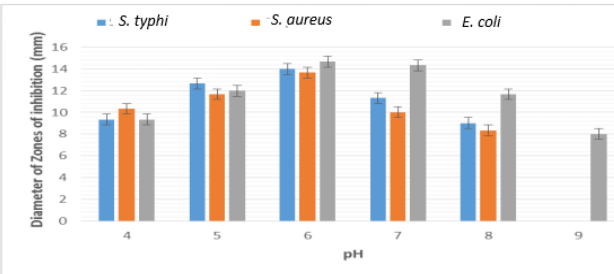

Figure 3a – Effect of pH on antimicrobial activity of bacteriocin extract from *Lactobacillus plantarum* FJ390111 against food spoilage pathogens

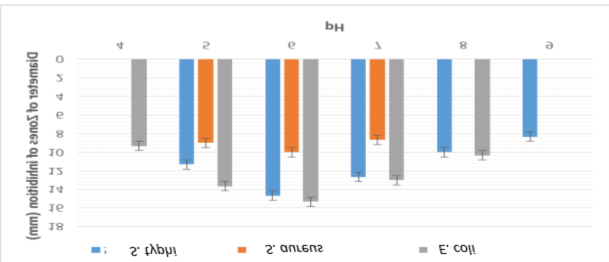

Figure 3b – Effect of pH on antimicrobial activity of bacteriocin extract from *Lactobacillus casei* CP14326 against food spoilage pathogens

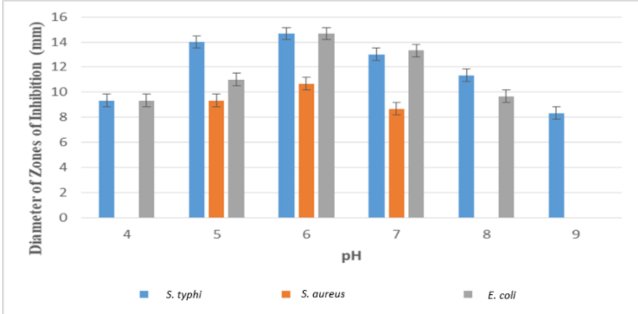

Figure 3c – Effect of pH on antimicrobial activity of Bacteriocin extract from *Enterococcus lactis* against food spoilage pathogens

Effect of Temperature: The optimum temperature for bacteriocin activity was determined to be around 35 °C. When exposed to temperatures of 40 °C and above, a decline in activity was observed, with almost complete loss of activity at 80–100 °C. Figures 4a–c graphically display these trends, confirming the thermosensitive nature of these antimicrobial proteins.

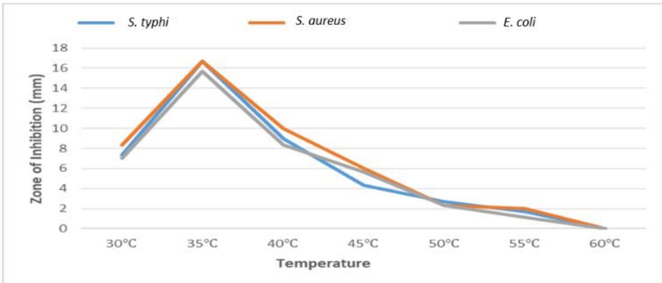

Figure 4a – Effect of temperature on antimicrobial activity of Bacteriocin extract from *Lactobacillus plantarium* FJ390111 against food spoilage pathogens

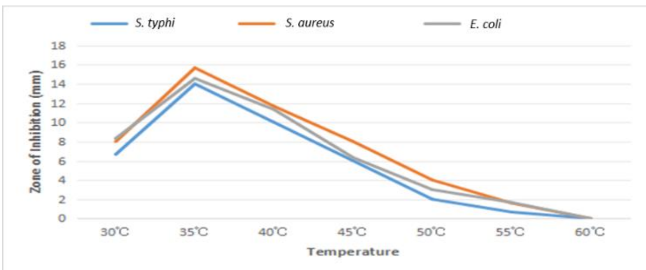

Figure 4b – Effect of temperature on antimicrobial activity of bacteriocin extract from *Lactobacillus casei* CP14326 against food spoilage pathogens

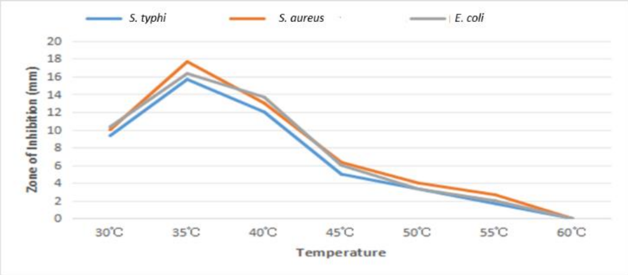

Figure 4c – Effect of temperature on antimicrobial activity of Bacteriocin extract from *Enterococcus lactis* NR117562 against food spoilage pathogens

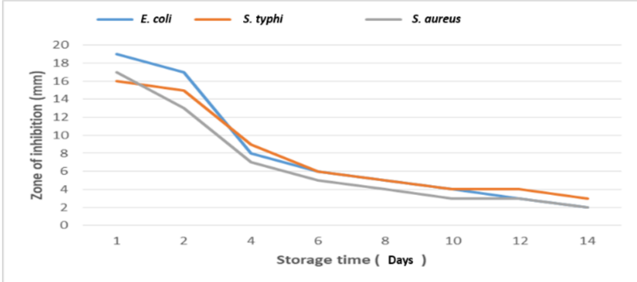

Figure 5c – Effect of storage time on the antimicrobial activity of the bacteriocin extract *of Enterococcus lactis* NR117562

Effect of Storage: Storage at 37 °C over a period of 336 hours (14 days) resulted in a gradual decline in bacteriocin potency. The inhibition zones reduced steadily over time; by day 10 to 14, the activity was nearly negligible. These findings underscore the need for enhanced preservation techniques if bacteriocins are to be effectively applied in real food systems. Figures 5a–c illustrate the time-dependent decrease in activity.

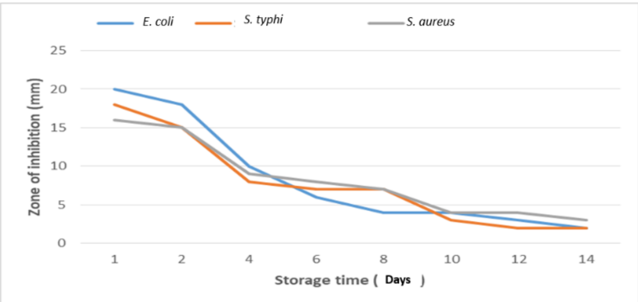

Figure 5a – Effect of storage time on the antimicrobial activity of the bacteriocin extract *Lactobacillus plantarum* FJ390111

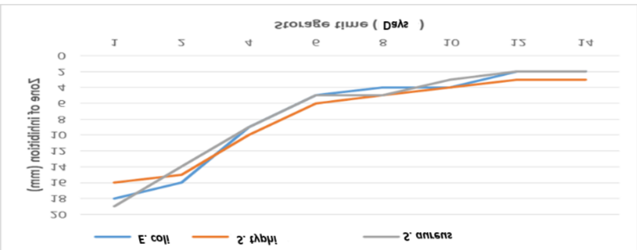

Figure 5b – Effect of storage time on the antimicrobial activity of the bacteriocin extract from *Lactobacillus casei* CP14326

The results confirm that fura da nono is a rich source of LAB, with several isolates exhibiting powerful antibacterial properties through bacteriocin production. The identification of predominant species such as *Lactobacillus plantarum* and *Lactobacillus casei* corresponds with previous studies on dairy fermentations. Their ability to rapidly acidify the medium and generate bioactive peptides is crucial for maintaining the product's quality.

The observed optimal activity at pH 6 and moderate temperatures is consistent with the natural environment of fermented milk products. However, the sensitivity of the bacteriocins to higher temperatures and prolonged storage indicates that while they are promising for immediate biopreservative applications, further work is required to enhance their stability in industrial settings.

The unexpected presence of *Lysinibacillus fusiformis* underscores the importance of rigorous hygiene and quality control during production. This environmental bacterium may influence overall microbial dynamics and serve as a marker for contamination.

Overall, the study supports the potential of using bacteriocin-producing LAB as natural preservatives. The findings pave the way for future research aimed at stabilising these compounds and optimising their use in real food matrices under variable processing conditions.

CONCLUSIONS

This study successfully isolated and screened lactic acid bacteria from fura da nono, identifying potent bacteriocin producers, including *Lactobacillus plantarum* FJ390111, *Lactobacillus casei* CP14326, and *Enterococcus lactis* NR117562.

These isolates exhibited rapid acidification and showed significant antibacterial activity against common foodborne pathogens. The bacteriocin activity was optimal at pH 6 and at moderate temperatures (35 °C) but declined drastically with elevated temperature exposure and with extended storage periods. The detection of *Lysinibacillus fusiformis* also underscored the importance of strict sanitation protocols.

Recommendations:

1) Enhanced Stabilisation Strategies: Future work should focus on methods to improve the thermostability and storage stability of bacteriocins—for example, through encapsulation or the use of stabilising additives—to ensure consistent antimicrobial activity in food applications.

2) Validation in Food Matrices: Since the results presented here were primarily obtained in laboratory media, further studies using model food systems are recommended to assess the real-world efficacy of these bacteriocins as bio-preservatives.

3) Quality Control Measures: Regular monitoring of production environments is necessary to prevent contamination with non-LAB species, such as Lysinibacillus fusiformis, thereby ensuring the safety and quality of the final product.

4) Mechanism and Synergy Studies: Investigate the detailed mechanism of bacteriocin action and explore possible synergistic effects with other natural preservation methods (e.g., organic acids or mild heat treatments) to develop a holistic approach for food preservation.

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