

Impact of Temperature Variations on Bacteriocin Activity Against Spoilage Bacteria in Melon and Its Fermented Product 'Ogiri'

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ABSTRACT

The efficacy of bacteriocins produced by *Lactobacillus plantarum* NRIC 0383 against spoilage bacteria isolated from melon seeds and their fermented product, Ogiri, was evaluated under various storage temperatures. Traditional fermented foods, such as Ogiri made from melon seeds (*Citrullus lanatus*), are significant protein sources in rural African communities but are susceptible to microbial contamination, posing public health risks. The study aimed to assess the antimicrobial activity of *L. plantarum* bacteriocins against identified spoilage bacteria: *Escherichia coli* 2013C-3342, *Staphylococcus aureus* CIP 9973, and *Enterobacter cloacae* AS10. Isolation and identification of these bacteria were performed using serial dilution and molecular techniques, respectively. Bacteriocin production by *L. plantarum* NRIC 0383 was confirmed via 16S rDNA sequencing and PCR amplification of bacteriocin genes. Partially purified bacteriocins, obtained through ammonium sulfate precipitation and dialysis, demonstrated significant inhibitory effects against the test microorganism, with inhibition zones ranging from 10-20 mm. Stability tests revealed that the bacteriocins retained more than 80% of their activity at temperatures between 30°C and 70°C, but activity declined at 90°C and was almost entirely lost at 121°C. These findings suggest that *L. plantarum* NRIC 0383 bacteriocins are potent bio-preservatives that could enhance the safety and shelf-life of melon seed-based fermented products, offering a natural alternative to chemical preservatives.

Keywords: Antibacterial activity, Bacteriocins, Fermented food, *Lactobacillus plantarum*, Ogiri,

1.0 INTRODUCTION

Traditionally fermented foods are a major contributor to the protein requirements of households across Africa (Käferstein *et al.*, 2019). These fermented foods are used as condiments in food due to their aroma and flavor. One such is Ogiri produced from melon seeds (*Citrullus lanatus*) and is the commonly used substrate for the production of ogiri, in Nigeria. Castor oil seeds ("*Ricinus communis*") and fluted pumpkin ("*Telferia occidentale*") have also been used in the production of this condiment (Ogbuonye, 2018, Käferstein, *et al.*, 2019). Contamination of these food products by pathogenic microorganisms remains a major concern (Ibeabuchi *et al.*, 2014). Food-borne diseases due to microbial spoilage have become a huge challenge to man; and microbes (Ibeabuchi *et al.*, 2014). Additionally, the growth of these microorganisms sometimes leads to high levels of toxins in food (Käferstein *et al.*, 2019, Rawat, 2015). Despite improvements in food preservation, and sanitary quality. Instances of food-borne diseases from contamination of fermented foods as a result of poor handling and hygiene have been recorded (Novais *et al.*, 2022).

Balancing the benefits of chemical preservatives in maintaining product safety and shelf life with potential risks to human health has been daunting (Novais *et al.*, 2022). Biopreservation is a healthier and more natural way of food preservation (Taylor *et al.*, 2019). It involves the preservation and improvement of the

safety of food and food products using biological agents and their metabolites to control spoilage and pathogenic microorganisms. Examples of biological agents used in the biopreservation of foods are the Lactic acid bacteria (LAB) (Miranda *et al.*, 2021).

Lactic acid bacteria comprise Gram-positive non-sporulating cocci and bacilli (Dordević *et al.*, 2021). They are significant organisms particularly for their fermentative ability and health and nutritional benefits (Pasolli *et al.*, 2020). The antimicrobial activity of Lactic acid bacteria has been attributed to the presence of antagonistic metabolites such as organic acids, ethanol, hydrogen peroxide, fatty acids, bacteriocins, and bacteriocin-like inhibitory substances. Examples of Lactic acid bacteria are *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, species, *etc.* (Dordević *et al.*, 2021, Imade., 2021).

Bacteriocins are antimicrobial peptides produced by bacteria that inhibit the growth of other bacteria. They have the Generally Regarded as Safe Status (GRAS), indicating that they are safe for food preservation based on a long history of common use in food and scientific research. These metabolites are part of the natural defense mechanism of the producing bacteria and provide a competitive advantage in their environment by suppressing rival microorganisms. Examples of Bacteriocin-producing bacteria include *Lactococcus lactis*, *Lactobacillus* species *Bacillus* species, *Pediococcus* species, *etc* (Ahsan *et al.*, 2022).

Bacteriocins are of interest in the food industry because they have the potential to preserve food, and also restore the nutritional properties of food (Ahsan *et al.*, 2022). This study therefore seeks to determine the activity of bacteriocins of *Lactobacillus* against spoilage bacteria from melon and Ogiri a fermented produce from melon.

2. METHODOLOGY

2.1 Test Isolates

Test bacterial (3) used in this study were previously isolated from Ogiri samples using the serial dilution agar plate technique (Ibeabuchi *et al.*, 2014) and obtained from the Microbiology laboratory of the Nigeria Stored Products Research Institute, Ilorin, Kwara state. The isolates were *Staphylococcus aureus* CIP 9973, *Enterobacter cloacae* AS10, and *Escherichia coli* 2013C-3342.

2.2 Processing of Fermented Melon (Ogiri)

The fermented melon sample (300 g) was reduced into smaller particles using a blender pre-sterilized using 70% alcohol. The homogenates were collected in labeled bottles and stored in sterile containers at 4°C in the refrigerator for further analysis.

2.3 Isolation and Purification of *Lactobacillus* Species From Yogurt Samples

Yogurt samples purchased from a supermarket in Ilorin were serially diluted, by transferring 1 ml of each sample separately into 9 ml of sterile water to make a stock mixture. A homogenized mixture (1 ml) was then taken into an appropriately labeled test tube to make 10⁻¹ of the mixture. The serial dilution was continued until 10⁻⁸ was obtained. An aliquot (0.1 ml) of the dilutions was spread over MRS (De Man, Rogosa, Sharpe) agar plates, and the plates were incubated in an anaerobic jar at 37 ± 2°C for 48 hours. Observed colony growth on incubated MRS plates after 48 hours (Ibeabuchi *et al.*, 2014) was subcultured on fresh MRS agar and incubated under the same conditions to get pure colonies. Colonies were subcultured twice on MRS agar and incubated in an incubator (Swiss model NU-5700, UK) at 37 ± 2°C for 48 hours to get pure colonies. Purified isolates were maintained at 4°C (Haier Thermocool, China) in MRS and nutrient broth medium respectively for further characterization and identification.

2.4 Molecular identification of *Lactobacillus* species producing bacteriocin

Twenty-four hours of culture of *Lactobacillus* on Man Ragosa Sharpe (MRS) were inoculated in sterile McCartney bottles containing 20 ml of MRS broth and incubated for 12 hours at 37 ± 2°C in molecular analysis. The selected species of *Lactobacillus* producing bacteriocin-like substance earlier identified by employing standard schemes of morphological, physiological, and biochemical characteristics was

further identified up to the genetic level as described earlier in section 3.3.4. A fresh culture of *Lactobacillus* on Man Ragosa Sharpe (MRS) plate was duplicated in sterile McCartney bottles containing 20 ml of MRS broth and incubated for 12 hours at 37 ± 2°C. Molecular identification was done using 16S rDNA gene sequencing. Deoxyribonucleic acid (DNA) extraction was carried out as earlier described. The polymerase chain reaction was also carried out using 16SrRNA gene universal primer (16SF GTGCCAGCAGCCGCGCTAA) and (16SR: AGACCCGGAACGTATTAC). Polymerase chain reaction was performed under the following conditions: denaturation: consisting of 30 cycles at 95 °C for 1 min, annealing: at 55 °C for 1 min, and extension: 72 °C for 1.5 min, followed by a final extension step for 5 min at 72 °C. After cycling, the PCR products were detected by electrophoresis on a 1% agarose gel, stained with ethidium bromide, and visualized under Ultraviolet (UV) light. Polymerase chain reaction amplified fragments were then sequenced using a Genetic Analyzer 3130 xl sequencer from applied biosystems using the manufacturers' manual while the sequencing kit used was that of a big dye terminator cycle sequencing kit. Bio-Edit software and MEGA 6 were used for the genetic analysis. The isolates were *Lactobacillus plantarum* NRIC 0383 (Tai *et al.*, 2015).

2.5 Determination of bacteriocin genes in *Lactobacillus plantarum* NRIC 0383

To determine the presence of bacteriocin genes, a PCR reaction was performed in a total volume of 30 µl containing 10 p mole/µl of each primer, and 200ng genomic DNA. The reaction mixture was amplified in a GTC thermal. Denaturation was carried out using the method of Ahsan *et al.* (2022).

2.6 Partial purification of crude extract produced by *L. plantarum* NRIC 0383

Bacteriocin produced by *Lactobacillus plantarum* isolate was purified using the method of Sharma *et al.* (2018) and Aslam *et al.* (2012). Ammonium sulfate was added to the *Lactobacillus plantarum* cell-free supernatant in MRS broth. The metabolite was centrifuged at 14,000 rpm for 30 min at 4°C and the precipitate obtained was adjusted to pH 6.5 with 1 M NaOH. The pellet containing the metabolite was suspended in 3 ml of 5 mM sodium phosphate buffer (pH 7.0) and dialyzed in the same buffer using a membrane with a 3.5 kDa cut-off for 24 hours at 4 °C to remove salts and low-molecular-weight impurities. The retentate was checked for its inhibitory activity against *Escherichia coli* 2013C-3342, *Staphylococcus aureus* CIP 9973, and *Enterobacter cloacae* AS10) isolated from Ogiri samples using the agar diffusion assay.

2.7 Determination of the Effect of Temperature on the Stability and Activity of Bacteriocin

The effect of temperature on the activity of purified metabolite against *Lactobacillus plantarum* isolates was determined by the method of Ihum *et al.* (2019) and Ogunbanwo *et al.* (2003). Purified bacteriocin (0.5 ml) was added to 9.5 ml of nutrient broth in test tubes. The test tubes were covered with paraffin oil to prevent evaporation, plugged with non-absorbent cotton covered with aluminum foil, and the water bath set according to temperatures of 30, 50, 70, and 90 °C at a time range of 15, 30, 45 and 60 minutes respectively also, at 121 °C for 15 minutes to check their activity at a very high temperature. Control was kept at room temperature (28 ± 2°C) and its activity was considered 100%. The test tubes were allowed to cool to room temperature and the activity of bacteriocin was determined against the test bacteria *E. coli* 2013C-3342, *S. aureus* CIP 9973, and *E. cloacae* AS10 using the agar well diffusion method.

Bacteriocin activity (%) was calculated.

$$\frac{\text{inhibition zone of the treated} \times 100}{\text{inhibition zone of the control (untreated) sample}}$$

Inhibition zone of the control (untreated) sample.

3.0 RESULTS

Polymerase chain reaction amplification of bacteriocin genes in *L. plantarum* NRIC 0383 showed the presence of Plantaricin S and Laf operon genes (Plate 1). Test bacteria isolated from Ogori samples and identified molecularly were *Staphylococcus aureus* CIP 9973, *Enterobacter cloacae* AS10, and *Escherichia coli* 2013C-3342 (Table 1). Inhibitory activity of partially purified bacteriocin using ammonium sulfate and dialysis showed that bacteriocin retained varying inhibitory activities against the test bacteria. Table 1 shows the inhibition zones were from 15-20 mm against *S. aureus* CIP 9973, and *E. cloacae* AS10 while for *E. coli* 2013C-3342 inhibition zones ranged from 10-15 mm (Table 1).

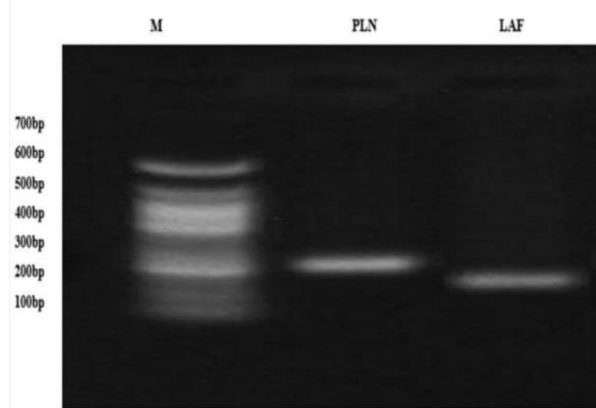


Plate 1: Polymerase chain reaction amplification of bacteriocin genes in *L. plantarum* NRIC 0383 showed the presence of Plantaricin S and Laf operon genes

Table 1: Inhibitory activity of Partially Purified Bacteriocin against the Test Bacteria

Test bacteria	Inhibitory activity of Purified bacteriocin
<i>Staphylococcus aureus</i> CIP 9973	+++
<i>Enterobacter cloacae</i> AS10	+++
<i>Escherichia coli</i> 2013C-3342	++
MRS broth	-

· -; No activity; +: Inhibitory zone diameter within 5-10 mm; ++: Inhibitory zone diameter within 10-15mm; +++: Inhibitory zone diameter within 15-20mm.

Table 2 shows the activity of bacteriocin of *L. plantarum* NRIC 0383 against *Enterobacter cloacae* AS10 at different temperatures. As shown bacteriocin retained more than 80% of its activity at temperatures of 30 to 70°C. Maximum activity of 99 ± % to 99. 84 against *E. cloacae* AS10 was observed for bacteriocin at temperatures of 30°C and exposure time of 15 to 60 min. Table 3 shows the activity of *L. plantarum* bacteriocin against *Escherichia coli* 2013C-3342. At temperatures of 30 to 90°C bacteriocin retained more than 50% activity against *Escherichia coli* 2013C-3342 at 15 to 45 minutes. A reduction in activity was observed after 45 minutes of exposure time and a temperature of 90°C.

Table 2: Activity of *L. plantarum* NRIC 0383 Bacteriocin against *Enterobacter cloacae* AS10 at Different Temperatures

Temperature (°C)	Activity of Bacteriocin (%) in minutes			
	15 min	30 min	45 min	60 min
30	99.84 ± 0.32 ^e	99.68 ± 0.16 ^e	99.52 ± 0.24 ^e	99 ± 0.87 ^d
50	91.65 ± 0.24 ^d	91.56 ± 0.16 ^d	90.95 ± 0.32 ^d	90.19 ± 0.24 ^c
70	88.33 ± 0.24 ^c	82.08 ± 0.16 ^c	81.33 ± 0.16 ^c	81.18 ± 5.78 ^c
90	49.84 ± 0.32 ^b	49.37 ± 0.24 ^b	48.42 ± 0.24 ^b	48.08 ± 0.24 ^b
121	27.05 ± 0.32 ^a			
*Control	100 ± 0 ^e	100 ± 0 ^e	100 ± 0 ^e	100 ± 0 ^d

*Control was processed at room temperature (28°C) and its activity was considered as 100%, Values are means of three replicates ± standard deviation.

Table 3: Activity of Bacteriocin against *Escherichia coli* 2013C-3342 at Different Temperatures

Activity of Bacteriocin (%) in minutes				
Temperature (°C)	15 min	30 min	45 min	60 min
30	99.20 ± 0.33 ^e	98.88 ± 0.25 ^e	99.19 ± 0.25 ^e	99.51 ± 0.16 ^e
50	90.84 ± 0.25 ^d	90.68 ± 0.24 ^d	90.91 ± 0.16 ^d	91.34 ± 0.25 ^d
70	60.77 ± 0.16 ^c	59.52 ± 0.24 ^c	59.42 ± 0.33 ^c	58.09 ± 0.17 ^c
90	51.05 ± 0.33 ^b	50.28 ± 0.16 ^b	50.41 ± 0.25 ^b	50 ± 0.25 ^b
121	28.78 ± 0.16 ^a			
*Control	100 ± 0 ^f	100 ± 0 ^f	100 ± 0 ^f	100 ± 0 ^e

*Control was processed at room temperature (28°C) and its activity was considered as 100%, Values are means of three replicates ± standard deviation.

Table 4 shows the activity of bacteriocin against *S. aureus* CIP 9973 at different storage temperatures. *L. plantarum* bacteriocin had over 50% of its activity (73.45%, 67.59%, 56.91%, and 42.24%) against *S. aureus* CIP 9973 at 90°C and exposure time of 15, 30, 45 and 60 minutes respectively. Statistical analysis showed that the activity of bacteriocin against *S. aureus* CIP 9973 differed significantly ($p < 0.001$) at different temperatures.

Table 4: Activity of bacteriocin activity against *Staphylococcus aureus* CIP 9973 at different temperatures

Activity of Bacteriocin (%) in minutes				
Temperature (°C)	15 min	30 min	45 min	60 min
30	96.91 ± 2.82 ^d	99.07 ± 0.93 ^e	98.41 ± 0.19 ^{de}	97.23 ± 0.48 ^d
50	97.45 ± 1.19 ^d	97.5 ± 0.37 ^d	97.23 ± 1.05 ^d	96.95 ± 1.42 ^d
70	78.18 ± 0.37 ^c	72.41 ± 0.19 ^c	69.16 ± 1.22 ^c	69.05 ± 2.38 ^c
90	75.45 ± 0.2 ^b	67.96 ± 0.37 ^b	58.04 ± 1.13 ^b	47.62 ± 0.38 ^b
121	39.09 ± 1 ^a			
*Control	100 ± 0 ^d	100 ± 0 ^e	100 ± 0 ^e	100 ± 0 ^d

*Control was processed at room temperature (28°C) and its activity was considered as 100%, Values are means of three replicates ± standard deviation.

4.0 DISCUSSION

This study investigated the impact of different storage temperatures on the activity of bacteriocin of *Lactobacillus plantarum* NRIC 0383 against spoilage bacteria in melon seeds and their fermented product, Ogiri. *E. coli* 2013C-3342, *S. aureus* CIP 9973, and *E. cloacae* AS10 were isolated and molecularly identified from Ogiri samples. Ogbuonye, (2018) isolated *Escherichia coli* spp., *Salmonella* spp., *Bacillus* spp., *Fusarium* spp., and *Lactobacillus* spp. Ibeabuchi *et al.*, (2014), also isolated and identified *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus* spp, *Saccharomyces cerevisiae*, *Rhizopus stolonifer*, *Penicillium* spp, and *Saccharomyces cerevisiae*. In his study, Ijabadeniyi (2007) isolated *Streptococcus* sp., *Lactobacillus* sp., *Pediococcus* sp., and *Bacillus* sp from Ogiri in Akure Metropolis. Molecular characterization of isolates revealed *E. coli* 2013C-3342, *S. aureus* CIP 9973, *E. cloacae* AS10, and *L. plantarum* NRIC 0383.

According to Ahsan *et al.* (2022), lactic acid bacteria found in fermented foods, produce a large volume of bacteriocins which can preserve food. In this study, *L. plantarum* NRIC 0383 metabolite showed the presence of genes that encode for bacteriocin and exhibited inhibitory activity against pathogenic isolates from Ogiri samples. According to Zacharof, *et al.* (2012) some species of bacteria may produce more than one type of bacteriocin and one bacteriocin may not be produced by a single species of bacteria. The study showed bacteriocin-encoding gene of *L. plantarum* NRIC 0383 exhibited inhibitory activity against the pathogenic bacteria isolated from Ogiri. Ahsan *et al.* (2022) opined that time-saving methods such as PCR must be used for characterization as it aids rapid identification. Using PCR bacteriocin-encoding genes were detected in this study. Purification of *L. plantarum* NRIC 0383 bacteriocin, using ammonium sulfate precipitation and dialysis did not deter the inhibitory activity of the metabolite against the test bacteria indicating, that the purification method applied though partial was effective. Wang *et al.* (2018)

reported increased antimicrobial using ammonium sulfate precipitation and dialysis. The purification step of bacteriocin is crucial for commercial applications especially where high purity is necessary. In this study, partial purification of bacteriocins did not diminish their antibacterial activity, suggesting the effectiveness of these purification methods (Zou *et al.*, 2018).

Bacteriocin production can be significantly influenced by pH, temperature, incubation time, and other environmental factors. Bacteriocins retained more than 80% of their activity between 30°C and 70°C, demonstrating high thermal stability within this range (Garsa *et al.*, 2014). This is important because it suggests that these metabolites can effectively inhibit spoilage bacteria under storage conditions for fermented foods in many rural settings (Garsa *et al.*, 2014). The highest activity was observed at 30°C, with inhibition percentages nearing 100% for *E. cloacae* AS10 and *E. coli* 2013C-3342, and slightly lower for *S. aureus* CIP 9973. This peak activity at 30°C aligns with typical ambient temperatures in these regions, suggesting applicability (Sahu *et al.*, 2021). However, at 90°C, bacteriocin activity saw a decline, retaining only about 50% of its original activity after 15 minutes of exposure, and almost entirely losing its efficacy at 121°C. It is important to note that while bacteriocins are effective at moderate heat, they might not be suitable for high-temperature processing. The results of this study agree with, Garsa *et al.*, (2014) which showed that the production and activity of bacteriocins can be significantly influenced by environmental factors such as temperature. For instance, Moonchai *et al.* (2005) and Sarika *et al.* (2010) affirmed that bacteriocin production was optimal at 30°C and decreased at higher temperatures, due to increased energy requirements for bacterial maintenance. At elevated temperatures and pH, bacteriocin production was lower because energy needs for metabolism are higher when temperature or pH increases (Peng, *et al.*, 2023). The results demonstrate that temperature plays a critical role in the stability and efficacy of these bacteriocins.

5.0 CONCLUSION

This study demonstrates the capability of bacteriocins produced by *L. plantarum* NRIC 0383 as effective bio-preservatives for melon seed-based fermented products, specifically Ogiri. The isolated bacteriocins exhibited significant inhibitory activity against common spoilage bacteria, including *Escherichia coli* 2013C-3342, *Staphylococcus aureus* CIP 9973, and *Enterobacter cloacae* AS10. Stability tests indicated that these bacteriocins maintain substantial activity across a wide range of temperatures, highlighting their suitability for use in diverse storage conditions. These findings underscore the potential of *L. plantarum* NRIC 0383 bacteriocins to enhance safety and extend the shelf-life of traditional fermented foods, providing a natural and

effective alternative to chemical preservatives. Future prospects include exploring the use of bacteriocins from *L. plantarum* NRIC 0383 in other food products. However, limitations such as the need for further studies on the long-term safety of these bacteriocins and their interactions with other food components must be addressed.

REFERENCES

- Ahsan, A., Mazhar, B., Khan, M. K., Mustafa, M., Hammad, M., & Ali, N. M. (2022). Bacteriocin-mediated inhibition of some common pathogens by wild and mutant *Lactobacillus* species and in vitro amplification of bacteriocin encoding genes. *ADMET and DMPK*, 10(1), 75-87.
- Aslam, M., Shahid, M., ur Rehman, F., Murtaza, M. A., Sharif, S., Ata, A., & Noor, S. (2012). Production optimization and characterization of a low molecular weight bacteriocin from *Lactococcus lactis* subsp. *lactis*. *Afr. J. Microbiol. Res.*, 6(30), 5924-5933.
- Dordević D., Jančíková S., Vítězová M., Kushkevych I. Hydrogen sulfide toxicity in the gut environment: Meta-analysis of sulfate-reducing and lactic acid bacteria inflammatory processes. *J. Adv. Res.* 27 (2021) 55-69. <https://doi.org/10.1016/j.jare.2020.03.003>
- Garsa, A. K., Kumariya, R., Sood, S. K., Kumar, A., & Kapila, S. (2014). Bacteriocin production and different strategies for their recovery and purification. *Probiotics and antimicrobial proteins*, 6, 47-58.
- Ibeabuchi, J. C., Olawuni, I. A., Iheagwara, M. C., Ojukwu, M., & Ofoedu, C. E. (2014). Microbiological evaluation of Iru and Ogiri-Isi used as food condiments. *Journal of Environmental Science, Toxicology and Food Technology*, 8(8), 45-50.
- Ihum, T. A., Iheukwumere, C. C., Ogbonna, I. O., & Gberikon, G. M. (2019). Bacteriocin Production by *Lactobacillus plantarum* NRIC 0383, Measured in Terms of Its Antagonistic Activity against Bacteria Pathogens of Selected Vegetables. *Microbiology Research Journal International*, 27(6), 1-7.
- Imade, E. E., Omonigho, S. E., Babalola, O. O., & Enagbonma, B. J. (2021). Lactic acid bacterial bacteriocins and their bioactive properties against food-associated antibiotic-resistant bacteria. *Annals of Microbiology*, 71, 1-14.
- Käferstein, F. K., Motarjemi, Y., Moy, G. G., & Quevado, F. (2019). Food safety: A worldwide public issue. In *International Food Safety Handbook* (pp. 1-20). Routledge.
- Miranda, C., Contente, D., Igrejas, G., Câmara, S. P., Dapkevicius, M. D. L. E., & Poeta, P. (2021). Role of exposure to lactic acid bacteria from foods

- of animal origin in human health. *Foods*, 10(9), 2092.
- Moonchai, S., Madlhoo, W., Jariyachavalit, K., Shimizu, H., Shioya, S., & Chauvatcharin, S. (2005). Application of a mathematical model and differential evolution algorithm approach to optimization of bacteriocin production by *Lactococcus lactis* C7. *Bioprocess and biosystems engineering*, 28, 15-26.
- Novais, C., Molina, A. K., Abreu, R. M., Santo-Buelga, C., Ferreira, I. C., Pereira, C., & Barros, L. (2022). Natural food colorants and preservatives: A review, a demand, and a challenge. *Journal of agricultural and food chemistry*, 70(9), 2789-2805.
- Ogbuonye, E. O. (2018). Chemical and microbial evaluation of 'ogiri'(A locally fermented food condiment) produced from kersting groundnut seeds. *Sci. Arena Pub. Spec. J. Bio. Sci*, 4(2), 7-13.
- Ogunbanwo, S. T., Sanni, A. I., & Onilude, A. A. (2003). Influence of cultural conditions on the production of bacteriocin by *Lactobacillus brevis* OG1. *African Journal of Biotechnology*, 2(7), 179-184.
- Pasolli E., De Filippis F., Mauriello I.E., Cumbo F., Walsh A.M., Leech J., Cotter P.D., Segata N., Ercolini D.(2020) Large-scale genome-wide analysis links lactic acid bacteria from food with the gut microbiome. *Nat. Commun.* 11 (2020) 2610. <https://doi.org/10.1038/s41467-020-16438-8>
- Peng, Z., Xiong, T., Huang, T., Xu, X., Fan, P., Qiao, B., & Xie, M. (2023). Factors affecting production and effectiveness, performance improvement and mechanisms of action of bacteriocins as food preservatives. *Critical Reviews in Food Science and Nutrition*, 63(33), 12294-12307.
- Rawat, S. (2015). Food Spoilage: Microorganisms and their prevention. *Asian journal of plant science and Research*, 5(4), 47-56.
- Sahu, M. M., & Dwivedi, M. V. (2021). Studies on *Lactobacillus* bacteriocin for production and characterization against some pathogenic and food spoilage bacteria. *Journal Homepage: http://mbsresearch.com*, 7(4).
- Sarika, A. R., Lipton, A. P., & Aishwarya, M. S. (2010). Bacteriocin production by a new isolate of *Lactobacillus rhamnosus* GP1 under different culture conditions. *Adv J Food Sci Technol*, 2(5), 291-7.
- Sharma, G., Dang, S., Gupta, S., & Gabrani, R. (2018). Antibacterial activity, cytotoxicity, and the mechanism of action of bacteriocin from *Bacillus subtilis* GAS101. *Medical Principles and Practice*, 27(2), 186-192.
- Tai H.F., Foo H.L., Abdul Rahim R., Loh T.C., Abdullah M.P., Yoshinobu K.. Molecular characterization of new organisation of plnEF and plw loci of bacteriocin genes harbour concomitantly in *Lactobacillus plantarum* I-UL4. *Microb. Cell Fact.* 14 (2015) 89. <https://doi.org/10.1186/s12934-015-0280-y>
- Taylor, T. M., Ravishankar, S., Bhargava, K., & Juneja, V. K. (2019). Chemical preservatives and natural food antimicrobials. *Food microbiology: Fundamentals and frontiers*, 705-731.
- Wang, Y., Shang, N., Qin, Y., Zhang, Y., Zhang, J., & Li, P. (2018). The complete genome sequence of *Lactobacillus plantarum* LPL-1, a novel antibacterial probiotic producing class IIa bacteriocin. *Journal of Biotechnology*, 266, 84-88.
- Zacharof, M. P., & Lovitt, R. W. (2012). Bacteriocins produced by lactic acid bacteria a review article. *Apchee Procedia*, 2, 50-56.
- Zou, J., Jiang, H., Cheng, H., Fang, J., & Huang, G. (2018). Strategies for screening, purification and characterization of bacteriocins. *International journal of s biological macromolecules*, 117, 781-78