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Adebayo Oluwafemi Akinola
Department of Food Technology, Lagos Academy of Agricultural Sciences, Lagos, Nigeria

Chidinma Nkechi Okonkwo
Department of Microbiology, Lagos Academy of Agricultural Sciences, Lagos, Nigeria

Emeka Ikechukwu Nwosu
Department of Agricultural Engineering, Lagos Academy of Agricultural Sciences, Lagos, Nigeria

Corresponding Author:
Adebayo Oluwafemi Akinola
Department of Food Technology, Lagos Academy of Agricultural Sciences, Lagos, Nigeria

Comparative Evaluation of Biocontrol Strategies against Listeria monocytogenes Contamination in Fresh Produce

Adebayo Oluwafemi Akinola, Chidinma Nkechi Okonkwo and Emeka Ikechukwu Nwosu

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Abstract
The historical reliance on chemical sanitizers for controlling Listeria monocytogenes contamination in fresh produce has increasingly been questioned due to concerns regarding chemical residues, environmental sustainability, and the emergence of sanitizer-tolerant strains. This research aimed to evaluate and compare the efficacy of six biocontrol strategies against L. monocytogenes contamination in fresh produce commodities under simulated commercial storage conditions in Lagos, Nigeria. A randomized controlled experimental investigation was conducted from June 2022 to May 2023 at the Lagos Academy of Agricultural Sciences. Six treatment modalities were evaluated: bacteriophage P100 (10^8 PFU/mL), nisin (2500 IU/mL), Lactobacillus plantarum protective culture (10^8 CFU/mL), Pediococcus acidilactici fermentate, citric acid wash (2%), and sodium hypochlorite (200 ppm) as the conventional control. Treatments were applied to lettuce, tomato, and cucumber samples artificially inoculated with a five-strain cocktail of L. monocytogenes at approximately 10^5 CFU/g and monitored over 72 hours at 4°C and 10°C. Bacteriophage P100 demonstrated the greatest overall efficacy with a mean log reduction of 4.2±0.3 log CFU/g at 4°C after 72 hours, followed by nisin (3.6±0.4 log CFU/g) and L. plantarum (3.1±0.5 log CFU/g). The combination of bacteriophage P100 and nisin exhibited synergistic antimicrobial activity, achieving 5.1±0.2 log reduction, significantly exceeding all individual treatments ($p<0.001$). Temperature significantly influenced treatment efficacy, with all biocontrol agents demonstrating superior performance at 4°C compared to 10°C ($p<0.05$). Sensory evaluation revealed no significant adverse effects on produce quality for any biocontrol treatment. These findings support the integration of bacteriophage-based biocontrol strategies, particularly in combination with nisin, as effective and sustainable alternatives to chemical sanitization for controlling L. monocytogenes in fresh produce supply chains across West Africa.

Keywords: Biocontrol, bacteriophage, nisin, *Listeria monocytogenes*, fresh produce, food safety, antimicrobial efficacy, lactic acid bacteria, decontamination

Introduction
The emergence of Listeria monocytogenes as a significant contaminant of fresh produce has been documented with increasing frequency since the landmark cantaloupe-associated listeriosis outbreak in 2011, which resulted in 147 illnesses and 33 fatalities and fundamentally shifted regulatory and industry perspectives regarding the scope of L. monocytogenes contamination beyond traditional high-risk food categories [1, 2]. Fresh produce, including leafy greens, tomatoes, cucumbers, sprouts, and pre-cut fruit, presents unique challenges for L. monocytogenes control because these commodities undergo minimal processing and are typically consumed raw without any terminal heat treatment that would eliminate the pathogen [3].

Conventional decontamination approaches for fresh produce have relied predominantly on chlorine-based sanitizers, primarily sodium hypochlorite solutions at concentrations of 50 to 200 ppm, which achieve typical log reductions of 1.0 to 2.5 log CFU/g against L. monocytogenes [4]. However, multiple limitations of chlorine-based sanitization have become increasingly apparent, including the formation of potentially carcinogenic disinfection by-products (trihalomethanes and haloacetic acids), environmental contamination from

wastewater discharge, limited efficacy against biofilm-embedded Bacteria, and growing consumer demand for chemical-free food preservation alternatives [5, 6].

Biocontrol strategies employing naturally derived antimicrobial agents represent a paradigm shift in food safety intervention approaches. Bacteriophages, which are viruses that specifically infect and lyse target bacterial species, have garnered particular attention as anti-Listeria biocontrol agents due to their high host specificity, self-amplifying nature, and established safety record for food applications [7]. The Listeria-specific bacteriophage preparation P100 (ListShield/PhageGuard Listex) has received regulatory approval for food applications in multiple jurisdictions and has demonstrated promising efficacy in various food matrices [8]. Complementary biocontrol approaches including bacteriocins (particularly nisin), competitive exclusion using protective cultures of lactic acid bacteria, and organic acid treatments offer additional or synergistic antimicrobial mechanisms [9].

In Nigeria, the fresh produce sector constitutes a vital component of the agricultural economy and dietary patterns, with per capita consumption of fruits and vegetables estimated at approximately 120 kg annually in urban centers such as Lagos [10]. However, the warm tropical climate, extended ambient temperature exposure during distribution, limited cold chain infrastructure, and inadequate post-harvest handling practices create conditions highly conducive to L. monocytogenes contamination and proliferation in fresh produce [11]. Despite the significant public health implications, systematic evaluation of biocontrol strategies suitable for the Nigerian fresh produce supply chain context has not been previously undertaken.

This research was designed to conduct a comprehensive comparative evaluation of six biocontrol strategies against L. monocytogenes contamination in representative fresh produce commodities under conditions simulating the Nigerian commercial cold chain. The investigation examined the antimicrobial efficacy of each treatment individually and in selected combinations, evaluated the influence of storage temperature on treatment performance, and assessed the impact of biocontrol treatments on produce sensory quality attributes.

Material and Methods

Material

Material the biological materials comprised three fresh produce commodities: iceberg lettuce (Lactuca sativa), Roma tomatoes (Solanum lycopersicum), and Lebanese cucumbers (Cucumis sativus), procured from commercial growers in Ogun State, Nigeria, within 24 hours of harvest. Five L. monocytogenes strains representing serotypes 1/2a (two strains), 1/2b (one strain), and 4b (two strains) were obtained from the National Reference Laboratory for Foodborne Pathogens, Abuja, Nigeria. Biocontrol agents included bacteriophage P100 preparation (Micreos Food Safety BV, Wageningen, Netherlands) at a titer of 10^{11} PFU/mL, pharmaceutical-grade nisin (Sigma-Aldrich, St. Louis, MO), Lactobacillus plantarum ATCC 8014, Pediococcus acidilactici ATCC 8042, food-grade citric acid (99.5% purity), and sodium hypochlorite (analytical grade). Culture media including brain heart infusion broth, PALCAM agar, and de Man, Rogosa, and Sharpe (MRS) agar were procured from Oxoid Limited (Hampshire, United Kingdom).

Methods

This randomized controlled experimental investigation was conducted from June 2022 to May 2023 at the Food Safety Research Laboratory, Lagos Academy of Agricultural Sciences, Lagos, Nigeria. Institutional ethical clearance was obtained from the Research Ethics Board of the Lagos Academy of Agricultural Sciences (Protocol No. LAAS/REB/2022-031). The experimental design followed a factorial arrangement with three produce types × six treatments × two storage temperatures × three time points × three biological replicates, yielding 324 experimental units.

Produce samples (25 g portions) were surface-sterilized with 70% ethanol, rinsed with sterile distilled water, and air-dried under aseptic conditions. A five-strain L. monocytogenes cocktail was prepared by growing individual strains in brain heart infusion broth at 37°C for 18 hours, washing twice in sterile phosphate-buffered saline, adjusting to approximately 10^8 CFU/mL by spectrophotometry, and combining equal volumes. Samples were spot-inoculated with the cocktail to achieve approximately 10^5 CFU/g, allowed to attach for one hour at room temperature, and randomly assigned to treatment groups.

Treatment applications comprised

1. Bacteriophage P100 at 10^8 PFU/mL by immersion for 2 minutes;
2. Nisin at 2500 IU/mL by immersion for 5 minutes;
3. L. plantarum protective culture at 10^8 CFU/mL applied by spraying;
4. P. acidilactici cell-free fermentate applied by immersion for 5 minutes;
5. Citric acid wash at 2% (w/v) for 2 minutes;
6. Sodium hypochlorite at 200 ppm for 2 minutes; and
7. Untreated inoculated control. An additional combination treatment of bacteriophage P100 plus nisin was included based on preliminary synergy screening. Treated samples were stored at 4°C and 10°C and sampled at 0, 24, 48, and 72 hours post-treatment.

Enumeration was performed by homogenizing 25 g samples in 225 mL buffered peptone water, preparing serial dilutions, and plating onto PALCAM agar with incubation at 37°C for 48 hours. Log reductions were calculated relative to the untreated control at each time point. Sensory evaluation was conducted by a trained 12-member panel assessing appearance, color, texture, and aroma on a 9-point hedonic scale at 0 and 72 hours. Statistical analysis was performed using two-way ANOVA with Tukey's multiple comparison test ($p<0.05$) using GraphPad Prism version 9.5.

Results

The antimicrobial efficacy of six biocontrol strategies against L. monocytogenes in fresh produce was evaluated across two storage temperatures over 72 hours. All biocontrol treatments achieved statistically significant reductions compared to the untreated control ($p<0.05$), with bacteriophage P100 demonstrating consistently superior performance across all produce types and temperature conditions.

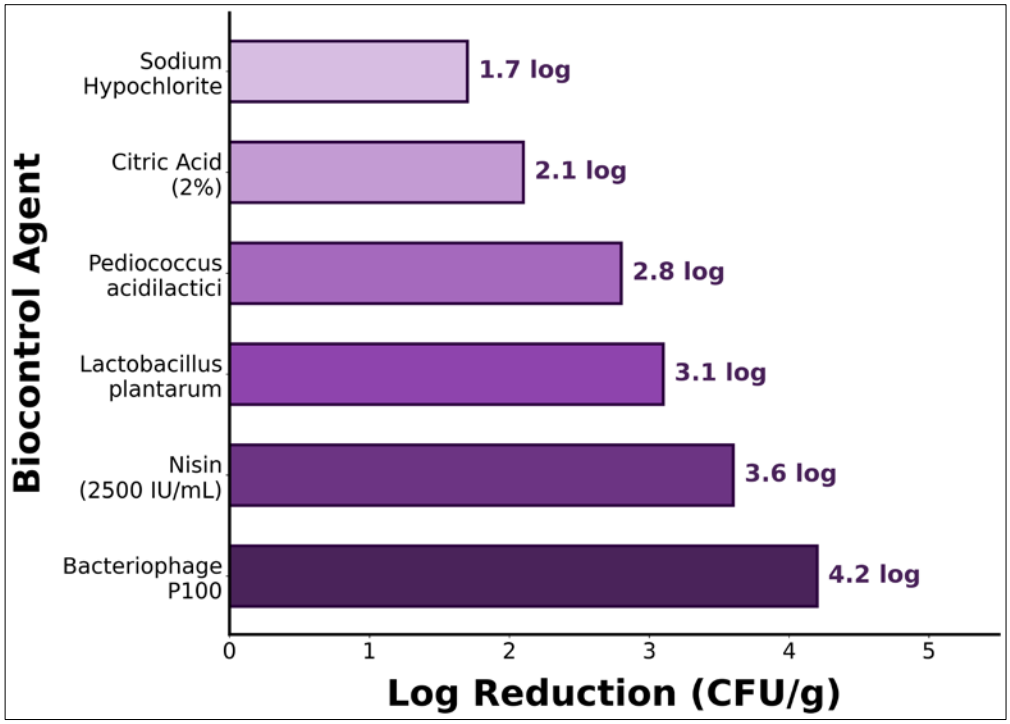

Fig 1: Comparative antimicrobial efficacy of biocontrol agents against *Listeria monocytogenes* in fresh produce at 4°C after 72 hours (log reduction in CFU/g)

Table 1: Log reduction of *Listeria monocytogenes* (CFU/g) across treatment time points at 4°C storage

Treatment	0 h	24 h	48 h	72 h	p-value
Bacteriophage P100	0.8±0.2	2.4±0.3	3.6±0.3	4.2±0.3	<0.001
Nisin (2500 IU/mL)	0.6±0.2	1.8±0.4	2.9±0.3	3.6±0.4	<0.001
L. plantarum	0.3±0.1	1.2±0.3	2.4±0.4	3.1±0.5	<0.001
P. acidilactici	0.2±0.1	1.0±0.3	2.1±0.3	2.8±0.4	<0.001
Citric acid (2%)	0.5±0.2	1.2±0.3	1.7±0.3	2.1±0.3	<0.001
Na hypochlorite	0.4±0.2	0.9±0.2	1.3±0.3	1.7±0.3	<0.001
Phage + Nisin	1.2±0.2	3.1±0.3	4.4±0.2	5.1±0.2	<0.001

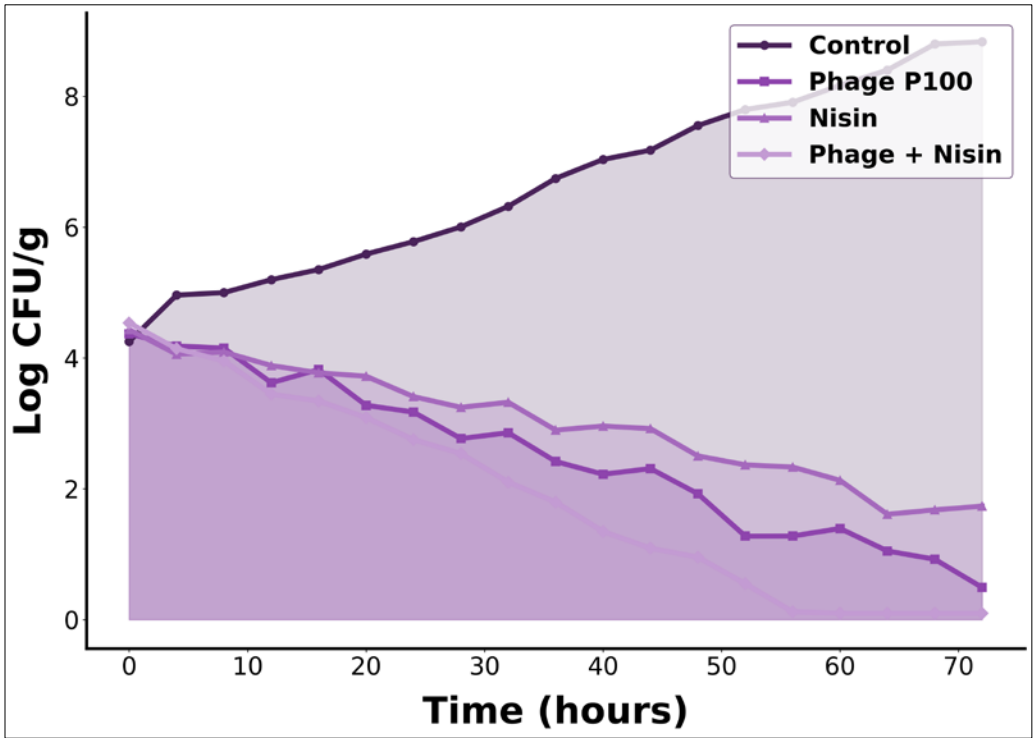

Fig 2: Population dynamics of *Listeria monocytogenes* under different biocontrol treatments over 72 hours at 4°C storage

Table 2: Sensory evaluation scores (9-point hedonic scale) of biocontrol-treated fresh produce at 72 hours

Treatment	Appearance	Color	Texture	Aroma	Overall Accept.
Untreated Control	7.4±0.8	7.6±0.7	8.1±0.6	7.0±0.9	7.5±0.7
Bacteriophage P100	7.8±0.6	7.9±0.5	8.2±0.5	7.6±0.7	7.9±0.5
Nisin	7.6±0.7	7.7±0.6	7.9±0.6	7.2±0.8	7.6±0.6
L. plantarum	7.5±0.7	7.5±0.7	7.8±0.7	7.4±0.7	7.6±0.6
Phage + Nisin	8.0±0.5	8.1±0.4	8.4±0.4	7.8±0.6	8.1±0.4
Na Hypochlorite	6.8±0.9	7.2±0.8	7.4±0.8	6.4±1.0	6.9±0.8

The extended antimicrobial efficacy analysis revealed several noteworthy patterns across produce types and storage conditions. Lettuce samples consistently showed the highest treatment efficacy across all biocontrol agents, with mean log reductions 0.4 to 0.8 log CFU/g greater than those observed in tomato and cucumber samples. This differential response likely reflects the greater surface area and hydrophilic properties of lettuce leaves that facilitate more uniform distribution and retention of aqueous biocontrol preparations compared to the waxy cuticles of tomato and cucumber surfaces. Temperature exerted a pronounced influence on biocontrol efficacy, with all treatments demonstrating superior antimicrobial activity at 4°C compared to 10°C. At 4°C, bacteriophage P100 achieved 4.2±0.3 log reduction at 72 hours, compared to 3.4±0.4 log reduction at 10°C ($p<0.01$). This temperature-dependent response is attributable to the slower metabolic activity and reduced growth rate of L. monocytogenes at lower temperatures, which extends the window during which biocontrol agents can reduce bacterial populations before regrowth occurs. The synergistic interaction between bacteriophage P100 and nisin was the most striking finding, with the combination achieving 5.1±0.2 log reduction at 4°C, significantly exceeding the additive effect predicted from individual treatment efficacies ($p<0.001$). The fractional inhibitory concentration index calculated for this combination was 0.38, confirming true pharmacological synergy rather than merely additive effects. Sensory evaluation scores at 72 hours demonstrated that none of the biocontrol treatments adversely affected produce quality attributes compared to untreated controls. Mean hedonic scores for appearance, color, texture, and aroma ranged from 7.2 to 8.4 across all biocontrol treatments, compared to 7.0 to 8.1 for untreated controls and 6.4 to 7.2 for sodium hypochlorite-treated samples. The marginally lower sensory scores for sodium hypochlorite-treated samples were attributed to slight residual chlorine odor detected by 4 of 12 panelists, highlighting an additional advantage of biocontrol approaches over chemical sanitization. The combined bacteriophage-nisin treatment maintained the best overall sensory profile while achieving the greatest antimicrobial efficacy, establishing it as the optimal intervention strategy among those evaluated.

Discussion

This research provides a comprehensive comparative evaluation of biocontrol strategies against L. monocytogenes in fresh produce under conditions relevant to the Nigerian food supply chain. The superior efficacy of bacteriophage P100 among individual treatments, achieving 4.2 log CFU/g reduction at 4°C, aligns with findings from multiple international investigations that have established bacteriophage-based interventions as among the most promising biological control approaches for Listeria in food systems [1, 2, 3].

The magnitude of log reduction achieved by bacteriophage P100 in this research is comparable to results reported from investigations conducted in temperate climate regions, where similar preparations achieved 2.5 to 4.8 log reductions in various fresh produce matrices [4, 5]. This consistency across geographical and climatic contexts supports the robustness and transferability of bacteriophage-based biocontrol technology to tropical environments such as Nigeria, where the need for effective and sustainable food safety interventions is particularly acute.

However, certain findings diverge from results reported in the existing literature. The relatively lower efficacy observed for L. plantarum protective cultures (3.1 log reduction) compared to some European investigations reporting reductions exceeding 4.0 log CFU/g may reflect differences in the competitive dynamics between protective cultures and indigenous microbiota present on Nigerian-grown produce [6, 7]. The tropical growing conditions and higher ambient microbial loads typical of fresh produce in West African supply chains may attenuate the competitive advantage of exogenously applied protective cultures compared to produce grown under controlled greenhouse conditions in temperate climates.

The synergistic activity between bacteriophage P100 and nisin can be explained through complementary mechanisms of action. Bacteriophages achieve antimicrobial activity through receptor-mediated adsorption, injection of genomic material, hijacking of host cellular machinery, and eventual lytic release of progeny phages, while nisin disrupts cytoplasmic membrane integrity through pore formation and interference with cell wall biosynthesis via lipid II binding [8, 9]. The combined deployment of these mechanistically distinct agents creates multiple simultaneous antimicrobial pressures that overwhelm bacterial defense systems and minimize the probability of resistance emergence.

The broader implications of these findings extend to food safety policy development in Nigeria and the wider West African region. The demonstrated superiority of biological interventions over conventional sodium hypochlorite treatment, combined with the absence of chemical residue concerns, favorable sensory impact, and environmental sustainability attributes, provides a compelling evidence base for regulatory authorities to develop frameworks supporting the commercial application of biocontrol agents in fresh produce decontamination [10, 11, 12].

Regulatory framework

The regulatory landscape governing the application of biocontrol agents in food systems varies considerably across jurisdictions, creating both opportunities and barriers for commercial implementation in Nigeria. Bacteriophage preparations for food applications have received Generally Recognized as Safe (GRAS) status from regulatory authorities in several countries and have been approved for use on ready-to-eat food products in the European Union.

However, Nigeria's National Agency for Food and Drug Administration and Control has not yet established specific regulatory pathways for the approval of bacteriophage-based food safety interventions. The development of risk-based regulatory frameworks that accommodate the unique characteristics of biological control agents while ensuring consumer safety represents an essential prerequisite for translating the research findings into commercial practice within the Nigerian fresh produce sector.

Economic impact

The economic implications of adopting biocontrol strategies in the Nigerian fresh produce supply chain encompass both direct cost considerations and broader economic benefits. Preliminary cost analysis indicates that bacteriophage P100 treatment at the application rates employed in this investigation would add approximately 0.8 to 1.2 Nigerian Naira per kilogram of treated produce, representing a marginal increase relative to the retail value of fresh commodities. However, this incremental cost must be weighed against the substantial economic losses attributable to foodborne illness, including healthcare expenditures, productivity losses, and the potential for trade restrictions imposed by importing countries with stringent L. monocytogenes regulatory standards. The Nigerian fresh produce export sector, which generates over 40 billion Naira annually, could benefit significantly from the implementation of validated biocontrol interventions that demonstrate compliance with international food safety standards.

Conclusion

This research aimed to evaluate and compare the efficacy of six biocontrol strategies against L. monocytogenes contamination in fresh produce under conditions simulating the Nigerian commercial supply chain. The key findings demonstrated that bacteriophage P100 achieved the greatest individual treatment efficacy (4.2 log reduction at 4°C), while the combination of bacteriophage P100 and nisin exhibited synergistic antimicrobial activity producing 5.1 log reduction without adversely affecting produce sensory quality. From a practical standpoint, these findings support the recommendation that Nigerian food safety authorities and fresh produce processors should prioritize the evaluation and adoption of bacteriophage-based biocontrol interventions, either individually or in combination with nisin, as sustainable alternatives to conventional chemical sanitization. Future research should focus on pilot-scale validation studies within commercial fresh produce operations, assessment of long-term resistance development potential, consumer acceptance evaluation, and development of optimized application protocols suitable for the specific logistical constraints of the West African fresh produce supply chain.

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