UKR Journal of Agriculture and Veterinary Sciences (UKRJAVS)
Homepage: https://ukrpublisher.com/ukrjavs/
Email: submit.ukrpublisher@gmail.com
Volume 1, Issue 1, May-June 2025 ISSN: XXXX-XXXX (Online)
OPEN ACCESS

Application Of Lactic Acid Bacteria-Derived Supernatants For Enhancing The Shelf Life And Preservation Of Tomatoes Postharvest

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ORGINAL ARTICLE

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Abstract

Lately, tomato (*Lycopersicon esculentum Mill.)* production has seen a global rise, driven by the crop's economic value and nutritional benefits. This widely consumed vegetable-fruit is a key ingredient in cooking and juice production due to its rich content of vitamins and minerals. These advantages have spurred various research efforts aimed at enhancing different stages of its production. However, postharvest preservation remains a major challenge, particularly concerning health safety and product management. In many parts of Africa, large harvests often fail to generate significant profits as a result of postharvest losses. This study aimed to evaluate the potential of certain Lactic Acid Bacteria (LAB) supernatants in preserving fresh-cut tomatoes postharvest. A total of 250 LAB strains were isolated from 25 samples of locally fermented dairy products collected in Kuje, Abuja, Nigeria. Standard microbiological methods were employed to screen the isolates, with *Lactobacillus plantarum* (LB69) and *Lactobacillus fermentum* (LBB6) exhibiting strong in vitro antagonistic activity against *Botrytis cinerea* and *Aspergillus niger*, two fungi previously identified on decaying tomatoes. The LAB supernatants were subsequently applied in vivo to healthy tomato fruits, using sterile distilled water as a control. Results demonstrated a significant delay in fungal growth in treated samples compared to the control group. Specifically, *L. plantarum* (LB69) provided 53% and 42% protection against *B. cinerea* and *A. niger*, respectively, while *L. fermentum* (LBB6) achieved 41% and 33% protection. These findings suggest that LAB-based treatments hold promise for safer, more effective fungal disease control in tomato postharvest management.

Keywords: Biocontrol, Postharvest Losses, Tomato Preservation, Lactic Acid Bacteria

Introduction

Tomato (*Solanum lycopersicum*), a member of the Solanaceae family (Seisuke & Neelima, 2008), is one of the most important horticultural crops worldwide, valued for its nutritional and economic contributions. Its widespread use spans from juice and soup preparation to inclusion in salads and sauces, with preferences varying by region and cultural practices (Schwarz et al., 2014). Numerous cultivars exist, differing in color—from yellow and green to deep red—and in flavor profiles, which are influenced by varying levels of water, sugars, fats, organic acids, vitamins, and minerals. Nutritionally, ripe tomatoes are composed of about 95% water, 4.3% carbohydrates, 1.1% proteins, 0.2% fats, 0.5% fiber, and a significant amount of vitamins (Raziard, 2020).

The high moisture and nutrient content make tomatoes especially susceptible to microbial invasion. These microorganisms, including bacteria and fungi, secrete enzymes that degrade the protective structures of the fruit, leading to softening, spoilage, and a decrease in both shelf life and market value (Forson et al., 2018). Additionally, some fungi produce harmful mycotoxins that pose serious health risks to humans and animals. Common pathogens identified in tomatoes include

Clostridium, *Staphylococcus*, Bacillus spp., and various fungal species (Mariga, 2012).

Postharvest storage of tomatoes is particularly challenging due to their delicate structure and susceptibility to bruising during conventional harvesting practices. These physical damages provide entry points for pathogens (Lemma et al., 2014). Tomatoes typically have a short postharvest life, ranging from 12 to 72 hours, limiting their commercial potential. While developed nations have implemented technological solutions to reduce postharvest losses, developing countries continue to experience significant waste due to inadequate storage, handling, and transportation practices (Akinyele & Akinkunmi, 2012). Much of the spoilage is driven by microbial contamination during various stages—harvesting, handling, processing, and distribution—often worsened by a lack of hygienic practices among handlers.

During sales in open markets, tomatoes are often displayed in baskets and tables (Mariga, 2012). Given the significance of tomatoes, various preservation methods have been proposed and implemented at different stages of value addition. The primary goal is to extend the shelf life of the fruit and reduce the economic losses caused by its frequent spoilage. In developed countries, cold storage facilities are widely used to preserve fresh tomatoes. However, these methods are often complex, requiring significant electrical power, which makes them inaccessible to many consumers in Africa (Kitinoja & AlHassan, 2013).

Furthermore, consumers are increasingly aware of the health risks associated with preservatives like sodium hypochlorite, sodium metabisulfite, and calcium chloride, commonly used in tomato preservation. As a result, the demand for these nutrient-rich fruits may continue to decline unless alternative preservation methods are explored (Kaminski & Christiaensen, 2014).

While plant extracts and other biological methods offer potential as cost-effective and multi-purpose alternatives, they have not received the attention necessary for widespread adoption. Although physical harvesting techniques significantly affect the postharvest quality and shelf life of tomatoes, the commercial use of bioactive materials for biological control remains limited (Dun-Chun et al., 2021). These bioactive materials are not only more affordable and readily available but are also safer for food use compared to chemical preservatives. Studies have indicated that the adoption of biological methods in postharvest tomato control can help prevent losses due to microbial spoilage.

This study aims to investigate the potential of using cell-free supernatants from certain Lactic Acid Bacteria for in vivo biological control of tomato postharvest spoilage.

Materials and Methods

Study Methodology

Collection and Handling of Samples

A total of 25 fermented milk product samples were collected early in the morning from dairy farms and households actively engaged in milk fermentation. The samples were placed into sterile 500 ml glass bottles, appropriately labeled, and transported in an ice-packed cooler to the microbiology laboratory for bacteriological analysis (Bello et al., 2013).

Isolation and Identification of Lactic Acid Bacteria (LAB)

The isolation procedure followed the method described by Etebu et al. (2013). Briefly, serial dilutions were performed using sterilized peptone water up to a dilution factor of 10^{-6}. From each dilution, 0.1 ml aliquots were plated onto de Man, Rogosa, and Sharpe (MRS) agar and M17 agar to selectively isolate LAB. The inoculated plates were incubated under anaerobic conditions at both 37°C and 45°C for 48 to 72 hours (Adebooye et al., 2006).

Following incubation, discrete colonies were sub-cultured to obtain pure isolates. These isolates were

subjected to morphological and biochemical characterizations, including colony appearance, Gram staining, catalase activity, glucose fermentation, and growth at various temperatures. Only Gram-positive, catalase-negative isolates were retained and further purified by repeated streaking.

Subsequent tests assessed the isolates' ability to grow at different temperatures and their fermentative properties—both homofermentative and heterofermentative—using various carbohydrate sources. These tests were performed in MRS broth supplemented with inverted Durham tubes to monitor gas production. Isolates meeting the criteria consistent with LAB, as outlined in Bergey's Manual of Determinative Bacteriology, were selected and preserved in 20% sterile glycerol for future use (Chuku et al., 2010).

Isolation and Identification of Spoilage Fungal Pathogens

Fungal pathogens associated with the decay of tomato fruits were isolated from visibly infected samples. One gram of the decayed tomato tissue was aseptically diluted in 9 ml of sterile peptone water and subsequently pour-plated onto Potato Dextrose Agar (PDA) amended with chloramphenicol to inhibit bacterial growth. The plates were incubated at 28°C for 3 to 5 days to allow for fungal growth and purification (Garg et al., 2013).

Isolates were sub-cultured on fresh PDA to obtain pure cultures. Based on macroscopic and microscopic features, preliminary identification was performed. *Aspergillus niger* was characterized by initially white colonies turning black over time, with dense outward radial growth and dark, septate mycelia bearing round spores. In contrast, colonies suspected to be *Botrytis cinerea* displayed variable mycelial colors—ranging from white to cream, grey, or light grey—with smooth to wavy margins. The reverse side of the cultures was mostly cream or white, occasionally grey. Microscopic examination revealed globose to ellipsoid, hyaline or pale brown conidia that were typically single-celled and non-septate.

Isolates resembling *B. cinerea* in morphology were selected for further analysis and cultured on 2% Malt Extract Agar (MEA) plates for six days at 20°C in dark conditions to confirm identification.

Pathogenicity Tests

To confirm pathogenicity, healthy, uninfected tomato fruits were inoculated with isolates of *A. niger*. Lesions began to appear on the fruits approximately five days after inoculation, presenting typical symptoms similar to those observed in naturally infected samples. The fungus was subsequently re-isolated from the decayed tissues, confirming its identity as *A. niger* based on morphological traits (Baiyewu et al., 2007).

For *B. cinerea*, ripe, unblemished tomatoes were surface-sterilized using 70% ethanol for two minutes and rinsed with sterile water. These were then inoculated with a conidial suspension (1.2×10^6 spores/ml) prepared in sterile distilled water. The inoculated fruits were placed in a humid environment and monitored for seven days. Control fruits were inoculated with sterile water under the same conditions.

Disease progression was assessed every 24 hours, and severity was rated on a scale of 1 to 4:

1: No visible infection
2: Infection covering up to 25% of the surface
3: Mycelial growth affecting 26–75%
4: Extensive infection, with more than 75% of the fruit covered by mycelia.

Experimental Setup for the Application of LAB Supernatant

Activation and Validation of LAB

Before conducting antagonistic tests, a loopful of each LAB isolate was streaked on MRS agar and incubated at 42°C for 24 hours. Afterward, individual colonies were observed, Gram stained, and checked to ensure the culture was pure.

Preparation of Cell-Free Supernatant

Each LAB culture was transferred into 10 ml of sterile MRS broth and incubated for 24 hours until cloudiness, indicating growth, was observed. The cultures were then centrifuged at 4000 RPM for 10 minutes to separate the supernatant from the pellet. The supernatant was decanted, and the pellet was washed three times with sterile peptone water. The washed pellet was reintroduced into 250 ml of fresh MRS broth and incubated for 48 hours with agitation at 125 RPM. After the incubation period, the resulting broth was centrifuged again, and the supernatant was used as the bio-active agent for fungal pathogen inhibition (Ajayi, 2013).

Application of the Cell-Free Supernatants

In Vitro Method

The agar well diffusion method was employed for testing the antifungal activity. Potato Dextrose Agar (PDA) modified with chloramphenicol was inoculated with the fungal suspension. Six millimeter wells were made in the agar using a sterile cork borer, and 0.5 ml of the LAB supernatant was added to each well. The plates were incubated for 7 days, after which the zones of inhibition around the wells were measured to determine the effectiveness against each pathogen (Ogundipe et al., 2012).

In Vivo Method

For the in vivo test, 40 fresh tomatoes were selected, washed with distilled water, and dipped in a hypochlorite solution for 2 minutes. After rinsing and air-drying, the tomatoes were inoculated with the spore suspension of the respective fungal pathogen and allowed to air-dry again. The tomatoes were then divided into two groups: one group (20 tomatoes) was treated with the LAB supernatant, while the other group (20 tomatoes) was treated with sterile distilled water as a control (Montet et al., 2014). All tomatoes were kept in a humid environment and observed over time for fungal infestation and decay rates. The control group was inoculated with sterile water.

Shelf-life Study of Tomato Fruits

Weight Loss

Weight loss in the tomato fruits was determined by measuring the weight before inoculation and again after storage. The percentage of weight loss was calculated using the formula:

Where A is the weight before inoculation and B is the weight after inoculation and storage (Tigist et al., 2013).

Firmness

The firmness of the stored tomatoes was visually compared with the control group and rated accordingly.

Microbial Analysis

Microbial counts were performed by taking 1g of both infected and control tomato samples, which were then diluted in 9 ml of sterile peptone water. Serial dilutions were plated onto different media: Total Plate Count Agar for bacterial counts, Chloramphenicol-modified Potato Dextrose Agar for fungal counts, and MRS agar for lactic acid bacteria counts. Plates were incubated at appropriate conditions, and the respective microbial counts were recorded (Workneh et al., 2012).

Results and Discussion

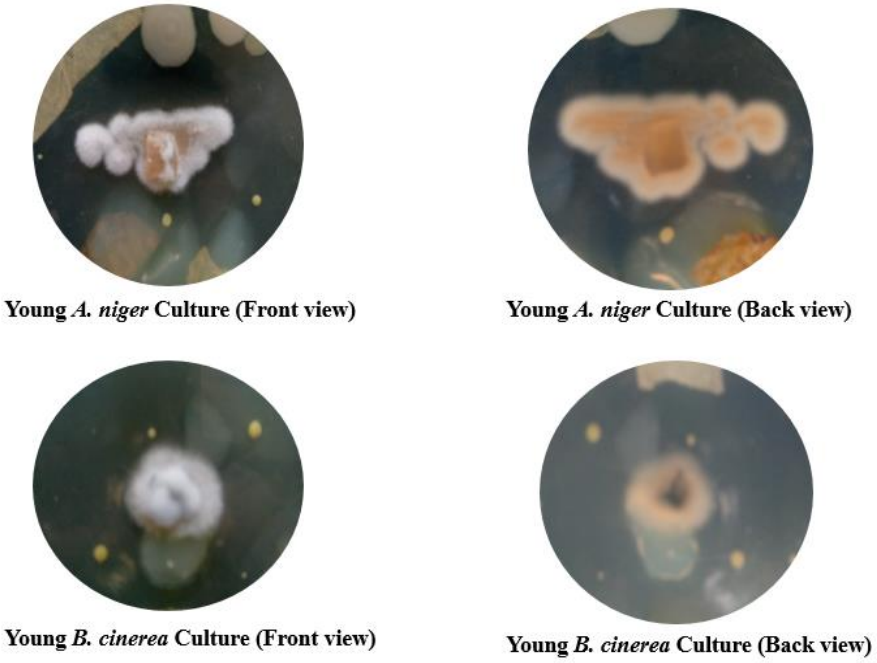
Isolation of LAB Strains

The LAB isolates were identified based on morphological characteristics and biochemical tests. All LAB isolates were small to medium in size, with whitish colonies and circular margins on MRS agar (Dessy et al., 2021). The isolates were Gram-positive and rod-shaped. A total of 250 bacterial isolates were screened, and two strains, *Lactobacillus plantarum* (LB69) and *Lactobacillus fermentum* (LBB6), were selected for further application. LAB from various fermented products have been widely studied for their antifungal potential, with some promising results, though additional research is needed. This study utilized MRS agar, which is a commonly used medium for isolating LAB strains. This approach is in line with the work of Purwati et al. (2019) on the antimicrobial

potential of *Pediococcus acidilactici* from the fermentation of sepat rawa fish.

Isolation and Identification of Pathogens

The fungal pathogens were isolated from spoiled tomatoes using standard mycological methods (Iranmanesh et al., 2014). The two primary pathogens identified in this study were *Aspergillus niger* and *Botrytis cinerea* while, pathogenicity potentials were confirmed respectively using Koch's Postulate method (Chitrabhanu et al., 2021).



Young *A. niger* Culture (Front view)

Young *A. niger* Culture (Back view)

Young *B. cinerea* Culture (Front view)

Young *B. cinerea* Culture (Back view)

Figure 1: Young Cultures of Isolated Pathogens on PDA

Cultural Characteristics of Selected Fungal Isolates

Aspergillus niger grown on PDA exhibited colony diameters ranging between 66–70 mm. Colonies initially appeared white but gradually turned black as spore production increased (Figure 1), consistent with findings by Ikhiwili (2012). The reverse side of the colonies ranged from colorless to pale yellow. Conversely, *Botrytis cinerea* colonies began white and transitioned to grey with age. The reverse side typically displayed a whitish-grey hue (Figure 1).

Effects of LAB Supernatant on Fungal Pathogens

In Vitro Antifungal Activity

The cell-free supernatants of LAB strains demonstrated antifungal effects against both pathogens based on the clear inhibition zones observed (Table 1).

Table 1: The *In vitro* Effects of LAB Supernatant on the Indicator Pathogens

LAB	Inhibition (mm)	
	Aspergillus niger	***Botrytis cinerea***
Lactobacillus plantarum	8.4	7.2
Lactobacillus fermentum	5.9	4.8
Distilled Water (control)	0.0	0.0

L. plantarum showed the highest inhibitory effect, producing inhibition zones of 8.4 mm and 7.2 mm against *A. niger* and *B. cinerea*, respectively. *L. fermentum* also displayed antifungal activity, though slightly less pronounced, with 5.9 mm and 4.8 mm inhibition zones. The strain showing the highest activity was selected for subsequent in vivo testing.

In Vivo Preservation Effect

Tomato fruits treated with the LAB supernatants showed notable protection compared to the untreated controls (Table 2).

Table 2: The *In vivo* Effects of LAB Supernatant on the Indicator Pathogens

Supernatant	Shelf-life (% Control/Protection)	
	Aspergillus niger	***Botrytis cinerea***
Lactobacillus plantarum	53	42
Lactobacillus fermentum	41	33
Distilled Water (control)	0.0	0.0

Weight loss and firmness assessments (not shown in detail) supported these findings. *L. plantarum* conferred the highest protection—53% and 42% against *A. niger* and *B. cinerea*, respectively—followed by *L. fermentum* with 41% and 33% protection.

Table 3: Microbial Analysis of Preserved Tomato Samples

Supernatant	TPC	FC	LAB Count
Lactobacillus plantarum	$3.3x10^2$	$2.2x10^1$	$4.2x10^1$
Lactobacillus fermentum	$4.1x10^3$	$3.5x10^2$	$1.8x10^1$
Distilled Water (control)	$1.6x10^7$	$4.2x10^4$	$2.1x10^3$

Table 3 presents the microbial analysis of tomato fruits after 10 days of preservation. The data indicates that the application of *Lactobacillus plantarum* and *Lactobacillus fermentum* significantly reduced the microbial load compared to the control (distilled water). The total plate counts (TPC) and fungal counts (FC) for tomatoes treated with *L. plantarum* and *L. fermentum* were considerably lower than the control, suggesting effective microbial management.

Discussion and Conclusion

The LAB strains tested demonstrated moderate to high antifungal potential using the agar well diffusion technique. Specifically, 62% of the strains inhibited *A. niger* and 57% inhibited *B. cinerea*. These results contrast with findings by Nicol et al. (2021), who reported that fewer than 2% of 900 LAB strains exhibited strong antifungal activity against spoilage molds like *Aspergillus*, *Penicillium*, and *Cladosporium*. This discrepancy highlights the strain-specific nature of LAB antifungal activity, as previously suggested by Salari and Almani (2020).

Since only the supernatants were applied (rather than whole LAB cells), the observed antifungal effects likely resulted from secondary metabolites rather than competitive colonization. This form of indirect antagonism aligns with the observations of Rana et al. (2016), who reported similar findings in their study on bacterial antagonism against *Phaeomoniella chlamydospora* in grapevines.

Our results further align with Maluleke et al. (2022), who found that non-conventional yeasts also exhibited antifungal properties in the absence of direct microbial competition. These findings support the growing view in food preservation science that bioactive microbial metabolites, even without viable cells, can be used to control spoilage organisms and extend the shelf-life of perishable fruits (Said et al., 2021).

Given increasing concerns over chemical preservatives and the need to ensure food safety and security for growing populations, LAB-derived supernatants offer a promising natural alternative. Moreover, since the use of live bacteria can sometimes lead to unintended fermentations and off-flavors, the success observed with supernatants alone encourages further exploration into their application in modern biocontrol strategies for food preservation.

The growth of filamentous fungi such as *Aspergillus niger* and *Botrytis cinerea* is a common cause of tomato spoilage, leading to rapid deterioration of quality and potential safety concerns due to mycotoxin production. Several studies have highlighted the role of fungi in food spoilage and safety issues, urging the search for more sustainable preservation methods. In this context, lactic acid bacteria (LAB) have shown significant promise due to their ability to inhibit fungal growth.

LAB, including *Lactobacillus plantarum* and *Lactobacillus fermentum*, are known for their antifungal properties, which can be attributed to the production of organic acids, bacteriocins, and other bacteriostatic metabolites. The cell-free supernatants (CFS) of these LAB strains were applied to artificially contaminated tomatoes and showed considerable efficacy in suppressing fungal growth and extending the shelf life of the fruits. This approach presents an innovative and environmentally friendly alternative to traditional chemical preservatives, aligning with the increasing demand for safer and more natural preservation methods in the food industry.

However, further studies are necessary to optimize the production of bioactive metabolites from LAB to ensure safety, enhance efficacy, and maintain the overall quality of the preserved products. Advancing research into these areas could lead to more efficient biocontrol strategies and the development of novel preservation technologies for the food industry.

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