

Preservative Potential of *Lactobacillus* Species for the Control of Post-Rot of Sweet Potatoes (*Ipomea Batatas*)

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ABSTRACT

This report presents a comprehensive investigation into the preservative potential of Lactobacillus species as a sustainable biological control agent against post-harvest rot in sweet potatoes (Ipomea batatas). The pervasive issue of sweet potato spoilage significantly contributes to global food insecurity and economic losses. Initial analyses revealed substantial nutritional degradation in rotten sweet potatoes, characterized by significant reductions in moisture, protein, ash, and crude fiber content compared to healthy tubers. Microbiological assessments confirmed high fungal loads in spoiled samples, with key pathogens identified as Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Fusarium oxysporum, and Penicillium sp. Pathogenicity tests empirically validated these fungi as primary causative agents of sweet potato rot. A particular concern arises from the high prevalence of Aspergillus flavus, known for its potential to produce mycotoxins, which poses a significant food safety risk. This research establishes a robust scientific foundation for Lactobacillus-based bio preservation as a promising, environmentally friendly alternative to conventional chemical treatments. Its successful implementation could significantly contribute to reduced food waste, enhanced food security, and improved public health by mitigating both spoilage and mycotoxin contamination.

I. INTRODUCTION

Background of the Study

Sweet potato (*Ipomea batatas*) holds immense global significance as a staple food crop, ranking as the fifth most important food crop in developing countries and seventh worldwide in terms of overall production. Beyond its caloric contribution, it serves as a crucial nutritional security crop, particularly in regions like sub-Saharan Africa, due to its drought tolerance and rich content of β -carotene (pro-vitamin A), which is vital for combating prevalent vitamin A deficiency (Anene et al., 2021). The crop also offers traditional medicinal value and versatility for industrial applications, including beverages and various food products.

Despite its importance, sweet potato, like other vegetables, is highly perishable, undergoing rapid deterioration post-harvest. This inherent perishability leads to staggering global losses, with approximately 45% of fruits and vegetables wasted annually, translating to economic losses ranging from \$10 billion to \$100 billion worldwide. The situation is particularly dire in developing countries, where inadequate handling facilities

exacerbate losses, often reaching up to 50%. Sweet potatoes, with their high-water content (70%), are especially vulnerable to wilting, discoloration, and decay, contributing to over 30% economic losses (Agrios, 2015). These substantial losses not only diminish food availability but also undermine the economic stability of farmers and value chains, underscoring the critical need for effective post-harvest preservation strategies. Post-harvest spoilage is predominantly caused by microbial contamination, primarily from fungi and bacteria. Key fungal pathogens include *Rhizopus stolonifer*, the main culprit behind soft rot, which can completely rot a sweet potato within days, typically acting as a wound parasite. Another significant fungal threat is *Ceratocystis fimbriata*, responsible for black rot, characterized by firm, dry, circular brown/black lesions. This fungus is highly persistent, spreading via various vectors including wind, water, soil, harvesting baskets, farm machinery, insects, and human clothing, and can survive for several years in environmental debris. Bacterial contaminants, such as *Pseudomonas*, *Clostridium*, and *Bacillus* species, also contribute to spoilage, entering through wounds and proliferating under favorable conditions (Chang and Coppola, 2009). Mechanical injuries sustained during harvesting, handling, packaging, or transportation are critical entry points for these pathogens, accelerating decay and serving as primary sites for infection. The diverse nature and pervasive spread mechanisms of these pathogens highlight the complexity of the challenge in controlling post-harvest rot.

Historically, the control of post-harvest diseases has relied heavily on conventional methods, including refrigeration, modified atmosphere packaging, and, most notably, synthetic fungicides. However, this approach faces severe limitations. Pathogens like *Rhizopus stolonifer* have developed increased resistance to conventional synthetic fungicides due to their widespread and long-term agricultural use. Furthermore, a substantial portion (70-99%) of applied fungicides fails to reach its target, leading to detrimental impacts on soil health, surface water quality, and terrestrial organisms. For black rot caused by *Ceratocystis fimbriata*, fungicides are only effective preventatively on seeds and slips, failing to control established infections once the pathogen has taken hold. These mounting challenges necessitate a critical shift towards sustainable, environmentally friendly, and safe alternatives that can effectively mitigate post-harvest losses without compromising ecological integrity or human health.

In response to these limitations, bio-preservation, which utilizes natural or beneficial organisms and their metabolites, has garnered significant interest as a sustainable and consumer-friendly approach. Lactic Acid Bacteria (LAB) are prominent in this field, recognized for their GRAS (Generally Recognized as Safe) status and their long history of safe use in food fermentation. LAB exert strong antagonistic effects on food-contaminating microorganisms by producing a range of antimicrobial metabolites, including organic acids (e.g., lactic acid, acetic acid), hydrogen peroxide, and bacteriocins. Beyond direct inhibition, they also compete with harmful bacteria for nutrients or adhesion sites and can disrupt biofilms, thereby enhancing shelf-life and food safety. The potential of LAB extends beyond mere preservation, as their biotransformation capabilities can also improve the nutritional and functional qualities of plant-based foods like sweet potatoes, such as enhancing antioxidant, anti-hyperglycemic, and anti-hypertensive properties (Griffiths et al., 2015). This dual benefit positions LAB as a highly promising avenue for addressing the complex challenges of sweet potato post-harvest rot, offering both spoilage control and value addition.

II. MATERIALS AND METHODS

A. *Study Area*

The study was conducted using samples collected from major markets within Anambra State, Nigeria. All laboratory work, including sample processing, microbial isolation, characterization, and in vitro assays, was meticulously carried out at the Biological Sciences laboratory located within the Alpha Research Laboratory at Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Anambra State. This defined geographical context is important for understanding the potential environmental factors influencing the microbial populations found on the sweet potato samples.

B. *Sample Collection and Preparation*

A total of 25 rotten and 25 healthy sweet potato tubers were procured from various major markets across Anambra State, specifically from Uli, Ihiala, Nkwo Nnewi, Eke Awka, and Nkwo Umunze. The selection of diseased sweet potato tubers adhered to the method described by Adetunji et al. (2014), focusing on tubers exhibiting clear symptoms of rot. These symptoms included black patches, browning, discoloration, softening, and the presence of a foul or offensive odor. In addition to sweet potato tubers, ten samples of locally made fruit juice were collected from Onitsha main market. All collected samples were immediately transported to the Biological Sciences laboratory at Alpha Research Laboratory for further analysis, ensuring minimal deterioration during transit.

C. *Proximate Analysis*

Proximate analysis was performed on both fresh and rotten sweet potato samples to determine their nutritional composition, following the gravimetric and chemical methods described by the AOAC (2015).

D. *Determination of Moisture Content:*

The moisture content was determined using the gravimetric method. A precisely weighed sample (5.0 g) was placed into a pre-weighed moisture dish. The dish containing the sample was then dried in an oven at 105°C for 3 hours. After drying, the sample was cooled in a desiccator and re-weighed. This drying, cooling, and weighing process was repeated at hourly intervals until a constant weight was achieved, indicating complete moisture removal. The percentage of moisture lost was calculated using the formula: Moisture content (%) = $(M2 - M3) / (M2 - M1) \times 100$, where M1 = Mass of empty moisture dish; M2 = Mass of empty dish + Sample before drying; M3 = Mass of dish + Sample dried to constant weight.

E. *Determination of Crude Protein:*

Crude protein content was determined using the Kjeldahl method. One gram of the sample was prepared in a micro Kjeldahl flask. To this, 25 mL of sulphuric acid (H₂SO₄), 1 gram of cupric acid (CuSO₄), and 10 grams of sodium sulphate (Na₂SO₄) were added. The flask was then heated gently at an inclined angle (60°C), initially at 70°C, with the temperature gradually increasing until the liquid turned bluish-green and was free from any brown or black coloration. An anti-bumping agent was included to prevent frothing. After cooling,

the content was diluted with 200 mL of distilled water and 60 mL of 40-50% NaOH. The flask was connected to a distillation apparatus, allowing ammonia gas to be trapped in a 250 mL conical flask containing 4% boric acid and 2 drops of screened methyl red indicator. The mixture was boiled at 80-90°C until the distillate volume reached 200 mL. The solution in the conical flask was then titrated against 0.1N H₂SO₄ from a burette until a light pink color was obtained. The percentage nitrogen and protein were calculated using the formulas: % Nitrogen = $(T_v \times 0.0014 \text{ g} \times 100) / \text{Weight of the sample}$; % Protein = % Nitrogen $\times$ protein factor.

F. Determination of Total Ash Content:

Total ash content was determined by the furnace incineration gravimetric method. Exactly 10 g of the sample was weighed into a previously weighed porcelain crucible. The sample was then incinerated in a muffle furnace at 550°C for three hours until it was completely ashed and turned grey. The crucible was then cooled in a desiccator and weighed. The weight of ash obtained was determined by difference and calculated as a percentage of the original sample weight: Ash (%) = $(M_2 - M_1) / \text{Mass of sample} \times 100$, where M₁ = Mass (g) of empty crucible; M₂ = Mass of crucible + Ash.

G. Determination of Crude Fibre Content:

Crude fiber content was determined by weighing 2 grams of the defatted sample into a conical flask. To this, 200 mL of 1.25% or 0.127N H₂SO₄ was added, and the mixture was boiled on a heating mantle at 80°C for 30 minutes. The hot solution was then filtered through muslin cloth, and the residue was thoroughly washed with boiled water. The residue was then transferred back into the conical flask using 200 mL of 1.25% or 0.313M NaOH and boiled again at 80°C for 30 minutes. The mixture was filtered into a pre-weighed filter paper (M₁). The filter paper and residue were dried in an oven at 80°C until constant weight (M₂) was achieved, then cooled in a desiccator and weighed. The paper containing the residue was then transferred to a pre-weighed, washed, dried, and cooled crucible (M₄). The crucible was placed in a muffle furnace and allowed to burn for 5 hours at 600°C. After ashing, it was cooled and weighed as M₅. The percentage fiber was calculated using the formula: % Fibre = $(M_7 \times 100) / M$, where M = mass of sample; M₃ = M₂ - M₁; M₆ = M₅ - M₄; M₇ = M₃ - M₆.

H. Determination of Crude Fat Content:

Crude fat content was determined using the Soxhlet extraction method. A 5-gram sample was wrapped in porous Whatman filter paper and placed into a thimble. The thimble was then inserted into a Soxhlet reflux flask, which was mounted onto a weighted extraction flask containing 250 mL of petroleum ether. The apparatus was assembled with a water condenser connected to the upper part of the reflux flask. The solvent (petroleum ether) was heated, vaporized, and condensed into the reflux flask. Once the thimble containing the sample was covered with the solvent, the solvent siphoned over, carrying the extracted oil down to the boiling flask. This extraction process was allowed to continue repeatedly for 4 hours. After extraction, the defatted sample was removed, the solvent was recovered, and the oil extract remained in the flask. The flask containing the oil extract was dried in an oven at 60°C for 30 minutes to remove any residual solvent, then cooled in a desiccator and weighed. The weight of the oil (fat) extract was determined by difference and

calculated as a percentage of the original sample weight: $\text{Fat (\%)} = (\text{M2} - \text{M1}) / \text{Mass of sample} \times 100$, where M1 = Mass (g) of empty extraction flask; M2 = Mass of flask + oil (fat) extract.

J. Determination of Carbohydrate Content:

The carbohydrate content was determined by difference. This involved deducting the mean values of the other determined proximate parameters (moisture content, crude protein, fat, crude fiber, and ash) from 100. Calculation: $\% \text{ Carbohydrate} = 100 - (\% \text{ Mc} + \% \text{ Cp} + \% \text{ Fat} + \% \text{ Crude fibre} + \% \text{ Ash})$, where Mc = moisture content; Cp = crude protein.

III. STERILIZATION OF MATERIALS

All materials utilized in the study, including glassware such as Petri dishes, conical flasks, round bottom flasks, measuring cylinders, and pipettes, as well as paper towels and inoculating loops, were meticulously prepared for sterilization. These items were individually wrapped with paper foil and subjected to sterilization according to the methods described by Singleton (2017). Sterilization was achieved in a high-pressure steam sterilizer (autoclave) at 121°C for 15 minutes. This rigorous sterilization protocol was essential to ensure aseptic conditions throughout the experiments, thereby preventing contamination and ensuring the reliability and validity of the obtained results.

A. Preparation of Culture Media

For the growth and maintenance of fungal isolates, Potato Dextrose Agar (PDA) was used as the primary culture medium. The PDA was prepared strictly in accordance with the manufacturer's instructions. Thirty-nine grams (39g) of dehydrated PDA powder were weighed into a 1-liter glass conical flask and dispersed in approximately 800ml of distilled water. The mixture was then heated in an electric water bath until the agar completely melted. Subsequently, the volume was adjusted to 1 liter with distilled water, and the pH was measured using a digital pH meter to ensure compliance with the standard range of 5.4 to 5.8. The prepared PDA medium was then transferred to a Pyrex media bottle and sterilized in an autoclave at 121°C, under a pressure of 15 Psi, for 15 minutes. After sterilization, the medium was allowed to cool to approximately 45°C, and 1ml of a 10% concentration of lactic acid solution was aseptically added to suppress bacterial growth and contamination. The medium was mixed thoroughly before being aseptically dispensed into 15ml aliquots into sterile glass Petri dishes. These plates were allowed to cool and gel, then aseptically wrapped externally with masking tape and stored in the refrigerator until needed for use. For the isolation of lactic acid bacteria, de Man, Rogosa and Sharpe (MRS) agar was employed. The preparation of MRS agar followed standard protocols to ensure optimal conditions for LAB growth.

B. Isolation and Identification of Sweet Potato Rot Pathogens

The collected rotten sweet potato tubers were initially rinsed thoroughly in sterilized distilled water to remove any superficial dirt. Subsequently, each tuber was surface sterilized by dipping it in 70% ethanol for 10 seconds. Following surface sterilization, the tubers were cut open using a sterile scalpel. Three pieces of infected tissue, approximately

3mm in diameter, were carefully removed from the advancing edge of the rot using a sterile cork borer. These tissue pieces were then aseptically inoculated onto solidified Potato Dextrose Agar (PDA) in Petri dishes. Three replicates were prepared for each sample. The inoculated plates were incubated at room temperature ($28 \pm 2^\circ\text{C}$) for 48 hours, with daily observations for the emergence of fungal growth. Subcultures were performed to obtain pure cultures of the isolated fungi. The identification of these fungal pathogens was based on their distinct morphological features observed both on the culture plates and under a compound microscope. Reference was made to Fungi Families of the World mycological monographs by Postgate (2022) and standard identification guides such as Sangoyomi (2004). Morphological characteristics such as colony color, extent of growth, presence or absence of aerial mycelia, spore morphology, and the nature of the colony surface were meticulously observed. For microscopic examination, slide mounts of the test isolates were prepared and stained with Lactophenol Cotton Blue Stain (LCBS). Stock cultures were prepared using PDA slants in McCartney bottles and stored at 5°C in a refrigerator for future use.

C. *Determination of Percentage Frequency Occurrence of the Fungal Isolates*

To determine the incidence of occurrence of the various fungal isolates associated with sweet potato tubers, the percentage frequency of occurrence was calculated. This involved recording the total number of times each specific fungal isolate was encountered across all screened samples. The percentage frequency of occurrence was then calculated using the formula described by Okigbo and Igwe (2007): Percentage frequency of occurrence = (Number of times a fungus was encountered $\times$ 100) / Total fungal isolations. This method provided quantitative data on the prevalence of specific pathogens, which is crucial for guiding the focus of subsequent biocontrol efforts towards the most common and impactful threats to sweet potato post-harvest quality.

D. *Pathogenicity Test*

To confirm that the isolated fungal species were indeed the causative agents of sweet potato rot, a pathogenicity test was conducted. Ten healthy sweet potato tubers were initially washed thoroughly under running tap water to remove any soil or dirt. Each tuber was then surface sterilized by dipping it into a 10% concentration of sodium hypochlorite for 2 minutes, followed by two rinses in sterile distilled water (SDW). The tubers were then placed on sterile paper towels and allowed to air dry for 20 minutes. Cylindrical holes were aseptically drilled at both the proximal and distal ends of each sweet potato tuber using a sterile 10mm cork borer. Discs of five-day-old pure pathogen cultures were then plugged into the created holes. The corresponding tuber disc removed by the cork borer was carefully placed back into the hole to seal the inoculation site. Vaseline was applied around the point of inoculation to prevent desiccation and external contamination. The inoculated tubers were then incubated at room temperature. Three replicates were prepared for each fungal isolate. After 72 hours of incubation, the sweet potatoes were cut transversely, and the symptoms produced were observed and recorded. The pathogens were then re-isolated from the infected tissues to fulfill Koch's postulates, as described by Agrios (2015). This test is critically important for validating the causal relationship between the isolated fungi and the observed sweet potato rot, thereby strengthening the scientific basis for targeting these specific organisms with biocontrol agents.

E. In Vitro Inhibition of Fungi Isolates by Lactobacillus

The in vitro antifungal activity of *Lactobacillus* isolates against the sweet potato rot pathogens was assessed using two primary methods: the cup plate method for determining the zone of inhibition and the agar dilution method for determining the Minimum Inhibitory Concentration (MIC).

F. Determination of Zone of Inhibition using the Cup Plate Method:

Single strength Potato Dextrose Agar (SDA) was prepared (20ml per plate), melted, and aseptically poured into sterile Petri dishes, allowing them to solidify. The agar surface of each plate was then flooded with a standardized spore suspension of the fungal pathogens at a concentration of 10^6 cfu/ml. A sterile cork borer (6mm diameter, number 4) was flamed red hot, allowed to cool, and used to bore holes in the solidified agar. Subsequently, varying concentrations (2000, 1500, 1000, 500, 250, and 100 µg/ml) of the different *Lactobacillus* culture supernatants (implied as the "anti-fungal agents") were prepared. One hundred microliters (100µl) of each varying concentration were dispensed into the respective holes on the SDA plates. The plates were allowed to stand for one hour to allow for diffusion of the antimicrobial agents into the agar, and then incubated at 30°C for periods ranging from 48 to 192 hours. The zones of inhibition, indicative of antifungal activity, were measured using a well-calibrated transparent meter ruler.

G. Determination of Minimum Inhibitory Concentration (MIC) using Agar Dilution method:

The Minimum Inhibitory Concentration (MIC) was determined using the agar dilution method. Ten milliliters (10ml) of double strength SDA were melted and aseptically mixed with 10ml volumes of varying concentrations of the test "anti-fungal agents" (presumably *Lactobacillus* culture supernatants or extracts). The concentrations tested included 2, 5, 10, 20, 40, 60, 100, 200, 500, 1000, 2000, and 4000 µg/ml. Each admixture was aseptically poured into sterile plates and allowed to set. Standardized spores of the test fungi (10^6 cfu spores/ml) were aseptically inoculated (10.0 µl) in duplicates onto sterile filter paper discs, which were then placed at equidistance on the SDA plates containing the test antifungal agents. The inoculated organisms were allowed to diffuse for a period of 30 minutes before the plates were incubated at 30°C for 48 hours. The lowest concentration at which no visible growth of the inoculated test fungi spores was observed was considered as the MIC of the test anti-fungal agent.

IV. DATA ANALYSIS

All collected data were initially entered into an Excel spreadsheet for organization and preliminary review. For comprehensive statistical analysis and inference, the Statistical Package for Social Science (SPSS) version 20 was utilized. Descriptive statistics, including the calculation of group means of inhibition zone diameters as mean $\pm$ SEM (Standard Error of the Mean), were employed to summarize the data. One-way ANOVA (Analysis of Variance) was performed to determine if there were statistically significant differences among the group means. Following a significant ANOVA result, Tukey's post hoc test was conducted to identify specific significant differences between individual group means.

Additionally, linear regression and the coefficient of determination (R^2) were used to explore relationships and the strength of associations between variables. This robust statistical approach ensured that the findings were rigorously analyzed and that any conclusions drawn were statistically sound.

V. RESULTS

Proximate Composition of Fresh and Rotten Sweet Potato Tubers. The proximate analysis of sweet potato tubers revealed significant changes in their nutritional composition upon spoilage. Table 1 presents the average proximate composition of both fresh and rotten sweet potato samples.

Table 1: Average Proximate composition of the fresh and rotten sweet potato tubers sampled

PROXIMATE	FRESH SWEET POTATO	ROTTEN SWEET POTATO
MOISTURE CONTENT (%)	58.00 ± 0.36	20.50 ± 0.22
ASH CONTENT (%)	3.86 ± 0.30	3.11 ± 0.10
PROTEIN CONTENT (%)	5.20 ± 0.33	3.30 ± 0.36
CRUDE FIBER CONTENT (%)	9.10 ± 0.50	3.80 ± 0.10
FATS AND OIL (%)	0.75 ± 0.30	0.62 ± 0.35
CARBOHYDRATE (%)	23.09 ± 0.20	68.67 ± 0.55

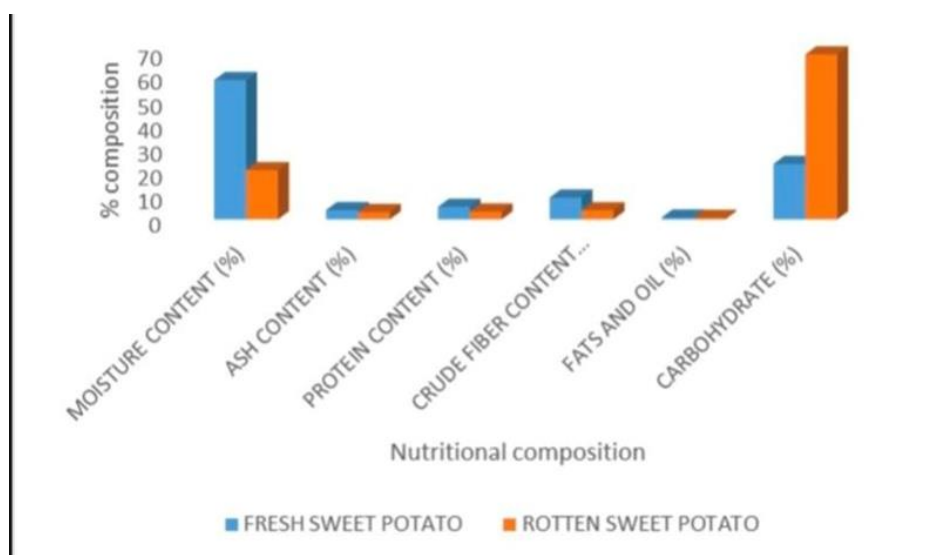


Fig 1: Nutritional composition of fresh and rotten sweet potatoes

The data clearly indicate a substantial reduction in moisture content, decreasing from 58.00% in fresh sweet potatoes to 20.50% in rotten samples. This significant loss of water is a hallmark of spoilage, leading to wilting and textural degradation. Similarly, there was a noticeable decrease in protein content (from 5.20% to 3.30%), ash content (from 3.86% to 3.11%), and crude fiber content (from 9.10% to 3.80%) in the rotten tubers compared to their fresh counterparts. These reductions signify the extensive degradation of essential

nutrients and structural components by microbial activity during the rotting process. Such changes directly translate to a diminished nutritional value and reduced palatability, highlighting the severe impact of post-harvest rot on food quality and its contribution to food insecurity.

Conversely, the carbohydrate content appeared to increase significantly, from 23.09% in fresh samples to 68.67% in rotten samples. This apparent increase in carbohydrate content, which was determined "by difference," does not represent an absolute gain in carbohydrates. Instead, it reflects a relative concentration effect. As other components like moisture, proteins, and fibers are consumed, degraded, or lost due to microbial spoilage, the remaining carbohydrates constitute a larger proportion of the total dry matter. This phenomenon underscores the comprehensive nature of spoilage, where the overall integrity and balanced nutritional profile of the sweet potato are severely compromised, leading to substantial economic and nutritional losses. The empirical data from this proximate analysis provides a quantitative foundation for understanding the extent of damage caused by rot, thereby validating the problem statement and emphasizing the urgency of effective preservation strategies.

A. *Fungal Counts and Identification from Rotten Sweet Potato Samples*

The analysis of rotten sweet potato samples revealed significant fungal contamination, with varying mean total fungi counts across different market locations in Anambra State. Table 2 presents these findings.

Table 2: Mean fungi count of rotten sweet potato samples

LOCATION	Mean total fungi count ($\times 10^4$ cfu/g)
Uli market	12.50 ± 0.22
Total Ihiala	10.80 ± 0.10
Nkwo Nnewi	13.00 ± 0.50
Eke Awka	17.45 ± 0.25
Nkwo Umunze	11.90 ± 0.20

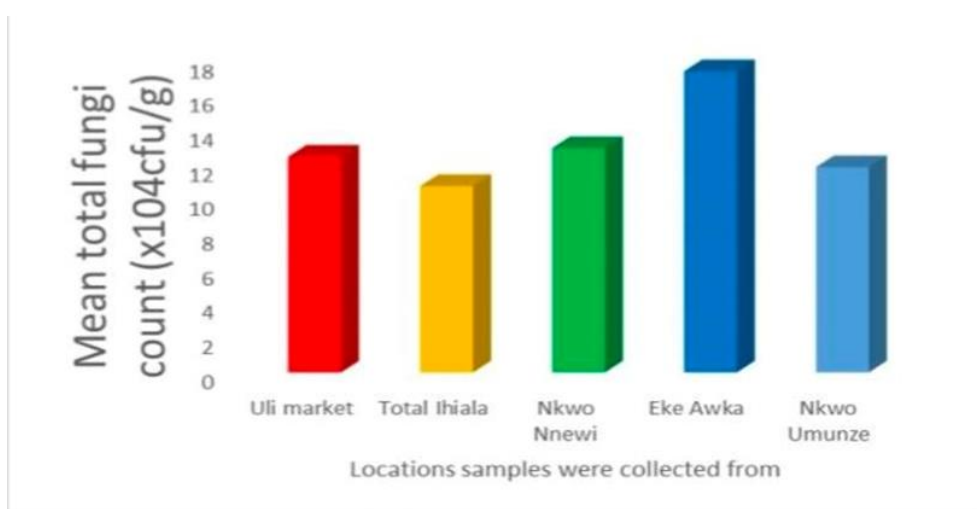


Fig 2: Mean fungi count of rotten sweet potato sample

The mean total fungi count ranged from 10.80×10^4 cfu/g in Total Ihiala to 17.45×10^4 cfu/g in Eke Awka. These high counts underscore the pervasive microbial load associated with spoilage in sweet potatoes, confirming that fungal proliferation is a primary driver of post-harvest deterioration. Further characterization of the fungal isolates revealed distinct morphological features, as detailed in Table 3.

Table 3: Morphological Characteristics of Fungal Isolates

S/N	Isolate Code	Color of Spores	Reverse of the agar	Aerial hyphae	Abundance	Growth	Pigmentation
1	AF1	Black	Light green	Powdery, spores embedded	Abundant	Fast	No
2	AF2	Black	Light green	Powdery, spores embedded	Abundant	Fast	No
3	AF3	Blue green –	Cream	Powdery, spores embedded	Abundant	Fast	No
4	AF4	White	Cream	Fluffy, raised a little	Abundant	Fast	No
5	AF5	White	Cream	Fluffy not raised	Abundant	Fast	No

Based on these morphological characteristics, the fungal isolates were identified to the genus and species level, as presented in Table 4

Table 4: Identification of Fungi

Isolate code	Description	Probable identity
AF1	They are typically powdery black, Conidiophores arising from long, broad, thick-walled, sometimes branched foot cell, it has tall conidiophores. Conidia are large with radiating heads, mostly globose and irregularly roughed.	<i>Aspergillus niger</i>
AF2	Colonies spread thinly, blue-green with strictly columnar conidial heads. Pigmented conidiophores present with clavate vesicles arising from clearly differentiated thick-walled foot cells. Conidia are absent.	<i>Aspergillus flavus</i>
AF3	Colonies smoke-grey in dark, yellowish brown in the light: odour aromatic, it has wide sporangiopore and a denser layer of short repeatedly branched sporangiopores. Sporangiopores thick-walled with granular contents.	<i>Mucor spp</i>
AF4	Colonies are fast growing, aerial mycelium sparse to abundant and floccose, becoming felted, white or peach, but with a violet tinge. Characteristic aromatic odour suggesting lilae.	<i>Fusarium sp.</i>
AF5	Colonies are fast growing conidiophores in fresh isolate typically loosely synematous, giving the	<i>Penicillium sp.</i>

	colony a zonate appearance. Colonies are light green, reversed colourless, yellow-brown conidiophores usually smooth walled, penicilli 2-3 staged branched with numerous usually oppressed mutulae, conidia sub-globose to ellipsoidal smooth-walled, odour aromatic, fruity and suggesting apples.	
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The identified fungal species included *Aspergillus niger*, *Aspergillus flavus*, *Mucor spp*, *Fusarium sp.*, and *Penicillium sp.*. The prevalence of these fungi in rotten sweet potato tubers was further quantified by their percentage frequency of occurrence, as shown in Table 5.

Table 4.5: Percentage frequency of occurrence of fungal isolate on the tubers

Isolates	% Occurrence
<i>Aspergillus niger</i>	88.00
<i>Rhizopus stolonifer</i>	55.00
<i>Fusarium oxysporum</i>	20.00
<i>Aspergillus flavus</i>	76.00
<i>Penicillium sp.</i>	55.00

Aspergillus niger was the most frequently occurring isolate, detected in 88.00% of the samples, followed by *Aspergillus flavus* at 76.00%. *Rhizopus stolonifer* and *Penicillium sp.* were found in 55.00% of samples, while *Fusarium oxysporum* had a 20.00% occurrence. The high percentage occurrence of *Aspergillus niger* and *Aspergillus flavus* is particularly noteworthy. While *A. niger* is a common spoilage agent, the significant prevalence of *A. flavus* raises a critical food safety concern due to its known potential to produce aflatoxins, which are potent mycotoxins harmful to human health. This observation suggests that effective biocontrol by *Lactobacillus* would not only reduce spoilage and economic losses but also potentially mitigate risks of mycotoxin contamination, thereby offering a broader public health benefit. The identification of these specific, prevalent pathogens provides clear targets for the development of effective biocontrol strategies.

Pathogenicity of Isolated Fungi

The pathogenicity test confirmed the ability of the isolated fungal species to induce rot in healthy sweet potato tubers, thereby validating their role as causative agents of post-harvest spoilage. Table 4.6 summarizes the symptoms, percentage infection, and severity ranking observed after 14 days of inoculation.

Table 4.6: Symptoms and severity ranking of pathogens causing rot in sweet potato

Fungal isolate	% Infection after 14 days	Symptoms of Infection	Pathogenicity	severity ranking
<i>Aspergillus niger</i>	100	Dry purple rot to yellowish brown firm rot with	+++	8.00

		black –charcoal like margin.		
<i>Rhizopus stolonifer</i>	61.00	Light brown to yellowish brown soft rot	++	6.00
<i>Fusarium oxysporum</i>	75.00	Cream to brown dry rot	+++	6.00
<i>Aspergillus flavus</i>	88.00	Dark brown soft rot	++	6.00
<i>Penicillium sp.</i>	50.00	Yellowish brown soft rot	++	5.00
<i>Geotrichum spp</i>	65.00	Dark brown soft rot	+++	6.00

Note: +++ = Highly pathogenic (> 50mm in diameter) ++ = moderately pathogenic (>10) Ranking based on 0-9 scale; 0 = least severe and 9 = most severe

All tested fungal isolates demonstrated pathogenicity, inducing varying degrees of rot in the inoculated sweet potato tubers. *Aspergillus niger* exhibited the highest virulence, causing 100% infection and manifesting as a dry purple rot to yellowish-brown firm rot with a characteristic black-charcoal like margin, receiving a severity ranking of 8.00. This high infection rate and severity underscore *A. niger* as a major threat to sweet potato post-harvest quality. *Aspergillus flavus* also showed significant pathogenicity with 88.00% infection, leading to dark brown soft rot. The high prevalence of *A. flavus* combined with its confirmed pathogenicity further emphasizes the dual concern of spoilage and potential mycotoxin production. *Fusarium oxysporum* caused 75.00% infection, resulting in cream to brown dry rot, and was categorized as highly pathogenic. *Rhizopus stolonifer* and *Geotrichum spp* both induced soft rot with infection rates of 61.00% and 65.00% respectively, while *Penicillium sp.* caused yellowish-brown soft rot with 50.00% infection. The empirical confirmation of these fungi as causative agents of sweet potato rot provides direct evidence supporting the rationale for developing targeted biocontrol strategies. The varying degrees of pathogenicity among the isolates also highlight the need for broad-spectrum or multi-strain biocontrol agents to effectively manage the diverse fungal threats in sweet potato post-harvest environments.

NB:

Lactobacillus species was collected from locally made fruit juice. Furthermore, they tested positive for starch hydrolysis, gelatin hydrolysis, casein hydrolysis, and dextran production. This indicates that these LAB strains can break down complex molecules. This metabolic versatility could contribute significantly to their competitive exclusion capabilities by enabling them to utilize a wider range of nutrients, potentially outcompeting spoilage pathogens. It also hints at their potential for value addition through enzymatic biotransformation, aligning with the study's broader objectives of enhancing sweet potato quality. The comprehensive characterization confirms the successful isolation and identification of promising *Lactobacillus* strains for further evaluation as biocontrol agents.

Table 4.7: Minimum Inhibitory Concentration (MIC) BIOFUNGICIES on isolated fungi from sweet potatoes

Biofungicides		<i>Aspergillus niger</i>	<i>Rhizopus stolonifer</i>	<i>Fusarium oxysporum</i>	<i>Aspergillus flavus</i>	<i>Penicillium sp.</i>
LABS 1 (Isolated from beverages)		1000.00	750.00	125.00	500.00	1000.00
LABS 2 (Isolated from beverages)		250.00	500.00	750.00	500.00	250.00
<i>Lactobacillus acidophilus</i> (reference strain)		125.00	250.00	500.00	125.00	1000

Lower MIC values indicate higher inhibitory potency. Consistent with the zone of inhibition data, *Lactobacillus acidophilus* generally demonstrated superior potency, particularly against *Aspergillus niger* (MIC of 125.00 µg/ml) and *Aspergillus flavus* (MIC of 125.00 µg/ml). LABS 2 also showed strong potency against *A. niger* and *Penicillium sp.* with MICs of 250.00 µg/ml. Conversely, *Fusarium oxysporum* required a relatively higher concentration of *L. acidophilus* (MIC of 500.00 µg/ml) for inhibition compared to *A. niger* and *A. flavus*, indicating its comparatively lower susceptibility to the reference strain. These MIC values complement the inhibition zone data by providing a more precise quantitative measure of the concentration of *Lactobacillus* metabolites required to inhibit fungal growth, which is vital information for practical application and formulation development. Overall, these *in vitro* findings strongly validate the premise that *Lactobacillus* species possess significant potential as biological control agents against sweet potato rot.

While the *in vitro* efficacy of *Lactobacillus* species against sweet potato pathogens is clearly demonstrated by the zone of inhibition and MIC data, the current study does not include specific results pertaining to the *in vivo* impact of *Lactobacillus*-based biopreservation on the nutritional, functional, and organoleptic qualities of sweet potatoes during storage, nor does it include data on shelf-life extension. Similarly, specific results related to the optimization of application methods and conditions for *Lactobacillus* species in post-harvest sweet potato preservation are not presented. These represent recognized gaps in fully understanding the practical applicability and comprehensive benefits of *Lactobacillus* in real-world sweet potato preservation, highlighting areas for future research.

VI. DISCUSSION

The proximate analysis conducted in this study clearly demonstrated significant nutritional degradation in sweet potatoes affected by post-harvest rot. A substantial reduction in moisture content was observed, decreasing from 58.00% in fresh tubers to 20.50% in rotten samples. This considerable water loss is a characteristic indicator of spoilage,

leading to the wilting and textural deterioration commonly associated with decay. Concurrently, there were noticeable decreases in protein content (from 5.20% to 3.30%), ash content (from 3.86% to 3.11%), and crude fiber content (from 9.10% to 3.80%) in the rotten tubers compared to their fresh counterparts. These reductions collectively signify the extensive degradation of essential nutrients and structural components, primarily driven by microbial activity during the rotting process. Such changes directly translate to a diminished nutritional value and reduced palatability, underscoring the severe impact of post-harvest rot on food quality and its contribution to food insecurity (Ezeibekwe and Ibe, 2010).

A nuanced observation from the analysis was the apparent increase in carbohydrate content, from 23.09% in fresh samples to 68.67% in rotten samples. This increase, calculated "by difference," does not reflect an absolute gain in carbohydrates. Instead, it represents a relative concentration effect: as other components, particularly moisture, proteins, and fibers, are consumed, degraded, or lost due to microbial spoilage, the remaining carbohydrates constitute a larger proportion of the total dry matter (Hawaz, 2014). This phenomenon highlights the comprehensive nature of spoilage, where the overall integrity and balanced nutritional profile of the sweet potato are severely compromised, leading to substantial economic and nutritional losses.

When comparing these findings with existing literature of Isibor *et al.* (2021), a complex picture emerges regarding the precise shifts in proximate composition during spoilage. The observed decrease in moisture and protein content is generally consistent with other studies on sweet potato deterioration. Microbial spoilage is known to involve the enzymatic degradation of carbohydrates, proteins, and lipids, leading to tissue softening, off-flavors, and odors. However, some external reports present a contrasting view on other components. For instance, Moyo *et al.*, (2024) suggests that fungal attack can lead to a depletion of carbohydrates and crude fiber, while *increasing* crude protein, fat, and ash. This directly contradicts the observed decreases in protein and ash in the current study. Similarly, while the current study noted a relative increase in carbohydrates, other research indicates a decrease in total starch during sweet potato storage, often coupled with an increase in simpler sugars like sucrose and glucose. These discrepancies underscore that the specific impact of fungal rot on proximate composition is not universally uniform. Such variations can be attributed to several factors. Firstly, the dominant fungal or bacterial species present in the spoilage process may employ different enzymatic degradation pathways. For example, some fungi might primarily consume carbohydrates, while others might lead to a relative increase in protein content due to the accumulation of fungal biomass or selective degradation of other components. Secondly, the degree and type of spoilage (e.g., soft rot versus dry rot, or early versus advanced stages of decay) can influence nutrient changes. The "relative concentration effect" for carbohydrates in this study suggests that even if the absolute amount of carbohydrates has decreased due to microbial consumption, their proportion increases as water and other components are lost. Thirdly, subtle differences in sample preparation, analytical techniques, or the specific methods of calculating proximate components (especially "by difference") across studies could contribute to varying results.

The microbiological analysis confirmed significant fungal contamination in rotten sweet potato samples collected from markets across Anambra State, with mean total fungi

counts reaching up to 17.45×10^4 cfu/g in some locations. The most frequently occurring fungal isolates were *Aspergillus niger* (88.00%), *Aspergillus flavus* (76.00%), *Rhizopus stolonifer* (55.00%), *Penicillium sp.* (55.00%), and *Fusarium oxysporum* (20.00%). Pathogenicity tests empirically validated that all these isolated fungi were indeed causative agents of sweet potato rot, inducing varying degrees of decay in healthy tubers. *Aspergillus niger* demonstrated the highest virulence, causing 100% infection and severe dry purple to yellowish-brown firm rot. Studies specifically in Nigeria have linked *Aspergillus* infection in sweet potatoes to aflatoxin production. Therefore, the prevalence of *A. flavus* in the samples elevates the significance of controlling this pathogen beyond mere spoilage reduction to a vital public health intervention, offering a dual benefit of mitigating economic losses and ensuring safer food products (Muhialdn *et al.*, 2011).

The identified pathogens are broadly consistent with those reported in other studies of Udemezue (2019) on sweet potato post-harvest rot both globally and within Nigeria. Research conducted in Jos, Ile-Ife, and Bida metropolis, for instance, also corroborates the frequent occurrence of *Aspergillus*, *Rhizopus*, *Fusarium*, and *Penicillium* species as major culprits. This consistency across various studies (Okeke *et al.*, 2020, Sanusi *et al.*, 2016) and locations strengthens the generalizability of the current findings regarding the primary fungal threats to sweet potato, confirming that the research addresses widespread and well-recognized challenges in post-harvest management.

While *Rhizopus stolonifer* caused 61% infection in this study, its impact in real-world scenarios is often more devastating. It is widely considered one of the most destructive post-harvest diseases of sweet potato, capable of causing complete decay of an infected root in as little as three days. Its ubiquity and ability to spread rapidly through wounds and direct contact contribute to substantial yield losses, estimated at 20–40% annually in some regions. This rapid and destructive nature of *Rhizopus stolonifer* soft rot underscores the urgent need for fast-acting and highly effective biocontrol agents. The confirmation of its pathogenicity in this study reinforces the works of Tamura (2011) the importance of targeting this pathogen to achieve significant reductions in immediate post-harvest losses and prevent widespread spoilage during storage and transport. The observed prevalence of these pathogens in different markets is not random but likely a direct consequence of local post-harvest handling practices, storage conditions, and the environmental presence of these ubiquitous fungal spores. Mechanical injuries during harvesting, handling, packaging, and transportation are critical entry points for pathogens, and storage conditions like temperature and humidity significantly influence disease progression and sporulation. This indicates that effective biocontrol strategies must be integrated with improved post-harvest management practices, such as minimizing wounds, proper curing, maintaining sanitary conditions, and optimizing storage temperatures, to achieve maximum efficacy. Understanding these interconnected factors is essential for developing holistic and region-specific disease management programs.

Furthermore, the locally made fruit juice tested positive for starch hydrolysis, gelatin hydrolysis, casein hydrolysis, and dextran production. This broad metabolic capability is a significant attribute for biocontrol. By hydrolyzing complex substrates like starch and proteins, these *Lactobacillus* strains can effectively outcompete spoilage pathogens for a wider range of available nutrients, thereby contributing to competitive exclusion. This enzymatic activity also serves as a value-addition mechanism: by breaking down complex

sweet potato components, *Lactobacillus* could potentially enhance the bioavailability of nutrients, improve digestibility, or even generate new bioactive compounds (e.g., peptides from protein hydrolysis). This positions *Lactobacillus* not just as a preservative, but as a bio-transformative agent for developing functional foods, aligning with the study's broader objective of enhancing sweet potato quality (Krieg *et al*, 1984).

The *in vitro* assessment of the antifungal activity of these *Lactobacillus* strains (LABS 1 and LABS 2) and the reference strain (*Lactobacillus acidophilus*) against the identified sweet potato rot pathogens yielded compelling results, demonstrating their inhibitory potential.

VII. CONCLUSION

Based on the findings of this study, it can be concluded that *Lactobacillus* species, particularly the reference strain *Lactobacillus acidophilus*, possess significant *in vitro* preservative potential against major post-harvest rot pathogens of sweet potato, including *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Penicillium sp.*, and *Fusarium oxysporum*. The successful isolation and comprehensive characterization of indigenous *Lactobacillus* strains, confirming their identity and metabolic capabilities (such as acid production and hydrolytic activities), provide a strong foundation for understanding their antimicrobial mechanisms. The empirical evidence of extensive nutritional degradation in rotten sweet potatoes, coupled with the confirmed pathogenicity of prevalent fungal isolates, underscores the urgent need for effective intervention. This research provides a robust scientific basis for considering *Lactobacillus*-based bio-preservation as a sustainable, environmentally friendly, and safe alternative to conventional chemical treatments for mitigating sweet potato post-harvest rot. The potential for *Lactobacillus* to not only prevent spoilage but also to reduce risks associated with mycotoxin-producing fungi like *Aspergillus flavus* adds a crucial food safety dimension to its application. While the *in vitro* results are highly promising, the full realization of *Lactobacillus*'s preservative and value-adding capabilities in real-world scenarios necessitates further investigation.

RECOMMENDATIONS

To build upon the promising *in vitro* findings of this study and facilitate the translation of *Lactobacillus*-based bio-preservation into practical applications, the following comprehensive recommendations for future research are proposed:

Comprehensive *in Vivo* Efficacy Evaluation: This is the most critical next step, directly addressing the recognized gap in the current study. Future research must prioritize extensive *in vivo* trials using selected *Lactobacillus* strains on whole sweet potato tubers. These trials should simulate and, ideally, replicate actual post-harvest storage and transport conditions, including varying temperatures, humidity, and handling stresses. Beyond merely observing rot incidence, the evaluation must meticulously quantify shelf-life extension (e.g., days or weeks of marketable quality). Crucially, it must include a thorough assessment of the impact on nutritional qualities (e.g., moisture, protein, carbohydrates, vitamins, minerals), functional properties (e.g., antioxidant capacity, anti-

hyperglycemic effects), and organoleptic qualities (e.g., taste, texture, appearance, aroma) throughout the storage period. This will provide a holistic understanding of the practical benefits, as *in vitro* results, while foundational, do not fully account for the complex biological and environmental interactions in a whole tuber. *In vivo* studies are essential to confirm if the observed antimicrobial effects translate into tangible benefits in a real-world system, considering factors like host defense responses, wound healing, and microbial competition on the sweet potato surface.

Detailed Mechanism Elucidation: While the study identified general antimicrobial mechanisms such as acid production and hydrolytic activities, future research should precisely characterize the specific antimicrobial compounds produced by the most effective *Lactobacillus* strains. This includes isolating and identifying specific bacteriocins, quantifying the exact types and concentrations of organic acids (e.g., lactic, acetic, propionic), and understanding their synergistic effects. Additionally, deeper studies on competitive exclusion mechanisms *in vivo* (e.g., how *Lactobacillus* outcompete pathogens for nutrients and adhesion sites) and their ability to disrupt or prevent biofilm formation by spoilage organisms would provide valuable insights for optimizing application strategies. Understanding these specific molecular mechanisms will allow for targeted strain selection, potential genetic enhancement, and the development of more potent and stable *Lactobacillus*-based formulations, moving beyond simply observing what works to understanding how it works, enabling rational design of biocontrol agents.

Strain Optimization and Genetic Enhancement: Conduct more extensive screening of indigenous *Lactobacillus* strains from diverse sources to identify novel strains with superior antifungal activity, broader spectrum inhibition, and enhanced stress tolerance (e.g., to varying temperatures, pH, or desiccation during storage). Furthermore, exploring advanced techniques such as genetic modification or metabolic engineering could enhance the biocontrol capabilities, improve the production of antimicrobial metabolites, and increase the viability and persistence of promising *Lactobacillus* candidates in challenging post-harvest environments. While the current isolates are promising, the vast diversity of *Lactobacillus* suggests that even more effective strains might exist, and genetic approaches could overcome natural limitations, leading to "designer" biocontrol agents tailored for specific sweet potato varieties or storage conditions.

Optimization of Application Methods and Conditions: This directly addresses another recognized gap in the current study. Practical implementation requires extensive research into optimal application methods for *Lactobacillus* on sweet potatoes. This includes evaluating different techniques such as dipping, spraying, edible coatings, or encapsulation technologies to ensure uniform coverage and sustained release of the biocontrol agent. Determine the optimal concentration of the *Lactobacillus* inoculum, the ideal timing for post-harvest treatment (e.g., immediately after harvest, after curing), and the most favorable environmental conditions (e.g., temperature, humidity) for maximum efficacy and sustained protection during storage and transport. Laboratory *in vitro* efficacy does not automatically translate to practical application; factors like inoculum load, contact time, and environmental interaction are crucial for real-world success. Optimizing these parameters is essential for scalability, cost-effectiveness, and consistent performance in diverse agricultural settings.

Mycotoxin Analysis: Given the high prevalence and confirmed pathogenicity of *Aspergillus flavus* (76.00% occurrence, 88.00% infection), and its known ability to produce harmful aflatoxins, future studies must explicitly quantify the reduction of mycotoxin contamination (e.g., aflatoxins B1, B2, G1, G2) in *Lactobacillus*-treated sweet potatoes compared to untreated controls. This would involve analytical chemistry techniques to measure mycotoxin levels. This goes beyond mere spoilage reduction to a critical food safety and public health benefit. Demonstrating a significant reduction in mycotoxin load would immensely strengthen the justification for *Lactobacillus*-based bio-preservation, providing tangible evidence of its value in ensuring safer food products, particularly in regions where aflatoxin exposure is a major health concern.

Economic Feasibility and Commercialization: While the study focuses on scientific understanding, practical adoption requires a strong economic case. Future research should conduct thorough studies on the economic viability, cost-effectiveness, and scalability of *Lactobacillus*-based biopreservation for commercial adoption by farmers and food industries. This would involve assessing production costs of *Lactobacillus* formulations, application costs, potential market acceptance of treated sweet potatoes, and the overall return on investment compared to conventional methods. Scientific efficacy is one part; economic viability and ease of adoption are crucial for widespread impact. Understanding the commercialization pathway is essential for translating research into real-world solutions that benefit farmers and consumers.

Cultivar-Specific Responses: The study acknowledges that sweet potato varieties may exhibit cultivar-specific responses. Future research should investigate the differential efficacy of selected *Lactobacillus* strains across various commercially relevant sweet potato cultivars. This would involve testing the biocontrol agents on different varieties to identify if certain *Lactobacillus* strains are more effective for particular sweet potato types, or if broad-spectrum formulations are necessary. Sweet potato is a diverse crop, and a "one-size-fits-all" solution might not be optimal. Tailoring biocontrol strategies to specific cultivars could maximize efficacy and ensure broader applicability across different agricultural practices.

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