Bindura University of Science Education

Faculty of Science

Biological science department

Isolation and characterization of potential antibiotic producing bacteria from rotting vegetables.

By

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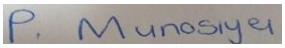
A research project submitted in partial fulfilment of the requirements of the Bachelor of Science Honours degree in Biotechnology.

June 2026

Approval form

The undersigned certify that they have read the dissertation titled ‘isolation and characterization of potential antibiotic producing bacteria from rotting vegetables’ and confirm that it is suitable for submission to the Biological Sciences Department, Faculty of Science and Engineering, for assessment.

Signature of student:  Date: 17/06/26

Signature of Supervisor:  Date: 17/06/26

Signature of Chairperson of Department:  Date: 12 August 2026

Declaration

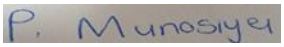
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Supervisor

I, P. Munosiyei, declare that I have supervised this thesis and I am satisfied that it can be submitted to the Biological Sciences Department, Faculty of Science and Engineering, at Bindura University of Science Education.

Signature:  Date:17/06/26

Dedication

I dedicate this project to God Almighty for giving me power and knowledge to succeed and my parents.

Acknowledgements

Firstly, I would want to thank my supervisor Mr. P. Munosiyei for all his maximum advice, assistance and suggestions to improve the research and for shaping this project. I thank him wholeheartedly, not only for tremendous academic support, but also for encouraging me to grow as an independent and confident researcher. My great appreciation to my workmates at City of Harare for all the support during my studies as well as the Bindura University of Science Education lab technicians for their input. Last, but not least, I would like to thank my family and friends, which is the main source of my life energy and my parents (Mr. and Mrs. Musonza) who gave me their unconditional love and encouragement, and supported me in all my pursuits. They have also made me stronger, better and more fulfilled than I could have ever imagined. I love you to the moon and back.

List of abbreviations

AMR	Antimicrobial Resistance
DNA	Deoxyribonucleic Acid
g	Gram
GC-MS	Gas Chromatography-Mass Spectrometry
H_0	Null Hypothesis
H_1	Alternative Hypothesis
HPLC	High Performance Liquid Chromatography
LCC	Long Cream Colony
MCC	Medium Cream Colony
MDR	Multidrug Resistant
MHA	Mueller Hinton Agar
ml	Millilitres

mm	Millimetres
NA	Nutrient Agar
NB	Nutrient Broth
°C	Degrees Celsius
rpm	Revolutions Per Minute
rRNA	Ribosomal Ribonucleic Acid
spp.	Species
WHO	World Health Organization

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Abstract

The escalating global crisis of antimicrobial resistance (AMR) has rendered many conventional treatments ineffective, creating an urgent need for the discovery of novel bioactive compounds. This project aimed to isolate and characterize bacteria from rotting vegetables with the potential to produce antimicrobial compounds. This research explored rotting vegetables as an underutilized ecological niche for isolating potential antibiotic-producing bacteria. The study hypothesized that the intense microbial competition for nutrients in decaying organic matter serves as a powerful evolutionary driver for the production of antimicrobial secondary metabolites. Samples of rotting tomato, carrot, lettuce, and potato were collected from a local market in Bindura. Bacteria were extracted using serial dilution and cultured on Nutrient Agar. Primary screening for antagonistic activity was performed using the cross-streak method against *Staphylococcus aureus.* Promising isolates underwent crude extract production by solvent extraction using hexane and ethanol. Secondary screening using the Kirby-Bauer disc diffusion method to evaluate potency at varying concentrations followed this. A total of 14 morphologically distinct bacterial colonies were isolated. 35.7% of the isolates demonstrated clear zones of inhibition against *S. aureus* during the primary screening. The Long Cream Colony (LCC) filamentous colony from carrot and as well as the long cream spore forming colony from lettuce exhibited the largest initial zones of 12 mm. the potato "Medium Cream Colony" (MCC) extract showed the highest inhibition, maintaining a 22 mm zone of inhibition at 100% concentration and remaining effective even at a 25% dilution during the secondary screening. Morphological and biochemical characterization, including gram staining, catalase, citrate utilization and oxidase tests, identified the successful isolates as belonging to the *Bacillus* and *Streptomyces* genera. The findings suggest that rotting vegetables are a rich and sustainable source of antibiotic-producing microorganisms. The identification of potent strains from organic waste supports a circular bio-economy approach, transforming common disposal problems into valuable resources for pharmaceutical discovery and the fight against multidrug-resistant pathogens.

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Chapter 1

Introduction

This study aimed to investigate the antibiotic production potential of bacteria isolated form rotting vegetables. The chapter introduces the research study on the isolation and characterization of potential antibiotic producing bacteria from rotting vegetables. The rationale behind this chapter is to cover the background of the study, the statement of the problem illustrating reasons which drove the need for research, justification of the study and research objectives of the study.

1.1 Background

The rapid and global spread of antimicrobial resistance (AMR) poses an existential threat to modern medicine. The World Health Organization (WHO) consistently lists AMR among the top global public health threats, emphasizing that the decline in effective antibiotics is undermining our ability to treat common infectious diseases, and minor injuries could once again become fatal (WHO, 2022). This crisis is fueled by the overuse of antibiotics in humans, animals and agriculture, coupled with a critical shortage of new drugs in the developmental pipeline. The pipeline of new antibiotics is running dry, partly because the most readily microorganisms that can be cultured from common environments like soil have been extensively mined, leading to the rediscovery of known compounds (Sitotaw et al., 2022). Therefore, there is an urgent and critical need to explore novel, underutilized, and extreme ecological niches as sources for isolating unique microorganisms with the potential to produce new antimicrobial compounds

Rotting vegetable matter represents a highly competitive and dynamic microbial ecosystem. As vegetables decay, they release a rich nutrient pool of carbohydrates, vitamins, and minerals. This nutrient-rich environment becomes a battlefield for a diverse consortium of microorganisms, including bacteria, fungi, and yeast, all competing for limited resources (Balogun et al., 2021). In this competitive environment, the production of antimicrobial secondary metabolites is a strategy for certain bacteria to inhibit or kill other microbes in order to secure their niche and access to nutrients. The intense competition for survival is a powerful driving force for the evolution of

antibiotic production. The microbial diversity found in this niche is likely to include genera with a well-established history of antibiotic production, such as *Bacillus, Pseudomonas*, and *Streptomyces*, as well as novel, less-studied species that have adapted to this specific environment (Sitawtow et al., 2022).

Soil has been the traditional source for antibiotic discovery and recent studies have validated the potential of alternative sources. Research has successfully isolated potent antibiotic-producing bacteria from unique sources such as mangrove sediments, rhizosphere soil of specific plants, insect guts, and indeed, decomposing organic matter (Iftikhar and Khalid, 2023). The rationale is rooted in microbial ecology and the concept of competing for resources for survival. In the confined space of decaying plant tissue, bacteria cannot evade their competitors and must produce antibacterial (and antifungal) compounds to secure their niche. Therefore, it is hypothesized that rotting vegetables harbor a high density of antibiotic-producing bacteria, and that these bacteria can be isolated and characterized through appropriate microbiological techniques.

1.2 Research problem statement

The escalating global crisis of antimicrobial resistance, characterized by the rapid emergence of multidrug-resistant pathogens, underscores a critical failure in the modern antibiotic pipeline and poses a severe threat to public health. A primary driver of this crisis is the stagnation in discovering novel antibiotics, largely due to the continual re-isolation of known compounds from convectional sources like soil. This necessitates the urgent exploration of unexplored and highly competitive ecological niches as reservoirs for new antimicrobial producers. Rotting vegetables represent one such promising yet underutilized environment. The decomposition process fosters intense microbial warfare for resources, creating a strong selective pressure for the production of potent antibacterial secondary metabolites. However, the specific potential of this common organic waste micro biome as a source for novel antibiotic producing bacteria remains systematically uninvestigated. Therefore, this project aims to address this gap by isolating and characterizing bacteria from rotting vegetables to identify potent strains with activity against clinically relevant, resistant pathogens, thereby contributing to the vital search for new therapeutic agents.

1.3 Justification

The pipeline for new antibiotics has dangerously dwindled, with few novel classes discovered in recent decades. This project directly addresses this crisis by embarking on the essential first step in the antibiotic discovery pipeline. Finding a single novel bioactive molecule could have profound implications for future therapeutic development. The process of decay involves a succession of diverse microbial communities including bacteria and fungi. Rotting vegetables are a nutrient-rich battleground where bacteria and fungi fiercely compete for resources. This project moves beyond conventional sources to target a unique and extreme microbial environment: rotting vegetables. Using rotting vegetables as a source material is a sustainable and cost-effective strategy. Vegetable waste is abundantly available and often discarded without any value. This project proposes to utilize this waste transforming a common disposal problem into a valuable resource for scientific discovery.

1.4 Purpose of the study

1.4.1 Aim

This project aimed to isolate and characterize bacteria from rotting vegetables with the potential to produce antimicrobial compounds.

1.4.2 Specific objectives

- To isolate pure cultures of bacteria using microbiological techniques
- To identify promising antibiotic-producing isolates using biochemical tests
- To characterize the crude antimicrobial extract from the most potential isolates
- To evaluate efficacy against antibiotic-resistant pathogens

1.5 Research questions

1. What is the diversity and abundance of bacteria present in different types of rotting vegetables?
2. What percentage of the isolated bacterial strains demonstrate antagonistic activity against Gram-negative indicator bacteria

3. How effective is the crude extract and what are the inhibition zones they produce at different concentrations against the test pathogen?

1.6 Hypothesis

H_0: rotting vegetables are a poor source of antibiotic producers

H_1: Rotting vegetables are a rich and competitive environment for discovering bacteria with significant antimicrobial potential

1.7 Significance of the study

The most critical significance of this study lies in its direct contribution to the fight against antimicrobial resistance. The rapid emergence of multidrug-resistant (MDR) "superbugs" is rendering our current arsenal of antibiotics ineffective, threatening to return modern medicine to a pre-antibiotic era. This project actively seeks new antimicrobial compounds, potentially providing a lead for developing new drugs to which pathogens have not yet developed resistance. The pharmaceutical pipeline for new antibiotics is alarmingly dry (WHO, 2022). This research represents the essential first step in the drug discovery process. By identifying a bacterial strain that produces a novel compound, this work can provide a crucial starting point for pharmaceutical development, potentially leading to a future therapeutic agent. Any antibiotic discovered could be developed to treat infections caused by MDR pathogens, saving lives and reducing healthcare costs associated with prolonged illnesses and complex treatments.

The study explores a unique and under-investigated ecological niche, the rotting vegetable micro biome, contributing to our understanding of microbial diversity, bacterial competition, and the ecological role of secondary metabolites like antibiotics in natural decomposition processes (Baltz, 2019). This project embodies the principles of a circular bio-economy. It transforms a common waste product (rotting vegetables) into a valuable resource for high-value discovery. This approach adds economic potential to organic waste, redefining it from a disposal problem to a raw material for biotechnology. The source material for this research is ubiquitous, rapidly renewable making it an exceptionally sustainable and scalable approach.

Chapter 2

Literature review

Introduction

This chapter reviews existing literature that is related to antibiotics, antimicrobial resistance and antibiotic-producing microorganisms. It also discusses the definition and applications of antibiotics, the increasing problem of antimicrobial resistance and the importance of exploring alternative habitats for novel antibiotic producers.

2.1 What are antibiotics?

The most important and commonly used secondary metabolite that is produced by microorganisms is the antibiotic. An antibiotic is a naturally occurring, semisynthetic or synthetic type of agent that destroys or inhibits the growth of microorganisms. It is a substance produced by one living microorganism and inhibits the growth of or kill other living organisms. Anti means against and bio refers to life. Bacteria are the most abudant producers of antibiotics. Antibiotics derived from natural sources are used to treat bacterial infections (Iftikhar and Khalid, 2023). They kill or prevent the bacteria from replicating or inhibit their growth. Antibiotics are effectively obtained by isolating them from microbes than by chemical synthesis (Yadav and Dubey, 2023). The discovery of penicillin from a fungus, *penicillium notatum,* started the golden age of antibiotics discovery (Castello, et al., 2025). This finding began the development of antibacterial substances made from living organisms.

Antibiotics have many applications. They are used to stop infection, also used in aquaculture and agriculture. Majority of antibiotics produced by fungi are used in chemotherapy (Yadav and Dubey, 2023).

2.2 Antimicrobial resistance

Antimicrobial resistance (AMR) occurs when microorganisms like bacteria, viruses, fungi and parasites no longer responds to antimicrobial medicines. This makes antibiotics ineffective making infections difficult or even impossible to treat (WHO, 2023). AMR is a process caused by genetic changes in pathogens. With the increase in population, bacterial infections have also intensified claiming many lives. The rise of antimicrobial resistance (AMR) has necessitated a

renewed search for novel bioactive compounds, refocusing attention on under-explored environmental niches and natural sources. Antibiotic resistant bacteria emerge at a high rate and it has been a major international health concern and threat (Sitotaw et al., 2022). The rise of new infectious diseases and multidrug resistant pathogens is on the rise. The rate of discovering new compounds has lowered, but the rate of isolation of known compounds has increased (Shrestha et al., 2021). In most of the classes of antibiotics, bacteria develops resistance (Shrestha et al, 2021). The ratio of resistant bacteria is on the high and it is threatening treatment methods of developed modern medicine (Mandal et al., 2019). So, there is a greater need of searching for new antibiotics that can kill and destroy the resistant bacteria. Therefore there is need to identify new groups of bacteria from underexplored environments.

2.3 Rotting vegetables as a source of antibiotic producing microorganisms

Antimicrobial producing bacteria have been isolated from different several environments. Soil is one of the hot spots to easily get antimicrobial producing bacteria. It is a very complex habitat which is rich in different types of microorganisms. High variation in the biotic and abiotic conditions in the soil challenges the micro biota (Sitotaw et al., 2022). Soil is the reservoir for antibiotic producing microbes and most of the antibiotics used were discovered by screening for antimicrobial activities of microorganisms isolated from soil (Balogun et al., 2021). Historically, soil-dwelling Actinomycetes have been the most prolific source of clinical antibiotics. However, the frequent re-isolation of known species and compounds from soil has led to a diminishing rate of discovery (Baltz, 2019).

As soil has been the most common source of antibiotic producing microorganisms, similarly, rotting vegetables are potential sources of antibiotic-producing microbes. While traditional sources like soil have been extensively studied for antibiotic-producing bacteria (Sitotaw et al., 2022), decaying organic matter represents an underexplored and rich niche for microbial diversity and secondary metabolite production. The exploration of microorganisms that produce antibiotics found in decaying organic matter such as rotting vegetables is one of the promising avenues. Rotting vegetable matter is an ecosystem of competitive microorganisms. It provides optimum conditions such as pH, temperature, organic matter and moisture that support the growth of microorganisms. For groth and other cellular activities, microorganisms produce

primary metabolites. Secondary metabolites are also produced for defense and survival (Sarkar et al., 2014). These secondary metabolites are the most promising resources for antibiotics (Mandal et al., 2019). It represents a promising source for the discovery of antibiotic-producing bacteria. Because of varying temperatures and climatic conditions rotting vegetation is most likely to harbor unusual types of microorganisms. Being a poorly studied and underexplored habitation increases chances of finding microorganisms capable of producing antibiotics (Bhuyan et al., 2017). Using rotting vegetables and fruits as sources of antibiotic producing bacteria can make waste useful and be beneficial in the food industry as well as pharmaceutical industry (Sagar et al., 2018). Bacteria produce an antibiotic to destroy and inhibitother microorganisms in case of limited nutrients and resources (Bhayun et al., 2017).

2.4 Different antibiotic producing microorganisms

Bacteria

Bacteria are unicellular, prokaryotic organisms without chlorophyll and nucleus. Spherical (cocci), rod (bacilli), spiral (spirillia), comma (vibrio) and spirochetes are classes of bacteria (Yadav and Dubet, 2023). Predominantly, *Bacillus* and *Actinomycetes* are producers of Bacitracin and Actinomycin. *Actinomycetes* are well known sources of biologically active microbial products, and also most medically and commercially important antibiotics (Ezebialu et al, 2020). *Streptomyces griseus*, a soil microorganism, produces Streptomycin that acts against *Mycobacterium tuberculosis* (Sarkar et al., 2014).

Fungi

Fungi are filamentous mycelium-containing soil microorganisms and makes antibiotics naturally. Fungi help in the initial decomposition of fresh organic compounds. The most common soil fungus include *Alternaria, Aspergillus, Botrytis, Cladosporium, Cephalosporium, Haetomium, Mucor, Monilia, Penicillium, Fusarium, Rhizopus, Gliocladium, Trichoderma, Pythium, and others* (Yadav and Dubey, 2023). Fungi is considered the best source of antibiotics.

The theoretical approach underpinning this project is one of bio prospecting guided by ecological principle. The theory of competitive exclusion suggests that antibiotic-producing bacteria may

have a survival advantage in nutrient-rich environments like rotting vegetables. This study aimed to isolate bacteria that has the potential to produce antibiotics from rotting vegetable samples. The methodology involved sample collection, serial dilution, culture-dependent isolation on selective media, primary (The cross-streak against safe relative pathogens like *Staphylococcus aureus* or *Escherichia coli*) and secondary screening(subjecting active isolates to submerged fermentation, and testing the cell-free supernatant via disc diffusion) for antimicrobial activity, and phenotypic and biochemical characterization of active isolates.

Chapter 3

Materials and methods

Introduction

This chapter describes the protocols followed to investigate the antibiotic-producing potential of bacteria isolated from rotting vegetables. It explains the study area, sample collection procedures, laboratory preparation techniques, bacterial isolation methods and screening procedures for antimicrobial activity. In addition, the chapter outlined the methods used for crude extraction, secondary screening and also biochemical characterization of the isolates.

3.1 Research area

The research study was conducted at the Biological Science laboratory (lab) at Bindura University of Science Education Astra Campus. All the chemicals, reagents and apparatus used were procured from the Bindura University of Science Education Astra Campus. The vegetable samples were collected aseptically form the Bindura vegetable market place. The market place was chosen because a variety of vegetables is found there. The test organisms, *Staphylococcus aureus*, were collected from the stock culture in the Biological science lab at Bindura University of Science Education.

3.2 Sample collection

Four rotting vegetable samples (tomato, carrot, lettuce and potato) showing visible signs of decay were collected from the Bindura local vegetable market. Using sterile gloves and tongs, samples were collected into clean and sterile sample bottles (Shrestha et al., 2021). The collected samples were transported to the university lab for further analysis.

3.3 Pre-treatment

Samples were processed upon arrive at the lab. The rotting parts were separated using a sterile knife and washed with distilled water to remove dirt. The separated rotting part was homogenized using sterilized mortar and pestle with distilled water (Chaudhary and Dhaka, 2016).

3.4 Isolation and serial dilutions

The bacteria from the rotting vegetable samples were extracted using a serial dilution method. 1g of each homogenized sample was weighed and mixed with 9 ml of sterile saline solution (0.85% sodium chloride) in a test tube and vortexed for 1 minute (Iftikhar and Khalid, 2023). Serial dilutions were made for each sample up to 10^{-9}. From the serial dilutions, 10^{-3}, 10^{-5} and 10^{-9}, for each of the 4 sample, 0.1ml of the suspension was spread onto the prepared Nutrient Agar (NA) (OXOID, Hampshire, United Kingdom) plates using a bent glass spreader. For the preparation of the NA, 31g was weighed and dissolved in 1000ml distilled water, heated and autoclaved at 121^0C for 15 minutes and then poured into the petri dishes and left to solidify at room temperature. The inoculated plates were properly labeled and incubated in an inverted position for 24 hours at 30^o C in an incubator (IncoThem, Gauteng, South Africa) (Ezebialu et al., 2020).



Figure 1:serial dilutions

3.5 Isolation of pure colonies

After the incubation period, the petri dishes were observed for clear zones of inhibition. The colonies showing antagonistic features against other bacteria, that displayed inhibitory zones, were picked. They were sub-cultured on fresh NA plates followed by incubation for 24 hours at 30˚C (Balogun et al., 2021). Pure cultures were obtained by using the streak plate method. Each colony from the pure culture was stored in an agar slant at 4^o C for further analysis and identification.

3.6 Primary screening of the isolates using cross streak method

The primary screening was done according to Shrestha et al., (2021). After incubation, the sub-cultured bacterial isolates were streaked vertically on the Mueller Hinton Agar (MHA) (OXOID, Hampshire, United Kingdom) and incubated at 30^{o}C for 24 hours. The MHA was prepared by suspending 38g in 1000ml, heating and autoclaving it at 121^{o}C for 15 minutes using an autoclave (SCITEK, United Kingdom). After sterilization by autoclaving, 20ml of the culture medium was poured into each petri dish and left to cool at room temperature. During the primary screening, the streaked vertical lines of the bacterial isolates were screened against *Staphylococcus aureus* by drawing perpendicular lines to the previous line of the isolates. The results showing the zone of inhibition were observed within 24 hours after proper incubation.

3.7 Crude extraction

Isolates that showed antibiotic properties in the preliminary screening were used for crude extraction which was used for the secondary screening. Fresh nutrient broth (NB) was prepared by dissolving 13g in 1000ml distilled water. In each properly labeled 250ml flask, 100ml of NB was dispensed and a loopful of 24 hour grown bacterial isolates were inoculated in the NB. The cultures were then put on a shaker at 200 rpm and room temperature for 3 days. After shaking, the contents of the flasks that had been incubated were filtered by use of a filter paper (Whatman). The culture filtrates were added to an equal amount of hexane and ethanol and shook hard for 1 hour. The aqueous phase was separated from the solvent phase, which is thought to contain an antibiotic compound. Evaporation was then used to concentrate the antibiotics in the solvent phase (Balouiri et al., 2016). Stock solutions of crude extract were diluted with 0.85% saline solution.

3.8 Secondary screening: the Disc diffusion method

Antimicrobial was done using the disc diffusion assay as the method of Kirby Bauer. Muller Hinton Agar (MHA) was used for the disc diffusion method (Shrestha et al., 2021). The filtered cell-free extracts of each isolate were selected for antibacterial activity using the disc diffusion method. 6mm discs were prepared and sterilized by autoclaving at 121^{o}C together with boiled 38g of MHA dissolved in 1000ml of distilled water. The culture was cultured for 24 hours in the

NB medium and incubated at 30°C. After incubation and adjusting turbidity, the bacterial strains, *S. aureus*, were swabbed on sterile MHA medium using a sterile cotton swab, and left for 10 minutes. Sterile discs having a 6 mm diameter were immersed in each cell-free extract for 30 minutes. Discs treated with the cell-free extracts and the selected standard antibiotic disc were applied on pre-inoculated MHA medium and left for 15 minutes to allow the diffusion of the metabolite, and then incubated at 30°C for 24 hours without inverting the plates (Sitotaw et al., 2022). Streptomycin antibiotic was used as the positive control. After incubation, the zone of inhibition (mm) around each disc was measured and recorded.

3.9 Characterization

The potential anti-biotic-producing bacterial isolates were characterized by microscopic observation, colony morphology on the media with color variation and texture of the colony and biochemical tests. Gram staining, oxidase test, catalase test, indole production test and citrate utilization tests were conducted.



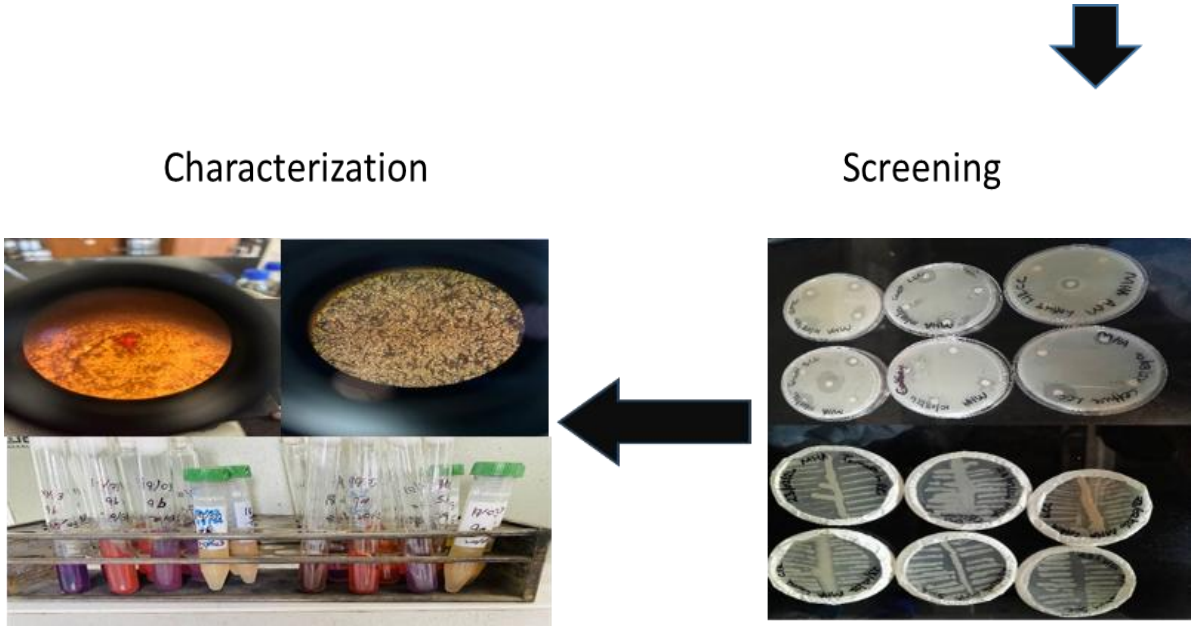


Figure 2: summarized methodology

Chapter 4

Results

Introduction

This chapter presents the findings obtained from the isolation, screening and characterization of bacteria from rotting vegetable samples. The results shows the number and morphology of bacterial colonies isolated, the outcomes of primary and secondary antimicrobial screening and the biochemical characteristics of the successful isolates. Statistical analysis was also conducted to determine the significance of the antimicrobial activities observed at different extract concentrations.

4.1 Isolation of bacteria

Plates containing more than 200 colonies with at least a colony showing halo around it were selected. 14 bacteria colonies showing zones of inhibition around them following the spread plate technique were gotten from 4 vegetable samples, namely tomato, carrot, lettuce and potato, out of 5. None of the isolates from samples from cabbage showed zone of inhibition. The isolates exhibited different colony sizes, color, texture, and shape.

Sample number	Sample name	Isolated pure colonies	Number of colonies per sample
01	Tomato	Large regular white Small cream colony Large irregular cream colony	3
02	Carrot	Medium yellow colony Long cream colony Clear irregular colony Small clear colony	4

03	Lettuce	Large cream colony Small clear colony Large irregular clear colony	3
04	Potato	Medium cream colony Long cream colony Large cream colony Small cream colony	4

Table 1: Morphological structure of the bacteria

4.2 Primary screening for antibiotic production

There were four-teen (14) morphological different bacterial colonies selected from different vegetable samples. The colonies were cross streaked against *S. aureus* to primarily screen them for any antagonistic activity. For the primary isolation of potential isolates, 5 out of 14 isolates (35.7%) showed clear areas of inhibition against the test pathogen *S. aureus*. The carrot LCC and lettuce LCC exhibited the largest zones, while the potato MCC and LCC also demonstrated considerable activity.

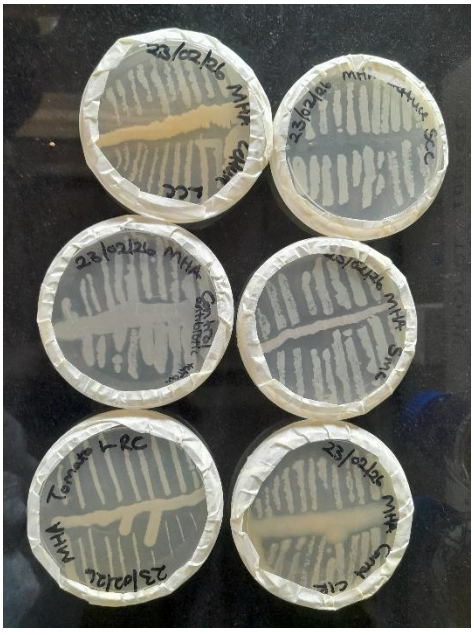


Figure 3: cross streak

Sample number	Sample name	Isolated pure colonies	Zone of inhibition in mm
01	Tomato	Large regular white	-
		Small cream colony	8
		Large irregular cream colony	-
02	Carrot	Medium yellow colony	-
		Long cream colony	12
		Clear irregular colony	-
		Small clear colony	-
03	Lettuce	Large cream colony	12
		Small clear colony	-
		Large irregular clear colony	-
		Medium cream colony	9
		Long cream colony	11

04	potato	Large cream colony	-
		Small cream colony	-

Table 2: Zones of inhibition (cross streak method).

4.3 Secondary screening

Crude extracts for secondary screening were obtained by solvent extraction. At this stage, the isolate's potency was evaluated at different concentrations (100%, 75%, 50% and 25%). The potato MCC extract maintained significant potency at lower concentration. The gradual decrease in inhibition zones with decreasing concentration demonstrates a dose-dependent relationship. Higher concentrations contained greater amounts of active antimicrobial compounds, resulting in stronger bacterial inhibition.

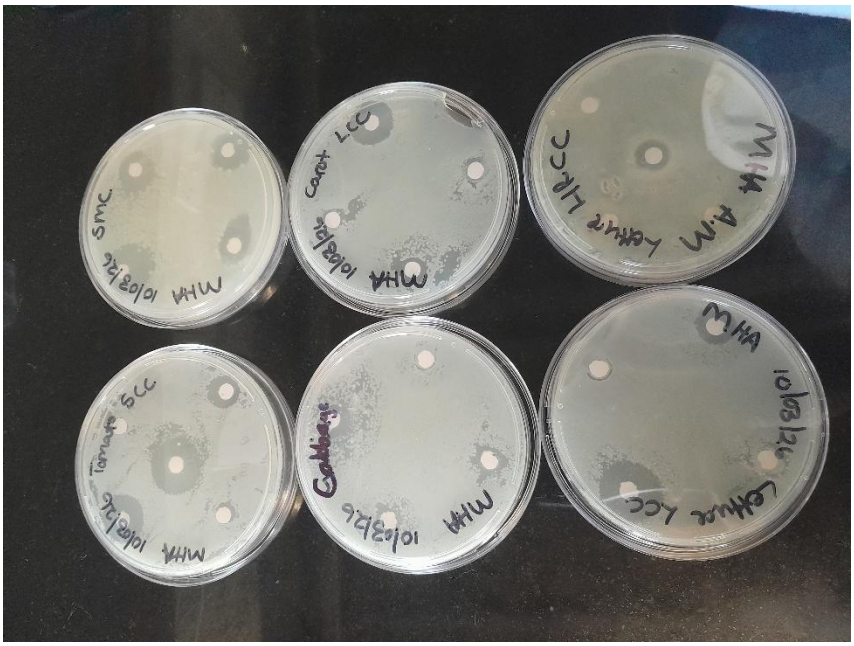


Figure 4: disc diffusion

Sample name	isolate	100% concentration	75% concentration	50% concentration	25% concentration
Tomato	Small creamy colony	20 20 19	14 13 12	8 7 7	0 0 0
Carrot	Long cream filamentous colony	19 19 18	12 11 10	8 7 7	0 0 0
Lettuce	Long creamy spore forming colony	16 15 15	9 8 8	6 6 5	0 0 0
Potato	Medium cream colony	22 22 21	20 20 19	15 15 14	11 11 10
	Long cream colony	12 11 12	9 10 10	8 7 8	0 0 0
Streptomycin (control)		30 29 29			

Table 3: Secondary screening (disc diffusion method).

4.4 Morphological and biochemical characterization

The successful isolates underwent gram staining and biochemical testing. The isolates showed different colony appearances such as circular, smooth, fluffy, cream-colored and raised colonies. Biochemical characterization showed that all successful isolates were Gram-positive and catalase-

positive. Some of the isolates were oxidase-positive and citrate-positive, while all were indole-negative. Based on these characteristics, the isolates were identified as belonging mainly to the genera *Bacillus* and *Streptomyces*.

Sample name and isolate	characteristics
1. Tomato: small cream colony	Circular, cream, smooth,
2. Carrot: long cream colony	Long, cream,
3. Lettuce: long cream colony	Long , cream, fluffy
4. Potato: medium cream colony	Circular, cream, raised
: long cream colony	Long, cream

Table 4 : Isolates morphology

Sample name	Isolate	Catalase test	Oxidase test	Indole test	Citrate utilization test	Gram staining
tomato	Small creamy colony	+	+	-	+	+
carrot	Long creamy colony	+	-	-	+	+
lettuce	Long creamy colony	+	-	-	+	+
potato	Medium cream colony	+	+	-	-	+

	Long cream colony	+	-	-	-	+

Table 5: Biochemical characteristics

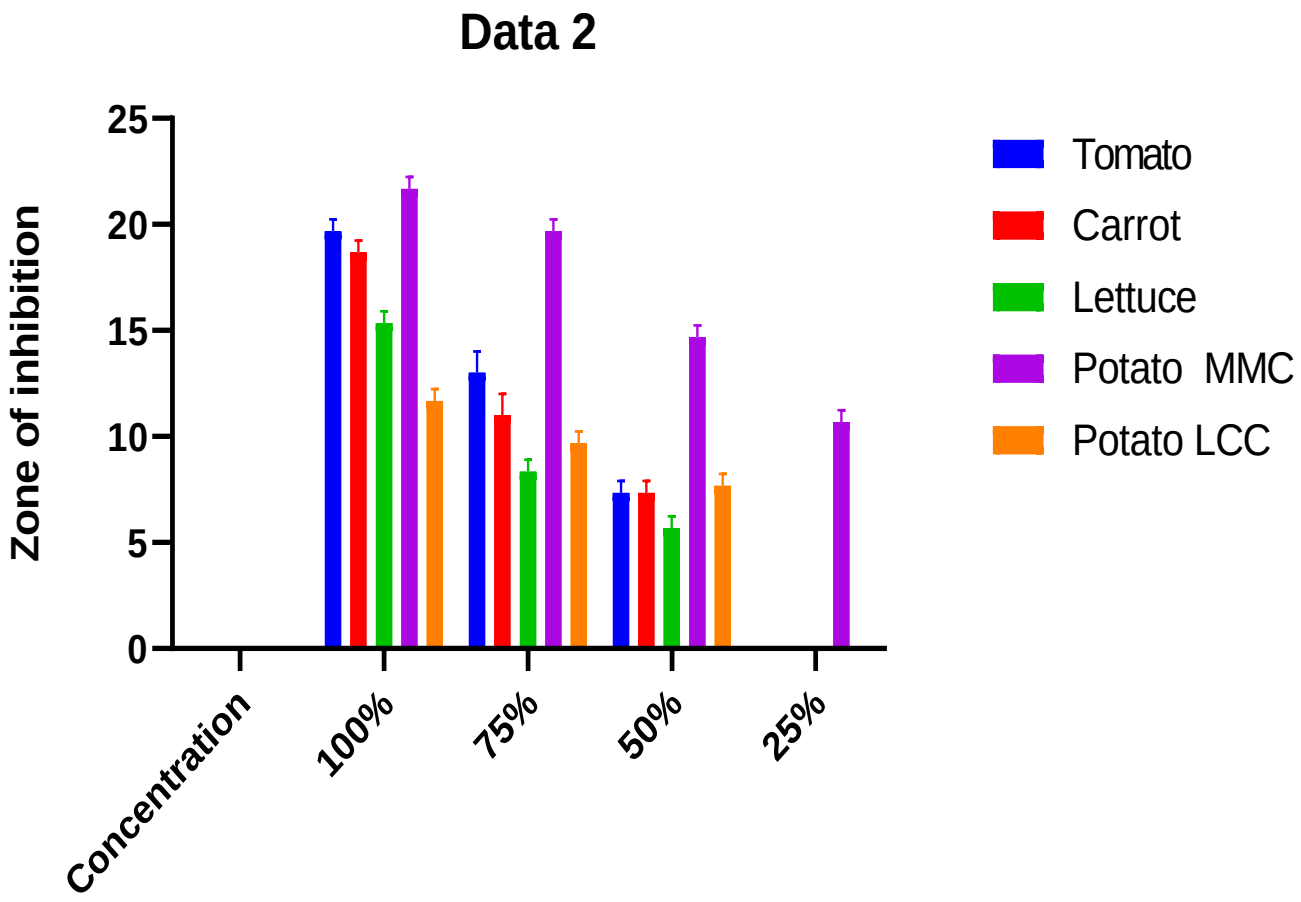


Figure 5: Barchart

4.5 Statistical analysis of the results

The One-Way ANOVA results showed statistically significant differences between treatment concentrations and the control groups in all tested isolates with p-values less than 0.001. This means that the antibacterial effects observed were not caused by chance. The concentration of crude extract significantly influenced the size of the inhibition zones.

The Dunnett post hoc tests further confirmed that all treatment concentrations differed significantly from the control group. As concentration decreased, inhibition zones also decreased significantly.

The statistical findings strengthen the reliability and validity of the experimental results because they demonstrate that the antimicrobial effects were scientifically significant.

Tomato one way ANOVA

Descriptive statistics

Group	N	Mean ± SD)
Control	3	29.67 ± 0.58mm
100%	3	19.67 ± 0.58 mm
75%	3	13.00 ± 1.00 mm
50%	3	7.33 ± 0.58 mm
25%	3	0 ±0

Table 6: Descriptive

Test for homogeneity of variance

		Levene statistic	df1	df2	p value

Zone	Based on mean	2.000	4	10	0.171

Table 7: Test for homogeneityof variety

After test for homogeneity of variance one way ANOVA was done .The results were as below.

	Sum of squares	df	Mean square	F	P-value
Between groups	1556.933	4	389.233	973.083	<0.001
Within groups	4.000	10	0.400		

Table 8: One way ANOVA

Post hoc analysis was done using Dunnett's test to compare each treatment concentration with the control group.

Comparison(Vs control)	Mean difference	Std error	p-value	Significance
100%vs control	-10.000	0.516	<0.001	<0.001
75%vs control	-16.667	0.516	<0.001	<0.001
50%vs control	-22.333	0.516	<0.001	<0.001
25%vs control	-29.667	0.516	<0.001	<0.001

Table 9: Dunnet's test

Carrot one way ANOVA

Descriptive statistics

Group		N	Mean ± SD)
Control		3	29.33 ± 0.58 mm

100%		3	18.67 ± 0.58 mm
75%		3	11.00 ± 1.00 mm
50%		3	7.33 ± 0.58 mm
25%		3	0 ± 0mm

Table 10

Test for homogeneity variances

		Levene statistic	df1	df2	p value
Zone	Based on mean	2.000	4	10	0.171

Table 11

After test for homogeneity of variance one way ANOVA was done .The results were as below.

	Sum of squares	df	Mean square	F	p-value
Between groups	1510.933	4	377.733	944.333	<0.001
Within groups	4.000	10	0.400		

Table 12

A Dunnett post hoc test was used to identify whether concentration groups differed significantly from the control as the ANOVA results were significant.

Comparison(Vs control)	Mean difference	Std error	p-value
100%vs control	-10.667	0.516	<0.001
75%vs control	-18.333	0.516	<0.001
50%vs control	-22.000	0.516	<0.001
25%vs control	-29.333	0.516	<0.001

Table 13

Lettuce one way ANOVA

Descriptive statistics

Group	N	Mean ± SD)
Control	3	29.33 ± 0.58mm
100%	3	15.33 ± 0.58 mm
75%	3	8.33 ± 0.58mm
50%	3	5.67± 0.58mm
25%	3	0± 0mm

Table 14

Test of homogeneity of variance

		Levene statistic	df1	df2	p value
Zone	Based on mean	4.000	4	10	0.034

Table 15

One way ANOVA for ethanol extract of streptococcus spp

	Sum of squares	df	Mean square	F	p-value
Between groups	1526.267	4	381.567	1430.875	<0.001
Within groups	2.667	10	0.267		

Table 16

Dunnet

Comparison(Vs control)	Mean difference	Std error	p-value
100%vs control	-14.000	0.422	<0.001
75%vs control	-21.000	0.422	<0.001
50%vs control	-23.667	0.422	<0.001
25%vs control	-29.333	0.422	<0.001

Table 17

Potato medium cream colony one way ANOVA

Descriptive statistics

Group	N	Mean ± SD)
Control	3	29.67 ± 0.58mm
100%	3	21.67 ± 0.58mm

75%	3	19.67± 0.58mm
50%	3	14.67 ± 0.58mm
25%	3	10.67± 0.58mm

Table 18

Test of homogeneity of variance

		Levene statistic	df1	df2	p value
Zone	Based on mean	0	4	10	1.00

Table 19

One way ANOVA

	Sum of squares	df	Mean square	F	p-value
Between groups	627.600	4	156.900	470.700	<0.001
Within groups	3.333	10	0.333		

Table 20

Post hoc analysis was performed using Dunnett's test to compare each treatment concentration with the control group.

Dunnet

Comparison(Vs control)	Mean difference	Std error	p-value
100%vs control	-8.000	0.471	<0.001
75%vs control	-10.000	0.471	<0.001
50%vs control	-15.000	0.471	<0.001
25%vs control	-19.000	0.471	<0.001

Table 21

Potato medium cream colony one way ANOVA

Descriptive statistics

Group	N	Mean ± SD)
Control	3	29.33± 0.58mm
100%	3	11.67± 0.58 mm
75%	3	9.67 ± 0.58 mm
50%	3	7.67 ± 0.58mm
25%	3	0 ± 0 mm

Table 22

Test for homogeneity of variance

		Levene statistic	df1	df2	p value
Zone	Based on mean	4.000	4	10	0.034

Table 23

One way ANOVA

	Sum of squares	Df	Mean square	F	p-value
Between groups	1404.677	4	351.167	1316.875	<0.001
Within groups	2.667	10	0.267		

Table 24

Post hoc analysis was performed using Dunnett's test to compare each treatment concentration with the control group.

Comparison(Vs control)	Mean difference	Std error	p-value
100%vs control	-17.667	0.422	<0.001
75%vs control	-19.667	0.422	<0.001
50%vs control	-21.667	0.422	<0.001
25%vs control	-29.333	0.422	<0.001

Table 25

Overall, the study successfully demonstrated that rotting vegetables contains bacteria that is capable of producing antimicrobial compounds. The results support the hypothesis that decaying organic matter is a rich ecological niche for discovering antibiotic-producing microorganisms.

The potato medium cream colony was identified as the most promising isolate due to its strong and stable antimicrobial activity. The identification of *Bacillus* and *Streptomyces* species further confirms the pharmaceutical importance of these isolates because both genera are historically associated with antibiotic production. The study also highlights the importance of converting vegetable waste into valuable biotechnological resources. Instead of being viewed solely as waste, rotting vegetables can serve as inexpensive and sustainable sources for discovering new antimicrobial agents that may help combat antimicrobial resistance.

Chapter 5

Discussion, summary, recommendations and conclusions

Introduction

The chapter discusses the significance of the findings in relation to existing literature and the objectives of the study. It interprets the antimicrobial activities observed among the isolates and explains the ecological importance of microbial competition in rotting vegetables. The chapter also summarizes the major findings, provided recommendations for future research and highlights the implications of the study in the search for novel antibiotics.

5.1 Discussion

Isolating and screening antibiotic producing bacteria is important to the global effort in overcoming the challenges from antimicrobial-resistant pathogens. The primary goal of this study was to isolate and characterize bacteria from rotting vegetables capable of producing antimicrobial compounds to address the global crisis of antimicrobial resistance (AMR). The vegetables samples were investigated according to Balogun et al., (2021) and Sitotaw et al., (2022). The results obtained from the research confirm the hypothesis that decaying organic matter is a competitive and rich reservoir for discovering potent microbial antagonists. From the four vegetable samples that showed signs of decay, tomato, carrot, lettuce, and potato, a total of 14 morphologically distinct bacterial colonies were isolated (Table 2). Samples from cabbage did not yield any isolates with visible zones of inhibition during the initial spread plate method. This variation suggests that the specific nutrient composition (carbohydrates, vitamins, and minerals) released during the decay of different vegetables influences the types of microbial communities and the intensity of the microbial warfare occurring within that niche.

The primary screening via the cross-streak method revealed that 35.7% (5 out of 14) of the isolates possessed antagonistic activity against the Gram-positive test pathogen *Staphylococcus aureus.* The Long Cream filamentous and spore forming Colonies from carrot and lettuce

produced the most significant initial inhibition zones of 12 mm. During secondary screening using the disc diffusion method, the crude extract from the potato, Medium Cream Colony (MCC), emerged as the most potent. It maintained a 22 mm zone of inhibition at 100% concentration. Notably, this isolate maintained a 11 mm zone even at a 25% dilution. This suggests that the produced antibiotic compound is very stable and effective even at lower concentrations. While these zones were smaller than the 30 mm zone produced by the Streptomycin control, they represent significant bioactivity for unpurified crude extracts.

Biochemical and morphological tests were conducted and categorized the successful isolates into the genera *Bacillus* spp and *Streptomyces* spp. The recovery of members of these genera was also reported in the previously conducted studies, Kalam and Ahmad (2021), isolated antifungal bacteria from rotten fruits and vegetables. Czajkowski et al., (2011), worked on characterizing bacterial isolates from rotting potato tuber tissue showing antagonism to *Dickey sp*. All five successful isolates tested positive for catalase and gram staining, which is consistent with the resilient, spore-forming nature of *Bacillus* species often found in soil and decaying matter. Bacillus species have many physiological capacities. They survive in extreme conditions because of their endospores and are capable of creating metabolites with antagonistic effects on nearby microorganisms (Sitotaw et al., 2022). The identification of these isolates aligns with historical data identifying Actinomycetes as the most prolific producers of clinical antibiotics, such as Streptomycin.

These findings support the waste-to-wealth concept by demonstrating that common vegetable waste can be transformed into a valuable resource for drug discovery. The isolation of strains with high potency against *S. aureus* provides a crucial starting point for further pharmaceutical development. The next logical steps for this research include purification using high performance liquid chromatography (HPLC) to move beyond crude extracts to identify the specific bioactive molecules. These isolates also need to be tested against a wider panel of gram-negative and multidrug-resistant bacteria.

5.2 Summary

The research successfully investigated the potential of bacteria isolated from rotting vegetables to produce antibiotics. The global threat of antimicrobial resistance and the stagnation of the conventional antibiotic discovery pipeline gave rise to this research. From the collection of rotting tomato, carrot, lettuce, and potato samples, 14 morphologically distinct bacterial colonies were isolated. Primary screening using the cross-streak method identified five isolates (35.7%) with antagonistic activity against gram-positive test pathogen *Staphylococcus aureus*. Secondary screening by the disc diffusion method highlighted the potato medium cream colony (MCC) as the most potent isolate, maintaining a 22 mm zone of inhibition at full concentration and demonstrating stability even at lower dilutions. Morphological and biochemical characterization identified these successful isolates as members of the *Bacillus spp.* and *Streptomyces spp.*

5.3 Recommendations

Future studies should utilize High-Performance Liquid Chromatography (HPLC) or Gas Chromatography-Mass Spectrometry (GC-MS) to purify the crude extracts and determine the exact chemical structures of the antimicrobial compounds produced by the potato MCC and other potent isolates. Secondly, the isolates should also be screened against a wider panel of indicator organisms, specifically focusing on Gram-negative bacteria and multi-drug resistant (MDR) clinical superbugs to determine the full spectrum of their activity. Also research should be conducted to optimize the fermentation parameters such as pH, temperature, and nutrient media to maximize the yield of the antimicrobial secondary metabolites. 16S rRNA sequencing should be performed on the most promising isolates to provide definitive species-level identification beyond biochemical characterization. Last but not least, in vitro and in vivo toxicity assays are recommended to evaluate the safety and biocompatibility of the crude and purified extracts for potential pharmaceutical applications.

5.4 Conclusions

According to the experimental findings, it is concluded that rotting vegetables are a rich and competitive environment for discovering bacteria with significant antimicrobial potential. The hypothesis that this common organic waste harbors a high density of antibiotic producers was

supported by the successful isolation of bioactive strains. The identification of *Bacillus* spp and *Streptomyces* species genera with a well-established history of antibiotic production validates the use of decaying vegetable matter as a viable, sustainable, and cost-effective source for bio-prospecting. This study successfully transforms a common disposal problem into a valuable resource for scientific discovery.

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