PHYSICOCHEMICAL AND PLANT GROWTH PROMOTING PROPERTIES OF BACTERIA ISOLATED FROM SELECTED FARMLAND IN BENIN CITY, EDO STATE, NIGERIA.

BY

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DEPARTMENT OF MICROBIOLOGY

UNIVERSITY OF BENIN

BENIN CITY.

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY.

FEBUARY, 2025

CERTIFICATION

This is to certify that this project work was carried out by **Juliet Ekinadose UYIGUE** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

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(Project Supervisor)

APPROVAL

This project work was carried out by **Juliet Ekinadose UYIGUE** in partial fulfilment of the award of a Bachelor of Science, B.Sc (Hons) degree in the Department of Microbiology, University of Benin, Benin City.

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PROF. (MRS.) F. I. AKINNIBOSUN **DATE**

(Head of Department)

DEDICATION

This project work is dedicated to God Almighty, for bringing me this far in life. I am truly grateful.

ACKNOWLEDGEMENT

I wish to begin by expressing my profound gratitude to God Almighty, whose endless love, grace, and mercy have been my constant source of strength and guidance throughout this academic journey. His divine favor has been instrumental in the successful completion of this project.

I am deeply indebted to my supervisor, **DR. A.G. OGOFURE** for her invaluable support, insightful guidance, and constructive feedback throughout this research. Her expertise and encouragement have greatly contributed to the success of this project, and I am truly grateful for her mentorship and patience.

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Lastly, I would like to express my sincere appreciation to my friends for their steadfast encouragement, support, and companionship throughout this academic endeavor. Their presence made this journey more meaningful and fulfilling. Your kindness, guidance, and support will forever be remembered. Thank you.

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ABSTRACT

Agricultural soil harbors a diverse array of microorganisms that play crucial roles in plant growth and soil fertility. In this study, we investigated the physicochemical properties and plant growth-promoting potential of bacteria isolated from selected farmlands in Benin City, Edo State, Nigeria. Soil samples were collected from various agricultural locations, and the total

heterotrophic bacterial counts ranged from 1.34 ± 0.37 to 2.48 ± 0.85 (x10^4 cfu/ml), with the highest counts found in the Capitol Area. Nine bacterial isolates were identified, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter aerogenes*, and *Bacillus subtilis*. *Bacillus subtilis* was present in all locations, while *P. aeruginosa* and *E. coli* were more localized. *B. subtilis* and *E. coli* were the most prevalent isolates, each accounting for 26.32% of the total isolates. The physicochemical properties of the soils showed variability across locations, with the pH ranging from slightly acidic to neutral. The highest electrical conductivity (EC) was observed in VC Quarters, suggesting a higher concentration of dissolved salts. Organic matter (OM), organic carbon (OC), and nitrogen content were notably higher in VC Quarters, indicating better soil fertility. Phosphorus (P) levels were also highest in VC Quarters, supporting nutrient availability for plant growth. Other essential nutrients such as calcium (Ca), potassium (K), and magnesium (Mg) varied across the locations, with Capitol Area exhibiting the highest calcium content, while potassium and magnesium were abundant in VC Quarters and Capitol Area, respectively. These findings underscore the role of soil microbiota in promoting plant growth, as well as the importance of soil nutrient composition for agriculture. The presence of diverse bacterial isolates with potential plant growth-promoting characteristics, alongside varying soil properties, offers valuable insights into improving agricultural practices and soil management in Benin City.

CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

Agriculture is a cornerstone of human survival and a primary driver of economic development in many regions of the world. As the global population continues to rise, achieving and maintaining

food security has become a critical priority. However, the capacity to sustain high levels of agricultural productivity is constantly challenged by the prevalence of plant diseases caused by a wide array of pathogens, including fungi, bacteria, and viruses. These diseases significantly impact crop yield and quality, posing a threat to global food systems. For instance, it is estimated that plant diseases are responsible for up to 40% of global crop losses annually (Savary *et al*., 2019).

Chemical pesticides have been the mainstay of plant disease management for decades. Their use has undeniably contributed to increasing agricultural productivity. However, their over-reliance has introduced a cascade of adverse consequences, including environmental degradation, contamination of water bodies, bioaccumulation in the food chain, and disruption of beneficial soil microbial communities. Additionally, the indiscriminate use of these chemicals has accelerated the emergence of resistant pathogen strains, rendering many pesticides ineffective over time (Alori *et al*., 2017).

In response to these challenges, there is a growing interest in sustainable and eco-friendly alternatives for disease control in agriculture. Biocontrol, which involves the use of living organisms to suppress plant diseases, has emerged as a promising approach. Antagonistic microorganisms, naturally present in agricultural soils, play a significant role in this process. These microorganisms exhibit various mechanisms, such as competition for nutrients and space, production of antimicrobial compounds, and direct parasitism of pathogens, which make them effective biological control agents (BCAs) (Compant *et al*., 2010).

Microorganisms such as *Bacillus*, *Pseudomonas*, and *Trichoderma* are well-known BCAs with proven efficacy against several plant pathogens. For example, species of *Trichoderma* have

demonstrated the ability to suppress soilborne fungal pathogens such as *Rhizoctonia solani* and *Fusarium oxysporum* through mechanisms like mycoparasitism and the secretion of lytic enzymes (Mojica-Marin *et al*., 2008). Agricultural soils, with their immense microbial diversity, serve as a reservoir for discovering new and effective BCAs, paving the way for innovations in sustainable agriculture.

Despite the benefits of chemical pesticides in controlling plant diseases, their long-term impacts on the environment, human health, and agricultural sustainability are becoming increasingly evident. The widespread and often indiscriminate application of these chemicals has led to serious ecological problems, including the contamination of soil and water resources, a decline in non-target beneficial organisms, and a disruption of the natural ecological balance (Aktar *et al*., 2009; Carvalho, 2017).

Another significant concern is the rise of pesticide-resistant pathogens, which diminishes the effectiveness of traditional chemical control measures and exacerbates crop losses (Hawkins *et al*., 2019). This problem is particularly severe in developing countries, where farmers may lack access to newer, more effective pesticides or integrated pest management practices (Pretty and Bharucha, 2015). The economic burden of continuously purchasing chemical inputs adds to the challenges faced by smallholder farmers, who form the backbone of agricultural production in many regions (Tilman *et al*., 2011).

Given these challenges, there is an urgent need to explore sustainable alternatives that are both environmentally friendly and economically viable. Biological control, leveraging the antagonistic potential of microorganisms isolated from agricultural soils, offers a promising solution. These naturally occurring microorganisms can suppress pathogens effectively, reduce

dependence on chemical pesticides, and promote soil health (Beneduzi *et al*., 2012; Compant *et al*., 2010). However, there is a gap in the identification and characterization of these beneficial microorganisms, particularly in local soils, which underscores the need for further research in this area.

Aim

The primary aim of this study is to investigate the biocontrol potential of antagonistic microorganisms isolated from agricultural soil .

The specific objectives of this research were:

1. enumerate and isolate and identify antagonistic bacterial from agricultural soil.
2. identify the bacterial isolates from the agricultural soil.
3. evaluate the antagonistic activity of the isolated microorganisms against selected plant pathogens.
4. determine the optimal conditions for the growth and antagonistic activity of the identified microorganisms.
5.

CHAPTER TWO

LITERATURE REVIEW

2.1. Plant Pathogens and Their Impact on Agriculture

Plant pathogens significantly threaten global agriculture, causing substantial losses in crop yield and quality. These pathogens, including fungi, bacteria, viruses, and nematodes, infect various crops, leading to diseases such as root rot, wilts, blights, leaf spots, and rusts. Notable fungal

pathogens include *Fusarium oxysporum*, responsible for wilt diseases; *Rhizoctonia solani*, which causes damping-off and root rot; *Pythium spp.*, associated with seedling damping-off; and *Alternaria spp.*, known for causing leaf spots and blights. Bacterial pathogens such as *Xanthomonas* and *Pseudomonas* species and viral pathogens like the Tomato Yellow Leaf Curl Virus further exacerbate the challenge of managing plant diseases.

The impact of these pathogens is reflected in the significant global crop losses they cause. Savary *et al*. (2019) reported that plant diseases account for 20–40% of crop losses annually, affecting staple crops such as rice, wheat, and maize. These losses not only threaten food security but also result in severe economic consequences, particularly in developing regions where agricultural practices often rely on traditional methods and where access to modern disease management technologies, such as resistant crop varieties and effective pesticides, is limited.

Moreover, climate change is exacerbating the situation by altering pathogen life cycles, geographical distribution, and host-pathogen dynamics. For instance, higher temperatures and humidity levels have been linked to increased incidences of fungal diseases such as downy mildew and late blight. These environmental changes are expected to further compromise agricultural productivity, particularly in vulnerable regions of Africa and Asia, where smallholder farmers dominate food production.

To mitigate the impact of plant pathogens, integrated pest management (IPM) strategies have been advocated. IPM combines cultural, biological, chemical, and genetic approaches to reduce pathogen pressure while minimizing environmental harm. According to Oerke (2006), adopting IPM and advancing biotechnological solutions, such as disease-resistant crop varieties and

microbial biocontrol agents, are essential for ensuring sustainable agriculture and mitigating the economic burden of plant diseases.

2.2. Biological Control: A Sustainable Alternative

Biological control (biocontrol) represents a promising and eco-friendly method for managing plant diseases. This approach utilizes natural organisms, predominantly microorganisms, to control or suppress the growth of harmful plant pathogens. By tapping into the power of naturally occurring microbes such as bacteria, fungi, actinomycetes, and even viruses, biocontrol offers a sustainable alternative to chemical pesticides, promoting environmental sustainability and reducing the associated risks of pesticide use in agriculture. As concerns about the harmful effects of chemical pesticides on human health, wildlife, and the environment grow, biocontrol is gaining recognition as a safer and more sustainable method for disease management in agricultural systems (Gohar *et al*., 2020; Higa and Wididana, 1991).

The core concept behind biological control is to harness the natural antagonistic properties of microorganisms that are present in the soil and other ecological niches. These microorganisms have evolved over time to combat plant pathogens in their environment through a variety of sophisticated and highly effective mechanisms. Biocontrol agents (BCAs) can inhibit pathogens through competition for essential resources, production of antimicrobial compounds (antibiosis), and even direct parasitism of the pathogens. These natural mechanisms make biological control a holistic and self-sustaining approach to disease management, as BCAs can continue to provide disease suppression over time with minimal human intervention (Cook and Baker, 1983; Compant *et al*., 2010).

For instance, competition involves the BCA outcompeting pathogens for nutrients, space, or other resources in the soil. This competitive exclusion can significantly reduce the ability of pathogens to establish and proliferate on the plant surface or within the soil environment. Antibiosis, on the other hand, involves the production of inhibitory compounds by BCAs, such as antibiotics, enzymes, or volatile organic compounds, that directly interfere with pathogen growth or kill the pathogens without harming the plants or beneficial organisms (Raaijmakers *et al*., 2002). Another mechanism, parasitism, occurs when the BCA actively invades and feeds on the pathogen, thereby limiting its ability to cause disease (Tian *et al*., 2016).

Biological control is not only about employing a single agent but is often implemented as part of Integrated Pest Management (IPM) strategies. IPM is an approach that combines various methods of pest and disease control to minimize the environmental impact while maximizing effectiveness. Biological control agents are a key component of IPM systems because they provide targeted, specific control of pathogens without the broad-spectrum effects often seen with chemical pesticides. This makes biocontrol agents safer for beneficial organisms, such as pollinators and non-target species, which may be negatively affected by chemical pesticides (Baker *et al*., 2009). The integration of biological control into IPM systems contributes to long-term pest management sustainability by enhancing the overall resilience of agricultural ecosystems (Altieri, 1999).

The incorporation of BCAs in IPM strategies is particularly important in the context of modern sustainable agriculture. With the increasing problem of pesticide resistance, the use of biocontrol can serve as an essential tool to reduce reliance on chemical treatments, thus preserving the efficacy of traditional pesticides for longer. Moreover, the growing concerns over the impact of

synthetic pesticides on biodiversity, soil health, and water contamination have led to a greater emphasis on environmentally friendly alternatives, such as biocontrol (Eilenberg *et al*., 2001). Biological control is increasingly seen as a key component of the global push toward more sustainable and ecologically friendly farming practices.

The diversity of microbial life in soil ecosystems plays a crucial role in the success of biological control. Soils host a vast array of microorganisms, many of which have evolved antagonistic interactions with pathogens. By isolating and identifying specific biocontrol agents from the soil, researchers and agricultural practitioners can discover new and more effective agents capable of controlling a broad range of plant pathogens. This involves isolating bacteria, fungi, and other microorganisms from soil samples, followed by laboratory testing to evaluate their efficacy in suppressing specific plant diseases. Such natural solutions are increasingly being developed and commercialized as biological control products, often in the form of biofungicides, bioinsecticides, or biopesticides (Glick, 2012; Hussain *et al*., 2017).

Furthermore, biocontrol agents tend to be more specific to their target pathogens than chemical pesticides, which can affect a wide range of organisms, including beneficial ones. This specificity is beneficial because it ensures that BCAs only target the harmful pathogens, preserving the overall health and biodiversity of the ecosystem. In contrast, chemical pesticides can disrupt beneficial microbial communities, harming soil fertility and even leading to the development of resistant pathogen strains (Huang *et al*., 2021).

2.3. Importance of Biocontrol in Agriculture

The increasing demand for sustainable and eco-friendly agricultural practices has led to a paradigm shift from the excessive use of chemical pesticides to the adoption of biological control methods. Biocontrol involves the use of natural antagonists to suppress plant diseases, offering several advantages over conventional chemical methods. These advantages include reduced environmental pollution, preservation of soil health, and a lower risk of pesticide resistance among pathogens. Furthermore, biocontrol agents can enhance soil fertility and promote plant growth, making them a vital component of integrated pest management (IPM) systems (Köhl *et al*., 2019).

The role of antagonistic microorganisms in biocontrol is particularly significant because of their ability to colonize the rhizosphere and protect plants from soil-borne pathogens. Unlike chemical treatments, which may have non-specific effects and harm beneficial organisms, biocontrol agents target specific pathogens while maintaining ecological balance. This specificity, coupled with their ability to adapt to diverse environmental conditions, makes antagonistic microorganisms an indispensable tool for modern agriculture.

2.4. Role of Soil Microbiota in Biocontrol

Soil is a dynamic ecosystem teeming with a diverse array of microorganisms, including bacteria, fungi, actinomycetes, and archaea. These microorganisms play crucial roles in nutrient cycling, organic matter decomposition, and the suppression of plant pathogens. Antagonistic microorganisms, in particular, constitute a vital component of soil microbiota and contribute to the natural defense mechanisms of plants.

2.4.1. The Rhizosphere as a Hub for Biocontrol Agents

The rhizosphere, the narrow zone of soil surrounding plant roots, is a hotspot for microbial activity and interactions. Plant roots release a variety of exudates, including sugars, amino acids, and organic acids, which serve as nutrients for soil microorganisms. These exudates not only promote the growth of beneficial microbes but also create a competitive environment that favors antagonistic species over pathogens (Philippot *et al*., 2013). For instance, *Pseudomonas fluorescens* and *Bacillus subtilis* thrive in the rhizosphere and exhibit robust biocontrol properties due to their ability to form biofilms and produce secondary metabolites.

2.4.2. Microbial Diversity and Soil Health

The diversity of soil microbiota is directly linked to soil health and its capacity to suppress plant diseases. High microbial diversity ensures functional redundancy, meaning that multiple species can perform similar roles in nutrient cycling and pathogen suppression. This diversity also reduces the likelihood of pathogen dominance, as antagonistic microorganisms continuously outcompete pathogens for resources and niches. For example, soils with high populations of *Trichoderma* species are less likely to support the growth of fungal pathogens such as *Fusarium* and *Rhizoctonia* (Harman *et al*., 2004).

2.5. Commonly Used Biocontrol Agents

Biocontrol, or the use of natural organisms to control pests and diseases, has emerged as a crucial component of sustainable agriculture. It offers an environmentally friendly alternative to chemical pesticides, aiming to reduce harmful impacts on ecosystems and human health. One key aspect of biocontrol is the isolation and application of antagonistic microorganisms, which

inhibit the growth of plant pathogens and promote healthier crop production. These microorganisms—including bacteria, fungi, and viruses—are naturally present in soil, where they engage in competitive interactions with harmful pathogens. Isolating and identifying such microorganisms from soil is essential in developing effective biocontrol agents that can be used in integrated pest management (IPM) strategies.

2.5.1. Bacteria as Biocontrol Agents

Bacteria are among the most commonly used biocontrol agents due to their ability to suppress plant pathogens through a variety of mechanisms. These mechanisms include the production of antimicrobial compounds, competition for space and nutrients, and the induction of systemic resistance in plants. Bacterial genera such as *Pseudomonas*, *Bacillus*, *Streptomyces*, and *Enterobacter* have shown significant biocontrol activity against a wide range of plant pathogens.

2.5.1.1. Pseudomonas *species*

Pseudomonas spp., particularly *Pseudomonas fluorescens* and *Pseudomonas putida*, are widely recognized for their biocontrol potential. These bacteria produce a variety of antimicrobial substances such as antibiotics, hydrogen cyanide, and siderophores, which inhibit the growth of plant pathogens. *Pseudomonas* spp. are also efficient competitors in the rhizosphere, where they outcompete harmful microbes for nutrients and space, effectively preventing pathogen colonization (Glick, 2012). Their ability to produce volatile organic compounds and biofilms further enhances their antagonistic activity (Compant *et al*., 2005).

2.5.1.2. Bacillus *species*

The genus *Bacillus* includes well-known biocontrol agents such as *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Bacillus thuringiensis*. These bacteria are effective against a broad spectrum of pathogens, including fungi like *Fusarium* and *Rhizoctonia*, due to their production of biocidal compounds such as lipopeptides, bacteriocins, and enzymes (Raaijmakers *et al*., 2009). Some *Bacillus* species, such as *B. thuringiensis*, also produce insecticidal proteins that target specific insect pests, making them dual-purpose biocontrol agents (Schnepf *et al*., 1998).

2.5.1.3. Streptomyces *species*

Streptomyces spp., a genus of soil-dwelling bacteria, are prolific producers of antibiotics like streptomycin, chloramphenicol, and tetracycline, which inhibit the growth of both fungal and bacterial plant pathogens (Berdy, 2005). In addition to their antimicrobial properties, *Streptomyces* species contribute to the decomposition of organic matter, enhancing soil health and fostering plant growth. Their diverse metabolic capabilities and high genetic potential make them promising candidates for biocontrol applications (Lemos *et al*., 2009).

2.5.1.4. Enterobacter *species*

Enterobacter spp., such as *Enterobacter cloacae*, have shown biocontrol activity by suppressing the growth of bacterial pathogens like *Xanthomonas* and *Pseudomonas* spp. (Haas and Defago, 2005). These bacteria also produce plant growth-promoting substances like indole-3-acetic acid (IAA) and are capable of enhancing plant resistance to biotic stresses through the induction of systemic acquired resistance (SAR) (Pieterse *et al*., 2014).

2.5.2. Fungi as Biocontrol Agents

Fungi, especially species of *Trichoderma*, *Beauveria*, and *Metarhizium*, have also garnered attention as biocontrol agents due to their ability to parasitize or outcompete plant pathogens. Their mechanisms of action include the production of enzymes that degrade fungal cell walls, the release of volatile and non-volatile compounds that inhibit pathogen growth, and direct parasitism of pests.

2.5.2.1. Trichoderma *species*

Trichoderma spp., particularly *Trichoderma harzianum* and *Trichoderma viride*, are well-established biocontrol agents due to their ability to parasitize pathogenic fungi and produce enzymes like chitinase and glucanase that degrade fungal cell walls (Howell, 2003). These fungi also produce volatile and non-volatile secondary metabolites that inhibit the growth of plant pathogens such as *Fusarium*, *Rhizoctonia*, and *Pythium* (Vinale *et al*., 2008). Additionally, *Trichoderma* species can induce systemic resistance in plants, enhancing their defense mechanisms against a broad spectrum of pathogens (Benitez *et al*., 2004).

2.5.2.2. Beauveria *species*

Beauveria bassiana is an entomopathogenic fungus that has shown effectiveness in controlling insect pests, particularly those that damage crops. *B. bassiana* infects insects by penetrating their exoskeleton and proliferating within the host, eventually killing it (Feng *et al*., 1994). This fungus has been successfully used in integrated pest management (IPM) systems for controlling pests like aphids, whiteflies, and caterpillars. The specificity of *B. bassiana* for insect pests makes it an eco-friendly alternative to chemical pesticides (Goettel *et al*., 2005).

2.5.2.3. Metarhizium *species*

Metarhizium spp., such as *Metarhizium anisopliae*, are another group of entomopathogenic fungi used in biocontrol. These fungi infect a variety of insect pests, including termites, ants, and weevils, by penetrating their cuticle and growing inside the host. *M. anisopliae* has been shown to be particularly effective in controlling soil-dwelling pests (Roberts and St. Leger, 2004). Like *Beauveria*, *Metarhizium* is also employed in IPM for its selective toxicity to pests and minimal environmental impact (Bateman *et al*., 2000).

2.5.3. Viruses as Biocontrol Agents

Viruses, particularly bacteriophages and entomopathogenic viruses, are increasingly being explored as biocontrol agents. These viruses target specific bacteria or insects, offering a highly selective and environmentally safe approach to pest management.

2.5.3.1. *Bacteriophages*

Bacteriophages are viruses that infect and lyse specific bacteria, making them an ideal tool for controlling bacterial plant pathogens. Bacteriophage therapy has been used to target bacteria such as *Pseudomonas syringae* and *Xanthomonas* spp., which cause a variety of plant diseases (Keen, 1992). The specificity of bacteriophages ensures that they do not harm beneficial microorganisms in the soil, making them a promising alternative to broad-spectrum chemical pesticides (Sulakvelidze *et al*., 2001).

2.5.3.2. *Baculoviruses*

Baculoviruses, including *Autographa californica* nucleopolyhedrovirus (AcNPV), are highly effective in controlling insect pests, particularly caterpillars, aphids, and whiteflies. These viruses infect and kill their insect hosts by replicating inside their cells and ultimately causing the host to die. Baculoviruses are known for their host specificity, making them an ideal biocontrol agent for managing insect pests without affecting non-target organisms (Stark *et al*., 2004).

Biocontrol agents, including bacteria, fungi, and viruses, represent a promising alternative to chemical pesticides. These agents are particularly effective in suppressing plant pathogens and insect pests through various mechanisms such as the production of antimicrobial compounds, competitive exclusion, and direct parasitism. The continued exploration and development of biocontrol agents, particularly through the isolation and characterization of antagonistic microorganisms from soil, are essential for advancing sustainable agricultural practices. With increasing emphasis on environmental protection and food security, biocontrol represents a crucial component of integrated pest management strategies.

2.6. *Mechanism of Antagonism*

Antagonistic microorganisms, including bacteria, fungi, and actinomycetes, play a significant role in plant health by naturally inhibiting or suppressing the growth of plant pathogens. They provide an eco-friendly alternative to chemical pesticides, promoting sustainable agricultural practices through biological control. These microorganisms utilize various strategies to combat pathogens, which can be broadly categorized into direct and indirect mechanisms of antagonism.

Understanding these mechanisms is crucial for developing effective biocontrol strategies in agricultural systems, particularly in the context of integrated pest management (IPM).

2.6.1. Direct Mechanisms of Antagonism

Direct antagonism refers to the direct interactions between beneficial microorganisms and pathogens, where the antagonists actively inhibit or kill pathogens through physical, chemical, or biological processes. These mechanisms include the production of antimicrobial compounds, competitive exclusion, and mycoparasitism.

- **Antibiotic Production**: One of the most well-known and widely studied mechanisms of antagonism is the production of antibiotics by biocontrol agents. Many microorganisms, such as *Pseudomonas* spp., *Bacillus* spp., and *Streptomyces* spp., produce secondary metabolites that have antimicrobial properties. These compounds include antibiotics, enzymes, and other bioactive molecules that directly suppress pathogen growth. For example, *Pseudomonas fluorescens* produces phenazines and pyoluteorin, which are toxic to many fungal pathogens (Raaijmakers *et al*., 2002). Similarly, *Bacillus subtilis* synthesizes surfactin and bacillomycin, which have antifungal properties. The production of antibiotics not only hinders the growth of pathogens but also reduces their ability to infect plants.
- **Production of Hydrolytic Enzymes**: Hydrolytic enzymes such as chitinases, glucanases, and proteases are secreted by antagonistic microorganisms to break down the structural components of pathogenic cell walls. This mechanism is especially effective against fungal pathogens. *Trichoderma* species, for example, are well-known for producing chitinase and glucanase enzymes that degrade the fungal cell wall, leading to pathogen

death (Benitez *et al*., 2004). These enzymes specifically target components like chitin, glucans, and proteins, which are vital for the integrity of fungal cells. Similarly, *Bacillus* species can secrete proteases and lipases that degrade the cell membranes of pathogens, rendering them inactive.

- **Antibiosis and Volatile Organic Compounds (VOCs)**: Antagonistic microorganisms, particularly fungi and bacteria, can produce volatile organic compounds (VOCs) that have antimicrobial properties. VOCs can diffuse through the soil and inhibit the growth of pathogens, either by directly affecting the pathogen or by inducing stress responses that make pathogens more susceptible to other environmental factors. *Trichoderma* species, for example, release VOCs that not only suppress the growth of fungal pathogens but also promote plant growth (Compant *et al*., 2005). These VOCs can create a protective barrier around plant roots, effectively reducing the likelihood of pathogen infection.
- **Toxin Production**: Some antagonistic microorganisms produce specific toxins that can directly damage pathogens. For instance, *Fusarium* species can produce trichothecenes, which inhibit the growth of competing fungi by interfering with their protein synthesis mechanisms (Ghisalberti, 2005). Such toxins can be highly effective in reducing pathogen populations in the rhizosphere or soil environment, thus providing a competitive edge to the antagonistic microorganism.

2.6.2. Indirect Mechanisms of Antagonism

Indirect mechanisms of antagonism refer to the ways in which antagonistic microorganisms influence their environment, creating conditions that are unfavorable for pathogen growth, or

promoting plant health through ecological interactions. These mechanisms include competition for resources, induced systemic resistance (ISR), and the alteration of microbial communities in the soil.

- **Competition for Nutrients and Space**: In the soil environment, microorganisms compete for nutrients and physical space. Antagonistic microorganisms can outcompete pathogenic organisms for resources such as carbon, nitrogen, and other growth factors. This competition can limit the ability of pathogens to establish themselves in the rhizosphere. For example, *Bacillus subtilis* and *Pseudomonas fluorescens* are known to aggressively colonize the root surfaces of plants, occupying available niches and thereby preventing pathogenic microbes from establishing themselves (Mazzola, 2002). The ability to form biofilms, dense microbial communities that adhere to plant roots, enhances the antagonists' competitive advantage, ensuring long-term protection against pathogen colonization.
- **Induced Systemic Resistance (ISR)**: Another important indirect mechanism is the induction of systemic resistance in plants. When beneficial microorganisms colonize plant roots, they can trigger a plant's defense mechanisms, making it more resistant to subsequent pathogen attacks. This phenomenon, known as induced systemic resistance (ISR), involves the activation of a plant's immune system, often through the production of signaling molecules like jasmonic acid, salicylic acid, and ethylene. *Trichoderma harzianum* and *Pseudomonas spp.* are examples of microorganisms that can trigger ISR in plants, leading to the enhanced production of defensive proteins such as pathogenesis-related (PR) proteins, which protect plants from pathogen infection (Harman, 2006). This

form of resistance is long-lasting and can provide broad-spectrum protection against a variety of pathogens.

- **Mycoparasitism**: Mycoparasitism refers to the parasitic relationship between an antagonistic microorganism and a fungal pathogen. Certain biocontrol fungi, such as *Trichoderma* and *Coniothyrium minitans*, actively parasitize and kill pathogenic fungi by growing on their hyphae and feeding on their tissues. This process involves the formation of specialized structures, such as appressoria or hyphal coils, which allow the antagonist to penetrate and degrade the fungal pathogen (Djonović *et al*., 2007). Mycoparasitism is an effective strategy for controlling plant diseases caused by soil-borne fungal pathogens, as it reduces the pathogen population in the soil and prevents the spread of infection to plants.
- **Production of Plant Growth-Promoting Substances**: Many antagonistic microorganisms, particularly *Pseudomonas* and *Azospirillum*, produce plant growth-promoting substances such as phytohormones (e.g., auxins, cytokinins, and gibberellins). These compounds can stimulate plant growth, improving plant vigor and enhancing its ability to resist pathogens. For example, *Pseudomonas spp.* produce IAA (indole-3-acetic acid), which promotes root elongation and increases nutrient uptake, thereby enhancing plant growth and providing a stronger defense against pathogens. Furthermore, some biocontrol agents help in nitrogen fixation, enriching the soil and improving plant health, which indirectly reduces the vulnerability of plants to pathogens (Compant *et al*., 2005).

2.6.3. Synergistic Interactions Between Mechanisms

In many cases, antagonistic microorganisms employ multiple mechanisms that work synergistically to enhance their overall biocontrol potential. For instance, *Trichoderma* spp. may produce antimicrobial compounds, secrete hydrolytic enzymes, and engage in mycoparasitism, all of which combine to make the organism highly effective against a wide range of plant pathogens (Benitez *et al*., 2004). These synergistic effects are often enhanced when microorganisms form biofilms, which provide a physical barrier against pathogens and protect the antagonists from environmental stresses such as desiccation or nutrient scarcity. The ability to produce volatile organic compounds (VOCs) further complements these mechanisms, allowing the biocontrol agent to spread its protective effect across a larger area, creating an effective zone of inhibition.

2.7. Environmental Factors Affecting Antagonism

Antagonism among microorganisms is a phenomenon where one microorganism inhibits or adversely affects the growth and survival of another. This interaction is critical in agricultural soil ecosystems, where antagonistic microorganisms play a pivotal role in biological control, suppressing plant pathogens, and promoting plant health. Various environmental factors significantly influence the expression of antagonism in agricultural soils, affecting the efficacy of biocontrol agents and the overall microbial ecology.

2.7.1. Soil pH

The pH of the soil is one of the most critical factors affecting microbial antagonism. It determines the solubility of nutrients, the availability of ions, and the metabolic activity of

microorganisms. Antagonistic microorganisms, such as *Bacillus spp.*, produce metabolites like antibiotics that are sensitive to pH changes. For instance, acidic soils may suppress the production of certain antibiotics, while alkaline conditions may enhance their activity (Mazzola, 2004). The balance between bacterial and fungal antagonists is also influenced by soil pH, with fungi often thriving in acidic conditions and bacteria predominating in neutral to slightly alkaline soils.

2.7.2. Soil Moisture Content

Moisture levels directly impact the mobility of nutrients and the metabolic processes of soil microorganisms. Antagonistic interactions are more pronounced under optimal moisture conditions, as extreme dryness or waterlogging can inhibit microbial growth. Moisture also influences the dispersal of antagonistic microbes and the diffusion of antimicrobial compounds in the soil matrix (Kokalis-Burelle *et al*., 2002). For example, *Pseudomonas spp.* and *Trichoderma spp.*, common biocontrol agents, exhibit reduced antagonistic activities under drought stress conditions.

2.7.3. Temperature

Temperature affects the growth rate, enzymatic activity, and survival of antagonistic microorganisms. Most biocontrol agents exhibit optimal activity within a narrow temperature range, often corresponding to mesophilic conditions (20–30°C). Fluctuations outside this range can impair the production of secondary metabolites, such as antibiotics or antifungal compounds. For example, the production of iturin by *Bacillus subtilis* is significantly reduced at temperatures

above 35°C (Duffy *et al*., 1997). Temperature also affects pathogen survival, indirectly modulating antagonism by altering the host-pathogen balance.

2.7.4. Soil Nutrient Availability

The availability of nutrients, particularly carbon and nitrogen, plays a pivotal role in shaping antagonistic interactions. Competition for nutrients is a key mechanism of antagonism. Microorganisms like *Trichoderma spp.* and *Streptomyces spp.* thrive in nutrient-rich environments, where they outcompete pathogens by rapidly consuming available resources and producing antimicrobial compounds (Vinale *et al*., 2008). Conversely, nutrient-poor soils may reduce antagonistic activity as microbial growth and metabolite production are constrained.

2.7.5. Organic Matter Content

Organic matter enhances microbial diversity and activity in soil by providing a steady supply of nutrients and creating favorable conditions for microbial growth. High organic matter content fosters the proliferation of antagonistic microorganisms by supplying substrates for energy production and secondary metabolite synthesis. Additionally, the decomposition of organic matter generates volatile organic compounds (VOCs) that may have antimicrobial properties, further influencing antagonistic interactions (Whipps, 2001).

2.7.6. Soil Aeration

Oxygen availability is a crucial determinant of microbial activity in soil. Aerobic microorganisms, including many biocontrol agents such as *Pseudomonas spp.* and *Bacillus spp.*, rely on adequate aeration for growth and metabolite production. Poorly aerated soils, often

caused by compaction or waterlogging, limit the activity of aerobic antagonists, favoring facultative or obligate anaerobic pathogens instead (Weller *et al*., 2002).

The presence and abundance of other microbial species in the soil can influence antagonistic interactions. High microbial diversity typically enhances competition, leading to stronger antagonism against pathogens. Conversely, monocultures or reduced diversity may allow pathogenic microorganisms to dominate, reducing the effectiveness of biocontrol agents (Mendes *et al*., 2011).

2.8. Challenges in the Application of Biocontrol Agents

Biological control (biocontrol) has emerged as an environmentally sustainable alternative to chemical pesticides in agricultural practices. It involves the use of natural organisms such as bacteria, fungi, and nematodes to suppress plant pathogens, thus reducing the reliance on chemical interventions. Despite the promising advantages of biocontrol agents (BCAs), several challenges hinder their widespread application and commercialization in agriculture. These challenges are multifaceted, involving biological, ecological, technical, and economic factors, which must be addressed to enhance the effectiveness of BCAs.

One of the key challenges in biocontrol is the host specificity of the biocontrol agents. Many BCAs are highly effective against a limited range of pathogens, which restricts their broader applicability in diverse cropping systems (Singh *et al*., 2017). For example, a BCA that works well against a particular strain of a pathogen may not be effective against other variants, limiting its use in areas with varying pathogen populations. The efficacy of BCAs can also be influenced by environmental conditions, such as temperature, humidity, and soil pH, which may reduce their performance in the field (Mazzola *et al*., 2018).

The stability of biocontrol agents in the field is another critical challenge. Most BCAs are sensitive to environmental stressors such as UV radiation, drought, and soil acidity, which can significantly affect their survival and activity (Borriss, 2015). For instance, UV radiation can degrade microbial cells, reducing the concentration of viable BCAs in the soil. Similarly, extreme weather conditions, such as high temperatures or heavy rainfall, can interfere with the persistence of these agents. Thus, maintaining the biological activity of BCAs in dynamic field conditions is a major hurdle in their successful application. Agricultural soils contain a rich diversity of indigenous microorganisms that interact with biocontrol agents, sometimes to their detriment. These native microorganisms may outcompete the introduced BCAs for nutrients and niches, thereby diminishing their effectiveness (Chavez *et al*., 2017). In some cases, indigenous microbes might even have antagonistic effects on the BCAs themselves, undermining their ability to control pathogens effectively. Understanding and managing these complex microbial interactions is essential for improving the success of biocontrol in agriculture.

The large-scale production and formulation of BCAs pose another significant challenge. For biocontrol to be practical and cost-effective in agriculture, BCAs need to be produced in large quantities and formulated into stable products that are easy to apply. However, the production of BCAs on an industrial scale can be expensive and technically challenging, particularly for slow-growing or fastidious microorganisms (Jin *et al*., 2019). Furthermore, the formulation of biocontrol agents, including their delivery systems and compatibility with other agricultural inputs, is critical for maintaining their viability and activity during storage and application. The regulatory approval process for biocontrol agents is often time-consuming and costly. Many countries require rigorous testing and documentation of the safety, efficacy, and environmental

impact of BCAs before they can be approved for commercial use. This regulatory process can delay the introduction of new biocontrol products to the market (Wang *et al*., 2018). Additionally, there is often a lack of incentive for private companies to invest in the development of BCAs, given the competitive advantage and dominance of chemical pesticides in the agricultural industry.

Although biocontrol agents offer long-term sustainability benefits, their initial cost can be higher than chemical pesticides, making them less appealing to resource-poor farmers (Huang *et al*., 2017). The cost-effectiveness of BCAs is often influenced by factors such as the scale of production, formulation costs, and the need for repeated applications. Economic feasibility studies are essential to evaluate the long-term cost benefits of using biocontrol agents compared to conventional chemical pesticides, which are often subsidized by governments.

While biocontrol agents hold great promise for sustainable pest and disease management in agriculture, overcoming the challenges associated with their application is essential for realizing their full potential. Addressing issues such as environmental stability, host specificity, competition with indigenous microbiota, and cost-effectiveness will require interdisciplinary efforts in research, development, and policy-making. By improving the production, formulation, and regulatory processes, as well as enhancing farmer education, biocontrol agents can become a key component of integrated pest management systems, contributing to the reduction of pesticide use and promoting environmentally sustainable agriculture.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area/Sample Collection

The study was carried out in the Benin metropolis, where soil samples were obtained from Ugbowo axis. Samples were transported to the laboratory for analysis in sterile plastic Ziplock bags.

3.2 Sterilization of Materials

Materials such as Petri dishes, pipettes, glass containers (conical flask, round bottom flask), and bottles were washed, drained, and dried. They were wrapped with aluminum foil and sterilized in a hot-air oven at 160oC for an hour. They were allowed to cool after sterilization before usage. An aseptic working environment was achieved with the use of a Bunsen burner flame and the disinfection of work surfaces with alcohol.

3.3 Preparation and Sterilization of Media

All media used were obtained from Oxoid and were prepared according to manufacturers' instructions. The media used in this study include Nutrient agar, Bacillus cereus agar (BCA), eosin methylene blue agar (EMB), violet red bike glucose (VRBGA), hectoen enteric agar, triple sugar iron agar (TSI), Simmons citrate agar (SCA).

3.4 Enumeration and Isolation of Bacterial from Samples

A ten thousand fold dilution was used for the analysis where the samples were diluted by mixing 10 g of soil with 90ml of sterile saline water (SSW). After that, an inoculum volume of 1 ml from the tube was transferred to the next tube containing 9ml of water and continued till the 3rd test tube and 0.1ml was added from the last test tube to the petri dish, to which nutrient agar was added (supplemented with 1% fluconazole). Replicates of samples were prepared, and the pour plate technique was employed for the isolation and enumeration of heterotrophic and coliform bacteria (cultured on VRBGA or EMB plates). All samples were diluted using a ten thousand-fold, and a volume of 0.1 ml was plated. The formula employed for the dilution factor is given below in equation (1)

Enumeration of the bacterial isolates was carried out using the formula delineated by Willey et al. (2008), and it is shown in equation (2) below.

3.5 Phenotypic Identification of Bacteria

Pure cultures of the bacterial isolates were obtained from the subculture of a single colony and were characterized using cultural, morphological and biochemical methods. Several tests, such as Gram reaction, catalase, urease, indole, oxidase, citrate utilization and respective reactions of bacteria on triple sugar iron agar, were carried out to identify bacterial isolates presumptively (Holt et al., 1994).

3.6 Gram Staining Test

A Gram staining test was carried out to determine the presence of Gram-positive and Gram-negative isolates. Neat, grease-free and sterile-dried microscope slides with labels were smeared using a sterilized loop, and the organism was air-dried and heat-fixed over a flaming Bunsen burner. The fixed smear was saturated with drops of crystal violet, left for one minute to react and washed off with distilled water. Lugol's iodine, which serves as a mordant, was added, left for one minute, and washed off with distilled water. The smear was decolorized by flooding with 95% ethyl alcohol, went for 30 seconds and washed off with distilled water. It was counter-stained with safranin solution for one minute and then rinsed with distilled water. Lastly, the smear was allowed to air dry, and immersion oil was added for a microscopic view using an immersion objective lens light microscope. Colours, shapes, and arrangements were observed. Gram-positive organisms maintained the crystal violet's purple colour, while Gram-negative retained the pink colour of safranin.

3.7 Biochemical Tests

To better characterize these isolates, biochemical tests were conducted, which included:

3.7.1 Indole Test

An indole test was carried out to demonstrate the ability of certain bacteria to decompose amino acid tryptophane into indole. Several drops of Kovac's indole reagent were placed on a filter paper. A portion of a pure isolated colony was picked with an inoculating loop and smeared onto the reagent-saturated area of the filter paper. It was allowed to be examined and observed for colour development within 2 - 3 minutes. In this spot test, indole combined with the reagents in

the filter paper matrix produced a blue-to-blue-green colour change on the bacterial smear, and adverse reactions remained colourless or light pink.

3.7.2 Oxidase Test

The oxidase test was carried out to detect the presence of a cytochrome oxidase or indophenol oxidase that will catalyze electron transfer between electron donors in the bacteria and a redox dye known as tetramethyl-p-phenylene-diamine. The dye would be reduced to a deep purple colour if yielded to positive reactions.

Several reagents can be used for this study, but Kovacs oxidase reagent, 1% tetra-methyl-p-phenylenediamine dihydrochloride in water, was used. The filter paper was saturated with a Kovacs oxidase reagent solution, and a speck of the pure culture was smeared on it with a platinum loop. Colour development was allowed and observed within 10 - 60 seconds. The appearance of a deep purple-blue/blue colour indicated oxidase production, and the negative result was when no colour changed.

3.7.3 Catalase Test

This test was used to distinguish between bacteria that produce the catalase enzyme, such as Staphylococci, and bacteria that do not, such as Streptococci. Catalase catalyzes the breakdown of hydrogen peroxide (H_2O_2) to oxygen (O_2) and water (H_2O). In this test, 2mL of hydrogen peroxide solution was poured into a test tube, and some colonies of the test organism were picked and immersed into the H_2O_2 solution using a sterile glass rod. The bacteria that generated catalase (positive result) produced gas bubbles (oxygen), but those that did not possess catalase enzyme had none (negative result).

3.7.4 Citrate Utilization Tests

The citrate utilization test is a part of the test used to differentiate organisms on their ability to utilize citrate as the primary energy source. Simon's citrate agar contained citrate as an energy source and was prepared for injection on slants. Well-prepared and sterilized citrate agar slants were inoculated from the pure isolated culture by streaking the surface with a sterilized loop. The plates were then incubated at 37°C for 24 hours. There were changes in colour due to bacterial growth of the organisms on the medium due to citrate metabolism, which gave a positive citrate test. The shift in pH turns the bromothymol blue indicator in the medium from green to blue (positive result).

3.7.5 Urease Test

The urease test is used to identify bacteria capable of producing the urease enzyme. The organisms that secrete urease can hydrolyze urea to ammonia and carbon dioxide. This test was used to distinguish urease-positive bacteria from other Enterobacteriaceae. The isolated pure bacteria were inoculated into well-prepared and autoclaved Christensen-modified urea broth and incubated for 24 hours at 37°C. Urease-positive cultures produced a pink colour due to a change in the indicator's colour in the presence of ammonia. At the same time, the negative result remains no colour change or yellow-orange colour.

3.7.6 Triple Sugar Iron Agar (TSI) Test

TSI test is a biochemical test used to identify and differentiate bacteria based on their ability to ferment sugars, produce gas and produce hydrogen sulphide (Islam et al., 2014). The TSI test

contains three sugars (glucose, lactose and sucrose), along with a pH indicator (phenol red) and ferrous sulphate to detect the production of hydrogen sulphide.

Procedure:

TSIA medium was inoculated by streaking the selected organism on the agar slant in a test tube and stabbing the agar deep into the medium with a sterile inoculating loop. Slants were incubated at 37℃ for 18-24hrs. After the incubation period, the medium was observed for any visible changes in colour, gas production and growth patterns.

Examination for Sugar fermentation: observe for a colour change in the medium. TSIA agar contains three sugars (glucose, lactose and sucrose). If all three sugars are fermented, acid end products are produced, causing the medium to turn yellow (slant and butt), while if only glucose is fermented, alkaline end products are produced, causing the medium to turn red(slant) and yellow (butt). No change in colour indicates no fermentation of the sugars.

Examination for Gas production: observe for the presence of gas in the agar. Fermentation of sugar produces gas, a by-product. If gas is produced, there will be cracks or liftings of the agar. No presence of gas production indicates no fermentation of sugars.

Examination for Hydrogen sulphide: observe for the formation of black precipitate in the agar. Some bacteria can produce hydrogen sulphide gas by breaking down sulfur-containing amino acids. This gas reacts with the iron salts present in the medium.

3.8 Growth on Differential Media

3.8.1 Hektoen Enteric Agar (HEA)

Hektoen Enteric Agar (HEA), also known as HE agar or HEK agar, is a selective and differential agar used in microbiology laboratories to isolate and differentiate enteric pathogens, particularly Salmonella and Shigella, from fecal samples, food, and water suspected of containing these organisms. HEA inhibits the growth of most Gram-positive bacteria and other non-enteric Gram-negative bacteria due to the presence of bile salts and dyes. HEA allows for the differentiation of lactose-fermenting and non-fermenting enteric bacteria based on a pH indicator and the presence of additional carbohydrates. Lactose-fermenting colonies appear yellow or salmon-coloured due to acid production from lactose fermentation. Examples include some strains of E. coli. The non-fermenting colonies remain blue-green due to no significant pH change. Salmonella and Shigella fall into this category. H2S-producing colonies may have a black precipitate around them, indicating H2S production from the reduction of thiosulfate. Some Salmonella spp. can produce H2S. The medium is prepared by weighing 76g in 1 litre and soaked for 10 minutes. It is heated gently and allowed to boil for a few minutes to dissolve the agar. It is not advisable to autoclave as it could destroy some sensitive components of the medium.

3.8.2 Violet Red Bile Glucose Agar (VRBA)

This is a glucose-containing selective medium for the detection and enumeration of Enterobacteriaceae in food products. It is used for the detection and enumeration of bile-tolerant Gram-negative bacteria in food, water and other materials of sanitary importance. This medium complies with the recommendations of the harmonized method in the United States

Pharmacopoeia (USP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP). VRBA plates are prepared following the manufacturer's specification, and the medium is prepared by boiling (without autoclaving) until a homogenous solution is obtained.

3.8.3 Eosin Methylene Blue (EMB) Agar

Eosin Methylene Blue (EMB) agar is a differential medium that inhibits the growth of Gram-positive bacteria and is used to indicate Gram-negative pathogenic enteric bacteria by distinguishing between organisms that ferment lactose and those that cannot cope with a colour indication. A sterile petri plate was prepared with EMB, which was autoclaved at 121°C for 15 minutes, allowed to cool and inoculated with pure inoculums by streaking. Inoculated plates were incubated at 37°C for 24 hours and examined plates for colonial morphological changes. Lactose fermenting bacteria produced dark colonies with green metallic sheen or pink mucoid colonies (positive result), and lactose non-fermenters were colourless (negative result).

3.9 Data Analysis

Analysis of variance (ANOVA) and Dunnet's method was employed for data evaluation; $p<0.05$ was taken as statistically significant. The software package (SPSS v16) was used for data analysis.

CHAPTER FOUR

4.0. RESULTS

The results for the total heterotrophic bacterial counts obtained from the soil samples collected from various locations in Benin City are shown in Table 1 below. The counts ranged from 1.34 ± 0.37 to 2.48 ± 0.85 ($x10^4$ cfu/ml). The highest counts were observed in the Capitol Area with 2.48 ± 0.85 ($x10^4$ cfu/ml), while the lowest count was obtained from the Blocks of Flat sample with 1.34 ± 0.37 ($x10^4$ cfu/ml).

Table 4.2. present the cultural, morphological and biochemical characteristics of the isolated bacteria from the soil sample. The biochemical tests conducted include indole, urease, citrate, lactose, maltose, xylose, glucose, mannitol, gas production, H_2S and spore test. Nine (9) bacteria isolates were identifield which include*, Pseudomonas aeruginosa, E. coli, Enterobacter aerogenes* and *Bacillus subtilis.*

Table 4.3. presents the distribution of coliforms from agricultural farmlands or soil samples collected from various locations in Benin City. Bacillus subtilis was present in all the locations: Blocks of Flat, VC Quarters, Capitol Area, UDSS1, and UDSS2. Pseudomonas aeruginosa was found in VC Quarters, UDSS1, and UDSS2, but was absent in Blocks of Flat and Capitol Area. Escherichia coli was present in all locations: Blocks of Flat, VC Quarters, Capitol Area, UDSS1, and UDSS2. Enterobacter aerogenes was found in Blocks of Flat, Capitol Area, and UDSS1, but was absent in VC Quarters and UDSS2.

Figure 4.1. presents the percentage frequency of occurrence of bacterial isolates obtained from agricultural farmlands. Bacillus subtilis and Escherichia coli were the most prevalent isolates,

each accounting for 26.32% of the total isolates. Pseudomonas aeruginosa and Enterobacter aerogenes were detected at lower frequencies, each representing 15.79%.

Farmlands	Heterotrophic Counts in Standard form ($x10^4$ CFU/ml)

Table 4.1. Heterotrophic bacterial and coliform counts of soil samples

Blocks of Flat	16.40±1.70
VC Quarters	24.60±0.85
Capitol Area	24.80±4.53
UDSS1	13.80±2.55
UDSS2	13.40±3.68

Table 4.2 Cultural morphological and biochemical characteristics of coliforms from soil samples

Morphological				
Elevation	Flat	Raised	Flat	Flat
Margin	Undulate	Entire	Undulate	Undulate
Color	Cream	lemon	Cream	Cream
Shape	Irregular	Circular	Irregular	Irregular
Size	large	Medium	Large	Large
Gr. diff. agar	BCA	PCA	EMB	EMB
Colour	Straw	green	green	Pink
Staining				
Gram stain	+	-	-	-
cell type	Rod	rod	Rod	Rod
Arrangement	disperse	disperse	disperse	disperse
Color	purple	pink	pink	Pink
Spore staining	+	-	-	-
Biochemical				
KOH String Test	-	+	+	+
Catalase	+	+	+	+
Indole	-	-	+	-
Citrate	+	+	-	+
Oxidase	-	+	-	-
Motility	+	+	+	+
Urease	-	+	-	-
Glucose	+	-	+	+
Sucrose	+	-	-	+
Lactose	+	-	+	+
Mannitol	+	-	-	-
Gas formation	-	-	+	-
H_2S formation	-	-	-	-
TSI (Slant/Butt) reaction	A/A	K/K	A/AG	A/A(K*)G*
Esculin Hydrolysis	-	-	-	+
Identity	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Enterobacter aerogenes*

Key:

+= Present

-=Absent

Table 4.3. Distribution of coliforms from agricultural farmlands or soil samples

Isolates	Blocks of Flat	VC Quarters	Capitol Area	UDSS1	UDSS2
Bacillus subtilis	+	+	+	+	+
Pseudomonas aeruginosa	-	+	-	+	+
Escherichia coli	+	+	+	+	+
Enterobacter aerogenes	+	-	+	+	-

Key:

+= Present

-=Absent

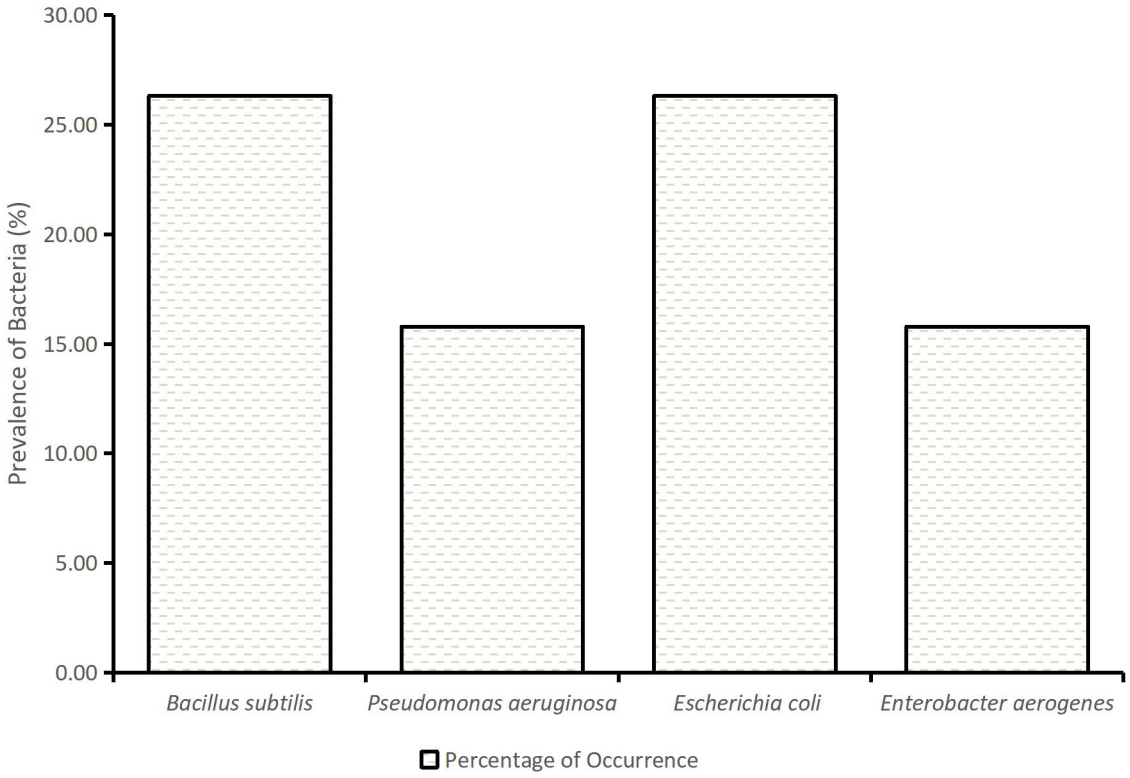

Figure 4.1. Percentage of occurrence of coliforms from soil samples in different locations

The physicochemical properties of soil samples collected from different locations in Benin City reveal notable variations across the sites. The pH values of the soils ranged from slightly acidic to neutral, with UDSS2 showing the highest pH (6.25 ± 0.23), indicating a more alkaline nature compared to other locations. Electrical conductivity (EC) was highest in VC Quarters (611.10 ± 30.56 µS/cm), suggesting a higher concentration of dissolved salts in that area.

Organic matter (OM) and organic carbon (OC) levels were highest in VC Quarters, at 3.35 ± 0.17% and 5.77 ± 0.29%, respectively, indicating relatively better soil fertility in that location. Nitrogen content was also elevated in VC Quarters (3.96 ± 0.20%), which contributes to soil fertility and plant growth. Phosphorus (P) content was higher in VC Quarters (7.85 ± 0.39 mg/kg) compared to other locations, reflecting better nutrient availability for plants.

Calcium (Ca) content peaked at Capitol Area (1.23 ± 0.06%), while potassium (K) levels were highest in VC Quarters (5.65 ± 0.28%), both essential for plant health. Magnesium (Mg) was most abundant in Capitol Area (15.08 ± 0.75%), an essential nutrient for photosynthesis. Sodium (Na) content remained low across all locations, with the highest being 0.43 ± 0.02% in VC Quarters, indicating that the soils are not saline.

The iron (Fe) content was highest in Capitol Area (446.49 ± 22.32 mg/kg), important for chlorophyll production, while zinc (Zn) was highest in Capitol Area as well (61.26 ± 3.06 mg/kg), essential for enzyme activity in plants. Lead (Pb) levels, which could pose an environmental concern, were highest in VC Quarters (3.28 ± 0.16 mg/kg), while copper (Cu) content was greatest in UDSS2 (20.70 ± 1.04 mg/kg), vital for photosynthesis and plant metabolism

Table 4.4. Physicochemical properties of the soil samples from different locations in Benin City

Parameters	Blocks of Flat	VC Quarters	Capitol Area	UDSS1	UDSS2
Ph	5.49±0.22	5.62±0.21	5.75±0.21	5.95±0.20	6.25±0.23
EC	527.80±26.39	611.10±30.56	486.50±24.33	505.40±25.27	542.88±27.14
OM	1.02±0.05	3.35±0.17	2.14±0.11	1.81±0.09	1.05±0.05
OC	1.76±0.09	5.77±0.29	3.68±0.18	3.12±0.16	1.81±0.09
P	6.83±0.34	7.85±0.39	5.36±0.27	7.20±0.36	7.02±0.35
N	2.44±0.12	3.96±0.20	2.58±0.13	2.98±0.15	2.51±0.13
Ca	0.69±0.03	0.88±0.04	1.23±0.06	0.59±0.03	0.71±0.04
K	3.57±0.18	5.65±0.28	5.53±0.28	2.93±0.15	3.67±0.18
Mg	10.00±0.50	12.34±0.62	15.08±0.75	9.63±0.48	10.28±0.51
Na	0.27±0.01	0.43±0.02	0.40±0.02	0.34±0.02	0.27±0.01
Fe	410.97±20.55	347.40±17.37	446.49±22.32	317.60±15.88	422.71±21.14
Zn	52.37±2.62	46.05±2.30	61.26±3.06	48.13±2.41	53.87±2.69
Pb	2.01±0.10	3.28±0.16	1.49±0.07	1.22±0.06	2.07±0.10
Cu	20.13±1.01	17.66±0.88	16.58±0.83	15.02±0.75	20.70±1.04

CHAPTER FIVE

5.0. DISCUSSION

Soil microbiology plays a crucial role in ecosystem stability, influencing nutrient cycling, soil fertility, and plant health. Microorganisms, particularly bacteria, contribute to soil quality through various biochemical processes, including organic matter decomposition, nitrogen fixation, and phosphate solubilization (Chen *et al*., 2024). In agricultural systems, plant growth-promoting (PGP) bacteria serve as an eco-friendly alternative to synthetic fertilizers, enhancing crop productivity while maintaining soil health. The microbial diversity in soil is largely influenced by environmental factors such as pH, organic matter content, and moisture levels, which determine the distribution and functionality of beneficial bacteria (Nannipieri *et al*., 2017). This study aimed to assess the physicochemical properties of soil from different locations in Benin City and identify bacterial isolates with PGP potential, offering insights into their possible applications in sustainable agriculture.

The total heterotrophic bacterial counts recorded in this study ranged from $1.34 \pm 0.37 \times 10^4$ CFU/ml to $2.48 \pm 0.85 \times 10^4$ CFU/ml, with the highest counts observed in the Capitol Area soil samples and the lowest in soil from the Blocks of Flat site. The variation in bacterial counts across different locations may be attributed to differences in soil physicochemical properties, such as organic matter content, pH, and moisture levels, all of which significantly influence microbial proliferation (Nannipieri *et al*., 2003; Sylvia *et al*., 2005).

Soils rich in organic matter generally support higher microbial populations due to the increased availability of nutrients and favorable growth conditions. In contrast, soils with low organic content or poor aeration may restrict microbial diversity and abundance (Jastrow *et al*., 2007).

The relatively high bacterial counts observed in some locations suggest that these soils may serve as reservoirs for beneficial microbes, including PGP bacteria that can enhance soil fertility and crop productivity (Vessey, 2003).

A total of nine bacterial isolates were identified from the different soil samples, including *Pseudomonas aeruginosa, Escherichia coli, Enterobacter aerogenes,* and *Bacillus subtilis*. The presence of these bacterial species in soil is well documented, and several of them have been reported to possess plant growth-promoting properties (Lugtenberg and Kamilova, 2009; Compant *et al*., 2010).

Among the isolates, *Bacillus subtilis* is particularly significant due to its well-established role in nitrogen fixation and phosphate solubilization. It has been widely used in biofertilizers because of its ability to enhance nutrient availability for plants (Ahemad and Kibret, 2014). Similarly, species of *Enterobacter* have been associated with the production of indole-3-acetic acid (IAA), a phytohormone known to promote root elongation and overall plant growth (Patten and Glick, 2002). The presence of these bacteria in the soil samples analyzed suggests their potential usefulness in sustainable agriculture by naturally improving soil fertility and reducing dependence on synthetic fertilizers (Bashan *et al*., 2004).

The distribution analysis of bacterial isolates across the different soil samples revealed that *Bacillus subtilis* and *Escherichia coli* were the most prevalent, each accounting for approximately 26.32% of the total isolates. The widespread occurrence of *Bacillus subtilis* across multiple locations highlights its adaptability to diverse soil conditions and its potential significance in enhancing soil health and promoting plant growth (Kloepper *et al*., 2004).

On the other hand, the presence of *Escherichia coli* in soil, while often linked to fecal contamination, can also be associated with its role in organic matter decomposition and nutrient cycling within the soil ecosystem (Ishii and Sadowsky, 2008). This suggests that its occurrence in these soils may be indicative of a complex microbial community contributing to soil health and fertility. The high prevalence of these bacterial isolates underscores the importance of further exploring their beneficial attributes in agricultural applications (Lugtenberg *et al*., 2001).

The physicochemical analysis of the soil samples revealed notable variations across the different locations. Soil from the VC Quarters exhibited the highest levels of organic matter (3.35 ± 0.17%) and organic carbon (5.77 ± 0.29%), indicating a relatively high soil fertility in this area. Organic matter plays a crucial role in maintaining soil structure, enhancing water retention, and providing essential nutrients for microbial activity and plant growth (Bot and Benites, 2005).

The pH values of the soil samples ranged from slightly acidic to neutral, with the highest pH recorded in the UDSS2 location (6.25 ± 0.23). Soil pH is a critical factor influencing microbial activity and nutrient availability. Most plant growth-promoting bacteria, including nitrogen-fixing and phosphate-solubilizing bacteria, thrive in neutral to slightly acidic conditions (Richardson *et al*., 2009). The findings from this study suggest that the soil conditions in these locations are conducive to the proliferation of beneficial microorganisms that support plant growth (Hayat *et al*., 2010).

The identified bacterial isolates exhibited several plant growth-promoting traits that can be leveraged to improve agricultural productivity. These include nitrogen fixation, phosphate solubilization, production of indole-3-acetic acid (IAA), and ammonia production (Vessey, 2003; Bashan and de-Bashan, 2010).

Nitrogen is an essential nutrient required for plant growth, but its availability in the soil is often limited. Certain bacteria, such as *Bacillus subtilis*, have the ability to fix atmospheric nitrogen, converting it into forms that can be readily utilized by plants. This process reduces the need for synthetic nitrogen fertilizers, making it an environmentally sustainable alternative for enhancing crop productivity (Kennedy *et al*., 2004). Phosphorus is another critical nutrient for plant development, yet it often exists in insoluble forms in the soil. Bacteria such as *Enterobacter aerogenes* have been reported to solubilize phosphate, making it more accessible to plants (Rodríguez and Fraga, 1999). This ability enhances root development and improves nutrient uptake, which is essential for healthy plant growth (Gyaneshwar *et al*., 2002). The phosphate-solubilizing potential of the isolates identified in this study indicates their suitability for use in biofertilizer formulations.

IAA is a key phytohormone involved in plant growth regulation, particularly in root elongation and branching. The production of IAA by bacterial isolates such as *Enterobacter* species stimulates root development, enhancing the plant's ability to absorb water and nutrients from the soil (Spaepen *et al*., 2007). Ammonia production is another important trait exhibited by some of the bacterial isolates. Ammonia contributes to soil nitrogen availability, which is crucial for plant metabolism and growth. The ability of these bacteria to produce ammonia suggests their potential role in enhancing soil fertility and supporting plant health (Bhardwaj *et al*., 2014).

The identification of soil bacteria with plant growth-promoting properties in this study highlights their potential for use as biofertilizers in sustainable agriculture. Utilizing these beneficial microbes can enhance soil fertility, improve crop yield, and reduce dependence on chemical fertilizers, which can have adverse environmental effects (Adesemoye *et al*., 2009).

Given the increasing need for sustainable farming practices, the application of native PGP bacteria offers an eco-friendly approach to improving soil health. Further research should focus on field trials to assess the efficacy of these bacterial isolates under various crop and environmental conditions. Additionally, exploring their synergistic interactions with other soil microorganisms may provide further insights into optimizing their use in agriculture (Compant *et al*., 2010).

5.1. Conclusion

This study underscores the diversity and potential of indigenous soil bacteria in promoting plant growth. The identified isolates exhibit multiple PGP traits, including nitrogen fixation, phosphate solubilization, IAA production, and ammonia production. These findings suggest promising avenues for enhancing crop productivity and soil health in Benin City and similar agro-ecological regions. Integrating these beneficial microbes into agricultural practices can contribute to sustainable food production and environmental conservation, ultimately supporting global efforts toward sustainable agriculture.

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