ISOLATION OF PLANT GROWTH PROMOTING BACTERIA SPECIES AND PHYSICOCHEMICAL PROPERTIES OF SELECTED AGRICULTURAL FARMLAND IN OLUKU VILAGE EDO STATE, NIGERIA.

BY

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UNIVERSITY OF BENIN

BENIN CITY.

FEBUARY, 2025

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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 **Precious Osasunwen AGBONTAEN** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

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DR. A.G. OGOFURE **DATE**

(Project Supervisor)

APPROVAL

This project work was carried out by **Precious Osasunwen AGBONTAEN** 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.

I extend my heartfelt gratitude to **PROF. (MRS.) F. I. AKINNIBOSUN**, the Head of the Department of Microbiology, for her exemplary leadership and support. I also appreciate all the staff members of the Department of Microbiology for their assistance, dedication, and commitment to my academic development.

A special and heartfelt thank you goes to my beloved mother, **MR** and **MRS AGBONTAEN** whose unwavering love, prayers, and sacrifices have been the bedrock of my success. Her words of encouragement and boundless support have been my inspiration throughout this journey. I am also immensely grateful to my siblings and my aunty **MRS VICTORIA EMMANUEL** for their constant love, care, and encouragement, which have motivated me to strive for excellence.

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.

TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

Soil health plays a crucial role in agricultural productivity, influencing microbial diversity, nutrient availability, and overall plant growth. The presence of plant growth-promoting bacteria (PGPB) in soil enhances crop yield by facilitating nitrogen fixation, phosphate solubilization, and the production of essential phytohormones This study focused on the isolation of PGPB from selected farmlands in Oluku and an assessment of the physicochemical properties of the soils. Bacterial counts varied across the farms, with the highest recorded in Farm 8 ($40.60 \pm 5.94 \times 10^4$ CFU/ml) and the lowest in Farm 6 ($11.20 \pm 0.57 \times 10^4$ CFU/ml). Nine bacterial species were identified, including *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Enterobacter aerogenes,* and *Bacillus pumilus.* Among these, *E. coli* exhibited all four key plant growth-promoting traits: nitrogen fixation, phosphate solubilization, ammonia production, and indole-3-acetic acid (IAA) production, making it the most versatile isolate. The physicochemical analysis of soil samples revealed pH values ranging from slightly acidic to nearly neutral (5.64–6.42). Electrical conductivity, organic matter, and organic carbon contents varied significantly, with Farm 2 exhibiting the highest values (EC: 873.00 ± 43.65 µS/cm, OM: 4.78 ± 0.24%, OC: 8.24 ± 0.41%). Essential nutrients such as phosphorus, nitrogen, potassium, calcium, and magnesium were also found in varying concentrations across the farmlands. Heavy metal analysis indicated the presence of iron (453.72–637.84 mg/kg), zinc (65.79–87.52 mg/kg), lead (2.87–4.69 mg/kg), and copper (21.45–28.75 mg/kg), with potential implications for soil quality and crop safety. These findings highlight the significance of beneficial soil bacteria in improving plant growth and emphasize the need for sustainable soil management practices. Regular monitoring of soil nutrients and heavy metal levels is essential for maintaining soil fertility and ensuring safe agricultural production.

CHAPTER ONE

INTRODUCTION

1.1.BACKGROUND OF THE STUDY

The agricultural sector remains fundamental to human survival and global economic stability, serving as a source of food, raw materials, and employment for billions worldwide. In many developing nations, agriculture is not only a means of livelihood but also a driver of socio-economic development (Mozumdar, 2012). However, this vital sector faces increasing pressure to meet the demands of a growing global population, projected to exceed 9 billion by 2050 (FAO, 2017). This population growth necessitates a corresponding increase in food production, but traditional agricultural practices often rely on methods that are unsustainable in the long term. Issues such as climate change, land degradation, loss of biodiversity, and over-reliance on chemical fertilizers and pesticides have exacerbated the fragility of agricultural ecosystems, calling for innovative and sustainable approaches to farming (Abebaw, 2019).

Plant growth-promoting bacteria (PGPB) have emerged as a viable alternative for enhancing agricultural productivity while maintaining environmental sustainability (Naik *et al*., 2019). These naturally occurring soil microorganisms enhance plant growth through various mechanisms, including improving nutrient availability, producing phytohormones, and providing protection against plant pathogens (Olanrewaju *et al*.,2017). The integration of PGPB into

agricultural practices represents a promising step toward addressing some of the most pressing challenges in modern agriculture (Naik *et al*., 2019)..

Plant growth-promoting bacteria (PGPB) are beneficial microorganisms that form intricate relationships with plants, primarily in the rhizosphere the region of soil that interacts directly with plant roots (Olanrewaju *et al*.,2017). This interaction is not merely incidental; it is critical for the nutrient uptake, growth, and overall health of plants. According to Vessey (2003), Plant growth-promoting bacteria (PGPB) directly promote plant growth by producing essential phytohormones such as auxins, cytokinins, and gibberellins, which influence root elongation and nutrient absorption. Additionally, Plant growth-promoting bacteria (PGPB) indirectly enhance plant health by inducing systemic resistance against pathogens, reducing the impact of abiotic stress factors like drought and salinity, and improving soil structure and fertility.

The soil microbiome, a diverse community of microorganisms within the soil ecosystem, plays a pivotal role in determining the efficacy of Plant growth-promoting bacteria (PGPB). Soil characteristics such as pH, temperature, organic matter content, and moisture levels influence the activity and survival of PGPB populations (Glick, 2012). For example, nitrogen-fixing bacteria such as *Rhizobium* thrive in specific soil pH ranges, while phosphate-solubilizing bacteria like *Pseudomonas* require adequate moisture to function effectively. These interactions underscore the importance of understanding local soil conditions when applying Plant growth-promoting bacteria (PGPB) in agricultural settings.

The integration of Plant growth-promoting bacteria (PGPB) into farming systems aligns with global efforts to reduce dependence on chemical fertilizers. Excessive use of synthetic fertilizers has led to significant environmental challenges, including eutrophication, greenhouse gas

emissions, and soil degradation (Tilman *et al*., 2002). In contrast, Plant growth-promoting bacteria (PGPB)-based biofertilizers offer an eco-friendly alternative by naturally enhancing nutrient availability and promoting sustainable agricultural practices.

Benin City, located in Edo State, Nigeria, offers a unique environment for studying Plant growth-promoting bacteria (PGPB) due to its tropical climate and diverse agricultural activities. The region is known for the cultivation of staple crops such as maize, cassava, and vegetables, which form the backbone of local food security and economic development. Despite the region's agricultural potential, declining soil fertility and limited access to chemical inputs remain significant challenges for smallholder farmers. Research into the microbial diversity of soils in Benin City could pave the way for developing biofertilizers tailored to local farming needs, as emphasized by Bashan *et al*. (2014). Such efforts align with the broader goal of promoting sustainable agriculture in developing regions.

Agricultural sustainability is under significant threat due to the growing global demand for food production. While chemical fertilizers have historically been effective in boosting crop yields, their long-term use has raised serious concerns about soil health and environmental degradation. The overuse of these fertilizers disrupts soil microbial diversity, reduces soil organic matter, and leads to the accumulation of harmful chemicals in the environment (Tilman *et al*., 2002). Additionally, the runoff of excess nutrients into water bodies contributes to eutrophication, threatening aquatic ecosystems and water quality.

For smallholder farmers in Nigeria, the reliance on chemical fertilizers is further complicated by economic constraints. High fertilizer costs often place these essential inputs out of reach for many farmers, leading to suboptimal crop productivity and economic hardship (Bhardwaj *et al*.,

2014). In a region like Benin City, where agriculture is a primary source of livelihood, these challenges are particularly acute.

Plant growth-promoting bacteria (PGPB) present a sustainable solution to these issues. By naturally enhancing nutrient uptake, improving soil fertility, and protecting plants from diseases, Plant growth-promoting bacteria (PGPB) offer a cost-effective and environmentally friendly alternative to chemical fertilizers. Despite their potential, the adoption of Plant growth-promoting bacteria (PGPB) in Nigerian agriculture remains limited. Several barriers, including a lack of research, inadequate awareness among farmers, and limited commercialization of biofertilizers, have hindered their widespread use (Adesemoye and Kloepper, 2009).

Addressing these challenges requires a comprehensive approach that includes isolating and characterizing native PGPB strains from local soils. Locally adapted strains are more likely to thrive under specific environmental conditions and provide optimal benefits to crops (Lugtenberg and Kamilova, 2009). Research in this area could lead to the development of biofertilizers tailored to the unique soil and climatic conditions of Benin City, enhancing agricultural productivity and sustainability.

The exploration of P Plant growth-promoting bacteria (PGPB) as a tool for sustainable agriculture is timely and essential, particularly in the face of global efforts to achieve the United Nations Sustainable Development Goals (SDGs), including Zero Hunger (SDG 2) and Climate Action (SDG 13). By promoting soil health and reducing reliance on chemical inputs, PGPB-based strategies align with these goals and offer a pathway toward sustainable agricultural systems.

Benin City serves as an ideal case study for exploring the potential of Plant growth-promoting bacteria (PGPB) due to its rich agricultural heritage and the pressing need for sustainable farming solutions. Smallholder farmers in the region face numerous challenges, including declining soil fertility and limited access to affordable inputs. Research into the isolation and application of native PGPB could provide locally relevant solutions, empowering farmers to improve crop yields while protecting the environment.

1.3 Aim and Objectives

The main objective of this study is to isolate and evaluate the physicochemical analysis of plant growth-promoting bacteria from soil samples collected from selected agricultural farmlands in Oluku Vilaage, Benin City Edo State, Nigeria.

The specific objectives of this research were:

1. To isolate and identify plant growth-promoting bacteria from the agricultural farmland.
2. To determine the physicochemical properties of the soil samples, including pH, organic matter content, and nutrient levels.
3. To evaluate the plant growth-promoting traits of the isolated bacteria, such as nitrogen fixation, phosphate solubilization, and production of phytohormones.
4. To assess the potential of the isolated bacteria for use as biofertilizers in sustainable agriculture.

CHAPTER TWO

LITERATURE REVIEW

Agriculture is a cornerstone of human sustenance, providing essential resources such as food, fiber, and raw materials. However, with the global population increasing at an unprecedented rate, the demand for agricultural products has also risen, placing immense pressure on existing agricultural systems. Traditional agricultural practices, particularly the extensive use of chemical fertilizers and pesticides, have resulted in significant environmental degradation. This includes soil erosion, nutrient depletion, water contamination, and loss of biodiversity (Adesemoye and Kloepper, 2009). These issues highlight the urgent need for sustainable agricultural practices that can enhance productivity without jeopardizing environmental health.

One promising approach to achieving sustainable agriculture is through the utilization of plant growth-promoting bacteria (PGPB). These naturally occurring microorganisms have shown tremendous potential in enhancing plant growth and soil health through a variety of direct and indirect mechanisms. The use of PGPB as biofertilizers represents an eco-friendly alternative to chemical inputs, contributing to both agricultural productivity and environmental conservation.

Plant growth-promoting bacteria are a diverse group of soil microorganisms that exert beneficial effects on plant growth and development. Unlike chemical fertilizers, which supply nutrients directly to plants, PGPB facilitate natural biological processes to enhance nutrient availability and uptake. For instance, nitrogen-fixing bacteria, such as *Rhizobium* and *Azospirillum*, convert atmospheric nitrogen into ammonia, making it available to plants in a usable form (Glick, 2012). Similarly, phosphate-solubilizing bacteria release organic acids that solubilize otherwise

inaccessible phosphorus compounds, making them bioavailable to plants (Bhattacharyya and Jha, 2012).

PGPB also produce phytohormones, including auxins, gibberellins, and cytokinins, which regulate critical plant growth processes. These hormones stimulate root elongation, cell division, and shoot development, improving the overall growth and productivity of plants (Spaepen *et al*., 2007). Additionally, some PGPB produce siderophores, which are iron-chelating compounds. These siderophores improve iron uptake by plants while inhibiting the growth of plant pathogens that depend on iron for survival (Vessey, 2003).

Another critical role of PGPB lies in their ability to protect plants from environmental stressors. They can mitigate biotic stress by producing antimicrobial compounds such as antibiotics and lytic enzymes, which suppress pathogenic microorganisms. Moreover, certain strains induce systemic resistance in plants, enhancing their ability to withstand abiotic stressors like drought, salinity, and heavy metal toxicity (Lugtenberg and Kamilova, 2009). This dual role of PGPB—promoting plant growth and mitigating stress—makes them invaluable to sustainable agriculture.

The global agricultural sector faces a significant challenge in balancing the demand for increased food production with the need to preserve environmental resources. The reliance on chemical fertilizers has led to several adverse effects, including soil acidification, reduced microbial diversity, and contamination of water bodies through nutrient runoff (Adesemoye *et al*., 2008). Furthermore, the production and application of these fertilizers contribute to greenhouse gas emissions, exacerbating climate change.

Biofertilizers, particularly those based on PGPB, offer a sustainable alternative to chemical inputs. These microbial inoculants enhance soil fertility, improve plant growth, and reduce environmental pollution. For example, nitrogen-fixing bacteria not only support plant growth but also enrich the soil with residual nitrogen, benefiting subsequent crops and reducing the need for synthetic fertilizers (Glick, 2012). Similarly, phosphate-solubilizing bacteria improve the availability of essential nutrients, enhancing crop productivity without depleting soil reserves.

The adoption of biofertilizers aligns with global initiatives to promote sustainable agriculture. For instance, the United Nations Sustainable Development Goals (SDGs) emphasize the need for environmentally sustainable practices to ensure food security and combat climate change. Specifically, Goals 2 (Zero Hunger) and 13 (Climate Action) underscore the importance of reducing dependency on chemical fertilizers and adopting eco-friendly alternatives like biofertilizers (UN, 2015).

Despite the numerous benefits of PGPB, their application remains underutilized, particularly in developing countries like Nigeria. Factors such as limited research, lack of awareness among farmers, and inadequate access to biofertilizer products have hindered their widespread adoption (Bhattacharyya and Jha, 2012). Addressing these challenges requires collaborative efforts from researchers, policymakers, and agricultural stakeholders to promote the use of PGPB in farming systems.

Plant growth-promoting bacteria represent a pivotal solution to the challenges of modern agriculture. Their ability to enhance plant growth, improve soil health, and reduce environmental impact positions them as a cornerstone of sustainable agricultural practices. By integrating PGPB

into farming systems, the agricultural sector can transition toward a more sustainable and resilient future, ensuring food security for generations to come.

2.2 Concept of Plant Growth-Promoting Bacteria (PGPB)

Plant growth-promoting bacteria (PGPB) are a diverse group of microorganisms that colonize plant roots, rhizospheres, and other plant tissues, contributing positively to plant growth and development. These bacteria are beneficial to plants in multiple ways, primarily by improving nutrient availability, inducing resistance to pathogens, and enhancing plant stress tolerance (Glick, 2012). PGPB can be categorized based on their specific mechanisms of action into two broad groups: **direct** and **indirect** promoters of plant growth.

- **Direct promoters** provide growth benefits through mechanisms such as nutrient acquisition (e.g., nitrogen fixation, phosphate solubilization) and the production of phytohormones (e.g., auxins, cytokinins, and gibberellins), which directly enhance plant development.
- **Indirect promoters** help plants by reducing or mitigating environmental stresses, such as biotic stress (e.g., pathogen attacks) and abiotic stress (e.g., drought, salinity). These bacteria achieve this by producing antimicrobial compounds, inducing systemic resistance, and outcompeting pathogens for nutrients and space in the rhizosphere (Barea *et al*., 2005).

The most well-known PGPB include *Azospirillum*, *Rhizobium*, *Pseudomonas*, *Bacillus* and *Enterobacter* spp. These organisms interact with plants in ways that are beneficial for both plant growth and soil health (Lugtenberg and Kamilova, 2009). The relationship between plants and

PGPB is considered mutualistic, as bacteria benefit from plant exudates, while plants gain essential nutrients and other advantages.

2.2.1. Mechanisms of Action: Direct and Indirect Promotion of Plant Growth

PGPB promote plant growth through various direct and indirect mechanisms, each contributing to plant health and productivity. Understanding these mechanisms is essential for harnessing PGPB in agricultural practices, particularly in sustainable and organic farming systems.

2.2.1.1. Direct Promotion of Plant Growth

Direct plant growth promotion primarily occurs through the enhancement of essential nutrients and the regulation of plant physiological processes via phytohormone production**.** Key mechanisms include:

2.2.1.1.1. Nutrient Cycling and Acquisition

PGPB contribute to the mobilization and availability of essential nutrients that plants require for growth, particularly nitrogen and phosphorus. Some PGPB species, such as *Rhizobium* and *Azospirillum*, are capable of biological nitrogen fixation**,** where they convert atmospheric nitrogen (N_2) into a form that plants can use (ammonia, NH_3). This process is vital in nitrogen-deficient soils, reducing the need for synthetic nitrogen fertilizers and improving soil fertility (Vessey, 2003). Nitrogen is an essential building block for amino acids, proteins, and chlorophyll in plants, making its availability crucial for plant health and productivity.

Similarly, phosphate solubilization is another important process by which PGPB facilitate the uptake of phosphorus by plants. Certain bacteria, including *Pseudomonas* spp. can solubilize

insoluble forms of phosphate in the soil, making them more accessible to plants (Barea *et al*., 2005). Phosphorus is a key nutrient required for energy transfer, cell division, and root development in plants, and the ability to increase its bioavailability can significantly boost plant growth.

2.2.1.1.2. Production of Phytohormones

One of the most prominent mechanisms of PGPB is the production of phytohormones**,** which are naturally occurring plant growth regulators that influence various developmental processes in plants. PGPB produce several classes of phytohormones, including:

- **Auxins**: The most common auxin produced by PGPB is indole-3-acetic acid (IAA), which promotes root elongation, lateral root formation, and overall root system development (Glick and Do, 2007). This is essential for plants to establish efficient root systems for nutrient and water uptake.
- **Cytokinins**: These hormones stimulate cell division and differentiation, particularly in the shoot system, which leads to better plant establishment and increased above-ground biomass (Barea *et al*., 2005).
- **Gibberellins**: These hormones are involved in stem elongation, seed germination, and flowering, which can enhance plant size, reproduction, and stress tolerance (Vessey, 2003).

These phytohormones not only help plants grow in favorable conditions but also enhance resilience under stress, contributing to overall plant health and yield.

2.2.2. Indirect Promotion of Plant Growth

In addition to direct mechanisms, PGPB can promote plant growth indirectly by protecting plants from pathogens, pests, and environmental stressors.

2.2.2.1. Biocontrol and Pathogen Suppression

PGPB can suppress plant pathogens through the production of **antimicrobial compounds**, such as antibiotics, lipopeptides, and hydrogen cyanide. For instance, *Pseudomonas* spp. produce the antibiotic 2,4-diacetylphloroglucinol (DAPG), which inhibits the growth of various soilborne pathogens, including *Fusarium* spp. and *Rhizoctonia* spp. (Maurhofer *et al*., 1992). *Bacillus* spp. produce a range of antimicrobial compounds, such as surfactin and iturin, which also show potent activity against fungal and bacterial pathogens (Loper and Henkels, 2004).

Table 2.1. Plant growth promoting bacteria and their common host plant family.

Sl. No	Family	Plant	Plant Growth-Promoting Bacteria	Reference
1	Fabaceae	Phaseolus vulgaris Mimosa pudica	*Rhizobium acidosoli, R. endophyticum, R. esperanzae, R. etli, R. hidalgoense, R. mesoamericanum, R. tropici, Acinetobacter Achromobacter* sp*., Brevibacillus* sp*., Cupriavidus* sp*.,* Ensifer sp., *Stenotrophomonas* sp., *Pseudomonas* sp., *Dyella* sp., *Bacillus* sp., *Moraxella* sp., *Rhizobium* sp.	Tapia-García *et al*. (2020)
2	Poaceae	Rice, wheat, maize, sorghum, sugarcane	*Azospirillum* sp.	Pedraza *et al*. (2020)
3	Asteraceae	Puticaria	*Bacillus cereus, Agrobacterium fabrum, Brevibacillus brevis, Bacillus subtilis, Paenibacillus, Acinetobacter radioresistant,*	ALKahtani *et al*. (2020)

Sl. No	Family	Plant	Plant Growth-Promoting Bacteria	Reference
4	Solanaceae	Artemisia annua	*Burkholderia* *Brevibacillus sp., Bacillus sp., Pseudomonas, Azospirillum, Klebsiella, Enterobacter, Alcaligenes, Azotobacter, Streptomyces* sp., *Pantoea, Bacteroides, Proteobacteria, Radiobacter* sp., *Stenotrophomonas* sp.	Husseiny *et al*. (2021)
5	Brassicaceae	Brassica oleracea	*Pseudomonas* sp., *Enterobacter, Arthrobacter* sp., *Pantoea*	Ferrari *et al*. (2023) and Gustab *et al*. (2024)
6	Crassulaceae	Echeveria laui	*Erwinia* sp., *Pantoea* sp.	Emmer *et al*. (2021)

2.2.2.1.2. Induced Systemic Resistance (ISR)

PGPB can activate induced systemic resistance **(**ISR) in plants. ISR is a defense mechanism in which plants, upon colonization by beneficial microbes, become more resistant to subsequent pathogen attacks. This response is mediated through the activation of plant defense pathways, such as the synthesis of pathogenesis-related (PR) proteins, which help combat pathogens more effectively (Lugtenberg and Kamilova, 2009). This mechanism is particularly valuable in organic and sustainable agriculture, where chemical pesticides are minimized.

2.2.2.1.3. Siderophore Production

Many PGPB produce siderophores**,** which are iron-chelating compounds that sequester iron from the environment. Iron is a critical nutrient for both plants and pathogens, and by producing siderophores, PGPB outcompete pathogenic microbes for this vital resource. This reduces pathogen growth and infection rates, thus improving plant health (Glick, 2012). Iron acquisition is a key aspect of plant immune responses, and siderophore production by PGPB enhances plant resistance to pathogen attacks.

2.2.2.4. Stress Tolerance

PGPB also help plants tolerate abiotic **stresses**, such as drought, salinity, and heavy metal toxicity. They do so by enhancing the plant's antioxidant systems, improving water retention, and influencing osmotic balance in plant tissues (Barea *et al*., 2005). The ability of PGPB to modulate plant stress responses makes them invaluable in regions prone to water scarcity, soil salinity, and other environmental challenges.

2.3. Nitrogen Fixation and Phosphate Solubilization

Nitrogen fixation by PGPB, particularly by *Rhizobium* and *Azospirillum* species, is one of the most significant contributions to plant nutrition. These bacteria form symbiotic relationships with legumes, where they fix atmospheric nitrogen into ammonia, which plants can directly assimilate. This process not only enhances nitrogen availability in the soil but also reduces the reliance on synthetic nitrogen fertilizers, which are costly and have environmental impacts (Vessey, 2003).

Phosphate solubilization is another critical process that enhances soil fertility. Many PGPB, such as *Bacillus* spp., secrete organic acids like citric and lactic acid that dissolve phosphorus from insoluble compounds, making it available to plants (Barea *et al*., 2005). Phosphorus is involved in key metabolic processes like energy transfer and root development, so its solubilization by bacteria significantly enhances plant growth and yield.

2.4. Production of Siderophores and Biocontrol Mechanisms

Siderophores are low-molecular-weight compounds that bind to iron and make it available for bacterial uptake, while depriving pathogenic microorganisms of this essential nutrient (Glick,

2012). In addition to iron chelation, PGPB often produce a variety of secondary metabolites, such as hydrogen cyanide (HCN), which possess toxic properties to many plant pathogens, thus acting as a natural biocontrol mechanism. The use of siderophores and antimicrobial compounds significantly reduces the need for chemical pesticides and fertilizers, making PGPB an essential tool for sustainable agricultural practices (Lugtenberg and Kamilova, 2009).

Plant growth-promoting bacteria (PGPB) are indispensable allies in promoting plant growth, improving soil health, and combating pathogens. Through both direct and indirect mechanisms, including nitrogen fixation, phosphate solubilization, phytohormone production, and biocontrol, these bacteria enhance crop productivity and contribute to sustainable farming practices. PGPB offer a promising approach to reducing dependence on chemical fertilizers and pesticides, leading to more environmentally friendly and economically viable agricultural systems.

2.5 Soil Microbial Ecology and Plant Growth-Promoting Bacteria (PGPB)

Soil microbial ecology plays a pivotal role in maintaining soil health, fertility, and plant growth, forming the foundation for sustainable agriculture. Within this complex environment, plant growth-promoting bacteria (PGPB) contribute significantly to plant health by enhancing nutrient uptake, facilitating disease resistance, and improving soil structure. Understanding the composition and diversity of microbial communities, as well as the factors that influence PGPB populations, is essential to harnessing the potential of these microorganisms for agricultural productivity.

2.5.1. Composition and Diversity of Microbial Communities in Agricultural Soils

Soil is a diverse habitat, hosting a myriad of microorganisms, including bacteria, fungi, archaea, viruses and actinomycetes. These microorganisms interact in complex ways that influence soil ecosystem processes, such as nutrient cycling, organic matter decomposition, and pathogen suppression. The microbial communities of agricultural soils are shaped by both natural and anthropogenic factors, including soil type, climate, land use, and agronomic practices.

Bacteria, which represent one of the most diverse groups within soil ecosystems, play a crucial role in the degradation of organic matter, nutrient cycling, and plant growth promotion. Among these, PGPB (Plant Growth-Promoting Bacteria) are of particular interest due to their beneficial effects on plant health. PGPB are typically free-living bacteria that can influence plant growth through direct or indirect mechanisms. They are commonly associated with the rhizosphere, the zone of soil surrounding plant roots, where they interact with the plant's root system to stimulate growth and development.

The diversity of microbial communities in agricultural soils can vary significantly based on several factors. Soil properties, such as texture, organic matter content, and moisture availability, determine the types of microorganisms that can thrive in a given environment. For instance, soils rich in organic matter tend to harbor a higher diversity of microbial species, including a greater number of PGPB, as these microbes utilize the organic matter for sustenance (Singh *et al*., 2018). Additionally, different agricultural practices, including crop types and management techniques, influence microbial community composition. It has been shown that the microbial diversity in soils subjected to monocropping is generally lower compared to soils with diversified crop rotations (Jousset *et al*., 2017).

The role of soil microbial diversity extends beyond the function of individual microorganisms. Higher microbial diversity in soils often correlates with enhanced ecosystem services, such as improved nutrient cycling and pathogen suppression (Van der Heijden *et al*., 2008). Thus, understanding the composition of soil microbial communities, particularly the abundance and diversity of PGPB, is crucial for developing sustainable agricultural practices that maximize plant productivity while maintaining soil health.

2.5.2. Factors Influencing PGPB Populations

Several environmental and management-related factors influence the abundance, diversity, and activity of PGPB in soils. These factors include soil physicochemical properties and agricultural practices, both of which significantly impact the health of soil microbial communities.

2.5.2.1. Physicochemical Properties of Agricultural Soils

Soil properties play a crucial role in determining the microbial activity and the overall growth of plants. They serve as the foundational support system for plant growth by influencing various ecological processes, including nutrient availability, microbial diversity, and root development. The interaction between soil characteristics and plant growth-promoting bacteria (PGPB) is particularly important in sustainable agricultural practices, as PGPB are vital for enhancing soil fertility, promoting plant growth, and protecting plants against pathogens.

The key physicochemical properties of soil that influence microbial activity and PGPB effectiveness include soil pH, organic matter content, and the availability of essential nutrients such as macronutrients and micronutrients.

2.5.2.1.1. Soil pH and Its Impact on Microbial Diversity

Soil pH is one of the most critical factors affecting both plant growth and microbial activity. It influences the solubility of nutrients and the availability of essential elements, which, in turn, affects microbial diversity and activity. The pH of soil determines the type of microorganisms that can thrive within it, as different microbes have varying pH tolerance ranges. For example, acidophilic microorganisms prefer lower pH values, while alkalophilic microorganisms thrive in more alkaline conditions (Singh and Tripathi, 2013).

A soil pH that is too high or too low can reduce the activity of plant growth-promoting bacteria (PGPB), which are often sensitive to extreme pH levels. Neutral to slightly acidic soils (pH 5.5 to 7.0) typically provide optimal conditions for the growth of beneficial microorganisms (Schreiner, 2016). For instance, PGPB such as *Rhizobium* spp. which are involved in nitrogen fixation, exhibit higher activity in soils with a pH between 6 and 7 (Kumar and Verma, 2017). In contrast, acidic soils (pH < 5.5) often limit the effectiveness of PGPB by making essential nutrients, such as nitrogen and phosphorus, less available to plants and microbes alike (Zhang *et al*., 2020).

2.5.2.1.2. Organic Matter Content as a Nutrient Source

Organic matter (OM) is another essential factor in soil health, directly influencing microbial populations and plant growth. Organic matter serves as a primary nutrient source for soil microorganisms, particularly PGPB. The decomposition of organic matter by soil microbes releases essential nutrients, such as nitrogen, phosphorus, and sulfur, in forms that are readily available for plant uptake (Rasmussen *et al*., 2018). Moreover, organic matter improves soil

structure by enhancing porosity, water retention, and aeration, which in turn supports healthy root development and microbial colonization around plant roots.

The presence of organic matter in the soil promotes the growth of beneficial bacteria, fungi, and other microorganisms that contribute to the nitrogen cycle, phosphorus solubilization, and disease suppression. Microorganisms, particularly those within the rhizosphere, form symbiotic relationships with plants, providing them with nutrients while receiving carbon compounds from plant roots (Bashan *et al*., 2014). A soil rich in organic matter fosters a diverse and active microbial community, which is essential for plant growth and protection against pathogens.

2.5.2.1.3. Availability of Macronutrients and Micronutrients

The availability of both macronutrients (such as nitrogen, phosphorus, and potassium) and micronutrients (such as iron, zinc, and manganese) is critical to soil fertility and microbial activity. Macronutrients are essential for microbial metabolism, growth, and reproduction, and their availability significantly impacts microbial populations in the soil. Nitrogen, for example, is a key nutrient for PGPB involved in nitrogen fixation, such as *Rhizobium* and *Azotobacter*. These bacteria convert atmospheric nitrogen into forms that plants can assimilate, promoting healthier growth (Tariq *et al*., 2018). Phosphorus is another crucial nutrient for plant development, as it supports energy transfer and cell division. Soil microorganisms, particularly those belonging to the genus *Bacillus*, can solubilize bound phosphorus, making it available to plants (Meena *et al*., 2015).

Micronutrients, although required in smaller quantities, are equally important in supporting plant and microbial functions. For instance, iron is essential for microbial respiration, and zinc is

involved in enzyme activity. The availability of these nutrients in the soil directly impacts the growth and efficiency of PGPB, which in turn benefits plant health and growth.

In addition to these primary nutrients, soil salinity, texture, and moisture content also affect microbial activity and nutrient availability. Soils with high salt concentrations can inhibit microbial growth by creating osmotic stress, while soils with poor drainage can lead to anaerobic conditions, limiting the diversity of microorganisms that can thrive (Abo-State, 2019). Therefore, maintaining balanced nutrient levels and good soil health is crucial for the effective functioning of plant growth-promoting bacteria and the overall productivity of agricultural soils.

The physicochemical properties of agricultural soils, including pH, organic matter content, and nutrient availability, significantly impact microbial diversity and the efficacy of plant growth-promoting bacteria. Understanding these factors allows for the optimization of soil conditions to enhance microbial activity, increase nutrient availability, and support sustainable plant growth. By managing soil properties effectively, farmers can improve soil health, boost crop yields, and reduce the need for chemical fertilizers, thus promoting environmentally sustainable agricultural practices.

2.5.2.2. Agricultural Practices

Agricultural practices, including fertilizer use, crop rotation, tillage, and irrigation, play a significant role in shaping soil microbial communities, particularly the populations of plant Growth-Promoting Bacteria (PGPB). The impact of these practices on soil microbiota can be both positive and negative, depending on how they are implemented.

1. **Fertilizer Use**: Fertilizers are commonly used to supplement soil nutrient levels and boost crop productivity. However, the excessive use of chemical fertilizers, particularly synthetic nitrogen fertilizers, can disrupt the balance of soil microbial communities. High fertilizer application often leads to a decrease in microbial diversity, including PGPB, by favoring certain bacteria over others (Venterea *et al*., 2005). Moreover, the over-application of fertilizers can lead to nutrient imbalances, creating an environment that is less conducive to PGPB activity. Conversely, the use of organic fertilizers or biofertilizers, such as those containing beneficial microbes, can promote PGPB growth and improve soil health (Khan *et al*., 2014).
2. **Crop Rotation**: Crop rotation is a key agricultural practice that helps maintain soil fertility and microbial diversity. By alternating the types of crops grown in a given field, farmers can disrupt pest cycles, reduce the buildup of soilborne pathogens, and enhance microbial diversity. Research has shown that diverse crop rotations can support a higher abundance of beneficial microorganisms, including PGPB, compared to monocropping systems (Rousk *et al*., 2010). Additionally, the inclusion of leguminous crops in rotation systems can enhance nitrogen fixation, benefiting both the plant and the microbial community.
3. **Tillage**: Tillage practices influence soil structure, aeration, and the distribution of microorganisms. While tillage can help aerate compacted soils and mix organic matter into the soil, it can also disturb the natural structure of the soil microbiota, including PGPB. Reduced tillage practices have been shown to support higher microbial diversity and maintain more stable PGPB populations compared to conventional tillage systems

(López *et al*., 2017). Minimum tillage, along with cover cropping, can help preserve beneficial microbial communities, including PGPB, by reducing soil disturbance.

4. **Irrigation**: The method and frequency of irrigation also affect microbial communities. Over-irrigation can lead to waterlogging, reducing oxygen availability in the soil and potentially harming aerobic plant Growth-Promoting Bacteria (PGPB) populations. On the other hand, inadequate irrigation can limit microbial activity due to drought stress, leading to a reduction in PGPB populations (Liu *et al*., 2015). Thus, optimal irrigation practices are necessary to maintain a favorable environment for microbial activity and plant growth promotion.

The composition and diversity of microbial communities in agricultural soils are essential components of soil health and fertility. Understanding the factors that influence the abundance and activity of PGPB, including soil physicochemical properties and agricultural practices, provides valuable insights for sustainable agricultural management. By optimizing these factors, it is possible to enhance Plant Growth-Promoting Bacteria (PGPB) populations and harness their potential to improve crop yields, reduce dependence on chemical fertilizers, and promote soil sustainability.

2.6 Role of Plant Growth-Promoting Bacteria (PGPB) in Sustainable Agriculture

In recent years, sustainable agriculture has emerged as a vital approach to maintaining global food security while preserving the environment. The adverse effects of conventional farming practices, such as the overuse of chemical fertilizers and pesticides, have spurred interest in alternative methods that are environmentally friendly, economically viable, and capable of enhancing crop productivity. Plant Growth-Promoting Bacteria (PGPB) have been recognized as

a promising solution to achieve these objectives. These beneficial microorganisms naturally colonize plant rhizospheres and offer a range of benefits to plants, such as improved growth, nutrient uptake, and resistance to environmental stressors. The role of PGPB in sustainable agriculture revolves around their potential to reduce dependency on chemical fertilizers, mitigate environmental impacts, and contribute to economic benefits for farmers.

2.6.1. Plant Growth-Promoting Bacteria (PGPB) in Reducing Chemical Fertilizer Usage

Chemical fertilizers, while effective in increasing crop yields, have significant negative impacts on soil health, water quality, and overall environmental sustainability. The excessive and often inefficient use of synthetic fertilizers has been associated with soil acidification, loss of soil biodiversity, and the contamination of water bodies through nutrient runoff, contributing to the eutrophication of aquatic ecosystems (Gianfreda *et al*., 2011). Furthermore, the production and application of chemical fertilizers contribute to greenhouse gas emissions, exacerbating climate change (Sharma *et al*., 2020). This underscores the need to reduce the reliance on these chemical inputs.

Plant Growth-Promoting Bacteria (PGPB) can play a pivotal role in alleviating the need for chemical fertilizers by enhancing nutrient availability to plants. These bacteria can solubilize essential nutrients like phosphorus, nitrogen, and potassium, making them more accessible to plants. For instance, nitrogen-fixing bacteria such as *Rhizobium* and *Azotobacter* can convert atmospheric nitrogen into forms that plants can utilize, thereby reducing the need for synthetic nitrogen fertilizers (Bashan *et al*., 2013). Similarly, phosphorus-solubilizing bacteria, such as *Pseudomonas* and *Bacillus*, can release phosphates from insoluble sources in the soil, making

them available to plants (Vassilev *et al*., 2010). By increasing nutrient availability, PGPB reduce the need for chemical fertilizers, leading to more sustainable agricultural practices.

2.6.2. Environmental and Economic Advantages of Biofertilizers

The environmental benefits of using Plant Growth-Promoting Bacteria (PGPB) as biofertilizers are profound. By minimizing the need for synthetic fertilizers, PGPB help reduce soil degradation, pollution, and greenhouse gas emissions. Biofertilizers, which consist of beneficial microorganisms like PGPB, contribute to soil health by improving soil structure, promoting the proliferation of beneficial soil organisms, and increasing organic matter content. This enhances soil fertility over time and supports a healthy and sustainable agricultural ecosystem (Glick, 2012). The reduction in chemical fertilizer usage also leads to decreased nitrogen and phosphorus leaching into groundwater and surface waters, preventing the harmful effects of nutrient pollution, such as algal blooms and aquatic dead zones (Craswell, 2021).

From an economic standpoint, the use of PGPB offers significant cost savings for farmers. By reducing or eliminating the need for expensive chemical fertilizers, farmers can cut their input costs while maintaining or even improving crop yields. Studies have shown that the use of PGPB-based biofertilizers can result in higher returns on investment due to increased agricultural productivity and reduced input costs (Ali *et al*., 2016). Moreover, biofertilizers are often less expensive than chemical fertilizers, making them more accessible to smallholder farmers in developing regions. This can enhance the economic viability of sustainable farming practices and improve the livelihoods of farmers.

2.7. Studies on the applications of PGPB in Crop Improvement

PGPB have been successfully applied in various agricultural systems to improve crop yield, soil health, and disease resistance, showcasing their potential as a viable alternative to chemical fertilizers. One of the most well-known examples is the use of nitrogen-fixing bacteria such as *Rhizobium* species in leguminous crops. *Rhizobium* establishes a symbiotic relationship with legume plants, forming nodules in the roots where atmospheric nitrogen is converted into ammonium, a form of nitrogen that the plant can absorb and utilize. This natural nitrogen fixation process significantly reduces the need for synthetic nitrogen fertilizers and contributes to sustainable agricultural practices (Gaur *et al*., 2015; Abd-Alla *et al*., 2023).

In addition to nitrogen fixation, PGPB also enhance plant growth by producing phytohormones such as indole acetic acid (IAA), which promote root development, improve water uptake, and increase plant resilience to environmental stressors like drought and salinity. For example, *Bacillus* and *Pseudomonas* species have been shown to produce plant growth regulators that enhance root elongation and improve nutrient uptake, leading to better growth and higher yields in crops like wheat, maize, and rice (Etesami and Maheshwari, 2018).

Moreover, PGPB have been utilized to enhance disease resistance in plants. For instance, *Bacillus subtilis* and *Pseudomonas fluorescens* have been used as biocontrol agents to suppress pathogenic fungi, bacteria, and nematodes that affect crops. These beneficial bacteria produce antimicrobial compounds that inhibit the growth of plant pathogens, thereby reducing the need for chemical pesticides. Studies have shown that PGPB-based biocontrol can effectively reduce the incidence of soil-borne diseases such as *Fusarium* wilt, root rot, and damping-off disease (Glick and Glick, 2020).

A notable success story in the use of PGPB in crop improvement is the application of *Azospirillum* and *Azotobacter* in cereal crops such as maize and wheat. These bacteria enhance nitrogen availability, stimulate root growth, and increase tolerance to abiotic stressors, leading to significant improvements in yield and quality. In addition, the use of *Azospirillum* in rice cultivation has been shown to increase productivity by improving nitrogen use efficiency and enhancing root architecture, which aids in water and nutrient uptake (Meena *et al*., 2017).

The use of PGPB has also been successful in improving the growth and yield of fruits and vegetables. For example, the application of *Bacillus megaterium* and *Pseudomonas putida* has led to improved growth in tomato plants, resulting in increased fruit size and yield. Similarly, PGPB have been used to enhance the resistance of crops like cucumbers, strawberries, and peppers to biotic and abiotic stress, further underscoring the versatility and potential of these microorganisms in sustainable agriculture (Kamilova *et al*., 2005).

PGPB represent a critical tool for promoting sustainable agriculture by reducing dependence on chemical fertilizers, improving soil health, and enhancing crop yields. The environmental and economic advantages of using biofertilizers are significant, as they reduce pollution, lower input costs, and promote more resilient farming systems. Successful applications of PGPB in crop improvement have been demonstrated across a wide range of crops, from legumes and cereals to fruits and vegetables. As research in this field continues to expand, the use of PGPB is expected to play an increasingly important role in sustainable agricultural practices, contributing to food security, environmental preservation, and economic sustainability for farmers worldwide.

2.7 Challenges and Prospects of Using PGPB

The use of Plant Growth-Promoting Bacteria (PGPB) in agriculture presents various opportunities for improving crop yield, enhancing soil health, and reducing dependency on chemical fertilizers. However, several challenges hinder the widespread adoption of PGPB-based biofertilizers. These challenges range from limitations in their commercial viability to difficulties in large-scale production and field application. Despite these challenges, advances in research and technology continue to open up prospects for the future use of PGPB in sustainable agriculture.

2.7.1. Limitations in the Adoption of PGPB-Based Biofertilizers in Agriculture

Despite the promising benefits of PGPB in agriculture, their widespread adoption faces several limitations. One major issue is the inconsistency in their performance across different environmental conditions. The effectiveness of PGPB varies depending on soil type, climate, and plant species, making it difficult to standardize their application. Studies have shown that while certain bacterial strains exhibit excellent growth-promoting properties in controlled laboratory conditions, they may not perform as effectively in the field due to environmental stressors such as temperature fluctuations, water availability, and soil salinity (Rashid *et al*., 2020).

Additionally, the market for biofertilizers remains limited due to farmers' lack of awareness and understanding of the benefits of PGPB. Traditional chemical fertilizers are often more readily available and easier to use, and many farmers are reluctant to invest in unfamiliar and less proven alternatives (Bashan *et al*., 2014). Moreover, the cost of producing PGPB-based biofertilizers and their low shelf life further discourage adoption. Commercial production of these biofertilizers is complex, and maintaining the viability of the bacteria during storage and transportation is a significant challenge (Glick, 2012). Furthermore, there is a lack of regulatory

frameworks to ensure the quality and consistency of biofertilizers, which raises concerns among farmers regarding their efficacy.

2.7.2. Challenges in Large-Scale Production and Field Application

Scaling up the production of PGPB and ensuring their effective field application presents significant challenges. One of the primary issues in large-scale production is the need for optimal growth conditions to maximize bacterial yield. Most PGPBs are cultured in liquid or solid media, and scaling these processes for mass production requires substantial investment in infrastructure and equipment. For example, maintaining sterile conditions, nutrient-rich media, and temperature control during mass cultivation can be expensive and resource-intensive (Abdellatif *et al*., 2020).

Another challenge is ensuring that PGPBs survive and remain effective during field application. Many beneficial bacteria are sensitive to environmental factors such as UV radiation, temperature extremes, and desiccation. To overcome this, various formulations such as microencapsulation, liquid formulations, and slow-release materials have been developed to protect PGPB from environmental stresses and improve their shelf life (Zhao *et al*., 2020). However, the development of such formulations adds to the complexity and cost of large-scale production.

Field application itself presents logistical challenges. For instance, the application of biofertilizers requires careful timing and precise dosage, which can be difficult to manage in large-scale farming operations. Moreover, the efficacy of PGPB may be affected by soil pH, moisture levels, and the presence of other microbial communities in the rhizosphere, which can complicate their use in diverse agricultural systems (Khalid *et al*., 2004).

2.8. Future Perspectives: Advances in Research and Technology for PGPB Utilization

Despite the challenges, significant progress is being made in the research and development of PGPB-based biofertilizers, offering promising prospects for their future use in sustainable agriculture. One area of active research is the identification of new, more robust bacterial strains with enhanced abilities to tolerate environmental stress and improve plant growth under diverse conditions. The application of advanced genetic and genomic tools, such as next-generation sequencing (NGS), is facilitating the discovery of novel PGPB strains with a broader range of beneficial traits, such as drought resistance, nitrogen fixation, and biocontrol of plant pathogens (Schulz-Bohm *et al*., 2018).

Moreover, advancements in synthetic biology and metabolic engineering hold the potential to improve the performance of PGPBs by enhancing their ability to produce plant growth hormones, solubilize phosphates, and degrade harmful substances in the soil. For example, the genetic modification of PGPBs to increase their resistance to environmental stressors like drought, salinity, and high temperatures could enable them to thrive in a wider range of agricultural conditions (Baek *et al*., 2020).

The development of more effective delivery systems is another promising avenue for improving the use of PGPB-based biofertilizers. Research into nanotechnology and nanoformulations could offer new methods for encapsulating PGPBs, ensuring their stable release and enhanced bioavailability in the soil. Additionally, combining PGPBs with other biocontrol agents, such as mycorrhizal fungi or plant resistance inducers, may lead to synergistic effects that improve plant health and yield in a more sustainable manner (Berg, 2009).

Lastly, the increasing use of precision agriculture and data-driven technologies offers exciting opportunities for optimizing the application of PGPBs. By utilizing sensors, GPS, and remote sensing technologies, farmers can monitor soil conditions in real-time and apply biofertilizers precisely where and when they are needed, thus maximizing their efficacy and minimizing waste (Kumar *et al*., 2020).

While the adoption of PGPB-based biofertilizers in agriculture faces several challenges, including inconsistent performance, production limitations, and logistical difficulties, ongoing advances in research and technology offer promising solutions. The identification of robust bacterial strains, the development of advanced delivery systems, and the integration of precision agriculture technologies will be key to overcoming these challenges and realizing the full potential of PGPBs in sustainable agriculture. Continued investment in research, education, and infrastructure will be essential to ensure the successful integration of PGPB-based biofertilizers into modern farming systems.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area/Sample Collection

The study was carried out in the Edo state, Benin City, where soil samples were obtained from Oluku. 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. Rhizobacterial Potential of Bacterial Isolates

3.9.1. Screening for Indole Acetic Acid (IAA) production

This was determined by reaction of liquid culture of rhizobacterial isolates grown in 500 mg/L L-Tryptophan (the precursor for IAA biosynthesis) placed in tryptic soy broth (1 g/L MES hydrate, pH 6) and Salkowki's reagent. Inoculated broth was incubated at 30°C for 72h in a rotary shaker. After incubation broth was centrifuged at 3000rpm for 15min. Then 1.0 ml of the supernatant was mixed with 2.0 ml of Salkowski reagent (50 ml of 35% Perchloric acid + 1 ml of 0.5 M $FeCl_3$ solution), and the mixture was then incubated at room temperature for 25mins. Development of pink color after incubation at room temperature indicated IAA production (Patten and Glick, 2002; Kumar et al., 2012; Ngoma et al., 2013).

3.9.2. Screening for Ammonia production

Freshly grown bacteria cultures were inoculated in 10 ml nutrient broth and incubated at 30°C for 48h in a rotator shaker. After incubation, 0.5 ml of Nessler's reagent was added to each tube. The development of a yellow to brown colour indicated a positive reaction for ammonia production (Kumar et al., 2012)

3.9.3. Screening for Nitrogen fixation activity

A day old culture of bacterial isolates grown on nutrient agar was streaked on a Jensen's Nitrogen free medium otherwise knows as NFM (formulated via the addition of: 20g/L sucrose, 1g/L K_2HPO_4, 0.5 g/L $MgSO_4.7H_2O$, 0.5 g/L NaCl, 0.1 g/L $FeCl_3$, 0.005g/L $Na_2MoO_4.2H_2O$, 2g/L $CaCO_3$, 15g/L agar). Plates were incubated at 28°C for 1- 7 days. Growth on nitrogen deficient medium confirms the ability to fix nitrogen (Weselowski et al., 2016).

3.9.4. Screening for Phosphate Solubilization activity

The bacterial isolates was cultured in replicates on prepared Pikovskya's agar (Micromaster) plates and incubated at 30°C for 3 days. A zone of clearing around the colonies after 1-3 days was scored as positive for phosphate solubilization. The diameter of the halozone and its bacteria colony from individual isolates was measured. The data obtained was used to calculate solubilization index (SI) using the formula below (Doilom et al., 2020).

3.10 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

Table 4.1. shows the total bacterial counts of soils from different farmlands in Oluku, expressed as $10^4 \times 10^4$ CFU/ml. The highest bacterial count was recorded in Farm 8 (40.60±5.94), while the lowest bacterial count was observed in Farm 6 (11.20±0.57).

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 *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, Enterobacter aerogenes* and *Bacillus pumilus.*

Table 4.3 Shows the plant growth-promoting properties of bacterial isolates from soil. *E. coli* exhibits all four traits: nitrogen fixation, phosphate solubilization, ammonia production, and IAA production, making it the most versatile isolate. *Enterobacter aerogenes* and *Pseudomonas aeruginosa* possess three traits but lack ammonia production. *Staphylococcus aureus* and

Bacillus pumilus show limited traits, lacking nitrogen fixation and IAA production. These variations indicate differing potentials for agricultural applications.

Table 4.1. Total bacterial counts of soils from different farmlands in Oluku

Farmlands	Bacterial Counts in Standard form ($x10^4$ CFU/ml)
Farm 6	11.20±0.57
Farm 7	20.20±1.98
Farm 8	40.60±5.94
Farm 9	16.20±1.41
Farm 10	11.40±4.81

Key: data presented were mean±SD from duplicate determinations

Table 4.2. Cultural, morphological and microscopic characteristics of bacterial isolates from soils

Morphological					
Elevation	Raised	Raised	Flat	Flat	Flat
Margin	smooth	Entire	Undulate	irregular	Undulate
Color	Cream	lemon	Cream	off-white	Cream
Shape	Irregular	Circular	Irregular	concave	Irregular
Size	Small	Medium	Large	large	Large
Gr. diff. agar	MSA	PCA	EMB	BCA	EMB
Colour	Yellow	green	green	Straw	Pink
Staining					
Gram stain	+	-	-	+	-
cell type	Cocci	rod	Rod	Rod	Rod
Arrangement	clusters	disperse	disperse	disperse	disperse
Color	purple	pink	pink	purple	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	K/A	A/A(K*)G*
Esculin Hydrolysis	-	-	-	+	+
Identity	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*E. coli*	*Bacillus pumilus*	*Enterobacter aerogenes*

Table 4.3. Plant growth promoting properties of bacterial isolates from soil

Isolates	*Nitrogen Fixation*	*Phosphate Solubilization*	*Ammonia Production*	*IAA production*
Staphylococcus aureus	-	+	+	-
Enterobacter aerogenes	+	+	-	+
Pseudomonas aeruginosa	+	+	-	+
E. coli	+	+	+	+
Bacillus pumilus	-	+	+	-

Key:

(+) =Presence

(-)=Absence

The soil samples from five different agricultural farmlands within Oluku village were analyzed to determine their physicochemical properties. The pH levels across the farms showed a range from slightly acidic to nearly neutral, with Farm 1 recording a pH of 6.42 ± 0.32, Farm 2 at 5.97 ± 0.30, Farm 3 at 6.13 ± 0.31, Farm 4 at 5.64 ± 0.28, and Farm 5 at 5.78 ± 0.29. Electrical conductivity (EC), which indicates the soil's salinity, varied significantly among the farms. Farm 1 had an EC of 754.00 ± 37.70 µS/cm, Farm 2 recorded the highest EC at 873.00 ± 43.65 µS/cm, Farm 3 had 695.00 ± 34.75 µS/cm, Farm 4 had 722.00 ± 36.18 µS/cm, and Farm 5 recorded the lowest at 678.60 ± 33.93 µS/cm.

Organic matter (OM) content was highest in Farm 2 with 4.78 ± 0.24%, indicating rich organic content, while Farm 1 and Farm 5 had the lowest OM levels at 1.46 ± 0.07% and 1.31 ± 0.07%, respectively. Organic carbon (OC), which is a component of organic matter, followed a similar trend with Farm 2 showing the highest percentage at 8.24 ± 0.41%, and Farm 5 the lowest at 2.27 ± 0.11%.

The concentration of essential nutrients like phosphorus (P) and nitrogen (N) also varied. Farm 1 had phosphorus levels of 9.75 ± 0.49 mg/kg and nitrogen levels of 3.49 ± 0.17%, whereas Farm 2 showed higher phosphorus at 11.22 ± 0.56 mg/kg and nitrogen at 5.66 ± 0.28%. Potassium (K) levels were also notably different across the farms, with Farm 2 showing the highest concentration at 8.07 ± 0.40 mg/kg and Farm 4 the lowest at 4.18 ± 0.21 mg/kg.

Calcium (Ca) and magnesium (Mg) levels, which are crucial for soil structure and plant health, were highest in Farm 2 with Ca at 1.25 ± 0.06 mg/kg and Mg at 18.63 ± 0.93 mg/kg. Sodium (Na) levels were relatively low across all farms, with Farm 1 recording 0.38 ± 0.02 mg/kg and Farm 5 the lowest at 0.34 ± 0.02 mg/kg.

The analysis of heavy metals revealed varying levels of iron (Fe), zinc (Zn), lead (Pb), and copper (Cu). Iron content was highest in Farm 3 at 637.84 ± 31.89 mg/kg and lowest in Farm 4 at 453.72 ± 22.69 mg/kg. Zinc levels peaked in Farm 3 at 87.52 ± 4.38 mg/kg, whereas Farm 1 had significantly lower levels at 65.79 ± 3.29 mg/kg. Lead and copper concentrations were found in trace amounts, with Farm 2 showing the highest lead concentration at 4.69 ± 0.23 mg/kg and

Farm 1 the lowest at 2.87 ± 0.14 mg/kg. Copper levels were relatively consistent, with Farm 1 having 28.75 ± 1.44 mg/kg and Farm 4 the lowest at 21.45 ± 1.07 mg/kg.

Implications for Heavy Metals in Agricultural Soils:

The presence of heavy metals such as lead (Pb) and copper (Cu) in agricultural soils is a concern due to their potential toxicity to plants, animals, and humans. The detected levels of lead in the soil samples, particularly in Farm 2 (4.69 ± 0.23 mg/kg), though not exceedingly high, could pose risks if crops take up these levels. Copper, while an essential micronutrient for plants, can become toxic at elevated levels, potentially leading to phytotoxicity and adversely affecting crop yields and quality.

Iron and zinc are essential for plant growth. Still, their high concentrations, especially in Farm 3, suggest the potential for both beneficial effects on plant nutrition and risks of toxicity depending on the crop species' tolerance. Monitoring and managing these metal levels are crucial to ensure they remain within safe limits for agricultural productivity and food safety. Regular soil testing and the implementation of best management practices can mitigate the risks associated with heavy metal contamination in soils.

Table 4.5. Physicochemical properties of the soil samples from agricultural farmlands in Oluku

Parameters	Farm 6	Farm 7	Farm 8	Farm 9	Farm 10
Ph	6.42±0.32	5.97±0.30	6.13±0.31	5.64±0.28	5.78±0.29
EC	754.00±37.70	873.00±43.65	695.00±34.75	722.00±36.10	678.60±33.93
OM	1.46±0.07	4.78±0.24	3.05±0.15	2.58±0.13	1.31±0.07
OC	2.52±0.13	8.24±0.41	5.26±0.26	4.45±0.22	2.27±0.11
P	9.75±0.49	11.22±0.56	7.65±0.38	10.28±0.51	8.78±0.44
N	3.49±0.17	5.66±0.28	3.68±0.18	4.25±0.21	3.14±0.16
Ca	0.98±0.05	1.25±0.06	1.76±0.09	0.84±0.04	0.88±0.04
K	5.10±0.26	8.07±0.40	7.90±0.40	4.18±0.21	4.59±0.23
Mg	14.28±0.71	17.63±0.88	21.54±1.08	13.75±0.69	12.85±0.64
Na	0.38±0.02	0.61±0.03	0.57±0.03	0.48±0.02	0.34±0.02
Fe	587.10±29.36	496.28±24.81	637.84±31.89	453.72±22.69	528.39±26.42
Zn	74.82±3.74	65.79±3.29	87.52±4.38	68.75±3.44	67.34±3.37
Pb	2.87±0.14	4.69±0.23	2.13±0.11	1.74±0.09	2.58±0.13
Cu	28.75±1.44	25.23±1.26	23.68±1.18	21.45±1.07	25.88±1.29

Keys: put the codes and units (mg/ml),

GHAPTER FIVE

5.1. DISCUSSION

Agriculture is a cornerstone of global food security and economic development, particularly in developing regions (Ruane and Sonnino, 2011). However, the continuous use of chemical fertilizers and pesticides in conventional farming practices has raised concerns about environmental sustainability, soil health, and food safety. In light of these challenges, there has been growing interest in alternative approaches to enhance soil fertility and promote crop growth. One promising solution lies in the utilization of plant growth-promoting bacteria (PGPB), which naturally enhance plant health and productivity through various mechanisms, such as nitrogen fixation, phosphate solubilization, and the production of growth-promoting hormones (Olanrewaju *et al*., 2017). This study focuses on the identification and characterization of PGPB from selected agricultural farmlands in Oluku Village, Edo State, Nigeria. By exploring the microbial diversity in these farmlands.

The soil samples collected from different farmlands demonstrated a variation in microbial population, reflecting the diversity of environmental and soil conditions across the study locations. The total viable bacterial counts ranged from 11.20×10^4 CFU/ml in Farm 6 to 40.60×10^4 CFU/ml in Farm 8. This wide range in bacterial abundance underscores the critical role of local soil conditions, such as organic matter content, nutrient availability, pH, and farming practices, in influencing microbial growth and activity (Mohammadi *et al*., 2011).

Soils with higher organic matter content tend to support more robust microbial populations due to the availability of carbon and other essential nutrients. Farms such as Farm 8, which exhibited

the highest microbial count, likely benefited from more favorable conditions such as organic inputs, adequate moisture, and minimal chemical disturbances. Conversely, the lower bacterial counts observed in Farms 6 and 10 could be attributed to factors such as nutrient depletion, soil compaction, or the presence of inhibitory substances. These findings align with the observations of Marschner *et al*. (2003), who reported a positive correlation between organic carbon levels and microbial abundance in long-term soil fertility studies.

The bacterial species isolated from the soil samples exhibited significant diversity, which is a hallmark of healthy soil ecosystems. The identified species included *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus pumilus* and *Enterobacter aerogenes*. Each of these isolates was characterized based on their morphological, biochemical, and functional properties, revealing their potential roles in promoting plant growth and maintaining soil fertility.

Gram-positive bacteria, such as *Bacillus pumilus*, are known for their ability to form endospores, enabling them to survive harsh environmental conditions (Nicholson *et al*., 2000). This resilience makes them valuable candidates for use as biofertilizers, particularly in resource-limited settings. Gram-negative bacteria, including *Pseudomonas aeruginosa* and *Enterobacter aerogenes*, demonstrated capabilities such as nitrogen fixation and hormone production, which are essential for plant development (Jha *et al*., 2011; Timofeeva *et al*, 2023). The presence of these diverse species highlights the ecological richness of the soil microbiome and its potential to enhance agricultural productivity. The functional properties of the bacterial isolates examined in this study highlight their significant potential as plant growth-promoting bacteria (PGPB) in sustainable agricultural systems. These isolates exhibited a range of beneficial traits that enhance soil fertility, nutrient availability, and plant productivity.

Among the isolates, species such as *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, and *Escherichia coli* demonstrated the ability to fix atmospheric nitrogen, converting it into bioavailable forms. This biological process is pivotal for reducing reliance on synthetic nitrogen fertilizers, which are not only expensive but also pose significant environmental challenges, including greenhouse gas emissions and waterway contamination. Nitrogen fixation by these bacteria supports crop growth by ensuring a steady supply of this essential nutrient in the root zone. This finding aligns with previous studies, such as those by Zablotowicz *et al*. (1991).

Phosphorus availability is a common limiting factor in many agricultural soils, often rendering plants unable to access this critical nutrient. The study identified isolates such as *Bacillus pumilus* and *Pseudomonas aeruginosa* as efficient phosphate solubilizers. These bacteria produce organic acids and enzymes that solubilize inorganic phosphates in the soil, making them available for plant uptake. This activity plays a crucial role in enhancing nutrient-use efficiency and improving crop yields, particularly in phosphorus-deficient soils .

The production of indole-3-acetic acid (IAA), a key phytohormone, was observed in several bacterial isolates, such as *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, and *Escherichia coli.* IAA is known to stimulate root elongation and branching, which significantly improves a plant's ability to absorb water and nutrients from the soil. Enhanced root development not only boosts crop growth but also increases resilience to environmental stressors such as drought. This finding underscores the potential application of these isolates as biofertilizers to enhance crop productivity and stress tolerance.

The production of ammonia by isolates such as *Staphylococcus aureus* and *Escherichia coli* adds another dimension to their plant growth-promoting capabilities. Ammonia contributes to soil

nitrogen enrichment, providing an additional nitrogen source for plants. This trait further underscores the ability of these bacterial isolates to improve soil nutrient status and support sustainable agricultural practices.

These findings are consistent with the work of Patten and Glick (2002), who highlighted the critical role of PGPB in nutrient cycling and plant growth stimulation. By facilitating key processes such as nitrogen fixation, phosphate solubilization, hormone production, and ammonia generation, these bacterial isolates offer a promising, eco-friendly alternative to chemical inputs in agriculture. Their functional properties underscore their potential as integral components of sustainable farming systems, promoting soil health, reducing environmental impact, and enhancing crop productivity.

The physicochemical properties of the soil samples analyzed in this study offered valuable insights into the environmental conditions that shape microbial diversity and activity. These properties, including soil pH, organic matter content, and heavy metal concentrations, play pivotal roles in influencing the soil's microbial ecosystem and its overall functionality in supporting plant growth and nutrient cycling.

The soil samples analyzed displayed pH values ranging from 5.64 to 6.42, indicating a slightly acidic to near-neutral range. This range is considered optimal for both microbial activity and plant growth, as it creates a balanced environment for nutrient availability. Slightly acidic to neutral pH levels enhance the solubility of essential nutrients, such as nitrogen, phosphorus, and potassium, while reducing the solubility of potentially harmful metals, such as aluminum and manganese, that can become toxic at lower pH levels. This favorable pH range fosters the proliferation of a wide variety of microbial species, including nitrogen-fixing bacteria and

phosphate-solubilizing microorganisms, thereby contributing to improved soil fertility and plant health (Weil and Brady, 2017).

The organic matter content of the soil samples emerged as a key determinant of microbial population density. Soils with higher levels of organic carbon and organic matter, such as those observed in Farm 8, supported a richer and more diverse microbial community. Organic matter serves as a critical energy source for soil microorganisms, driving their metabolic processes and promoting microbial interactions. This observation aligns with findings from Ahemad and Khan (2012), who demonstrated that the addition of organic amendments significantly boosts microbial activity and diversity. The presence of ample organic matter also enhances soil structure, water retention, and nutrient availability, creating an environment conducive to sustained microbial and plant productivity.

The analysis also revealed the presence of heavy metals, such as lead and copper, in the soil samples, although their concentrations were within acceptable limits. While these levels may not immediately pose a threat, the presence of heavy metals underscores the importance of regular soil monitoring. Prolonged exposure to even low concentrations of heavy metals can have cumulative effects on soil microbial communities, disrupting essential microbial functions such as nutrient cycling and organic matter decomposition (Abdu *et al*., 2017). Additionally, heavy metals can enter the food chain through bioaccumulation in crops, posing potential risks to human and animal health over time. Addressing heavy metal contamination through proper soil management practices, including phytoremediation and the use of organic amendments, can mitigate these risks and ensure the long-term sustainability of agricultural systems (Priya *et al*., 2023).

The findings from this study hold profound implications for the promotion of sustainable agriculture and the enhancement of soil management practices. The incorporation of plant growth-promoting bacteria (PGPB) into agricultural systems represents a viable and environmentally conscious approach to improving productivity while preserving soil health. These beneficial microorganisms contribute significantly to key aspects of sustainable farming, offering practical solutions to some of the pressing challenges faced by modern agriculture.

One of the most notable benefits of utilizing PGPB lies in their ability to improve soil fertility (Majeed *et al*., 2018). Through nitrogen fixation and phosphate solubilization, these bacteria ensure a steady supply of essential nutrients to plants. By converting atmospheric nitrogen into bioavailable forms and enhancing the availability of phosphorus in the soil, PGPB reduce the reliance on synthetic fertilizers, which are often expensive and environmentally detrimental. This not only lowers the financial burden on farmers but also minimizes the risks of nutrient leaching and chemical pollution in water bodies, thereby supporting a more balanced ecosystem (Nkwunonwo *et al*., 2020).

Furthermore, the activities of PGPB significantly enhance crop productivity. The production of growth-promoting hormones such as indole-3-acetic acid (IAA) facilitates root elongation and branching, improving the plant's ability to absorb water and nutrients (Bhattacharya and Bhattacharya, 2021). These mechanisms contribute to healthier, more robust crops, leading to increased yields and improved agricultural outcomes. This enhancement in productivity is crucial for addressing global food security challenges while reducing the environmental footprint of farming practices.

In addition to boosting fertility and productivity, the use of PGPB aligns seamlessly with the principles of environmental sustainability. Indigenous bacterial strains, which are well-adapted to local soil conditions, can be harnessed as biofertilizers, promoting eco-friendly agricultural practices. Unlike chemical fertilizers, which often disrupt soil ecosystems and degrade over time, biofertilizers derived from PGPB work in harmony with natural processes, maintaining soil health and biodiversity. This approach mitigates the adverse effects of intensive farming, such as soil erosion, nutrient depletion, and water contamination, thereby contributing to the long-term sustainability of agricultural (Alves *et al*., 2004 Adesemoye *et al*., 2009; Hungria *et al*., 2013; Maitra *et al*., 2021).

5.1 CONCLUSION

This study has successfully identified and characterized a diverse array of plant growth-promoting bacteria (PGPB) with significant potential for enhancing agricultural productivity and promoting sustainable farming practices. The bacterial isolates demonstrated a range of functional properties, including nitrogen fixation, phosphate solubilization, hormone production, and ammonia production, all of which contribute to improved soil fertility and enhanced plant growth.

The physicochemical analysis of the soil samples further revealed that factors such as pH, organic matter content, and heavy metal concentrations play crucial roles in influencing microbial diversity and activity. Soils with higher organic matter content supported greater microbial populations, while heavy metals, though within acceptable limits, highlighted the importance of monitoring soil health to mitigate potential risks. The integration of these indigenous bacterial strains into agricultural systems presents several advantages, including

reduced dependence on synthetic fertilizers, improved crop yields, and better environmental sustainability. The use of PGPB aligns with global efforts to reduce the environmental impact of chemical fertilizers and promote eco-friendly farming practices.

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