

ISOLATION AND CHARACTERIZATION OF INDIGENOUS SOIL BACTERIA
USING CALCITE PRECIPITATING ABILITIES.

A

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CERTIFICATION

DEDICATION

This research is dedicated to my wonderful parents, Mr. and Mrs. Abdulroshid whose hard work has greatly enhanced my life in every aspect.

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All praise and gratitude be to Almighty Allah, the Most Gracious, the Most Merciful, for His boundless blessings, guidance, and protection throughout this endeavour. Without His mercy and wisdom, this accomplishment would not have been achievable. Alhamdulillah for His innumerable favors, for enabling all things to happen, and for surrounding me with individuals who have been a true blessing in my life.

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ABSTRACT

The present study focused on the *Isolation and Characterization of Indigenous Soil Bacteria Using Calcite Precipitating Abilities*. The aim was to identify native soil bacteria capable of producing calcium carbonate (CaCO_3) through microbial-induced calcite precipitation (MICP), a process relevant to sustainable soil improvement and agricultural productivity. Soil samples were collected from cultivated farmlands within Usmanu Danfodiyo University Sokoto and subjected to serial dilution and nutrient agar plating techniques for bacterial isolation. The isolates were characterized based on colonial morphology, Gram staining, and biochemical tests such as catalase, citrate, urease, and oxidase reactions. Four bacterial species were identified: *Proteus mirabilis*, *Klebsiella aerogenes*, *Kocuria* species, and *Micrococcus* species. All isolates exhibited positive growth on urea- CaCl_2 medium, confirming their ability to precipitate calcium carbonate. *Proteus mirabilis* and *Klebsiella aerogenes* demonstrated the highest calcite formation, attributed to their strong urease activity and efficient hydrolysis of urea. The study establishes that indigenous soil bacteria in Sokoto possess significant bio cementation potential and can be utilized for eco-friendly soil stabilization and fertility enhancement in agricultural systems. This finding contributes to the development of biological alternatives to chemical soil stabilizers, aligning with global efforts toward sustainable agricultural practices and environmental conservation. Further molecular characterization and field application trials are recommended to explore the full potential of these bacterial isolates in large-scale soil bioremediation and stabilization.

CHAPTER ONE

1.0

INTRODUCTION

1.1 Background of the Study

Soil is a dynamic and living system that harbours a diverse range of microorganisms which play crucial roles in sustaining soil fertility, structure, and agricultural productivity. Among these microorganisms, bacteria are the most abundant and functionally diverse group, participating in several biogeochemical processes such as nutrient cycling, organic matter decomposition, and mineral formation. Recently, attention has been drawn to the ability of certain soil bacteria to precipitate minerals, particularly calcium carbonate (CaCO_3), through a process known as microbial induced calcite precipitation (MICP) (Yusuf *et al.*, 2023).

Microbial-induced calcite precipitation occurs when microorganisms produce metabolic by-products such as carbonate ions (CO_3^{2-}) and ammonium (NH_4^+) during processes like urea hydrolysis, leading to the formation of (CaCO_3) in the presence of calcium ions (Ca^{2+}). This biogenic process is not only of engineering importance for soil stabilization and self-healing concrete, but it is also gaining agricultural relevance in improving soil structure, reducing erosion, and promoting root stability (Meandira *et al.*, 2024).

In agriculture, the biological improvement of soil properties using indigenous bacteria offers an environmentally friendly alternative to chemical soil conditioners. Indigenous calcite-precipitating bacteria (CPB) are naturally adapted to local conditions and have been reported to enhance soil aggregation, reduce compaction, and increase moisture retention (Kumari *et al.*, 2022). These bacteria can also improve the mechanical strength of degraded soils, which is critical for sustainable crop production in regions prone to erosion and nutrient loss (Al-Sallami *et al.*, 2021).

Understanding the diversity and biochemical characteristics of these indigenous bacteria is fundamental for developing microbial-based soil improvement strategies. Through isolation and characterization of calcite-precipitating bacteria, researchers can identify species with high urease activity and calcite precipitation efficiency that could be applied to improve soil health and productivity in agricultural settings (Hadi and Saeed, 2022).

Therefore, this study focuses on isolating and characterizing indigenous soil bacteria capable of calcite precipitation from agricultural soils, thereby exploring their potential for sustainable soil management and agricultural enhancement.

1.2 Statement of the Problem

Conventional methods of soil stabilization and fertility improvement often rely heavily on chemical inputs, such as lime, gypsum, or synthetic fertilizers. While effective in the

short term, these inputs can lead to environmental degradation, soil acidification, and high long-term costs for farmers. In many developing agricultural systems, including Nigeria, there is also an increasing problem of soil degradation, erosion, and loss of organic matter, leading to declining crop yields.

Microbial-induced calcite precipitation (MICP) presents a sustainable biological solution to these problems. However, limited research has been conducted on identifying indigenous bacterial species in local agricultural soils that possess calcite-precipitating abilities. Most studies available have focused on foreign or laboratory strains, which may not survive or perform effectively under local soil and climatic conditions (Yusuf *et al.*, 2023).

This knowledge gap hinders the practical application of MICP in agricultural soil management. Therefore, there is a need to isolate and characterize native bacterial species that can precipitate calcite efficiently and potentially improve soil structure and fertility in an eco-friendly and cost-effective manner.

1.3 Justification of the Study

This study is significant because it contributes to the expanding field of bio mineralization in agriculture, an environmentally friendly approach that utilizes naturally occurring soil microorganisms to improve soil structure. Identifying indigenous calcite-

precipitating bacteria offers several benefits: it provides a sustainable and low-cost alternative to chemical soil stabilizers, enhances soil physical properties such as aggregation, porosity, and water retention—thereby supporting improved crop productivity and generates valuable data for the development of bio-based soil management strategies in Nigeria and other regions with similar environmental conditions. Overall, the study promotes sustainable agricultural practices and contributes to reducing the ecological footprint associated with intensive farming.

Moreover, isolating indigenous strains ensures that the bacteria are well adapted to the local soil and climatic conditions, increasing their survival and performance in field applications (Rani *et al.*, 2021).

1.4 Aim and Objectives of the Study

To isolate and characterize indigenous soil bacteria based on their calcite-precipitating abilities for potential agricultural applications. With the following objectives:

1. Isolate bacteria from agricultural soil samples using standard microbiological techniques.
2. Characterize the bacterial isolates based on their morphological and biochemical properties.

3. Determine the urease activity of the isolates to identify those with calcite-precipitating potential.

CHAPTER TWO

2.0

LITERATURE REVIEW

2.1 Soil Microorganisms and Their Ecological Roles

Soil hosts a complex and dynamic microbial community comprising bacteria, fungi, actinomycetes, protozoa, and algae. Among these, bacteria are the most abundant and functionally diverse group, with population densities ranging from $10^6 - 10^9$ cells g^{-1} of soil (Kumar and Singh, 2021). These microorganisms drive essential biogeochemical cycles, including carbon, nitrogen, sulfur, and phosphorus transformations, which sustain soil fertility and plant growth.

Bacteria influence soil structure by producing extracellular polymeric substances (EPS), which help in the aggregation of soil particles, improving aeration and water infiltration (Al-Sallami *et al.*, 2021). Some bacterial genera, such as *Bacillus*, *Pseudomonas*, *Sporosarcina*, and *Arthrobacter*, have been recognized for their ability to produce calcite through metabolic activities, linking microbial activity directly to soil stabilization and mineral formation (Hadi and Saeed, 2022).

2.2 Indigenous Soil Bacteria

Indigenous soil bacteria are microbial populations naturally adapted to local soil environments. They play critical roles in maintaining ecological balance and resilience

under environmental stresses such as drought, salinity, and nutrient limitation. Indigenous bacteria are more efficient in natural systems than introduced strains because they are well adapted to the physicochemical conditions of their native soils (Rani *et al.*, 2021).

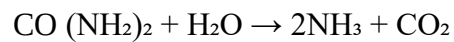
Studies have revealed that indigenous bacterial species such as *Bacillus subtilis*, *Sporosarcina pasteurii*, and *Bacillus cereus* are capable of urease production and (CaCO₃) precipitation in agricultural soils (Meandira *et al.*, 2024). Their utilization in soil improvement reduces dependency on synthetic conditioners while enhancing soil fertility naturally.

2.3 Concept of Microbially Induced Calcite Precipitation (MICP)

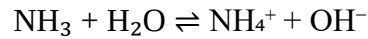
Microbially Induced Calcite Precipitation is a biogenic process by which microorganisms facilitate the deposition of calcium carbonate ((CaCO₃)) minerals. It typically involves the enzymatic hydrolysis of urea by urease-producing bacteria, leading to carbonate ion (CO₃²⁻) generation, which subsequently reacts with calcium ions (Ca²⁺) to form solid calcite crystals (Yusuf *et al.*, 2023).

The simplified chemical reaction sequence is as follows:

1. Urea hydrolysis:



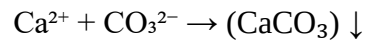
2. Ammonia equilibrium:



3. Carbonate formation:



4. Calcite precipitation:



Through these reactions, bacteria indirectly increase the local pH and carbonate ion concentration, promoting (CaCO₃) precipitation around cell surfaces and within the extracellular matrix (Kumari *et al.*, 2022).

2.4 Mechanisms of Calcite Precipitation by Bacteria

2.4.1 Urease-Mediated Pathway

The urease-mediated pathway is the most widely studied mechanism of calcite precipitation. Urease-positive bacteria hydrolyze urea to produce ammonia and carbon dioxide, resulting in pH elevation and carbonate ion formation. The carbonate ions then react with available calcium ions to precipitate (CaCO₃) (Hadi and Saeed, 2022). *Bacillus subtilis* and *Sporosarcina pasteurii* are among the most studied species exhibiting high urease activity.

2.4.2 Carbonic Anhydrase-Mediated Pathway

Some bacteria can precipitate (CaCO_3) through carbonic anhydrase (CA) enzymes that catalyze the reversible hydration of CO_2 into bicarbonate (HCO_3^-) ions. This pathway is important in environments with limited urea availability, such as natural agricultural soils (Meandira *et al.*, 2024).

2.4.3 Photosynthetic and Heterotrophic Pathways

Certain photosynthetic microorganisms (e.g., cyanobacteria) and heterotrophic bacteria can induce calcite precipitation indirectly by removing CO_2 through metabolic activities, shifting equilibrium towards carbonate formation (Al-Sallami *et al.*, 2021). These non-ureolytic pathways are more environmentally friendly, as they minimize ammonia release.

2.5 Factors Affecting Bacterial Calcite Precipitation

The efficiency of bacterial calcite precipitation is influenced by several physicochemical and biological factors:

- **pH:** Optimal (CaCO_3) precipitation occurs under alkaline conditions (pH 8 – 9).

- **Calcium Ion Concentration:** Adequate Ca^{2+} availability is essential for crystal formation.
- **Temperature:** Bacterial enzymatic activity increases temperature up to an optimum (usually 30–37 °C).
- **Urea Concentration:** Urea serves as the substrate for urease activity; too little or too much can inhibit precipitation efficiency.
- **Bacterial Density and Growth Phase:** Actively growing bacterial cells promote more nucleation sites for calcite deposition (Kumari *et al.*, 2022).

2.6 Agricultural Significance of Calcite-Precipitating Bacteria

The application of calcite-precipitating bacteria extends beyond engineering and construction into **agricultural soil management**. MICP has been shown to:

- **Improve soil structure and aggregation**, enhancing water retention and aeration.
- **Reduce erosion** by binding loose soil particles through (CaCO_3) deposition.
- **Enhance root anchorage**, leading to stronger plant establishment.
- **Improve nutrient availability** by stabilizing organic matter and reducing nutrient leaching (Rani *et al.*, 2021).

- **Promote carbon sequestration**, thereby contributing to climate-smart agriculture (Meandira *et al.*, 2024).

Recent field trials indicate that MICP-treated soils exhibit better mechanical strength and less susceptibility to erosion compared to untreated controls (Abdullahi *et al.*, 2023). In Nigeria and other developing countries, integrating indigenous calcite-precipitating bacteria into agricultural soil management could significantly enhance soil health and productivity in an environmentally sustainable manner.

2.7 Biochemical Characterization of Calcite-Precipitating Bacteria

Biochemical characterization helps to identify and differentiate bacterial species based on their enzymatic and metabolic activities. Common biochemical tests include:

- **Catalase test** – detects the presence of catalase enzyme, which breaks down hydrogen peroxide into water and oxygen.
- **Oxidase test** – identifies bacteria that produce cytochrome oxidase.
- **Citrate utilization** – determines the ability to use citrate as the sole carbon source.
- **Urease test** – assesses the bacteria's capacity to hydrolyze urea, a critical indicator of calcite-precipitating potential.

- **Sugar fermentation tests** – reveal carbohydrate utilization patterns (Hadi and Saeed, 2022).with

The data from these tests provide insight into the diversity and functional potential of indigenous bacterial isolates. Typically, *Bacillus* spp. and *Sporosarcina* spp. are dominant urease-positive and calcite-precipitating bacteria identified in soil studies (Kumari *et al.*, 2022).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area and Soil Sample Collection

3.1.1 Site Description

The sampling site is located at the Sokoto Energy Research Centre (SERC), Usmanu Danfodiyo University, Sokoto, situated on the university's main campus in Sokoto city, Sokoto State, Nigeria. The site lies in a semi-arid climate characterized by high solar radiation and hot temperatures (Tafinta and Habsatu 2017). The terrain is generally flat to gently undulating with patches of open ground used for solar energy demonstration plots and associated infrastructure. Surface cover alternates between bare soil, grassed lawns, and paved walkways associated with the research facilities. Soil types across the site are likely to be sandy to loamy, with sparse vegetative cover in parts due to exposure. For sampling, locations were selected to represent:

- i. Areas immediately adjacent to solar installation racks,
- ii. Open ground away from infrastructural influence.
- iii. Control zones under natural vegetation.

Each sampling point was recorded with GPS coordinates longitude 5° 11° 30° E and 5° 14° 30° E and latitude 13° 8° 30° N and 13° 7° 0° N. Surface cover noted, depth of sampling and observable site conditions (e.g., presence of equipment, drainage features, shading from panels). The site access is via the university property, and prior coordination with the centre management was arranged to ensure minimal disruption to research activities. Sample collection was timed to avoid the peak dry season when soils are overly desiccated and to capture post-rainfall moisture conditions for comparative analysis (Hamdani *et al.*, 2021).

3.1.2 Sample Collection

Approximately 500 grams of soil was collected at the 0 -5cm depth. The samples were placed in sterile, labelled polyethylene bags and transported to the laboratory within 24 hours. The samples were immediately refrigerated at 4°C when they arrived until further analysis could be performed.

3.3 Isolation of Soil Bacteria

3.3.1 Serial Dilution and Plating

One gram of soil was suspended in nine millilitres of sterile distilled water, and the mixture was agitated vigorously to separate the soil bacteria. After that, serial dilutions

were carried out until the dilution factor reached 10^{-6} . 0.1 mL of each dilution was uniformly distributed on nutrient agar plates. After that, these plates were incubated for 24 to 48 hours at 37°C to support the growth of bacterial colonies.

3.3.2 Colony Selection and Purification

Morphological distinct colonies were selected and streaked onto fresh nutrient agar (NA) plates. Pure isolates were then sub-cultured multiple times to ensure their purity. These purified isolates were preserved by storing them on slants at 4°C

3.4 Preliminary Screening for Urease Activity

3.4.1 Christensen's Urea Agar Test

For preliminary screening of urease activity, each isolate was inoculated onto Christensen's urea agar [Composition; peptone-1.0g, sodium chloride- 5.0g, potassium phosphate- 2.0g, glucose- 1.0g, phenol red- 0.012g, urea- 20.0g, agar- 15.0g] slants and incubated at 30°C for up to 72 hours. Positive urease activity was indicated by a color change in the medium from yellow to pink, resulting from alkalinisation.

3.5 Quantitative Urease Activity Assay

3.5.1 Preparation of Inoculum

The inoculum was prepared by isolating bacteria from soil samples. A small amount of soil was suspended in sterile saline solution and mixed thoroughly to create a soil slurry. This suspension was then serially diluted, and an aliquot was used to inoculate an appropriate growth medium. The culture was incubated under suitable conditions until it reached the desired cell density, at which point it was used for the quantitative urease activity assay.

3.5.2 Observation and Collection of Precipitate

The soil isolate culture was observed for the formation of any precipitate, which indicates enzymatic activity or biochemical changes. When a precipitate was visible, it was carefully collected by centrifugation or filtration, depending on the method used. The collected precipitate was then washed with sterile distilled water to remove any impurities before further analysis or quantification.

3.6 Morphological Characterization of Bacterial Isolates

The colonies were observed for color, shape, elevation, margin, and texture. Microscopic examination was carried out using Gram staining (for bacteria) and Lactophenol Cotton Blue staining (for fungi) to determine cell morphology and structure.

3.7 Biochemical Tests (for Bacteria)

Biochemical tests are essential tools in microbiology used to identify and differentiate bacteria based on their enzymatic activities and metabolic characteristics. These tests are designed to detect the presence or absence of certain enzymes, metabolic pathways, and the ability of bacteria to utilize or break down specific substrates. Each bacterial species possesses unique biochemical properties, and by analysing these reactions, microbiologists can accurately determine the identity of an unknown bacterial isolate.

3.7.1 The citrate utilization test

A slant of Simmons' Citrate Agar was prepared and marked for every test organism and control tubes. A pure colony (18 h old) of each isolate was transferred using a sterile inoculating needle to the slanted surface of the medium by streaking in a zigzag fashion; the butt was not stabbed. The tubes were incubated aerobically at 37 °C for 24 h, then examined; if there was no growth or color change, incubation was extended up to 48 h.

The test was positive if there was growth on the slant surface and/or the medium changed from green to blue (indicating an alkaline pH shift as a result of citrate metabolism). A negative result was recorded when there was no growth and the medium remained green. The degree of growth and intensity of colour change were semi-quantitatively recorded as light, moderate or heavy, and colour change graded as 0 (no change), 1 (slight bluish), 2 (full blue). *Klebsiella pneumoniae* (positive) and *E. coli* (negative) were employed as control organisms to validate the test.

3.7.2 The Urease Test

Dissolve 14 grams of urea in 200 mL of distilled water to create a concentrated urea solution that will be used as the supplement for the medium. Prepare the base agar medium by combining the standard components (peptone, glucose, sodium chloride, phosphates, phenol red indicator, and agar) in distilled water at the designated amounts and sterilize by autoclaving, then cool to approximately 50 -55 °C. Then, while cooled, aseptically add the sterilized urea solution to the cooled base medium, thoroughly mix and transfer to sterile test tubes (-5 mL per tube) so the contents are slanted. Label each slant and inoculate with a heavy loopful of an 18-24 h pure culture of bacteria on the slant portion only (do not stab the butt). Place the tubes at 35-37 °C for incubation with caps loosely fitted. If the organism produces urease, hydrolyses the urea to ammonia and carbon dioxide, resulting in alkalinisation of the medium and visible colour change of the

phenol red indicator, turning from yellow/orange to bright pink or fuchsia (positive test).

If no urease is produced, the medium remains its original colour and demonstrates little to no growth (negative test).

3.7.3 Triple Sugar Iron (TSI) agar test,

Distilled water, heating until the agar is completely melted and the ingredients are uniformly dispersed. After sterilising the prepared medium (e.g., autoclaving at 121 °C for 15 minutes), dispense into sterile test tubes 5 mL each and allow the tubes to cool in a slanted position. To prepare the TSI medium, weigh out 12.8 g of the agar powder (containing the required sugars, peptones, sodium thiosulfate, ferrous salt and phenol red) and dissolve it in 200 mL of position so as to form a short slanted position and deep butt. Using a sterile inoculating needle, stab the butt of the slant deeply and then streak the surface of the slant with a pure culture; loosely cap the tubes and incubate at 35-37 °C for 18-24 hours. After incubation, interpret the results by examining the colour of the medium and the presence of gas bubbles or black precipitate: a yellow slant and butt indicates fermentation of glucose, lactose and/or sucrose; a red slant with yellow butt indicates only glucose fermentation; blackening in the butt signifies hydrogen sulphide (H₂S) production; and cracks or lifts in the agar indicate gas production.

3.7.4 Gram staining

Gram staining is a differential staining process used for the grouping of bacteria based on their cell wall structure. A smear of bacteria is prepared initially on a clean slide and subjected to heat-fixation to secure the cells. The smear is flooded with crystal violet, the first stain, for about 30–60 seconds and then washed with water, which colors all the bacteria purple at first. The slide is incubated using Gram's iodine as a mordant for a further 30–60 seconds; it forms a crystal violet-iodine complex that becomes trapped in the dense peptidoglycan layer of Gram-positive cells. The slide is then washed in alcohol or acetone for a short time as a decolourizer. Gram-positive bacteria retain the purple stain because of their thick peptidoglycan layer, whereas Gram-negative bacteria, with a thin layer of peptidoglycan and an outer membrane, lose the dye and appear pale-colour. In order to make the decolorized cells visible, the smear is counterstained with safranin for 30–60 seconds, which red or pink stains Gram-negative bacteria, whereas Gram-positive bacteria remain purple. Gram-positive bacteria under the microscope are purple-colour and typically differentiated by a thick peptidoglycan cell wall, for example, *Staphylococcus* or *Streptococcus*, whereas Gram-negative bacteria are pink-colour and possess a thin peptidoglycan layer topped with an outer lipid membrane, for example, *Escherichia coli* or *Salmonella*. Decolourization timing and young cultures are necessary to obtain accurate results

3.8 Biochemical Tests Interpretation

Biochemical characterization yielded qualitative results (positive or negative reactions), no statistical analysis was applied. The results were interpreted based on established biochemical profiles for bacterial identification. Samples identified biochemically in the study were carried out in triplicates to ensure reliability and reproducibility of the results. All experiments conducted in triplicates. The biochemical characteristics of the bacterial isolates were determined based on standard reactions for citrate utilization, urease production, hydrogen sulfide formation, and carbohydrate fermentation patterns. The reactions were recorded as positive (+) or negative (-), and the results were used for species identification.

CHAPTER FOUR

4.0

RESULTS

4.1 Isolation and morphological characteristics of bacterial isolates

Bacterial colonies obtained from different soil sample exhibited varying morphological characteristics. Colonies differed in color, margin, shape and elevation, indicating the presence of diverse bacterial species within the soil. Most of the isolates produced circular, opaque and cream colored colonies typically *Bacillus* species, while other showed greenish or yellow pigmentation.

Distinct colonies were observed on nutrient agar after incubation. The colonies differed in colours, size, margin, and elevation, which served as preliminary identification criteria. Most colonies appeared creamy, circular, and raised, typical of soil bacteria such as *Bacillus* and *Pseudomonas*

Table 1: Isolation and morphological characteristics of bacterial isolates

Isolate	Colony size	Color	Texture
ERa	Large	Milk	Rough edge
ERb	Large	White	Smooth edge
ERc	Small	Milk	Smooth edge
ERd	Small	White	Smooth edge

Key: ER = Energy research

4.2 Biochemical Characterization of Bacterial Isolates

Isolates were subjected to a series of biochemical tests including urease, citrate utilization, and triple sugar iron (gas, glucose, lactose, sucrose H₂S, motility).

4.3 Identification of the Isolates

Based on biochemical reactions and comparison with Bergey's Manual of Determinative Bacteriology, the isolates were tentatively identified ERa (*proteus mirabilis*), ERb (*kocuria* species), ERc (*klebsiella aerogenes*), ERd (*micrococcus* species) suggests they are the major calcite-precipitating bacteria in the studied soil.

Proteus mirabilis is a Gram-negative, motile rod known for its characteristic swarming growth on agar and positive urease and H₂S production. Though primarily studied as a human pathogen, it plays a role in soil nutrient cycling and decomposition. *Kocuria* species are Gram-positive cocci arranged in tetrads or irregular clusters, forming pigmented colonies and showing catalase positivity; they are notable in agriculture for their ability to promote plant growth through phytohormone production, phosphate solubilisation, and bioremediation of heavy metals. *Klebsiella aerogenes*, a Gram-negative, mucoid rod, ferments lactose and shows positive citrate and Voges-Proskauer reactions; it contributes significantly to agriculture by fixing atmospheric nitrogen and producing phytohormones that enhance plant growth. *Micrococcus* species are Gram-

positive cocci occurring in tetrads with pigmented colonies, catalase and oxidase positive, and non-motile; they are important in soils for organic matter decomposition and maintaining soil health. Together, these bacteria demonstrate diverse roles in nutrient cycling, plant growth promotion, and soil ecosystem maintenance.

Table 2: Biochemical Characteristics of Bacterial Isolates

Isolate	Gram	Cit	ure	H ₂ S	Mot	Gas	Glu	Suc	Lac	Organism
ERa	–	+	+	+	+	–	+	–	–	<i>Proteus mirabilis</i>
ERb	+	+	+	+	+	+	+	+	+	<i>Kocuria spp</i>
ERc	–	+	+	–	+	–	+	+	+	<i>Klebsiella</i> <i>aerogenes</i>
ERd	+	+	+	+	–	–	+	+	+	<i>Micrococcus</i> species

Key:

(+) = Positive reaction; (–) = Negative reaction

Key: Cit = Citrate, Ure = Urea, H₂S= Hydrogen Sulphide, Glu = Glucose, Suc = Sucrose, Lac =Lactose

4.4 Calcite Precipitating Ability

The studies recognized that *Proteus mirabilis*, *Klebsiella aerogenes*, *Kocuria* species, and *Micrococcus* species are able to precipitate calcium carbonate with varying efficiencies. The Gram-negative bacteria *Proteus mirabilis* and *Klebsiella aerogenes* showed greater calcite precipitation, whose causes are perhaps due to their strong urease activity that allows for the hydrolysis of urea into carbonate ions that are incorporated with calcium to form calcium carbonate. *Kocuria* and *Micrococcus* species, Gram-positive bacteria, also contributed to calcite precipitation, though less effectively, implying their potential role in mineral enrichment of the soil. The ability of such microbes to induce calcite precipitation holds many applications in agriculture, including improving soil texture, reducing erosion, and augmenting water retention and nutrient provision. The findings show that Gram-positive and Gram-negative bacteria can be employed as bio-agents for sustainable soil fertility and biofertilization to develop healthier and more sustainable agricultural environments.

4.5 Limitations and Future Work

This study was limited to laboratory-scale isolation and characterization. Further molecular and genomic analyses are required to confirm specific urease genes and pathways involved in calcite precipitation. Field-scale experiments should also be conducted to assess the efficiency of these isolates under natural environmental

conditions. Future research could explore optimization of nutrient conditions, co-culturing of isolates, and the long-term durability of bio cemented soils.

CHAPTER FIVE

DISCUSSION

5.1 Discussion

The microbial analysis of the soil samples revealed the presence of four dominant bacterial isolates: *Proteus mirabilis*, *Klebsiella aerogenes*, *Kocuria* spp., and *Micrococcus* spp., indicating a metabolically and taxonomically diverse soil microbiome. The ability of these isolates to grow on urea-containing media and form visible calcium carbonate precipitates confirms their potential roles in Microbial Induced Calcite Precipitation (MICP).

The coexistence of both Gram-positive (*Kocuria* and *Micrococcus*) and Gram-negative (*Proteus mirabilis* and *Klebsiella aerogenes*) bacteria suggests that the soil environment supports microorganisms with diverse metabolic capabilities. These bacteria likely contribute to essential soil functions such as organic matter decomposition, nitrogen transformation, and carbonate mineralization. The observed calcite precipitation indicates the activity of urease-producing bacteria that can hydrolyse urea, increase local pH, and promote calcium carbonate crystallization — a process essential in soil stabilization and bio cementation.

The bacterial isolates identified in this study are consistent with those previously reported in MICP-related research. For instance, *Proteus mirabilis* and *Klebsiella aerogenes* have been described as efficient urease producers capable of inducing calcite precipitation in contaminated or alkaline soils (Ghosh et al., 2019; Patel et al., 2021). Similarly, *Micrococcus* and *Kocuria* spp. have been associated with bio calcification in nutrient-limited environments where their tolerance to stress conditions supports mineral formation (Prakash et al., 2025). These parallels strengthen the reliability of the findings and highlight the ubiquity of these bacteria in different soil types

Although *Micrococcus* species are often reported in low abundance in nutrient-rich soils, their notable presence in this study may indicate adaptation to nutrient-poor or alkaline environments. Such ecological versatility may enhance their potential for applications in bioremediation and bio-consolidation. Additionally, the relatively higher urease activity observed in *Klebsiella aerogenes* compared to *Proteus mirabilis* contrasts with previous studies, possibly due to differences in soil origin, nutrient availability, or incubation conditions

The MICP potential of these indigenous isolates suggests promising applications in eco-friendly construction materials, soil stabilization, and restoration of eroded lands. Their ability to precipitate calcium carbonate could also be explored in carbon sequestration and reduction of soil permeability for geotechnical engineering purposes. Moreover,

using native bacterial strains reduces ecological risks compared to introducing non-indigenous microorganisms, aligning with sustainable environmental management goals.

5.2 Conclusion

This study successfully isolated and characterized four indigenous bacterial species from agricultural soil with the potential for calcite precipitation. Among the isolates, *Proteus mirabilis* exhibited the highest urease activity and calcite precipitation ability, followed by *Klebsiella aerogenes*, *Kocuria* species, and *Micrococcus* species.

The results demonstrate that these indigenous bacteria can effectively precipitate calcium carbonate through biochemical pathways involving urease activity and pH modulation. This biological process offers a sustainable, non-toxic alternative to chemical soil stabilization techniques, making it highly relevant for agricultural and environmental management practices.

Therefore, the use of indigenous calcite-precipitating bacteria represents a promising biotechnological approach for improving soil physical properties and ensuring long-term agricultural sustainability.

5.3 Recommendation

1. Application in Agriculture: *Proteus mirabilis* and *Klebsiella aerogenes* should be further developed as bio-agents for enhancing soil aggregation, erosion control, and water retention capacity in farmlands.
2. Further Research: Future studies should include molecular identification (16S rRNA sequencing) to confirm bacterial taxonomy and assess genetic determinants of urease production.
3. Optimization of MICP Conditions: Studies should be carried out to optimize parameters such as calcium source, urea concentration, incubation time, and temperature for maximum calcite yield.
4. Pilot Field Trials: Bio-cementation trials should be conducted in agricultural fields to validate laboratory results and evaluate real-world performance.
5. Integration into Soil Fertility Programs: Agricultural extension services and soil research institutes should promote microbial soil stabilization as part of integrated soil fertility management and climate adaptation strategies.

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