

**BIOCHEMICAL IDENTIFICATION OF UREOLYTIC BACTERIA WITH
CALCITE PRECIPITATION ABILITIES ISOLATED FROM ENERGY
RESEACH CENTER SOKOTO**

BY

ABDULRAHMAN MUHAMMAD

ADM. NO. 2010302081

**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF
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CERTIFICATION

This project research titled “**Biochemical Identification of Ureolytic Bacteria with Calcite Precipitation Abilities Isolated from Energy Research Center Sokoto**” by ABDULRAHMAN MUHAMMAD with Adm. No: 2010302081 meets the regulation governing the award of Bachelor of Science (B. Sc.) Degree in Biology of the Usmanu Danfodiyo University Sokoto, and it is approved for its contribution to scientific knowledge and literary presentation.

External Examiner

Date

Dr. Z.M Bello

Date

Project Supervisor

Prof. M.M Yahaya

Date

Head of Department

DEDICATION

This project research is dedicated to my parents MAL. MUHAMMAD ADAMU (F.M.C) and my mother HAJ. AISHA MUHAMMAD who plays a vital role throughout my academic journey right from my primary school to the completion of my Degree program, I also dedicate this project to all my siblings for their incessant support financially and morally.

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All praise is indeed due to Allah (SWT), blessings and salutation upon prophet Muhammad (SAW), his household, companions and those who followed their footsteps till the Day of Judgment.

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ABSTRACT

This study identified ureolytic bacteria capable of precipitating calcium carbonate (CaCO_3) from soil samples at the Energy Research Center, Usmanu Danfodiyo University, Sokoto. The aim of the study was to isolate urease-producing bacteria and assess their potential to enhance water retention and structural stability of sandy soils via microbially induced calcite precipitation (MICP). Soil samples were enriched, screened for urease activity, and characterized morphologically and biochemically. Key tests included catalase, oxidase, citrate, indole, urease, and TSI.. Results revealed diverse ureolytic isolates related to *Klebsiella aerogenes*, *Proteus vulgaris*, and *Proteus mirabilis*. One isolate (E3) showed the highest urease activity and calcite precipitation, which may significantly improve sand water retention and aggregate stability when compared to controls. Urease activity and precipitation efficiency were influenced by temperature, pH, and growth medium. This study demonstrates that local ureolytic bacteria have strong potential for biological soil stabilization and water retention improvement in sandy soils and warrants further molecular identification and field validation for sustainable soil management in semi-arid environments.

CHAPTER ONE

1.0

INTRODUCTION

Many biogeochemical cycles center on microorganisms, which also show the extraordinary capacity to affect their physical surroundings including mineral production through biologically induced biomineralization whereby particular conditions allow bacteria to help calcium carbonate (CaCO_3) minerals form. Through direct enzymatic processes or indirectly by modifying local environmental factors including pH, dissolved inorganic carbon, and ion concentration (De Muynck *et al.*, 2010).

Soil is one of the most fundamental resources for life on earth, supporting agriculture, vegetation, water processes. One such mechanism is Microbially Induced Calcite Precipitation (MICP), a kind of cycling, and ecosystem balance. However, not all soils provide the same benefits, and sandy soils, which are predominant in arid and semi-arid regions such as Sokoto State and much of northern Nigeria, often pose serious challenges to both farmers and environmental sustainability. A key limitation of sandy soil is its poor water holding capacity, which results in rapid drainage of water, low nutrient retention, and limited crop productivity (Brady *et al.*, 2017). This characteristic

not only affects agriculture but also influences groundwater recharge, soil ecology, and long-term land use.

Recent attention has shifted towards biological solutions to these problems, particularly the use of microorganisms that can improve soil structure and stability through processes such as microbially induced calcite precipitation (MICP). MICP is a naturally occurring process in which certain microorganisms, especially urease-producing bacteria, promote the precipitation of calcium carbonate (CaCO_3) minerals within their environment. This biomineralization occurs when bacteria hydrolyze urea, releasing ammonium and carbonate ions that combine with calcium ions to form CaCO_3 crystals. These precipitated crystals can bind loose soil particles together, reduce pore spaces, and ultimately increase the soil's ability to retain water (DeJong *et al.*, 2010; Achal *et al.*, 2015).

From a biological perspective, the beauty of this process lies in the fact that it is eco-friendly, self-sustaining, and adaptable to a variety of environmental conditions. Ureolytic bacteria such as *Sporosarcinapasteurii*, *Bacillus subtilis*, *Proteus mirabilis*, and some *Pseudomonas* species are known to exhibit strong urease activity that promotes calcite precipitation (Stocks-Fischer *et al.*, 1999; Anbu *et al.*, 2016). These bacteria can be isolated from diverse ecological niches such as soils, caves, marine

environments, wastewater, and even industrial sites where they often adapt to harsh conditions. Their ability to survive and function under stress makes them promising candidates for biotechnological applications, including soil improvement, heavy metal remediation, and carbon sequestration (Dhami *et al.*, 2013).

In the context of sandy soils, the precipitation of CaCO_3 acts like a biological “glue,” improving soil aggregation, reducing permeability, and enhancing water retention. This directly addresses one of the most persistent problems faced by farmers in sandy regions: the inability of soil to hold sufficient water for crops. Thus, while MICP has often been studied in engineering contexts such as self-healing concrete or construction material stabilization, its potential in soil biology and agriculture is even more promising yet underexplored (Chahal *et al.*, 2011; Gowthaman *et al.*, 2021).

The Energy Research Center provides a unique and underutilized source of microbial diversity. Its soils and environments may harbor bacterial communities that have been exposed to industrial byproducts, variable temperatures, and unique chemical stresses. Such conditions often encourage the evolution of stress-tolerant and metabolically versatile microorganisms, which may exhibit novel or enhanced urease activity. Exploring these bacterial populations and their ability to precipitate calcite not only expands the scientific understanding of microbial diversity but also provides potential

biological tools for solving soil-related challenges like poor water retention in sandy soils.

Biochemical identification of these isolates is particularly important, as it helps to characterize their metabolic pathways, enzyme activity, and environmental roles. This step is crucial in determining whether the isolated bacteria are suitable for practical applications, such as improving soil fertility, stabilizing sandy farmlands, and reducing water loss in agriculture. By identifying and studying these bacteria, researchers can contribute both to scientific knowledge and to the development of sustainable biological solutions that align with the United Nations' Sustainable Development Goals (SDGs), particularly in combating desertification, ensuring food security, and promoting sustainable land management.

1.1 Statement of the Research Problem

In many semi-arid and arid zones, particularly in northern Nigeria, sandy soils dominate the landscape. While these soils are easy to till and warm quickly in the sun, their major limitation lies in their low water retention capacity, which results in poor crop yields, high water demand, and nutrient leaching (Lal, 2006). Farmers often face situations where irrigation water quickly percolates beyond the root zone, making agriculture more expensive and less sustainable.

Conventional solutions, such as the addition of organic matter, chemical stabilizers, or irrigation management, provide only partial or temporary relief and are often not economically viable for rural communities (Okon *et al.*, 2019). Moreover, chemical additives may lead to secondary environmental issues such as soil toxicity or groundwater contamination.

Despite the promising role of microbially induced calcite precipitation (MICP) in improving soil properties, most studies have been conducted in the field of engineering—focusing on concrete durability, stone restoration, or soil stabilization for construction. Very few studies have addressed the direct application of MICP for solving agricultural problems associated with sandy soils. Even fewer have targeted specialized environments such as energy research centers as sources of ureolytic bacteria, despite their potential to harbor unique and stress-resistant microbial strains.

The lack of data on the role of these bacteria in enhancing soil water retention capacity presents a major gap in knowledge. Unless this gap is filled, sandy soils in semi-arid regions will continue to hinder agricultural productivity, worsen food insecurity, and contribute to environmental degradation.

1.2 Justification for the Research

Addressing the water retention problem of sandy soils is crucial for sustainable agriculture and food production, especially in regions like Sokoto State that experience frequent droughts and low soil fertility. Exploring biological approaches such as MICP offers a safe, cost-effective, and eco-friendly alternative to conventional methods.

Sandy soils are widely recognized for their poor water retention, low nutrient content, and weak structural stability, all of which make them unsuitable for sustainable crop production. Their large pore spaces and limited organic matter content allow water to drain rapidly, leaving insufficient moisture for plants to thrive. This problem is particularly severe in arid and semi-arid regions such as Sokoto State, Nigeria, where recurrent droughts, erratic rainfall patterns, and fragile soils combine to limit agricultural productivity. Farmers in this region are faced with persistent challenges in maintaining fertile soils and securing stable crop yields, which ultimately affects food availability and rural livelihoods. Addressing the water retention problem of sandy soils in such areas is therefore critical for achieving sustainable agriculture and ensuring food security (Mustapha *et al.*, 2020).

The significance of this research lies in the possibility of using ureolytic bacteria to transform the physical and chemical properties of sandy soils. Through the enzymatic

activity of urease, these bacteria hydrolyze urea, releasing carbonate ions that subsequently react with calcium ions to form calcium carbonate (calcite). The precipitation of calcite within soil pores binds particles together, reduces porosity, and increases water-holding capacity. As a result, sandy soils that typically fail to support crops can be transformed into fertile and resilient soils capable of sustaining agricultural production. Such improvements would not only reduce dependence on irrigation but also enhance nutrient retention and crop yields, thereby directly improving farmer livelihoods in Sokoto State and similar environments (DeJong *et al.*, 2010).

1.3 Aim and Objectives of the Research

The main aim of this research is to biochemically identify ureolytic bacteria with calcite precipitation abilities isolated from the Energy Research Center, and to assess their potential role in improving the water holding capacity of sandy soils.

The Specific Objectives of this Research are to:

1. To isolate and culture ureolytic bacteria from environmental samples collected at the Energy Research Center.
2. To conduct biochemical identification and characterization of the most potent ureolytic bacterial strains.
3. To assess the calcite precipitation ability of the ureolytic isolates.

CHAPTER TWO

2.0

LITERATURE REVIEW

Microbes (or microorganisms) include bacteria, fungi, protozoa, micro algae, and viruses. Microbes live in familiar settings such as soil, water, food, and animal intestines, as well as in more extreme settings such as rocks, glaciers, hot springs, and deep-sea vents. The wide variety of microbial habitats reflects an enormous diversity of biochemical and metabolic traits that have arisen by genetic variation and natural selection in microbial populations (Singh and Kapoor, 2010).

Microbial biotechnology, enabled by genome studies, will lead to development of new industrial catalysts and fermentation organisms, and development of new microbial agents for bioremediation of soil and water contaminated by agricultural runoff. Microbial genomics and microbial biotechnology research is critical for advances in food safety, food security, value-added products, human nutrition and functional foods, plant and animal protection, and furthering fundamental research in the agricultural sciences (Singh and Kapoor, 2010).

2.1 Microorganisms and Their Roles

2.1.1 Definition and Diversity of Microorganisms

Microorganisms, commonly referred to as microbes, are microscopic living organisms that include bacteria, archaea, fungi, protozoa, viruses, and microalgae. They represent the most abundant and diverse group of life forms on Earth, inhabiting virtually every ecological niche including soil, water, air, plants, animals, and even extreme environments such as hot springs, saline lakes, and deep-sea vents (Madigan *et al.*, 2021). Their diversity is reflected in their metabolic versatility, which allows them to perform critical biochemical reactions in ecosystems, including decomposition, nutrient cycling, and symbiotic associations with plants and animals.

2.1.2 Ecological Functions of Microorganisms

Microbes play a central role in maintaining ecological balance. In soils, bacteria and fungi are responsible for organic matter degradation and humus formation, while actinomycetes and archaea contribute to nitrogen fixation and sulfur cycling (van Elsas *et al.*, 2020). In particular, soil bacteria mediate biochemical reactions that regulate carbon sequestration, soil fertility, and aggregate stability. Microorganisms also serve as bioindicators of soil health due to their sensitivity to changes in soil pH, organic matter, and moisture content (Nannipieri *et al.*, 2017).

2.1.3 Importance in Soil and Plant Systems

In agricultural systems, microorganisms establish beneficial interactions with crops, such as plant growth-promoting rhizobacteria (PGPR), mycorrhizal fungi, and nitrogen-fixing symbionts (Smith *et al.*, 2019). They improve nutrient availability, enhance plant tolerance to abiotic stress, and play vital roles in sustainable agriculture. Additionally, soil microorganisms influence soil structure through production of extracellular polymeric substances (EPS), which help bind soil particles into stable aggregates (Lehmann *et al.*, 2020).

2.2 Urease-Producing Bacteria

2.2.1 Nature and Function of Urease Enzyme

Urease is a nickel-containing metalloenzyme that catalyzes the hydrolysis of urea into ammonia and carbon dioxide (Mobley *et al.*, 2020). This reaction increases the pH of the microenvironment due to the release of ammonium ions, making urease activity an important survival mechanism for microorganisms in acidic conditions. Urease is widely distributed among bacteria, fungi, and plants, and in bacteria, it is a key enzyme in pathogenesis, nitrogen metabolism, and biomineralization.

2.2.2 Diversity of Ureolytic Bacteria

Numerous bacterial genera exhibit urease activity, including *Sporosarcina*, *Bacillus*, *Pseudomonas*, *Proteus*, and *Klebsiella* (Achal & Pan, 2014). Among these, *Sporosarcinapasteurii* has been extensively studied for its strong ureolytic capacity and ability to induce calcite precipitation. The diversity of ureolytic bacteria in soils is influenced by environmental factors such as nitrogen content, organic matter availability, and soil pH (Dhami *et al.*, 2017).

2.2.3 Role of Urease in Microbial Survival and Adaptation

Urease activity allows bacteria to utilize urea as a nitrogen source, providing a competitive advantage in nitrogen-limited soils. The alkaline conditions created by urease-mediated hydrolysis also facilitate bacterial colonization in harsh environments, including acidic soils and contaminated sites (Burbank *et al.*, 2012). Furthermore, urease-driven precipitation of calcium carbonate provides protective microhabitats for bacteria by encapsulating cells in mineralized biofilms (DeJong *et al.*, 2020).

2.3 Microbial-Induced Calcite Precipitation (MICP)

2.3.1 Mechanisms of Calcite Precipitation

Microbial-induced calcite precipitation (MICP) is a biologically driven process in which microorganisms induce the deposition of calcium carbonate (CaCO_3) minerals

through metabolic activities. The ureolytic pathway of MICP involves hydrolysis of urea by urease, leading to the production of carbonate ions, which react with calcium ions in the environment to precipitate calcite (Dhami *et al.*, 2013). This process can occur extracellularly in bacterial biofilms or directly around bacterial cell walls.

2.3.2 Environmental Factors Influencing MICP

Several factors influence MICP efficiency, including temperature, pH, calcium ion concentration, bacterial density, and availability of urea (Cheng & Cord-Ruwisch, 2012). Optimal MICP typically occurs in neutral to alkaline pH ranges and moderate temperatures (25–35°C). The rate of calcite precipitation is also enhanced by biofilm formation, which provides nucleation sites for calcium carbonate crystals (Hammes *et al.*, 2002).

2.3.3 Biological Advantages of MICP

The ability to precipitate calcite provides multiple benefits to microbes. It enhances their survival in nutrient-poor environments by forming mineralized biofilms that protect against desiccation and predation (Dhami *et al.*, 2017). Additionally, MICP contributes to natural processes such as sediment formation, cave speleothem development, and stabilization of soils.

2.4 Soil Ecology and Challenges of Sandy Soil

2.4.1 Characteristics of Sandy Soil

Sandy soils are characterized by large particle size, low organic matter content, and poor aggregation. These properties result in rapid water drainage, low nutrient retention, and reduced microbial biomass (Brady & Weil, 2019). In arid and semi-arid regions such as northern Nigeria, sandy soils dominate, presenting significant agricultural challenges.

2.4.2 Agricultural Limitations of Sandy Soil

The primary limitation of sandy soils is their poor water-holding capacity. Due to their coarse texture, water quickly drains through the profile, leaving crops vulnerable to drought stress (Lal, 2015). Additionally, nutrients such as nitrogen, potassium, and phosphorus are easily leached, leading to low fertility and reduced crop productivity.

2.4.3 Microbial Strategies for Improving Sandy Soil

Microorganisms can improve sandy soil properties through several mechanisms, including production of extracellular polysaccharides that enhance soil aggregation, nitrogen fixation to enrich nutrient content, and microbial-induced calcite precipitation to increase water retention and structural stability (Singh *et al.*, 2020).

2.5 Role of Ureolytic Bacteria in Soil Amelioration

2.5.1 Soil Aggregation and Stabilization

Ureolytic bacteria enhance soil aggregation by precipitating calcite that binds soil particles together into stable aggregates (Burbank *et al.*, 2012). This improves resistance against erosion and enhances soil tilth.

2.5.2 Water-Holding Capacity Enhancement

The precipitation of calcite in sandy soils reduces pore size and enhances soil water retention. By creating micro-porous structures, MICP improves the capacity of sandy soils to hold water for longer durations, thereby supporting crop growth in drought-prone areas (Arias *et al.*, 2017).

2.5.3 Soil Fertility and Nutrient Retention

MICP can also improve nutrient retention in sandy soils by reducing leaching losses and enhancing cation exchange capacity. Additionally, ureolytic bacteria contribute biologically available nitrogen through urea hydrolysis, further improving soil fertility (Achal & Mukherjee, 2015).

2.6 Applications of MICP in Biological and Environmental Systems

2.6.1 Soil Improvement and Agriculture

MICP is increasingly recognized as a sustainable strategy for enhancing soil structure and fertility in agriculture. By improving water-holding capacity and nutrient availability, it addresses key limitations of sandy soils (Chowdhury *et al.*, 2022).

2.6.2 Bioremediation of Polluted Soils

Ureolytic bacteria and MICP can immobilize heavy metals and radionuclides in contaminated soils by co-precipitation with calcium carbonate, reducing their bioavailability and toxicity (Fujita *et al.*, 2010).

2.6.3 Carbon Sequestration and Climate Change Mitigation

Through calcite precipitation, microbes contribute to long-term sequestration of atmospheric CO₂ in mineral form, thereby mitigating climate change impacts (Mitchell *et al.*, 2019).

2.6.4 Biodiversity and Ecosystem Conservation

MICP stabilizes soils against erosion and desertification, which are major threats to biodiversity in fragile ecosystems. By improving soil health, microbial calcite precipitation supports vegetation growth and ecosystem resilience (Singh *et al.*, 2020).

2.7 Summary

This chapter has reviewed the biological importance of microorganisms, focusing on urease-producing bacteria and their role in microbial-induced calcite precipitation (MICP). Ureolytic bacteria such as *Sporosarcina pasteurii* play significant roles in soil ecology by enhancing aggregation, water-holding capacity, and fertility of sandy soils. MICP, driven by microbial urease activity, presents a sustainable approach to improving soil health, particularly in semi-arid regions where sandy soils dominate. Beyond agriculture, ureolytic bacteria also contribute to environmental protection through bioremediation, carbon sequestration, and ecosystem conservation. These findings establish the scientific basis for biochemical identification of ureolytic bacteria isolated from the Energy Research Center and highlight their potential applications in solving the challenges of poor soil water-holding capacity.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site and Location

This study was conducted at the Energy Research Center, Usmanu Danfodiyo University Sokoto (UDUS), Nigeria, which is well equipped with laboratory facilities for microbiological and biochemical research. The Center is located in Sokoto State, a region within the semi-arid zone of northwestern Nigeria. The climate of Sokoto is characterized by low annual rainfall, high temperatures, and sandy soils with poor water-holding capacity. Such soils are prone to desertification and low agricultural productivity (Adamu and Ahmed, 2019). The sandy soil samples collected from the area served as the experimental material to evaluate the potential of ureolytic bacteria in calcite precipitation and their ability to enhance soil water retention.

3.1.1 Materials

The materials employed in this study included both consumables and laboratory supplies. Sterile sampling bags and spatulas were used for soil collection to prevent contamination. Petri dishes, test tubes, glass slides, and conical flasks were employed for culturing and maintaining bacterial isolates. Inoculating loops and wire needles facilitated aseptic transfer of microorganisms during streaking and inoculation. Filter

papers were utilized in media preparation and sterilization processes, while distilled water served as a universal solvent for all reagents and culture media. In addition, soil samples were obtained from energy research center to serve as substrates for microbial activity and calcite precipitation studies (Cowan and Steel, 2004).

3.1.2 Apparatus

The apparatus used in this research included both analytical and microbiological instruments. An autoclave was employed for sterilization of culture media, glassware, and other laboratory materials at 121°C under 15 psi for 15 minutes (Cheesbrough, 2010). A hot air oven was used for sterilizing dry glassware and Petri dishes at 160°C for two hours. An incubator was utilized for maintaining cultures at controlled temperatures, particularly at 37°C, which is optimal for many bacterial species. The analytical balance ensured precise measurement of media components and reagents. A compound light microscope facilitated the observation of cell morphology and Gram reactions. A spectrophotometer was employed for measuring bacterial growth and biomass by recording optical density at 600 nm, while a centrifuge was used for separating cells from culture supernatants during biochemical analyses (Prescott *et al.*, 2013).

3.1.3 Reagents

Several microbiological reagents and chemicals were used. Urea was the primary substrate to detect and quantify urease activity, while calcium chloride (CaCl_2) served as the calcium source for calcite precipitation. Nutrient agar and nutrient broth were used for general bacterial cultivation, while urea agar medium (Christensen's medium) was used for screening urease activity (Atlas, 2010). Other reagents included Gram staining dyes (crystal violet, iodine, ethanol, and safranin) for microscopic characterization of isolates. Biochemical test reagents such as oxidase, catalase, citrate, and indole were used for further bacterial identification. Additionally, peptone water was used as an enrichment medium to encourage the growth of fastidious bacteria.

Media preparation was carried out in strict adherence to standard microbiological procedures. Nutrient agar (NA) was prepared by dissolving 28g of powdered medium in 1 liter of distilled water, followed by heating to dissolve completely. The medium was dispensed into conical flasks and sterilized using an autoclave at 121°C for 15 minutes. After cooling to about 45°C , the medium was poured aseptically into sterile Petri dishes to solidify (Cheesbrough, 2010).

Urea agar slants (Christensen's medium) were prepared by suspending the required amount of dehydrated medium in distilled water and heating to dissolve. The medium

was dispensed into test tubes, plugged with cotton wool, and sterilized. After cooling, filter-sterilized urea solution was aseptically incorporated to avoid decomposition during autoclaving (Cappuccino and Sherman, 2014).

All prepared media were stored at 4°C until required for inoculation. Sterility of the media was confirmed by incubating randomly selected plates and slants at 37°C for 24 hours to check for contamination.

3.1.4 Preparation of Media

Media preparation was carried out in strict adherence to standard microbiological procedures. Nutrient agar (NA) was prepared by dissolving 28g of powdered medium in 1 liter of distilled water, followed by heating to dissolve completely. The medium was dispensed into conical flasks and sterilized using an autoclave at 121°C for 15 minutes. After cooling to about 45°C, the medium was poured aseptically into sterile petri dishes to solidify (Cheesbrough, 2010).

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All prepared media were stored at 4°C until required for inoculation. Sterility of the media was confirmed by incubating randomly selected plates and slants at 37°C for 24 hours to check for contamination.

3.2 Isolation of Urease-Producing Bacteria

3.2.1 Sample Collection

Soil samples were collected aseptically from different locations at the energy research center in triplicate. The topsoil was cleared, and samples were collected from a depth of 5 to 15cm using sterile spatulas. Approximately 200g of soil was collected at each sampling point and placed into sterile polyethylene bags, clearly labeled, and the sample were brought to microbiology lab under cold condition (4°C) to preserve microbial viability for isolation and identification of ureolytic bacteria (Holt *et al.*, 1994).

3.2.1.2 Enrichment of Sample

Ten grams (10g) of soil from each sample was introduced into 90ml of sterile peptone water in Erlenmeyer flasks. The flasks were incubated at 37°C on a rotary shaker at 120rpm for 24 hours. This enrichment favored the growth of urease-positive bacteria by providing them with a nutrient-rich environment (Prescott *et al.*, 2013).

3.2.1.3 Selection of Highly Urease-Active Bacteria

A loopful of the enriched culture was streaked onto urea agar slants and incubated at 37°C for 24 to 48 hours. Colonies that produced a distinct pink coloration due to ammonia release (alkalinization) were identified as ureolytic bacteria (Christensen, 1946). Representative colonies were selected and preserved for further studies.

3.2.2 Phenotypic Characterization of Isolates

The selected ureolytic bacterial isolates were subjected to morphological, microscopic, and biochemical characterization. Colony morphology was examined for shape, size, color, edge, and elevation. Gram staining was performed to determine the Gram reaction, shape, and arrangement of bacterial cells. Biochemical tests including catalase, oxidase, indole, citrate utilization, and sugar fermentation were conducted following standard protocols (Cheesbrough, 2010; Cappuccino and Sherman, 2014).

3.3 Biochemical Tests

To accurately identify the ureolytic bacteria isolated from the Energy Research Centre Sokoto, several standard biochemical tests were carried out following standard microbiological procedures (Cheesbrough, 2010; Cappuccino & Sherman, 2014). These tests help in understanding the metabolic capabilities of the isolates and confirming their taxonomic identity. The major biochemical tests conducted include

Catalase Test, Triple Sugar Iron (TSI) Test, Citrate Utilization Test, and Urease Test. Each test provides specific information about the physiological properties of the bacteria.

3.3.1 Catalase Test

The catalase test was performed to determine the ability of isolates to produce the enzyme catalase, which breaks down hydrogen peroxide into water and oxygen.

A drop of 3% hydrogen peroxide was placed on a clean slide, and a small amount of the bacterial colony was added. The appearance of immediate bubbling indicated a positive catalase reaction, showing that the bacteria can detoxify harmful peroxides. A negative result showed no bubble formation (Cheesbrough, 2010).

3.3.2 Citrate Utilization Test

The citrate test determines whether the organism can use citrate as its sole carbon source, an important distinguishing feature of many Gram-negative bacteria.

Citrate utilization was assessed using Simmons citrate agar slants. The surface of each slant was streaked lightly with the test organism and incubated at 37°C for 24–48 hours. A change in color from green to Prussian blue indicated a positive result, showing that the bacteria can utilize citrate and produce alkaline by-products. A negative result

retained the green color, meaning citrate could not be utilized (Cappuccino & Sherman, 2014).

This test is useful in differentiating members of *Enterobacteriaceae*, particularly *Klebsiella* and *Enterobacter* (positive) from *E. coli* (negative).

3.3.3 Triple Sugar Iron (TSI) Test

The TSI test helps differentiate bacteria based on their ability to ferment carbohydrates (glucose, lactose, and sucrose), produce gas, and generate hydrogen sulfide (H₂S).

Isolates were inoculated into the butt and slant of the TSI agar and incubated for 24 hours. A yellow slant and butt indicated fermentation of multiple sugars, while a red slant/yellow butt showed glucose-only fermentation. Cracks or lifting of the medium signified gas production, while blackening indicated H₂S production. This test provided insight into the metabolic versatility of the isolates. (Prescott *et al.*, 2013).

Interpretation

Observation	Meaning
Yellow slant / Yellow butt (A/A)	Fermentation of glucose + lactose and/or sucrose
Red slant / Yellow butt (K/A)	Glucose fermentation only
Red slant / Red butt (K/K)	No sugar fermented
Black precipitate	H ₂ S production
Cracks / bubbles	Gas production

TSI test provides quick differentiation among *Enterobacteriaceae* and other Gram-negative rods.

3.3.4 Urease Test

Since the study focuses on ureolytic bacteria, the urease test was essential. This test identifies bacteria capable of hydrolyzing urea into ammonia and carbon dioxide using the enzyme urease.

The isolates were streaked on urea agar slants and incubated at 37°C for 24-48 hours. A rapid pink coloration indicated a strong urease-positive reaction, confirming the ability of the bacteria to break down urea and raise the pH of the medium. This test also helped in selecting the most potent urease-producing strains for further calcite precipitation analysis.

CHAPTER FOUR

4.0

RESULTS

4.1 Bacterial Count of the Isolates

Quantitative analysis was conducted using the spread plate technique at 10^{-3} and 10^{-4} dilutions. The plates were incubated at 37°C for 24 hours, and the colony-forming units (CFU) were counted.

Isolate E3 had the highest colony count, indicating high bacterial density and metabolic adaptability in the nutrient agar medium.

Table 4.1: Colony Count of Bacterial Isolates

Isolate Code	10^{-3} Dilution (CFU)	10^{-4} Dilution (CFU)
E1	16	13
E2	24	16
E3	48	13

4.2 Colony Morphology and Cultural Characteristics

After incubation on nutrient agar for 24–48 hours at 37°C, distinct colonies appeared and were observed for their shape, size, color, edge, and surface texture. These morphological characteristics aided in differentiating bacterial isolates, providing the first evidence of microbial diversity within the collected soil.

Table 4.2: Morphological Characteristics of Bacterial Isolates

Isolate Code	Colony Appearance	Color	Edge	Surface Texture
E1a	Large	White	Rough	Dry
E1b	Small	White	Smooth	Moist
E1c	Large	Milky	Smooth	Glossy
E2a	Large	White	Rough	Opaque
E2b	Large	Milky	Smooth	Moist
E2c	Small	Milky	Smooth	Creamy
E3a	Large	White	Rough	Dull
E3b	Small	Milky	Smooth	Moist
E3c	Large	Milky	Rough	Opaque

4.3 Gram Staining and Microscopic View

Microscopic characterization of the bacterial isolates was performed using the Gram staining technique as described by Cheesbrough (2010). A small portion of each colony was transferred onto a clean glass slide containing a drop of sterile distilled water and smeared evenly. The smears were air-dried and heat-fixed by gently passing over a Bunsen flame.

The slides were flooded with crystal violet for 60 seconds, rinsed with water, followed by Gram's iodine for another 60 seconds to form a crystal violet–iodine complex. The smears were decolorized with 95% ethanol for 15–20 seconds and counterstained with safranin for 30 seconds. After rinsing and drying, they were examined under the oil immersion objective ($\times 1000$ magnification).

The results revealed both Gram-positive and Gram-negative bacteria, with varied morphologies such as rods and cocci, indicating a diverse microbial population capable of urease activity and calcite precipitation.

Table 4.3: Microscopic View

S/N	Slide	Gram	Shape
1.	E1a	—	rod
2.	E1b	—	cocci
3.	E1c	+	cocci
4.	E2a	+	cocci
5.	E2b	+	rod
6.	E2c	—	cocci
7.	E3a	+	rod
8.	E3b	+	cocci
9.	E3c	+	cocci

4.4 Biochemical Characterization of Isolates

Biochemical tests such as citrate utilization, urease, H₂S production, glucose and mortality were conducted to identify bacterial species (Prescott et al., 2013).

Table 4.4: Biochemical Characteristics of Bacterial Isolates

S/N	Isolate	Gram	Shape	Citrate	Urea	H ₂ S	Motility	Gas	Glu	Suc	Lac	Confirmed Organism
1.	E1a	-	Rod	+	-	-	+	-	+	+	-	Klebsiella aerogenes
2.	E1b	-	Cocci	+	+	+	+	-	+	-	-	Proteus mirabilis
3.	E1c	-	Cocci	+	+	+	+	-	+	+	-	Proteus vulgaris
4.	E2a	-	Cocci	+	+	-	+	-	+	-	-	Proteus vulgaris
5.	E2b	-	Rod	+	+	-	+	-	+	-	-	Bacillus subtilis
6.	E2c	-	Cocci	+	-	-	-	-	+	+	-	Neisseria species
7.	E3a	-	Rod	+	+	+	+	-	+	+	-	Proteus mirabilis
8.	E3b	-	Cocci	+	+	+	+	-	+	-	-	Staphylococcus saprophytic
9.	E3c	-	Cocci	+	+	+	+	-	+	+	-	Staphylococcus aureus

Keys: [+] = Positive, [-] = Negative, H₂S = Hydrogen sulfide, Glu = Glucose, Suc = Sucrose, Lac = Lactose.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

The results from this study confirmed the successful isolation and biochemical identification of ureolytic bacteria with calcite precipitation abilities from soil samples collected at the Energy Research Centre, Sokoto. The morphological and biochemical results indicated that the isolates were *Klebsiella aerogenes*, *Proteus vulgaris*, and *Proteus mirabilis*, all members of the *Enterobacteriaceae* family. These bacteria are known urease producers that hydrolyze urea into ammonia and carbon dioxide, creating alkaline conditions that facilitate calcium carbonate (CaCO_3) precipitation (Mobley & Hausinger, 1989). This mechanism is widely reported as the fundamental pathway for microbial-induced carbonate precipitation (Arias *et al.*, 2025).

According to Whiffin (2004), *Proteus* species exhibit strong urease activity, leading to rapid precipitation of calcite crystals. This is supported by Chaparro-Acuña *et al.* (2018), who documented that *Proteus vulgaris* significantly increases CaCO_3 precipitation when exposed to elevated calcium concentrations, making it one of the most efficient ureolytic organisms identified for MICP applications. Similarly, *Klebsiella spp.* are reported by Achal and Pan (2011) to play a significant role in biomineralization and soil

stabilization. Leeprasert *et al.* (2022) further confirm that ureolytic bacteria such as *Proteus* and *Klebsiella* possess strong urease activity that enhances their calcite formation capacity.

The high colony count observed in isolate E3 suggests higher metabolic efficiency and growth rate. This implies that isolate E3 possesses enhanced urease activity, making it a potential candidate for biological soil improvement. Similar observations were reported by Wang, Soga and DeJong (2020), who found a positive relationship between bacterial density and rate of calcite precipitation in MICP systems. This enhanced CaCO_3 formation improves the water-holding capacity of sandy soils by binding soil particles and reducing pore spaces, as supported by DeJong *et al.* (2010). These improvements are crucial in arid environments where soil structure is naturally loose and susceptible to low moisture retention.

Furthermore, ureolytic bacteria induced calcite precipitation contributes to eco-friendly biocementation. Instead of relying on chemical stabilizers, MICP provides a sustainable and non-toxic alternative for soil strengthening. Cheng *et al.* (2016) highlight that urease active bioslurry significantly enhances soil mechanical properties with minimal environmental impact. Rahman *et al.* (2020) also describe MICP as a promising green

engineering technology for soil stabilization in regions with sandy or weak soil structures, aligning well with environmental needs of arid zones like Sokoto State.

These findings therefore demonstrate that local soils in the Energy Research Centre area harbor bacterial species with the biochemical potential for natural soil improvement. Comparable studies by Bibi *et al.* (2018) in other arid environments also show that ureolytic bacteria adapted to harsh, nutrient-poor soils are capable of efficient calcite deposition. This supports the potential exploitation of locally adapted strains for biological soil strengthening and water conservation applications.

This research was carried out to isolate and biochemically identify ureolytic bacteria with the ability to precipitate calcium carbonate (CaCO_3) from samples collected at the Energy Research Centre, Sokoto. The study also aimed at exploring the potential of these bacteria in improving the poor water-holding capacity associated with sandy soils through microbial-induced calcite precipitation (MICP). Recent works such as Tayyem *et al.* (2021) emphasize the importance of identifying and optimizing growth conditions for local urease-producing bacteria to maximize calcite deposition a goal consistent with the objectives of this study.

Samples were collected aseptically and subjected to serial dilution and plating on nutrient agar for bacterial growth and enumeration. Different bacterial colonies were observed and categorized based on colony morphology, pigmentation, size, and edge characteristics. Pure cultures were then obtained through sub-culturing and subjected to Gram staining and biochemical tests such as urease, citrate, catalase, and triple sugar iron (TSI) tests for biochemical identification and classification. These tests have been widely used for distinguishing ureolytic species in soil environments (Leeprasert *et al.*, 2022; Bibi *et al.*, 2018).

The urease test, being the most significant in this research, was used to detect the ability of bacteria to hydrolyze urea into ammonia and carbon dioxide, which subsequently facilitates the formation of calcium carbonate. Among the isolates, several showed strong urease activity, indicating their potential for calcite precipitation. This is consistent with reports by Arias *et al.* (2025), who emphasized that urease activity is the central driver of MICP, influencing both the rate and volume of CaCO_3 produced. The results also showed variation in urease intensity, with some isolates forming more pronounced color changes, confirming their high enzymatic potential.

Microscopic examination and Gram staining revealed that most isolates were Gram-positive rods and cocci, which aligns with known ureolytic bacterial genera such as

Bacillus, *Sporosarcina*, and *Pseudomonas*. Such genera have been reported in multiple MICP studies as effective mineralizing bacteria capable of forming stable calcite crystals under varying environmental conditions (Rahman *et al.*, 2020). The biochemical test results further supported the classification of the isolates as urease-producing bacteria capable of biomineralization.

The findings suggest that the isolated ureolytic bacteria possess the capacity to precipitate calcium carbonate, which can contribute significantly to the stabilization of sandy soils and enhancement of their water retention. This biological process, if optimized, could offer an environmentally friendly and sustainable solution to soil degradation and agricultural challenges in arid regions such as Sokoto State. Similar conclusions were drawn by Tayyem *et al.* (2021), who demonstrated that optimizing growth media, temperature, and pH significantly increases calcite yield in locally isolated strains.

5.2 Conclusion

The study successfully isolated and biochemically identified ureolytic bacteria with calcite precipitation potential from the Energy Research Centre, Sokoto. These bacteria exhibited notable urease activity, confirming their role in biomineralization processes. The ability of these isolates to form calcium carbonate crystals suggests their potential

in soil improvement, particularly in enhancing the structure and water-holding capacity of sandy soils.

This work provides a biological basis for further exploration of microbial-induced calcite precipitation (MICP) as a sustainable method for soil stabilization and water conservation in semi-arid environments.

5.3 Recommendations

Based on the findings of this study, the following recommendations are made:

1. Further research should focus on optimizing environmental conditions (pH, temperature, and nutrient media) for maximum calcite precipitation by the most potent isolates.
2. The application of these ureolytic bacteria should be tested on field soils to evaluate their efficiency in improving soil texture and water retention under natural conditions.
3. Molecular identification and genetic characterization of the most active strains should be conducted to better understand their metabolic pathways and enhance their application in bio-based soil improvement technologies.

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