*ejpmr*, 2017,4(9), 30-38.

**SJIF Impact Factor 4.161**

**EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH**

www.ejpmr.com

**Research Article**

**ISSN 2394-3211**

**EJPMR**

# ISOLATION AND CHARACTERIZATION OF SOIL-BORNE UREASE PRODUCING BACTERIA

**Dr. Anyadoh-Nwadike S. O.*[1], Ezeanyika C.[1] and Okorondu S. I.[2]**

[1]Department of Biotechnology, School of Biological Sciences, Federal University of Technology, Owerri, Imo State, Nigeria.

[2]Department of Microbiology, School of Biological Sciences, Federal University of Technology, Owerri, Imo State, Nigeria.

***Corresponding Author: Dr. Anyadoh-Nwadike S. O.**

Department of Biotechnology, School of Biological Sciences, Federal University of Technology, Owerri, Imo State, Nigeria.

**Article Received on 23/06/2017** **Article Revised on 14/07/2017** **Article Accepted on 04/08/2017**

## ABSTRACT

Urease producing bacteria are significant for their increasing roles in human pathogenicity, biocementation and as soil fertilizer. This study was therefore aimed at isolation of such urease producers from urea-rich soil samples collected from various locations in Federal University of Technology Owerri. Sample collection and processing were done using standard microbiological methods. Soil samples were aseptically collected from seven different locations. Isolation of the various bacterial species was done using Nutrient, MacConkey, Eosin Methylene Blue and *Salmonella Shigella* agar. Identification of isolates was done using morphological characteristics as well as standard microbiological and biochemical procedures. The urease producing strains of bacteria were obtained using the urease hydrolysis test. Urease tolerance level was obtained using the optical density on a spectrophotometer. Results revealed the presence of twenty one bacterial strains of seven species; *Baccilus* sp, *Staphylococcus aureus, Shigella* sp, *Escherichia coli, Proteus* sp, *Klebsella* sp and *Enterobacter* sp. Four of the isolates designated as C1, F2, C2, and D1 were selected on the basis of qualitative and quantitative screening analysis for urease activity. Isolate C1 was identified as *Proteus* sp., C2 was identified as *Klebsiella* sp. Isolate D1 was also identified as *Proteus* sp. while F2 was also identified as *Klebsiella* sp. Urease tolerance test revealed D1 (*Proteus sp*) having higher growth rate of 0.063 at a urea concentration of 1M with the growth rate decreasing as urea concentration increased. Isolate C2 *(Klebsiella* sp*)* also showed high growth rate of 0.042 at a urea concentration of 1M. Growth rate decreased with increasing urea concentration. These organisms with high urease tolerance level can be used for diverse purposes ranging from enzyme production, medical uses to calcite precipitation in biocementation.

**KEYWORDS:** Urease, Bacteria, Soil, Urea-rich, Biocementation.

## INTRODUCTION

Soil contains a variety of microorganisms including bacteria. Microorganisms play important roles on nutritional chains that are important for biological balance in the life on our planet being essential for the closing of nutrient and geochemical cycles such as the carbon, nitrogen, sulfur and phosphorous cycle.[1] Without bacteria, soil would not be fertile and organic matters such as straw or leaves would accumulate within a short time.[2,3] Microorganisms are widely present in the worl.[4-7] Hence it is essential to explore, preserve, conserve and utilize the unique soil microbial flora in fulfilling emerging needs of society, industries and clean environment.

Urease belongs to the super family of amidohydrolases and phosphotriesterases.[8-10] Urease is a nickel-dependent metalloenzyme of high molecular weight synthesized by plants, some bacteria and fungi.[11-13] Urease enzyme is responsible for the hydrolysis of urea fertilizers applied to the soil and due to this role, urease enzyme from soil microflora have received a lot of attention, since it was first reported by Rotini.[14]

More specifically, urease catalyzes the hydrolysis of urea to produce ammonia and carbamate. The carbamate produced, is subsequently degraded by spontaneous hydrolysis to produce ammonia and carbonic acid.[10,15] Urease activity tends to increase the pH of its environment as it produces ammonia a basic molecule.[9,16,17] Urease activity (UA) defines the urea hydrolysis activity produced by the enzyme urease per minute.[16]

Urease from jack bean has been widely studied being the first nickel metalloenzyme identified and crystallized.[16,18-20] The ability to produce urease is widespread among microbial populations and the enzyme has been well studied from a clinical perspective as it can indicate increased virulence properties in

pathogenic bacteria[10,21,22] and its use in agricultural soils has also been severally reported especially in nitrogen volatilization.[23–27] Microbial enzymes are more stable and have properties which are more diverse than other enzymes derived from plants and animals.[5,7] Earlier studies have shown that there are increasing number of microorganisms being screened from extreme environments with capability to produce essential enzymes useful for various industrial applications.[10,28–30]

The C terminal portions of plant and fungal chains resemble the large subunits of bacterial ureases (e.g. α chain of *S. pasteurii urease*). The high sequence similarity of all ureases indicates they are variants of the same ancestral protein and are likely to possess similar tertiary structures and catalytic mechanisms.[11]

**Importance of Urease**

The biological roles of ureases as enzymes and catalysts have been variously studied.[10,16,22,30-32] The following importance have been ascribed to Ureases in relation to the organisms that produce them enables bacteria and fungi to use urea as a nitrogen source for their development while contributing to defense mechanisms of the microorganisms through the release of toxic ammonia. The release of ammonia to the environment results in increase in the pH of the surrounding medium.[10,16,32] This is essential for survival of urease-producing microorganisms in acidic media, example the pathogenic bacterium *Helicobacter pylori*, that colonizes the stomach of humans causing gastritis and eventually gastric cancer.

On the other hand, urease provide an alternative route for nitrogen disponibilization to plant embryos in synergistic activity to arginase. They also aid precipitation of calcium salts interfering on the process of soil mineralization.[13] Diverse bacterial species participate in the precipitation of mineral carbonates in various natural environments, including soils, geological formations, freshwater biofilms, oceans and saline lakes. Recently, biocementation; a microbial mineral precipitation process resulting from metabolic activities of some specific microorganisms to improve the overall behavior of concrete has become an important area of research.[10,13] The use of this biocementation concept leads to the potential invention of a new material called biocement.[10,28,30,33]

Urease enzyme of microbial sources has a significant role towards human pathogenicity and urease enzyme is used as a vaccine on the basis of its catalytic inhibition activity for protection against microbial infection.[10,11,31] Ureases can thus be applied for the treatment of many health disorders like gastrointestinal infection and hypertension. Therefore, urease producing soil microorganisms have received a lot of attention. In addition to internally generated urea, externally applied urea can also be utilized by plants. Urea is a widely used fertilizer because of its low costs, ease in handling and high nitrogen content.[32] In plants, urease is the only enzyme that is able to recapture nitrogen from urea.[34] Fertilization with urea through leaves could be an efficient method of plant feeding and any modifications leading to increased urease activity in leaves could result in more effective assimilation of this fertilizer.[25-27] Such an increase might have a positive impact on the nitrogen metabolism in plants since more ammonia would be available for assimilation *via* glutamine into a variety of nitrogenous compounds.

Currently, urease is only available in industrial quantities from Roche companies for use in the diagnostic and high technology specialist ceramics fields.[35] It is thus expensive. The purity of the enzyme may higher than is required for other uses such as biocementation.

**AIM OF STUDY**

The aim of this study is to isolate and characterize strains of urease-producing bacteria from soil.

**OBJECTIVES**

1. To culturally isolate and identify urease producing local bacterial species from urea rich soil.
2. Screen and select high urease producer strain.
3. Analyze the physiochemical properties of soil used in the study

**Significance of the study**

1. This study will possibly reveal some urease producing bacteria that can be used to produce clinically and industrially useful urease enzyme.
2. The study explored the possibility of isolating local bacterial isolate that will possibly be used in strengthening cement via calcite precipitation and other possible uses (dust suppression, against soil erosion, etc). It may be of great importance in soil stabilization in sand areas.
3. Data generated from this study may aid the enzyme and construction industries which are currently suffering from closures and siege. In addition, it would pave the way for a new frontier with a wide range of possible applications using local strains of bacterial isolate that are acclimatized to local conditions.

**METHODOLOGY**

The methods used to achieve the objectives are as follows:

**Sample collection and preparation**

Soil samples from seemingly urea rich locations were used for this study. A total of 14 samples were aseptically collected randomly from various locations. During collection, a clean stick was used to clear the debris away and 10 cm hole was dug. The soil sample was collected using sterile spatula and put in a sterile bag. The samples were transported to the laboratory within one hour for analysis.

**Study area/sample location**

The samples were collected from various locations in Federal University of Technology Owerri. (FUTO). All sample collection points are frequently used by students and other members of FUTO community as urinating/waste disposal sites.

These locations included:
1) Back of Hostel A (SA)
2) Back of Hostel B (SB)
3) Edge of FUTO Bus Park (SC)
4) Edge of School of Science Extension (SD)
5) Edge of FUTO market (SE)
6) Back of School of Engineering (SF)
7) Behind Hostel D (SG)

**Isolation of microorganisms**

All media used in culturing, sub-culturing and biochemical tests were prepared according to the manufacturers guidelines and instructions. They were sterilized by autoclaving at 121 psi for 15 minutes and aseptically poured into clearly labeled sterile petri dishes. The media used were nutrient agar (NA), MacConkey agar (MA), S*almomella Shigella* agar (SSA), Eosin methylene agar (EMB), Mannitol salt agar (MSA), Methyl-red-Voges prokauer medium, Triple Salt Iron agar (TSI), and half strength nutrient agar.

**Serial Dilution**

This was done prior to inoculation using as follows:
1. A ten-fold serial dilution was carried out first by dissolving 1g of the test samples into 9ml of sterile water; the mixture was swirled clockwise and anti-clockwise to obtain a homogenous mixture.
2. Subsequent dilution was made by transferring 1ml (mixture of soil sample in water) from the first bijou bottle to the second bijou bottle and in that order until the tenth bottle.

**Inoculation**

Aliquots (0.1 ml) of the 3rd, 4th and 5th dilutions were inoculated in duplicate plates of freshly prepared sterile solid NA, MA, EMB, SSA. After the inoculation, a sterilized L shaped spreader was used to spread the innoculum evenly on the surface of the media. Then the plates were inverted and incubated at the temperature of 37$^{o}$C for 24 -48 hours following the methods described by Cheesbrough.[36]

**Viable Bacterial Count**

Viable bacterial count was done using a gallenkamp colony counter. Total colony count was expressed as colony forming units per ml (cfu/ml) of the sample analyzed. The standard formula was used to obtain the viable count as follows:

Total count (cfu/ml)

$$= \frac{\text{Av. no of the colonies on duplicate plates X dilution factor}}{\text{Volume plated}}$$

N.B: dilution factor = $\frac{1}{\text{Dilution}}$

The various isolates obtained were sub-cultured to obtain pure cultures. After sub-culturing, plates were incubated at appropriate conditions. Finally observations were made and all the morphological characteristics of the isolates were recorded.

**Identification/Characterization of Isolates**

All the isolates were further identified using biochemical tests such as Gram staining, motility test, citrate test, indole test, urease test, methyl red test etc. all the biochemical tests were carried out using the procedures described by Cheesbrough[36] and ogbulie *et al.,*[37] The biochemical tests done included:

Gram Staining, Indole Test, Citrate Utilizing Test, Methyl Red and Voges Proskauer's Test (MRVP), Methyl Red / Voges Proskauer test, Motility Test, Coagulase test, Catalase Test, Sugar fermentation test.

**Identification of Urease Producing Isolates**

This was done using the Urease test as described by Ogbulie et al.[37] The isolates that were Urease test positive were then further subjected to Urea tolerance assay to further investigate the possibility of their use for calcite precipitation.

The Urea Tolerance Assay was done using inocula appropriately prepared as follows:
1. The bacterial isolates were grown to mid exponential phase in nutrient broth (Lab M) on a rotatory incubator (150 rpm) at room temperature (28$^{o}$C) for 24 hrs.
2. The cells were harvested by centrifugation at 3000 rpm for 10 mins. The supernatant was decanted and the cell sediment washed twice in sterile distilled water by centrifugation at 3000 rpm for 10 mins.
3. The washed cells were re-suspended in sterile distilled water and turbidity adjusted to an optical density of 0.1 at 540 nm using a spectrophotometer.
4. An aliquot of 0.1 ml of the cell suspension was used as inoculum in the Urea tolerance assay.

**Determination of Physicochemical Properties of the Soil Samples**

The physicochemical properties of the soil were determined using standard protocols. They include; soil pH, organic carbon, phosphorus, Particle Size, Aluminium + Hydrogen and Calcium + Magnesium levels.

**Result Presentation and Analysis**

Results are presented in tables. Simple statistical methods (means and standard deviation) were used for result analysis.

## RESULTS

The results obtained are as follows:

**Result of Viable Bacterial Count**

Table 1 shows the mean bacterial count of the duplicate plates of soil sample analyzed. Growth was observed on all plates with sample A, sample C, Sample D and sample E.

**Table 1: Mean Bacterial Count (Cfu/ml) for Duplicate Sample Analyzed.**

| Sample | THBC | TCC | TSSC |
|---|---|---|---|
| A | $3.0 \times 10^7 \pm 0.803$ | $2.2 \times 10^5 \pm 0.707$ | $2.8 \times 10^5 \pm 0.619$ |
| B | $6.2 \times 10^7 \pm 0.799$ | $5.8 \times 10^5 \pm 0.115$ | 0 |
| C | $2 \times 10^7 \pm 0$ | $7.9 \times 10^5 \pm 0.900$ | $2.7 \times 10^5 \pm 0.639$ |
| D | $2.8 \times 10^7 \pm 0.399$ | $1.1 \times 10^5 \pm 0.056$ | $6.4 \times 10^5 \pm 0.316$ |
| E | $4 \times 10^7 \pm 0.190$ | $1 \times 10^5 \pm 0.124$ | $4 \times 10^5 \pm 3.961$ |
| F | $4 \times 10^6 \pm 0$ | $4 \times 10^4 \pm 3.254$ | 0 |
| G | $4.5 \times 10^6 \pm 0.069$ | 0 | 0 |

N/B:Values are expressed in mean ± standard deviation of duplicate determination.

**KEY:**

THBC = Total Heterobacteria count

TCC = Total Coliform count

TSSC = Total *Salmonella Shigella* count.

**Results of Identification/Characterization of Pure Bacterial Isolates**

The morphological and biochemical properties of the various bacterial species used for characterization are shown in Table 2 below. Isolates C1, C2, D1 and F2 were shown to have hydrolyzed urea.

**Table 2: Colonial and Biochemical Characterization of Pure Culture of Bacterial Isolates.**

| sample | Code no | Colonial characteristics | Gram Reaction | Catalase | Coagulase | Citrate | Indole | Methyl red | Vogues/prostauer | Motility | Glucose | Lactose/sucrose | $H_2S$ | Gas | Urease | Probable organism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | $A_1$ | Irregular, cream, moderate, raised, dull, dry, undulate, translucent, rough | +ve rods chains | + | + | + | - | - | + | + | + | - | + | + | - | *Bacillus sp* |
| | A2 | Round, shiny, yellow, raised, small, entire, smooth | +ve cocci in clusters | + | + | + | - | - | + | - | + | + | - | - | - | *Staphylococcus aureus* |
| | A3 | Round, clear, colourless, shiny, small, Raised, entire, transparent, smooth | -ve rods | + | + | + | - | + | - | - | + | - | - | - | - | *Shigellasp* |
| B | B1 | Irregular, cream, dry, moderate, dull, translucent, rough | +ve rods in pairs | + | + | + | - | - | + | + | + | - | - | - | - | *Bacillus sp* |
| | B2 | Round, shiny, yellow, raised, small, entire, smooth | +ve cocci in clusters | + | + | + | - | - | + | - | + | + | - | - | - | *Staphylococcus aureus* |
| | B3 | Round, purple with green metallic sheen, opaque, moderate, entire, shiny, Raised | -ve rods | + | + | + | + | + | - | + | + | + | - | + | - | *Escherishia coli* |
| C | C1 | Irregular, undulate, raised, colourless, dry, dull, moderate | -ve rods in chains | + | + | + | + | + | + | + | + | - | + | - | + | *Proteus sp* |
| | C2 | Round, pink, Raised, entire, shiny, translucent, moderate, smooth | –ve rod | + | + | + | - | - | + | - | + | + | - | + | + | *Klebsiella sp* |
| | C3 | Round, pinkish with dark centre, entire, raised opaque, moderate translucent, shiny, colony. | -ve rod | + | + | + | - | - | + | + | + | + | - | + | - | *Eaterobecter sp* |
| | C4 | Round, colourlesswithout dark centre, moderate, raised, entire, opaque, shiny, colony. | -ve rod | + | + | + | - | + | - | + | + | - | + | + | - | *Salmonella sp* |
| | C5 | Round, colourless without dark centre, Raised, centre, translucent, moderate, shiny, colony. | -ve rod | + | + | + | - | + | - | - | + | - | - | - | - | *Shigella sp* |
| | C6 | Irregular, yellow, small, shiny, translucent, entire, raised, smooth, colony. | +ve cocci in clusters | + | + | + | - | - | + | - | + | + | - | - | - | *Staphylococcus aureus* |
| D | D1 | Irregular colourless, undulate, raised, translucent, dry, dull, moderate, colony. | -ve rod in pairs | + | + | + | + | + | + | + | + | - | + | - | + | *Proteus sp* |
| | D2 | Round, yellow, small, shiny, translucent, entire, raised, smooth, colony. | +ve in cocci in clusters | + | + | + | - | - | + | - | + | + | - | - | - | *Staphylococcus aureus* |
| | D3 | Clear, round, colourless, shiny, entire, raised, shiny, transparent, colony. | -ve rod | + | + | + | - | + | - | - | + | - | - | - | - | *Shigella sp* |
| E | E1 | Round, large, entire, raised, fairly, smooth, translucent, colony. | –ve tiny rods in pairs | + | + | - | - | + | + | + | + | - | + | - | - | *Bacillus sp* |
| | E2 | Round, yellow, shiny, small, entire, raised, smooth, translucent, colony. | +ve cocci in clusters | + | + | + | - | - | + | - | + | + | - | - | - | *Staphylococcus aureus* |
| F | F1 | Round, punctiform, flat, entire, cream, transparent, shiny, colony. | +rods in pairs | + | + | + | - | - | + | + | + | - | + | + | - | *Bacillus sp* |
| | F2 | Round, pink, moderate, entire, raised, smooth, shiny, translucent, colony. | -ve rods | + | + | + | - | - | + | - | + | + | - | + | + | *Klebsiella sp* |
| G | G1 | Irregular, cream, moderate, raised, dull, dry, undulate, translucent rough, colony. | +rod in pairs | + | + | + | - | - | + | + | + | - | + | + | - | *Bacillus sp* |
| | G2 | Round, punctiform, flat, entire, cream, transparent, slimy, colony. | +rod in pairs | + | + | + | - | - | + | + | + | - | + | + | - | *Bacillus sp* |

**KEY:** + = Positive ; – = Negative; TS1 = Triple Sugar Iron agar ; $H_2S$ = Hydrogen sulphide.

**Result of Urea Tolerance Assay of the Bacterial Isolate**
This is shown in Table 4 below:

**Table 4: Urea Tolerance Assay Result.**

| **Mean optical density of isolate (OD)** | | | | |
|---|---|---|---|---|
| **Urea Concentration (mg/ml)** | **C1** | **C2** | **D1** | **F2** |
| 0 | 0.075 ± 0.088 | 0.08 ± 0.007 | 0.086 ± 0.006 | 0.069 ± 0.004 |
| 1 | 0.053 ± 0.001 | 0.042 ± 0.002 | 0.063 ± 0.009 | 0.039 ± 0.006 |
| 1.5 | 0.036 ± 0.004 | 0.033 ± 0.006 | 0.061 ± 0.03 | 0.029 ± 0.004 |
| 2 | 0.033 ± 0.004 | 0.027 ± 0.002 | 0.04 ± 0.001 | 0.026 ± 0.001 |
| 2.5 | 0.033 ± 0.002 | 0.026 ± 0.005 | 0.034 ± 0 | 0.0245 ± 0.011 |
| 3 | 0.028 ± 0.001 | 0.022 ± 0.004 | 0.029 ± 0.002 | 0.018 ± 0.003 |

KEY: Values are expressed in means ± standard deviation of duplicate determination

**Results of the Physiochemical Analysis of the Soil Sample**
These are shown in Table 5 below for the various soil samples. pH tended towards neutral/alkalinity. Calcium was within the high optimal range.

**Table 5: Physiochemical Analysis of the Soil Sample.**

| Samples | pH | OC (%) | TN (%) | P (ppm) | Ca (ppm) | Mg Mol/kg-1 | Al +H Mol/ kg-1 |
|---|---|---|---|---|---|---|---|
| A | 7.59 | 0.6983 | 0.049 | 28.42 | 3520 | 0.38 | 0.68 |
| B | 6.67 | 0.9377 | 0.047 | 28.14 | 880 | 0.27 | 3.8 |
| C | 7.39 | 0.4389 | 0.022 | 25.7 | 3480 | 1.6 | 0.76 |
| D | 6.20 | 0.5399 | 0.040 | 28.2 | 3220 | 0.52 | 0.65 |
| E | 6.70 | 0.6061 | 0.037 | 26.9 | 3390 | 0.34 | 0.58 |
| F | 6.90 | 0.6217 | 0.049 | 29.0 | 3000 | 0.49 | 0.60 |
| G | 7.11 | 0.0599 | 0.004 | 55.37 | 2240 | 0.74 | 0.52 |

**KEY**
OC = Organic carbon
TN = Total particle size
Ca = Calcium
Mg = Magnesium
Al = Aluminum
H = Hydrogen
P = Phosphorous

**DISCUSSION**
This study has revealed the soil as habouring diverse types and numbers of viable bacteria as shown in Tables 1 and 2 even at 10 cm depth. This however collaborates earlier authors who reported that microorganisms including bacteria are ubiquitous and that many are free-living in the soil as an important habitat for microorganisms.[3–5] According to JoVE[38] and Sylvia,[5] soils are a heterogeneous mixture of inorganic and organic particles that combine together to form secondary aggregates. Within and between the aggregates are voids or pores that visually contain both air and water. These conditions create an ideal ecosystem for bacteria, so all soils contain vast populations of bacteria, usually over 1 million per gram of soil. Infact the microbial content of the soil is a measure of soil 'health'.

The identified bacterial isolates in this study; *Baccilus* sp, *Staphylococcus aureus, Shigella* sp, *Escherichia coli, Proteus* sp, *Klebsella sp* and *Enterobacter* sp. have also been reported for soil samples.

Urease test still remains confirmatory for bacteria with urea-hydrolyzing ability. Similar isolates to the four urease-producing isolates obtained; C1 (*Proteus* sp), C2 (*Klebsiella* sp), D1 (*Proteus* sp) and F2 *(Klebsiella* sp*)* have been earlier reported to have ability of breaking down urea into ammonia and carbon-dioxide.[13,36,39-40] Urease producing bacteria It use urea as an energy source and produce ammonia which increases the pH in the environment and generate carbonate, causing $Ca^{2+}$ and $CO_3{}^{2-}$ to be precipitated as $CaCO_3$.[10,30,40-41]

*Proteus* sp is a Gram negative, facultative anerobic, rod shaped bacterium. it shows swarming motility, urease activity.[4,42] It is widely distributed in soil and water. This rod shaped bacterium has the ability to produce high level of urease which hydrolyses urea to ammonia. It is an important candidate for biocementation.[13]

*Klebsiella* species are found everywhere in nature. They can be found in water, soil, plants, insects, animals, and human. *Klebsiella* is a genus of non-motile, Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule. In particular, the medically most important are *Klebsiella* species, *Klebsiella pneumonia.*[4,36,42] *Klebsiella* sp have been variously noted to produce the enzyme urease.

The *Bacillus* sp isolated is an aerobic spore forming, rod shaped bacterium that has been shown to use urea as an energy source to increase the pH of the environment and generate carbonate.[40,43] In microbial induced carbonate precipitation by urea hydrolysis, the enzyme urease catalyses substrate urea and precipitates carbonate ions in presence of ammonium. These carbonate ions in the presence of a calcium source readily precipitate $CaCO_3$. This urea hydrolysis is the result of metabolic processes, which depends on the type of *Bacillus* sp being used.

The physiochemical properties of the soil showed different characteristics of the soil. Most importantly was the enhancement in pH value of the soil samples (ranging from 6.67 to 7.59) which are within the range of optimal soil pH (6.0 – 7.5) and tends to alkalinity. This could be in relationship to the urease activity in the soil which tends to improve the pH of the soil while producing ammonia, a basic molecule.[10,13,30,40] The calcium level of almost all the soil samples used were on the high (>2000 ppm) optimal range (600 – 4000 ppm) side for fruit trees nutrition.[44] This is a possible pointer to calcite precipitation as a result of activities of ureases.

From Table 4, the capacity of four of the bacterial isolates to withstand/hydrolyze urea as a result of the urease they produced was revealed. It is worthy to note that in microbial induced carbonate precipitation by urea hydrolysis, the enzyme urease catalyses substrate urea and precipitates carbonate ions in presence of ammonium. These carbonate ions in the presence of a calcium source readily precipitate $CaCO_3$, a process known as biocementation.[13] The four isolates CI, C2, D1 and F2 from this research can therefore be considered as potential local candidates for production of urease enzymes. The import of this cannot be overemphasized because as local microbial isolates, they will be more adapted to the local environmental conditions especially in case of use in biocementation.

Urease tolerance test also revealed D1 (*Proteus sp*) having higher growth rate/ tolerance of 0.063 at a urea concentration of 1M. Isolate C2 *(Klebsiella sp)* also showed high growth rate of 0.042 at a urea concentration of 1M. *Proteus* species therefore may be the species of choice for commercial urease production against its *Klebsiella* sp. Counterpart this corroborates the study by Varalakshmi and Anchana.[13] It is also note worthy that for all isolates, growth rate decreased with increasing urea concentration. This may imply that high urea concentration and cell aging affects multiplication in batch culture hence there may be need for use of continuous culture for production of urease by bacterial cells.

Furthermore, the isolates can be standardized for use in production of urease enzymes for commercial and industrial purposes. Ureases are useful in drug production, medicine/health care,[11,21] agriculture[23–27] and even civil engineering via biocementation.[10,33,45] According to Qin and Cabral,[31] application of urease encompasses these areas; urea content analysis in blood, urine, alcoholic beverages, natural water and environmental wastewaters; analysis of heavy metal content in natural waters, wastewaters and soil; determination of creatinine, arginine and IgG; urea removal from artificial kidney dialyzates, alcohol beverages and fertilizer wastewaters; wastewater reclamation for life support systems in space; pH control or shift for multi-enzyme reaction system; and urea hydrolysis as sources of ammonia or carbon dioxide in special cases.

## CONCLUSION

Microbes are known to produce urease enzyme. This study has revealed that urease producing bacteria can be locally isolated from soil. The commercial demand for urease is currently not high and urease is only available in industrial quantities from Roche companies for use in the diagnostic and high technology specialist ceramics fields.[35] It is thus expensive and is of a higher purity than is required for some applications example, biocementation. Hence production and commercialization of the enzyme from local bacterial isolates will generate a cheaper, more stable and sustainable source of ureases in large scale which will in turn be of great economic value. It is worthy to note that microbial enzymes are more stable and have properties which are more diverse than other enzymes derived from plants and animals.[7]

## REFERENCES


1. Madsen, E.L. *Environmental Microbiology: From Genomes to Biogeochemistry*. Malden, MA: Wiley/Blackwell, 2008.
2. Kummerer K. Resistance in the environment. *J. Antimicrob Chemoth*, 2004; 45: 311–320.
3. Sylvia, C.M., David, M., Jeffry, J., Fuhrmann, G., Peter, G., Hartel, U. and David, A. *Principles and Applications of Soil Microbiology*. Upper Saddle River: Prentice Hall, 2008.
4. Prescott M.L., John P.H. and Donald A.K. *Textbook of Microbiology*, 6th edition,. McGraw Hill, New York, 2005.
5. Singh, U. and Kapoor, K. *Microbial Biotechnology*. Oxford Book Company, 2010; 1.
6. AnyadohNwadike S.O., Eri O., Nwaokoro J.C. and Nwadike P.O. Prevalence of *Staphylococcus aureus* within the Hospital Environment. *Asian J. Med. Pharm. Res*, 1(1): 17-21.

7. Alves, P.D., Siqueira Fde, F., Facchin, S., Horta, C.C., Victoria, J.M. & Kalapothakis, E. Survey of microbial enzymes in soil, water, and plant microenvironments. *The Open Microbiology Journal*, 2014; 8: 25-31.
8. Holm, L. and Sander, C. An evolutionary treasure, unification of a broad set of amidohydrolase related to urease. *Proteins,* 1997; 28: 72-82.
9. Krajewska B., I. Ureases Functional, catalytic and kinetic properties: a reviewJ Mol Catal B Enzym, 2009; 59(1): 9-21.
10. Modolo L.V., de Souza A., Horta L.P., Araujo D.P., Ângelo de Fátima, An overview on the potential of natural products as ureases inhibitors: A review. *Journal of Advanced Research*, 2015; 6(1): 35-44.
11. Follmer, C. Insights into the role and structure of plant ureases. *Phytochemistry of Science,* 2008; 69: 18-28.
12. Krajewska, B., Van-Eldik, R. and Brindell, M., Temperature- and pressure-dependent stopped-flow kinetic studies of Jack Bean urease. Implications for the catalytic mechanism. *Journal of Biological Inorganic Chemistry*, 2012; 17: 1123-1134.
13. Varalakshmi and Anchana devi, Isolation and Characterization of Urease Utilizing Bacteria to Produce Biocement. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, Apr. 2014; 8(4): 52 – 57.
14. Rotini, O.T. La trasformazione enzimatica dell'urea nel terreno, *Ann Labor Ric Ferm Spallanrani,* 1935; 3: 143-154.
15. Zimmer, M. Molecular mechanics evaluation of the proposed mechanisms for the degradation of urea by urease. *Journal Biomolecule Structure Dyn,* 2000; 17(5): 787–97.
16. Lorenc A. Investigating the action of urease. Science in Schools; the European Journal for Science teachers, 2008; 9. http://www.scienceinschool.org/2008/issue9/urease.
17. Maroney M.L. and Ciurl S. Nonredox nickel enzymes. *Chem Rev*, 2014; 114: 4206-4228.
18. Blakeley, R.L. and Zerner, B. Jack bean urease, the first nickel enzyme. *Journal of Molecular Catalysis,* 2004; 23: 263-292.
19. Dixon, N. E., Gazzola, C., Blakeley, R. L., & Zerner, B. Jack bean urease (EC 3.5.1.5). A metalloenzyme. A simple biological role for nickel? *Journal of the American Chemical Society*, 1975; 97: 4131-4133.
20. Sumner, J. B. The isolation and crystallization of the enzyme urease. *Journal of Biological Chemistry*, 1926; 69: 435-441.
21. Provorov, N and Vorobyov, N. Population genetics of Rhizobia: Construction and analysis of an "infection and release" model. *Journal of Theoretical Biology*, 2000; 205(1): 105-119.
22. Banerjee Sujoy. Potential clinical significance of urease enzyme. *European Scientific Journal*, 2013; 9(21). ISSN: 1857 – 7881 (Print) e - ISSN 1857-7431 94.
23. Nielsen, T., Bonde, T and Sorensen, J. Significance of microbial urea turnover in N cycling. FEMS Microbiology Ecology, 1998; 25(2): 147-157.
24. Akiyama H, Yan X.Y., Yagi K. Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for $N_2O$ and NO emissions from agricultural soils: meta-analysis. *Glob Change Biol*, 2010; 16: 1837-1846.
25. Canfield D.E, Glazer A.N. and Falkowski P.G. The evolution and future of Earth's nitrogen cycle. *Science*, 2010; 330: 192-196.
26. Artola E., Cruchaga S., Ariz I, Moran J.F., Garnica M, Houdusse F., *et al.* Effect of N-(*n*-butyl) thiophosphoric triamide on urea metabolism and the assimilation of ammonium by *Triticum aestivum* L. *Plant Growth Regul*, 2011; 63: 73-79.
27. Cameron K.C., Di H.J., and Moir J.L. Nitrogen losses from the soil/plant system: a review. *Ann Appl Biol*, 2013; 162(2013): 145-173.
28. Elmanama, A.A. & Alhour, M.T. Isolation, characterization and application of calcite producing bacteria from urea rich soils. Journal of Advanced Science and Engineering Research, 2013; 3: 377-399.
29. Krishnapriya, S., Venkatesh, B.D.L. & Arulraj, P.G. Isolation and identification of bacteria to improve the strength of concrete. *Microbiological Research*, 2015; 174: 48-55.
30. Omoregie A.I, Nurnajwani Senian, Phua Ye Li, Ngu Lock Hei, Dominic Ong Ek Leong, Irine Runnie Henry Ginjom and Nissom P.M. Screening for Urease-Producing Bacteria from Limestone Caves of Sarawak Borneo Journal of Resource Science and Technology, 2016; 6(1): 37-45. [accessed Jun 27, 2017].
31. Qin Y and Cabral J. M.S. Review Properties and Applications of Urease. *Biocatalysis and Biotransformation,* 2002; 20(1).
32. Mobley, H.L.T. and Hausinger, R.P. Microbial Ureases: significance, regulation and molecular characterization. *Microbial Reviews,* 2000; 53: 85-108.
33. Jonkers, H.M. and Schlangen, E. Development of a bacteria-based self-healing concrete. *Tailor made concrete Structures - new solutions for our society*. Taylor & Francis Group, London, 2008.
34. Polacco, J.C. and Holland, M. A. Roles of urease in plant cells. *International Revised Cyto*logy, 2003; 145: 56-103.
35. Gauckler, L.J., Graule, Th and Baader, F. Ceramic forming using enzyme catalyzed reactions in three Danish agricultural soils. Materials Chemistry and Physics, 1999; 61(1): 78-102.
36. Cheesbrough M. *Medical laboratory manual for tropical countries vol. 11: Microbiology*. London: Butterwort-Hienemann, 2000.
37. Ogbulie, J.N., Uwaezuoke, J.C. and Ogiehar, S.J. *Introductory Microbiology Practical.* Springfield; Owerri, Nigeria, 1998. ISBN: 978-8001-07-6.

38. JoVE *Essentials of Environmental Microbiology. Culturing and Enumerating Bacteria from Soil Samples*. JoVE Science Education Database; JoVE, Cambridge, MA, 2017.
39. Andrews, R.K., Blakeley, R.L. and Zerner, B. Urease: a Ni (II) metalloenzyme. The bioinorganic chemistry of nickel. *VCH, New York,* 2009; 1: 141-166.
40. Chahal, N., Rajor, A. and Siddique, R. Calcium carbonate precipitation by different bacterial strains. *African Journal of Biotechnology,* 2011; 10(42): 8359-8372.
41. Stocks-Fischer, S., Galinat, J. and Bang, S. Microbiological precipitation of $CaCO_3$. *Soil Biology and Biochemistry,* 1999; 31(11): 1563-1571.
42. Todar K. Online Textbook of Bacteriology. *http://www.textbookofbacteriology.net*, Retrieved 30/05/2017, 2009.
43. Achal, V., Mukherjee, A and Reddy, S. Characterization of two urease -producing and calcifying Bacillus spp. isolated from cement. J. Microbiol. Biotechnol, 2010; 20(11): 1571–1576.
44. Tree Fruit Research & Extension Center. *Tree Fruit Soil and Nutrition*. Washington State University,1100 N Western Ave., Wenatchee, WA, 2004.
45. De Muynck, W.D., Cox, k., Belie, N.D. and Verstraete, W. Microbial carbonate precipitation in construction material. *A Review Ecological Engineering,* 2010; 36: 118-136.