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Isolation and screening of urease-producing bacteria from natural sources

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ABSTRACT

Urease is an enzyme made by ureolytic microorganisms that hydrolyzes urea into ammonia and carbon dioxide. Microbial urease has wide applications in biotechnology, agriculture, construction, and geotechnical engineering. Urease-producing microbes can be isolated from different ecosystems such as soil, oceans, and various geological formations. The aim of this study was to isolate and characterize urease-producing bacteria from different natural sources. Ten urease-producing bacterial isolates were screened and selected using a qualitative urease activity assay. Among these, four isolates showed rapid urease activity. The isolates were further characterized with respect to their morphological, characteristics.

Key words: *Urease, Ureolytic bacteria, MICP*

Introduction

Microorganisms influence mineral precipitation and alter the geological properties of the soil or any earth component. Biomineralization can improve soil quality by increasing soil stiffness through bacterial activities. Biocementation occurs through the action of different enzymes the most common is through urease-associated hydrolysis of urea in the soil yielding carbonate and ammonia. Ureolytic bacteria are bacteria capable of hydrolyzing urea (John *et al.,* 1974; Mekonnen *et al.,* 2021).

In construction, these bacteria are known to help improve soil stability. Currently, microbially induced mineral precipitation causing metabolic actions of specific urease-producing bacteria in concrete to increase the strength of concrete has become a significant area of research. Mineral precipitation processes are active in nearly all environments on the Earth.

Microbial urease can exist in two possible states in soil. It occurs either intracellular, associated directly with ureolytic microorganisms, or extracellular, after being released from cells (Mekonnen *et al.,* 2021; Paulson *et al*., 1969). Urease-producing bacteria are of particular interest to producing complex bio enzymes and are known to produce other soil enzymes that lead to the stabilization of expansive clays through cation exchange and flocculation of the clay minerals.

Urease is an enzyme produced by ureolytic microorganisms which hydrolyzes urea into ammonia and carbon dioxide. Microbial urease has wide applications in biotechnology, agriculture, medicine, construction, and geotechnical engineering (Burbank *et al*., 2012).

The research work presented in this paper aims to characterize the soil bacterial populations exhibiting ureolytic activity and investigate their diversity and distribution in a region with harsh weather conditions. The innovation resides in the establishment of a screening program of isolated bacteria based on

([1]Research Scholar, [2]Dean and Head)

rapid estimation of urease activity.

Material and Methods

Collection of samples

Urease-producing bacterial strains were isolated from different natural soil. Two Soil samples were collected from different areas in the Karad region. Thesesoil samples were collected in screw-capped or zip-lock cover bags using a spatula and transported to the laboratory, maintained at ambient temperature, and then immediately used in the present study.

Enrichment of samples

To enrich urease-producing bacteria from soil samples, 1g of each soil sample was inoculated separately into 100ml of urea broth medium consisting of 1.0g/l peptone, 1.0g/l dextrose, 5.0g/l sodium chloride, 2.0 g/l disodium phosphate, 0.012g/l of phenol red, and20 g/l urea, pH 6.8 and incubated under aerobic batch conditions at 30 °C for 120h under shaking condition at 130rpm. After enrichment samples were screened for urea hydrolysis (Mekonnen *et al.,* 2021; Kang *et al*., 2016; Heba Abdel *et al*., 2019).

Isolation of ureolyticbacteria

For bacterial isolation, an aliquot of 1ml was serially diluted and from the last enrichment, 0.1mL of the sample was inoculated onto urea agar plates (1.0g/l peptone, 1.0g/l dextrose, 5.0g/l sodium chloride, 2.0 g/l disodium phosphate, 0.012g/l of phenol red, and 20 g/l urea, Agar 25g/l, pH 6.8 Colonies showing urea hydrolyzing potential were purified by subsequent culturing and plating until single bacterial colonies were obtained. Urease production was tested through visual observation of color changes. Thus, isolates with positive ureolytic potential turned the urea agar medium from pale yellow to a pink-red color (Bharathi *et al.,* 2014; Heba Abdel *et al*., 2019).

Results and Discussion

Collection of samples

The soil samples (Table 1) were subjected to enrichment media separately, after five days of incubation turbidity, as well as color change, was observed in a conicalflask. After the enrichment of samples, a total of 10 different isolates were obtained on urea agar. Outof these only four isolates gave color change i.e., pink color (Photo plate 1 and 2).

Table 1. Soil samples

S. No.	Sample type	Source of sample	pH
1.	Soil	Sadashiv gad (Karad)	8.0
2.	Soil	Karad	8.2

Photo plate 1. Enrichment of ureolytic organism before incubation.

Photo plate 2. Enrichment of ureolytic organism after incubation

The bacterial strains were named as isolates 1, 2, 3&4. The colonies of isolate 1 had whitish in color, circular in shape, and flat elevations. Gram staining determination showed isolate 1 was Gram-positive rod. Isolate 3 was a circular, entire, flat, creamy opaque, moist colony. When these isolates were gram stained to study gram nature and morphology, it was observed that all were gram-positive rods. On Christensen's agar media, these four isolates gavepink-colored colonies, which means isolates had the capacity to hydrolyze urea as carbon

Table 2. Summarizes the Morphological and colony characteristics of these four ureolytic isolates on Christensen's agar at 30^0 for 48 h incubation

Isolate No.	Size	Shape	Margin	Elevation	Color	Opacity	Consistency	Gram nature
1.	1 mm	circular	Entire	flat	whitish	opaque	moist	gram +verods
2.	Pinpointed	circular	Irregular	convex	Off white	translucent	moist	gram + vecocco bacilli
3.	1 mm	circular	Entire	flat	Creamy	opaque	moist	gram +ve rods
4.	1mm	circular	Entire	flat	whitish	opaque	moist	gram +ve small rods.

and nitrogen sources. (Table 2 and Photo plate -3)

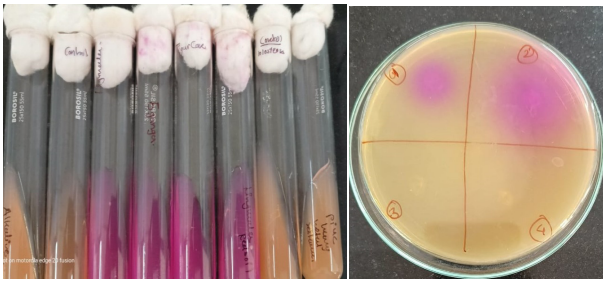

Photo plate 3. Ureolyticisolates

Conclusion

The results obtained from this research confirm the presence of ureolytic bacteria in old caves. After the screening of isolates, from that four isolates were promising to hydrolyze urea and create alkaline conditions. These promising isolates hydrolyzed urea within less than 24 h. In future work, this ureolytic property will be used in the precipitation of calcium carbonate to produce biocement.

Further work of this research includes

1. Screening of ureolytic activity of calcifying bacteria.
2. Characterization of isolates.
3. Quantification of bio cement produced by calcium-precipitating bacteria.

Conflict of Interest: no conflict of interest is there amongst the author.

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