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ORIGINAL ARTICLE **OPEN ACCESS**

Unique Enrichment Technique for Bio Cement Production by Native Microbial Isolates from Natural Sources

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ABSTRACT

The biomineralization of carbonates is a common and ubiquitous process. The calcareous material includes natural rocks, limestone, marble, and calcareous sandstones and exists in environments such as marine water, fresh water, caves, and soil. Most of the carbonate minerals have been found to be precipitated as calcium carbonates ($CaCO_3$).Calcium carbonate is one of the most common minerals widespread on Earth. Microbial-induced carbonate precipitation is a process by which living organisms produce inorganic solids. The ubiquity and the importance of microbes in inducing calcite precipitation make "Bio cement''. The present study investigates the potential of selectively enriched native calcium carbonate-precipitating bacteria. Two different soil samples were subjected to enrichment. The enriched samples were streak inoculated on calcium precipitation agar media., Four different isolates were obtained and out of which two isolates showed promising calcium precipitation.

***Key words**: Bio cement, Calcium precipitation, MICP (Microbial induced carbonate precipitation), urea lysis.*

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INTRODUCTION

In nature, manyrocks are cemented by the formation of calcium carbonate. Calcium carbonate is one of the most common minerals widespread on Earth. Microbial-induced carbonate precipitation is a process by which living organisms produce inorganic solids. The ubiquity and importance of microbes in inducing calcite precipitation make "Bio cement''.Microbially Induced Carbonate Precipitation (MICP) is a naturally occurring bio-mineralization process. The most widely explored MICP pathway is uerolysis. Generally, micro-cracks are not harmful but when aggressive material ingress it leads to an increase in porosity which is dangerous and decreases the life of the structure. Several microorganisms have properties of interest for various biotechnological applications.[1] A Japanese researcher first coined the term "Biomineralization" in 1940and interest began to grow in the study of this phenomenon. Many research papers show that Microbial Calcium Precipitation is used as Bio cement micro-cracks and bacterial-induced calcium precipitation by urease production[5]. Bio cement is also cost-effective and environment-friendly, non-pathogenic microorganisms are used to produce Bio cement. The main role of bacteria in the precipitation can able to create an alkaline environment under the influence of various physiological activities. The negatively charged nature of bacterial cells can bind with divalent cations such as ca2+ and mg2+ and makean ideal crystal nucleation site[2]. Thus, Microbial Induced Calcium Precipitation (MICP) can be used for the remediation and restoration of buildings. It is easy to produce Bio cement using microorganisms as it requires less time and requirement [10, 11].

MATERIAL AND METHODS

Collection of Samples

Samples such as soil (300 years old Ganesh temple), and waste from aera of the electroplating industry, dumping sites from the Karad region were used as sources for the isolation of bio-cement-producing microorganisms. The soil samples were collected from 0-30 cm depths in an alcohol-sterilized container. Out of the total samples, above three samples of soil, and two samples each of industrial wastewater 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.

Characterization of Soil.

Determination of pH

Air-dried soil (20 g) was taken in a 100 mL beaker and 50 mL of distilled water was added to it and thoroughly stirred for 2-3 min using a glass rod. Further, it was kept in shaking condition (120 rpm) for 3 hrs. The suspension was allowed to settle for 30 min. The instrument was calibrated with two buffer solutions of known pH viz. one acidic and another alkaline. The electrode was rinsed with distilled water and carefully wiped with filter paper. The pH of the soil was measured by immersing the electrode in a supernatant solution. The pH value was recorded when the reading was stabilized (usually after 1 min). The soil sample was mixed with water and stirred as described previously. KCl solution was used to calibrate the pH meter. While the pH of industrial waste was directly determined using a pH meter[3].

Enrichment of Samples

The samples were inoculated in 100 ml unique enrichment media (nutrient broth supplemented with sodium acetate 0.82%, Ammonium sulfate (1%), Urea (2%), and Glucose (0.1%). The ureais separately sterilized and then added to sterilized and cooled enrichment media. The broth was incubated at 30^0C temperature for 10 days on a rotary shaker. After enrichment, each sample was serially diluted in sterile distilled water up to 10^{-10} separately, followed by plating with 0.1 mL of final dilution on nutrient agar plates. Plates were incubated at an ambient temperature of 30 degrees for 24-48 h. After incubation well-isolated colonies were selected and transferred on nutrient agar slants, incubated, then preserved at refrigeration temperature, and further screened for bio cement formation ability[4].

Isolation of Bio-Cement-Producing Bacteria

The isolated different bacterial strains were plated on calcium precipitation agar (nutrient broth supplemented with Urea 20g/l, $NaHCO_3$ 2.12 g/l, NH_4Cl 10 g/l, $CaCl_2.H_2O$ 25g/l)where urea was separately sterilized and then add in medium to avoid decomposition of urea. The plates were incubated at ambient temperature and a precipitation zone was observed surrounding colonies[1, 3].

RESULT AND DISCUSSION

Determination of pH

pH of all samples was checked by using digital pH meter.It was found that different samples showed different pH such as follows (Table 1).

All samples were added to enrichment media separately, after ten days of incubation turbidity, as well as precipitate, was observed at bottom of the conical flask(Photoplate-1).After the enrichment of samples,a total of four different isolates were obtained on nutrient agar supplemented with 2% urea. These 4 organisms primarily screened for the precipitation of calcium carbonate; The 2 isolates were found efficient to precipitate calcium carbonate by observing the precipitation zone surrounding the colonies(Photoplates-2 and 3).Precipitation of calcium carbonate was tentatively tested by adding 1 N HCl tothe growth of bacteria. After the addition of 1N HCl bubble formation occurs which confirmed the precipitation of calcium carbonate.

As per Table 1, A total of 4 bacterial strains which able to precipitate calcium carbonate were isolated from the Karad regional area, out of which only 2strains were selected based on their ability to precipitate calcium carbonate. The bacterial strains were named isolates no.1,2,3,4.the colonies of isolate no.1 had a yellow color, circular shape, and flat elevations. Gram staining determination showed isolate no 1Gram-positive cocci. Isolate no.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 except isolate no.1 which was cocci in nature.

We isolated two isolates for precipitation of calcium carbonate from a 300 year sold temple located at Karad city and confirmed their ability to produce bio cement. Bharathi Reported that bacterial community plays a vital role in mineral precipitation in the environment everywhere possibly.

Table 1: pH of Samples

Sr. No	Sample type	Source of sample	pH
1.	Soil	300-year-old Ganesh temple.	8.0
2.	Industrial waste	Electroplating industry	8.2

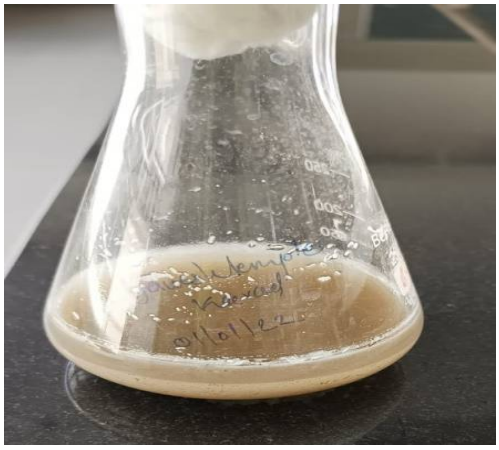

Photo plate no.1: Enrichment of soil sample in broth.

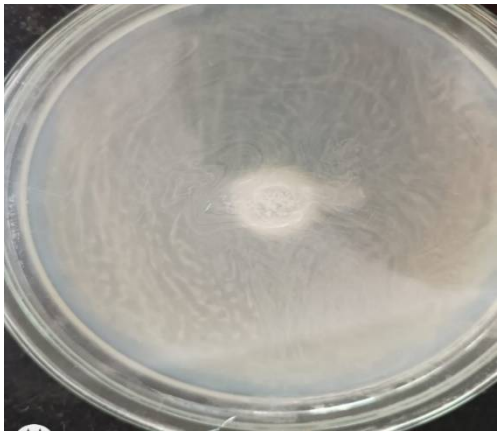

Photo plate no. 2: precipitation zone surrounding the growth.

Photo plate no 3: Isolation on calcium precipitation

CONCLUSION

The results obtained from this research confirm the presence of calcium-precipitating bacteria from Karad from that two isolates are more efficient to precipitate calcium carbonate. The urea in media after its hydrolysis creates alkaline conditions which help $CaCO_3$precipitation and glucose enhanced the growth. These two promising isolates have potential for production of bio cement, the enrichment and isolation method for bio cement producing organism is simple and unique one.

Further work of this research includes:

1. Screening of uerolytic activity of calcifying bacteria.
2. Characterization of isolate.
3. Quantification of bio cement produced by calcium precipiting bacteria.

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