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Calcium carbonate producing yeast from soil enhance chemical resistance on cement concrete specimen

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Abstract: Yeasts are ubiquitous in nature which is spread everywhere having specific biochemical pathways which enhance mineral formation. Calcium carbonate is one of the most common mineral which was precipitated by ureolytic yeast strain identified as *Candida tropicalis* confirmed by biochemical tests. Optimal growth conditions as pH and temperature, and mineralization conditions were carried. This work proved that the calcium carbonate precipitating ureolytic yeast enhance the chemical properties of cement concrete specimen.
Key words: *C.tropicalis*, urease, concrete, calcium carbonate, Yeast.

Introduction

Unicellular fungi are called yeasts. Over the years evidence accumulated that soil yeasts may exert a positive effect on soil structure, nutrient recycling and even plant growth. The role of some soil yeasts in soil aggregate formation has been known since 1970s. Some yeast especially affects soil structures as they are able to produce extracellular polymeric compounds that bind soil particles together (1). Yeasts are ubiquitous in their distribution and populations mainly depend on the type and concentration of organic materials. The distribution of species as well as their numbers and metabolic characteristics were found to be governed by existing environmental conditions.

Calcium carbonate is widely distributed mineral in nature. It can form loose crystals from rapid chemical reaction of carbonate and calcium ions. However its precipitation can also be triggered by microbial reactions. Microbial CaCO3 precipitation under appropriate conditions is a good phenomenon occurring in nature. Number of species associated with mineral formation dumbbells by slime-producing microbes.

Three main groups of microorganism that can induce the carbonate precipitation in nature: i. photosynthetic microorganism such as cyanobacteria and micro-algae that can remove CO2; ii. sulphate reducing bacteria that are responsible for dissimilatory reduction of sulphates; and iii. some species of microorganism participate in nitrogen cycle by any one of the methods such as oxidative deamination of amino acids, nitrate reduction or hydrolysis of urea.The involvement of microorganism evidenced in calcium carbonate precipitation, has lead the development of bioprocess technology in the field of construction material. The involvement of microorganism in CaCO3 precipitation can be described in three type of mechanism: i. spontaneous mechanism, usually by photosynthetic microorganism; ii.through nitrogen cycle; iii. through sulfur cycle (2).

One particular process by which significant amounts of calcite can be formed rapidly is by the bacterial release of carbonate from the hydrolysis of urea in the presence of calcium solution. In fact, an equimolar mixture of urea and calcium chloride solution can be considered as a cementation solution. In the presence of this cementation solution, ureolytic microbe will form $CaCO_3$ crystals at controllable rates and NH4+ ions which cause the pH to increase.

$CO(NH_2)_2 + Ca^{2+} + 2H_2O \longrightarrow NH_4^+ + CaCO_3$ (in presence of urease)

Carbonate precipitation process is a complex process which has described as involving metabolic pathways associated with photosynthesis, nitrogen and sulfur cycles and ion exchange. The microbial urease catalyzes the hydrolysis of urea into ammonium and carbonate.

One mole of urea is hydrolyzed intra-cellularly to 1 mol of ammonia and 1 mol of carbamate, which spontaneously hydrolyzes to form an additional 1 mol of ammonia and carbonic acid. CO(NH2)2 + H2O →

NH2COOH + NH3
NH2COOH + H2O → NH3 + H2 CO3
2NH3 +2H2 O → 2NH4 + 2OH
H2 CO3 ↔ HCO3 + H

Ammonium and carbonic acid form bicarbonate and 2 moles of ammonium and hydroxide ion in water which can produce hydroxide ions results in the increase of pH which in turn can shift the bicarbonate equilibrium increasing the formation of carbonate ions.

HCO3 + H+ 2NH4 + 2OH ↔ CO3 + 2NH4+2H 2O5
Ca2+ + CO3 2- → CaCO3

The produced carbonate ions precipitate in the presence of calcium ions as calcium carbonate crystals (3).

Materials and methods

Isolation of *C.tropicalis*

From agricultural field, collection of 1g of soil sample was serially diluted and plated on sabouraud dextrose broth. These plates were incubated at room temperature for 24 – 48hrs. After 48 hrs incubation the grown cells were used for further biochemical studies.

Gram staining

Gram staining is a technique used to detect the microbes whether yeast or Bacteria. Smear was prepared, on a glass slide and heat fixed. Smear was flooded with crystal violet for 60 sec. and then washed gently in water to remove excess crystal violet. Later it was flooded with Gram's iodine for 10 sec. and washed gently in water. Smear was decolourised with ethanol for 10 sec. and washed immediately in tap water. Counterstaining was done with safranin for 15 sec. and washed with water to remove the excessive stain. Finally samples were visualized under microscope at different magnification and observed for the Gram reaction and morphology of cells.

C.tropicalis medium for confirmation

CHROMagar Candida (CA), a yeast differential and selective medium, allows the recognition of mixed yeast cultures The medium permits the presumptive identification of C. *Albicans* from other *Candida* spp. Yeast populations are differentiated by colonial morphology and colours which are generated by a chromophore in the agar(4).

Urease test

The isolated strain was tested for urease activity which was done by streaking the purified culture on urease test agar and incubated 48 hrs at 350C. The media composition are; urea (20g/l), Na2HPO4 (9.5g/l), KH2PO4 (9.1g/l), SD broth (0.1g/l) and 0.01g phenol. pH was made to 6.8+/- 2. This test detects the ability of organism to produce urease enzyme. This enzyme converts urea to ammonia and CO_2.

Calcium carbonate precipitating medium

Urea 20g/l; NaHCO3 2.21g/l; NH4Cl 10g/l; CaCl2.2H2O 25g/l; and SD broth 3g/l.

Urease activity

The urease activity was determined by measuring the amount of ammonia released from urea according to the phenol-hypochlorite assay method. Ammonium chloride (100µg/ml) was used as the standard. Isolates were grown in corresponding media then re-inoculated into urease media and incubated at 37°C. After an interval of 24 hrs, the culture filtrate was added to a mixture containing 1 ml of 0.1 M Potassium Phosphate buffer (pH 8.0) and 2.5 ml of Urea (0.1 M), incubated at 37°C for 5 min followed by addition of Phenol Nitroprusside and alkaline hypochlorite, 1 ml each and incubated at 37°C for 25 min. Optical density was measured at 760 nm. Prepared standard graph (R2=0.992) was used. One unit of urease is defined as the amount of enzyme hydrolysing one µmole urea per min (5).

$$\text{Urease activity} = \frac{\text{Sample-Blank}}{\text{Slope} \times t} \text{ U/ml}$$

One unit of urease catalyzes urea to 1 µmole ammonia per min at pH 7.0 under the assay conditions.

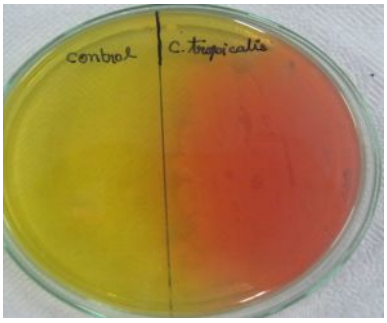
Preparation of concrete specimen

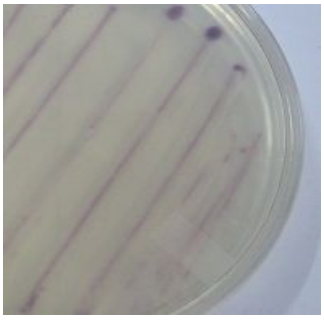
Specimen was prepared by 1:2:2 ratio which indicates by weight of1 part cement to 2 parts coarse aggregate to 2 parts fine aggregate

Results

Biochemical tests

Growth on Sabourd dextrose agar plate shows cream coloured with mycelial boarder.
No capsule was present using Indian ink preparation.
Confirmation test on CHROMagar medium shows bluish purple color indicates *C.tropicalis.*





Urease positive **Bluish purple colored *C.tropicalis***

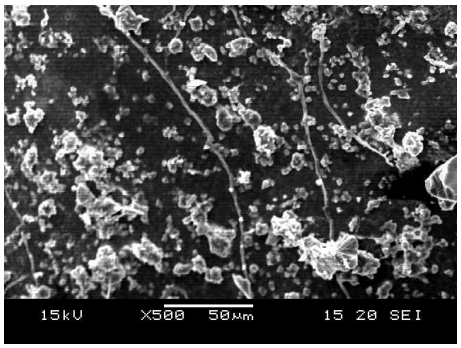
Germ tube test shows negative and urea hydrolysis test shows positive
Calculated urease activity was 6.2u/ml

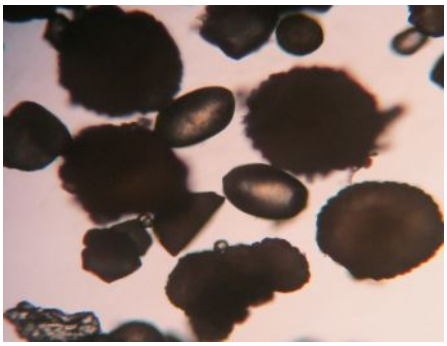
Growth conditions

Maximum biomass concentration was red spectrophotometrically 0.163 optical densities at 600nm.
Optimum temperature and pH was 35°C and 8.5 may withstand at 9 respectively.
The calcium carbonate producing compositions are: urea 20g/l; NaHCO3 2.21g/l; NH4Cl 10g/l; CaCl2.2H2O 25g/l; SD broth 3g/l.

Conditions for mineral precipitation

Optimum temperature and pH at 35°C and 8 respectively.
Under microscopic and SEM images confirm calcium carbonate crystals.





SEM image of Crystals **Microscopic view of crystals**

Immersion method

The prepared specimens were immersed into the respective calcium carbonate solution containing inoculates. The control specimens were immersed into water. After 48hrs the precipitation of calcium carbonate was observed on the surface of the concrete specimen which confirms the ability of *C.tropicalis* can able to precipitate calcium carbonate.

Immersed specimen **Precipitation on concrete specimen**

Water absorption test

Prepared specimen was weighed initially and allowed immersed into water overnight. The overnight saturated specimen was weighed accurately. Then dried oven with 30mnts and weighed. The absorption of water content was calculated by

$$\% \text{ water absorption} = \frac{W_{Saturation} - W_{ovendried}}{W_{ovendried}} \times 100$$

6% of water absorption was calculated when compared to control specimen which was absorbed 10%

Acid resistance test

A series of H2SO4 solution of different pH were prepared for testing the acid resistance of the cement specimen. The pH solutions were as follows; 1.0, 2.0, 3.0, 4.0, 5.0, and 6. A drop of H2SO4 solution was dripped on the concrete specimen from the beginning of the pH=5.5 If no air bubbles appeared, it could be concluded that the mortar could resist the corrosion of H2SO4 solution of this pH. Specimen showed the appearance of bubbles at 3.

Conclusion

This work concluded that the isolated yeast strain as *C.tropicalis* can able to hydrolyse urea and enhance the biochemical reaction for precipitating calcium carbonate. Optimal conditions of growth and

calcium carbonate precipitation was carried and applied on cement concrete specimen, the precipitates enhance the water absorption resistance and acid resistance. This work also proved that the precipitation on the surface of the concrete which protects from the parameters of water absorption and acid corrosion.

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