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Isolation and identification of bacteria to improve the strength of concrete

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ABSTRACT

The objective of this research work is to isolate and identify calcite precipitating bacteria and to check the suitability of these bacteria for use in concrete to improve its strength. Bacteria to be incorporated in concrete should be alkali resistant to endure the high pH of concrete and endospore forming to withstand the mechanical stresses induced in concrete during mixing. They must exhibit high urease activity to precipitate calcium carbonate in the form of calcite. Bacterial strains were isolated from alkaline soil samples of a cement factory and were tested for urease activity, potential to form endospores and precipitation of calcium carbonate. Based on these results, three isolates were selected and identified by 16S rRNA gene sequencing. They were identified as *Bacillus megaterium* BSKAU, *Bacillus licheniformis* BSKNAU and *Bacillus flexus* BSKNAU. The results were compared with *B. megaterium* MTCC 1684 obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh, India. Experimental work was carried out to assess the influence of bacteria on the compressive strength and tests revealed that bacterial concrete specimens showed enhancement in compressive strength. The efficiency of bacteria toward crack healing was also tested. Substantial increase in strength and complete healing of cracks was observed in concrete specimens cast with *B. megaterium* BSKAU, *B. licheniformis* BSKNAU and *B. megaterium* MTCC 1684. This indicates the suitability of these bacterial strains for use in concrete. The enhancement of strength and healing of cracks can be attributed to the filling of cracks in concrete by calcite which was visualized by scanning electron microscope.

1. Introduction

Concrete is the second most consumed material on earth, next only to water. But it is susceptible to micro crack formation and has pores in it. Micro cracks and pores in concrete are highly undesirable because they provide an open pathway for the ingress of water and other deleterious substances. This leads to corrosion of reinforcement and reduces the strength and durability of concrete. Large costs are incurred all over the globe to repair cracks in concrete. For repairing cracks, a variety of techniques are available but majority of traditional repair systems are chemical based, expensive and lead to environmental and health hazards. Recently, microbiologically induced calcite precipitation has been proposed as an effective alternative repair technique for plugging of micro cracks and pores in concrete. This bacterial remediation technique surpasses other techniques as it is bio based, eco friendly, cost effective and durable.

Urease positive bacteria have been found to influence the precipitation of calcium carbonate (calcite) by the production of an urease enzyme. This enzyme catalyzes the hydrolysis of urea to CO_2 and ammonia, resulting in an increase of the pH and calcite precipitation in the bacterial environment (Stocks-Fischer et al. 1999).

This innovative environmental friendly method was first used for the repair of cracks to prevent leaching in channels (Gollapudi et al. 1995). It can also be used for enhanced oil recovery, prevention of acid mine drainage, for remediation of granite, mortar, limestone and concrete (Ramakrishnan et al. 2001; Zhong and Islam 1995).

The calcite precipitation induced by *Bacillus pasteruii* and *Bacillus sphaericus* was found to be effective in remediating cracks of concrete and increased its compressive strength (Ramakrishnan et al. 2001; Van Tittelboom et al. 2010). The durability of concrete specimens treated with *B. pasteruii* exposed to alkaline, sulphate and freeze–thaw environments was also reported to increase (Ramakrishnan et al. 2005).

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Table 1
Electric conductivity [EC (mS/m)] of urease assay mixture at different time intervals.

BI 1		BI 2		BI 3		BI 4		BI 5		*Bacillus megaterium* MTCC 1684	
Time (s)	EC (mS/m)	Time (s)	EC (mS/m)	Time (s)	EC (mS/m)	Time (s)	EC (mS/m)	Time (s)	EC (mS/m)	Time (s)	EC (mS/m)
0	135.6	0	121.9	0	122.7	0	118.7	0	128.1	0	240
300	138.7	300	136.5	300	123.2	300	119.8	300	131.9	300	251
3960	142.1	3960	139.2	3960	125.0	3960	121.8	3960	141.2	3960	282
6360	161.2	6360	140.6	6360	129.2	6360	126.2	6360	145.9	6360	296

Considerable research work on concrete incorporating bacterial species *B. pasteruii* and *B. sphaericus* has been reported in the literature. But limited research has been done on other species of bacteria. The present study deals with the isolation and identification of indigenous calcite precipitating bacteria and checking the suitability of these bacteria for use in concrete. The influence of bacteria on the compressive strength and healing of cracks in concrete has also been studied. The calcite precipitates were visually examined by SEM. *Bacillus megaterium* MTCC 1684 obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh, India was used as the standard culture. This study also uses wheat bran as an alternative substrate for growth of bacteria which will result in reduction of cost of bacterial treatment.

2. Materials and methods

2.1. Bacterial strains and wheat bran as alternative substrate

Five pure alkali resistant bacterial strains were isolated from the alkaline soil samples of a cement factory at Coimbatore, Tamil Nadu, India. They were maintained constantly on nutrient agar slants. Contamination from other bacteria was checked periodically by streaking on nutrient agar plates.

Wheat bran samples were obtained from local market and were used as an alternative substrate for cultivating bacterial cultures to reduce the cost of substrate. Whenever required, few colonies of the pure culture were inoculated into nutrient broth of 25 ml in 100 ml conical flask and the growth condition was maintained at 37 °C temperature and placed in 125 rpm orbital shaker. Media composition used for the growth of culture was Yeast Extract 5 g/l, Beef Extract 5 g/l and Wheat Bran 20 g/l. pH was maintained alkaline at 8.

2.2. Quantitative urease assay by electric conductivity

Conductivity method for urease activity assay was conducted as per Hammad et al. (2013) and Marien et al. (2010). The urease reaction involved the hydrolysis of non-ionic substrate urea to ionic products thus generating a proportionate increase in conductivity under standard conditions. For enzyme assay, 1.0 ml of bacterial broth culture (nutrient broth-urea) was added to 9.0 ml of 1.11 M urea solution. Final conductivity record was taken after 5 min of incubation at 20 °C by electric conductivity meter. Urease activity is presented by the rate of conductivity increase as mS/min. Table 1 gives the electric conductivity [EC (mS/m)] of urease assay at different time intervals.

Based on the high urease activity, three bacterial isolates (BI 1, BI 2 and BI 5) and *B. megaterium* MTCC 1684 were selected for use in concrete. *B. megaterium* MTCC 1684 was used as the standard culture and was also used in concrete for comparing the results with the bacterial isolates.

2.3. Endospore staining and $CaCo_3$ (calcium carbonate) precipitation in broth state

The selected bacterial isolates and *B. megaterium* MTCC 1684 were tested for their ability to form endospores by Schaeffer–Fulton endospore staining procedure as per Geeta and Mehrotra (2009). The smears of bacterial isolates and *B. megaterium* MTCC 1684 were prepared and heat fixed. The smears were covered with a piece of absorbent paper cut to fit the slide and the slides were placed on wire gauze on a ring stand. The paper was saturated with malachite green and the slide was heated until steam could be seen rising from the surface. The slide was removed from heat and reheated to keep the slide steaming for about 3 min. As the paper began to dry, a drop of malachite green was added to keep it moist. The paper was removed with tweezers and the slide was rinsed thoroughly with tap water. The slide was drained and counterstained for 45 s with 0.5% safranin. It was then washed, blotted and examined under a research compound microscope (100×). The vegetative cells will appear red or pink and the endospores will appear green.

The selected isolates were further tested for $CaCO_3$ precipitation in broth state as per Hammad et al. (2013). For measurement of $CaCO_3$ precipitation in broth, nutrient broth supplemented with 2% urea and calcium chloride (NB-U/Ca) was used. 30 ml of NB-U/Ca was inoculated with 2% inoculum then incubated under shaking condition (at 130 rpm) at 30 °C for 7 days. Three replicates were tested. Precipitated $CaCO_3$ was filtered through filter paper (Whatman filter paper), which was dried in 60 °C oven for 3 h and then weighed. $CaCO_3$ precipitant weight (W_c) was determined from the equation:

$$W_c = W_{fc} - W_f$$

where (W_{fc}) is the weight of filter paper containing precipitant; and (W_f) is the weight of empty filter paper.

2.4. Molecular identification

Further identification of the isolate was performed using 16S rRNA gene sequencing. The DNA was isolated and the analysis of DNA sequences was performed by using the Blastx software (BLAST), National center for biotechnology information.

2.5. Concrete

Ordinary Portland cement of 53 grade available in the market was used. The physical and chemical properties of cement are given in Table 2. It satisfies the requirements as per IS:12269 (1987b). Locally available sand passing through 4.75 mm sieve and

Table 2
Physical and chemical properties of cement.

Physical property	
Color	Gray
Specific gravity	3.15
Chemical constituent (%)	
SiO_2	21
CaO	62
Al_2O_3	5.04
Fe_2O_3	3.16
MgO	4.56
Na_2O	0.08
Loss on ignition	1.29