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ORIGINAL ARTICLE

# Development on bio-based concrete crack healing in soil exposures: isolation, identification, and characterization of potential bacteria and evaluation of crack healing performance

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## Abstract

Undeniably, concrete cracks if not healed on time could cause various durability issues, resulting in high-cost crack repairing strategy. Recently, due to several environmental concerns, bio-based construction materials gained much attention among researchers. In this context, bacterial precipitation of calcium carbonate can be effectively exploited in concrete structures to heal cracks. With this in view, this study focused to isolate bacterial strains from five different locations towards examining the crack healing potential. The investigations were carried out for urease positive test and calcium carbonate precipitate test among the five different isolated bacteria of whom three bacterial species were found to have the needed potential. Using the 16S rRNA molecular gene sequencing, they were identified and subjected to concrete healing inspection using an appropriate nutrient solution. On the 7th day of conventional water curing, bacteria-added concrete cubes were pre-cracked and kept in soil exposures in four different pits for a healing period of 120 days. Filling soil material was supplemented with three different proportions of nutrient and bacterial solution ratios (7:3, 8:2, and 9:1) without any changes in the existing natural water content. A microstructure study revealed that the formation of significant calcium carbonate compounds in bacteria supplemented the concrete specimens. The bacterial pit, having a proportion of 9:1, had showed higher healing efficiency of about 88%, nevertheless while the remaining pits showed only 60% and 66% healing efficiency. The study implied bacteria addition with nutrient supply in soil environment could be highly beneficial for healing cracks in concrete specimens. Consequently, the study's inference can pave the way for futuristic bio-based processing in construction materials.

**Keywords** Calcium carbonate · Bacterial isolation · 16S rRNA sequencing · Crack healing · Soil exposures · Microstructure study

## Abbreviations

| | |
|---|---|
| $m^2/kg$ | Meter square per kg |
| mins | Minutes |
| $kg/m^3$ | Kilogram per meter cube |
| % | Percentage |
| °C | Degree celsius |
| h | Hours |
| g | Gram |
| v/v | Volume per volume |
| Nos | Numbers |
| ml | Milliliter |
| ID | Identification |
| NCBI | National Center for Biotechnology Information |
| rpm | Revolution per minute |
| SEM | Scanning electron microscopy |
| XRD | X-ray diffraction |
| $CaCO_3$ | Calcium carbonate |
| RNA | Ribonucleic acid |
| DNA | Deoxyribonucleic acid |
| pH | Potential of hydrogen |
| OD | Optical density |
| BP | Bacterial pit |
| mm | Millimeter |
| cm | Centimeter |

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## 1 Introduction

Concrete is a predominant material that is used for construction of bridges, tunnels, subways, and other structures [1, 2]. However, concrete is brittle and heterogenetic in nature with low tensile strength [3, 4] and easily cracks due to some reasons, such as compressive force, mechanical force, autogenous shrinkage, and free-thaw action [5, 6]. It is proven that the structural safety of concrete is significantly affected by one of the aforementioned reasons, cracks, which decrease durability and mechanical performance [7]. To overcome this issue, cracks can be treated by passive and active methods. In this light, passive treatment can be carried out to seal the external cracks using the application of appropriate polymers and chemical admixtures [8]. In another, by employing active treatment methods, both the internal and external cracks are sealed by adding suitable bacteria on to the concrete to heal the cracks [9]. In this respect, various experimental studies were carried out on the incorporation of bacteria in concrete under different nutrient and curing conditions [10, 11]. So far, several investigations have been carried out on addition of bacteria in the concrete specimens using proper nutrients that can induce the precipitation of calcite to heal the crack formation. In this context, some research outcomes have indicated that the addition of bacteria in soil stabilization with polymers could be potential implementation. Thus far, only limited investigations were carried out on the study concerning to crack healing in concrete specimens with soil interactions. However, in larger crack width, the potential healing performance fails to fill the cracks effectively due to inadequate nutrients requirement. In this context, Hesham et al. [12] isolated seven gram-negative bacterial species from contaminated soil to study on bio-degradation of polycyclic aromatic hydrocarbons. The results found that the bacterial species, *Pseudomonas alcaligenes*, *Arthrobacter oxydans*, and *Stenotrophomonas zoogloeoides*, showed better performance on bio-degradation. In another study, Mokhtar et al. [13] identified that incorporation of urease-positive bacterial species, *Bacillus wiedmannii* and *Bacillus paramycoides*, in the concrete specimens can increase self-healing efficiency and compressive strength. Canakci et al. [14] isolated seven urease-positive bacillus species from natural soil that enhance the shear strength in peat specimen through the mechanism of calcium carbonate crystals precipitations. Sumathi et al. [15] identified that the *Bacillus cohnii* which was isolated from soil in a concrete dump yard showed 90% and 88% surface healing, respectively, based on full-wet and wet-dry conditions while adding to the concrete specimens. Raut et al. [16] observed notable calcite precipitation using *Bacillus pasteurii* in the conventional bricks contained 80% clay, 15% ash, and 5% rice husk. After processing, their results demonstrated that the increase of compressive strength up to 83.9% and reduction of water absorption capacity up to 48.9% were achieved in the treated specimens. Kiranmaye et al. [17] evaluated the suitability of *Pseudomonas alcaligenes* in a concrete environment having the pH value of 13 and a temperature of 37 °C. The results revealed that the isolate *P. alkaligenes* was found to be suitable for construction industry which had a higher growth rate at an optimum temperature of 35 °C. Esaker et al. [18] studied the effect of *Bacillus subtilis* in healing mortar cracks under different pH environments in the soil. It was noted that the healing ratio increased to 82% after the addition of bacteria to mortar cubes. However, healing ratio of cubes decreased by 43% when it was incubated in acidic soil condition having the pH value, 4.5. Hamza et al. [19] studied on the performance of *Bacillus subtilis* under various exposure conditions in soil for an incubation period of 28 days. The results revealed that the naturally available bacteria in soil did not reveal healing in mortar specimens. Hoang et al. [20] incorporated *Sporosarcina pasteurii*–derived urease enzyme in bio-stabilization of non-plastic and low plastic silt sand. During their study, reduction in permeability was noticed in bacterial enzyme–induced calcite precipitation with the unconfined compression value of the same specimens being achieved during early calcite formation. In yet another study, Ramachandran et al. [21] used soil stabilization with xanthan gum and bacterial biopolymer. They detected that the treated sand exhibited lesser strength due to reinforcement unavailability. Nevertheless, addition of reinforcing material was led to attain high strength values. This result proves the binding property of bacterial polymer with clay soil can be a promising approach. Gowthaman et al. [22] evaluated on microbiological induced calcite precipitation of various native bacteria species in the stabilization of slopes. They looked on the *Psychrobacillus* sp. which exhibited an effective calcite precipitation. In addition, a comparative study was carried by Gowthaman et al. using nutrient supply made of low-grade and lab-grade chemicals. As a result, low-grade chemicals led to 96% cost reduction followed by doubled unconfined compression values. Mekonnen et al. [23] studied on the stabilization of black cotton soil with four bacterial strains. They found that the bearing ratio of soil treated with *Bacillus paramycoides* bacteria showed a significant result compared to the other species.

Keeping view in bacterial concrete healing attributes, this investigation focused on identifying the appropriate bacteria that could repair concrete cracks in various soil environments. The healing effectiveness of bacteria-amended concrete cubes which were incubated in soil medium and nutrient solution was evaluated. This

**Table 1** Physical properties of cement

| Physical properties of cement | Value |
|---|---|
| Fineness ($m^2$/kg) | 224 |
| Specific gravity | 3.14 |
| Initial setting time (min) | 69 |
| Final setting time (min) | 253 |
| Consistency (%) | 35 |

**Table 2** Physical properties of aggregates

| Property | Value of aggregates | |
|---|---|---|
| | Fine | Coarse |
| Specific gravity | 2.66 | 2.81 |
| Water absorption (%) | 1.15 | 1 |
| Bulk density (kg/$m^3$) | 1690 | 1596 |
| Fineness modulus | 3.28 | 5.16 |

investigation was laid to figure out an appropriate requirement of bacterial and nutrient solution to be amended in order to stimulate improved crack healing in soil environment. Further, the healing mechanism of existing concrete substructures with provision of nutrient and bacteria sources was ascertained and practically correlated.

## 2 Materials and methods

### 2.1 Cement, fine aggregate, and coarse aggregate

Ordinary Portland cement (53 grade) was used for experimental work with reference with IS. 269–2015 [24]. Fine aggregate of 4.75 mm maximum was collected from a nearby site and crushed as angular coarse aggregate of 20 mm for use with stipulation followed from [25, 26]. The physical properties of cement, fine aggregate, and coarse aggregate chosen for this study are presented in Tables 1 and 2.

### 2.2 Isolation of bacteria and morphological characterization

Soil samples were collected from five different locations with varying natural habitat as shown in Fig. 1. Due to the nitration of fertilizers, the soil could be acidic [27]. To avoid organic matters and debris, soil samples were collected below 15 cm at chosen location [28]. The samples were kept safe in zip lockable sterilized polythene covers. Soil samples were labeled with ID, S1, S2, S3, S4, and S5 that were gathered from soil habitats, such as river beds, roots of *Ocimum tenuiflorum*, a bio-gas plant, chlorination effluent pond, and concrete debris respectively as shown in Fig. 1.


**Fig. 1** Sample collections at various site location

The samples were serially diluted up to $10^{-6}$ and subjected to aseptic growth in petri dish plate having nutrient agar medium [29]. The plates were placed in bacteriological incubator for period of 24 h and maintained at 37.5°C. Pure individual colonies were isolated and preserved at 4 °C [30]. After incubation, five colonies of isolated species were identified and morphologically characterized based on gram staining results as shown in Fig. 2.

## 2.3 Efficiency of strains in concrete applications

To identify suitable bacteria to fill cracks in concrete, five isolated strains were selected and subjected to examine the urease enzyme production test and calcium carbonate precipitating test. Further, based on these results, the more effective bacterial strain was chosen and investigated as crack healing agent [31].

### 2.3.1 Urease enzyme production

The ability of an organism to split urea by urease enzyme production is determined by the urease test. Urease enzyme produced by urease positive bacteria converts urea into carbon dioxide and ammonia, which increases pH and calcite precipitations in concrete [13]. Urease enzyme is produced by bacteria that act as a biological catalyst by hydrolyzing urea to form carbonate ions without producing protons, which results in $CaCO_3$ precipitation in the presence of calcium ions [26]. In this context, negative-charged bacterial cell wall not only serves as a nucleation site for $CaCO_3$ precipitation but also produces an alkaline environment that promotes the subsequent growth of $CaCO_3$ crystals [8]. Equation (1) shows the intracellular hydrolysis of 1 mol of urea into 1 mol of ammonia and 1 mol of carbamate. As per Eq. (2), ammonia and carbonic acid are formed when carbamate hydrolyzes. Equations (3) and (4) show that former products subsequently equilibrate in water to create bicarbonate, ammonium, and hydroxide ions. The latter results in a rise in pH and the production of carbonate ions as shown in Eq. (5). Carbonate ions in the presence of calcium ion precipitates $CaCO_3$; Eq. (6) and Eq. (7) illustrate the overall reaction, showing that the system's additional urea and calcium produced byproducts of ammonium and calcium carbonate [9].

$$CO\left(NH_2\right)_2 + H_2O \rightarrow NH_2COOH + NH_3 \tag{1}$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \tag{2}$$

$$H_2CO_3 \rightarrow HCO_3^- + H^+ \tag{3}$$

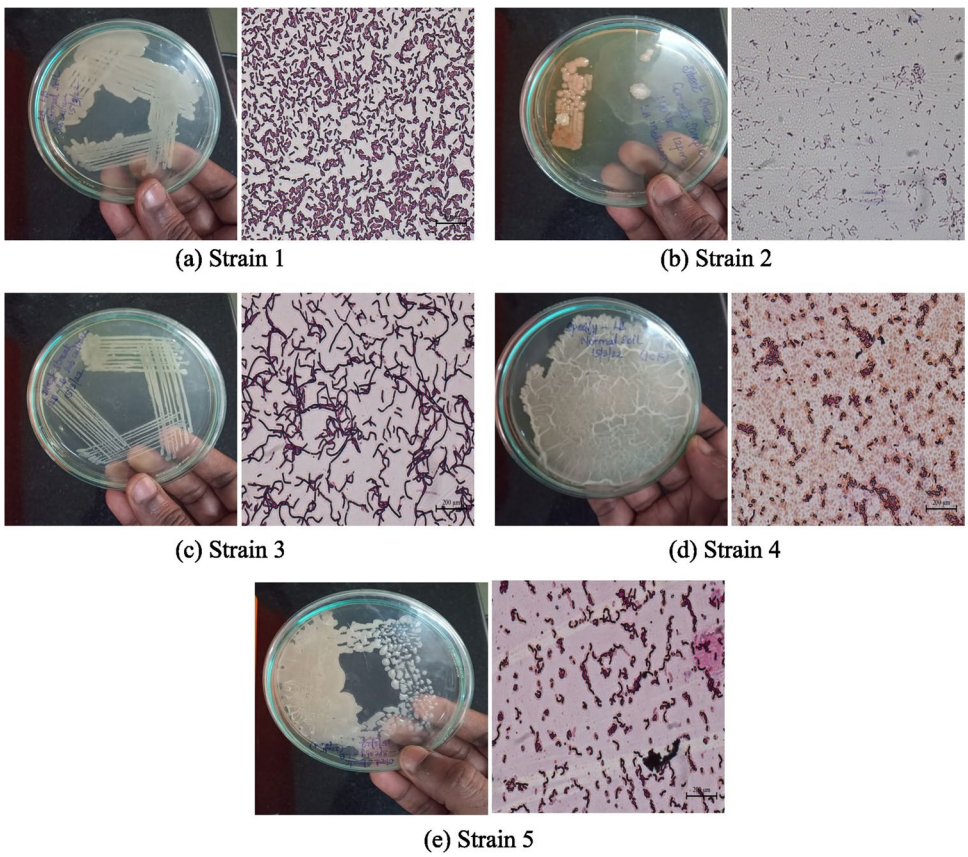


**Fig. 2** Isolated bacterial species and their microscopic observations

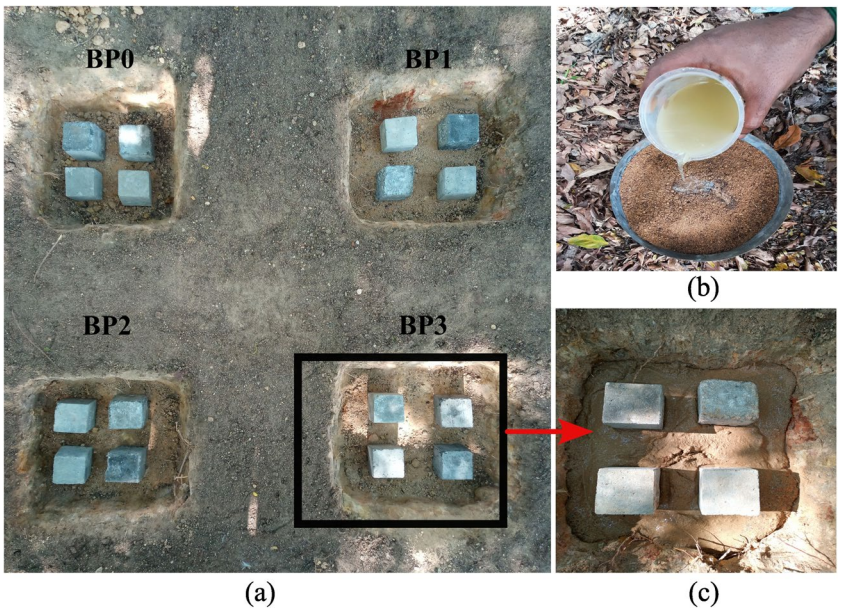


**Fig. 3** Incubation pit for single strain. (**a**) Pits showing with different coded IDs. (**b**) Addition of healing solution in soil. (**c**) Expanded view of single pit

**Table 3** Proportions of healing solution mixed in pit soil

| Pit ID | Healing solution | |
|---|---|---|
| | Bacterial solution | Nutrient solution |
| BP0 | – | – |
| BP1 | 30% | 70% |
| BP2 | 20% | 80% |
| BP3 | 10% | 90% |

$$2NH_3 + 2H_2O \rightarrow 2NH^{4+} + 2OH^- \quad (4)$$

$$HCO_3^- + H^+ + 2OH^- \rightarrow CO3^{2-} + 2H_2O \quad (5)$$

$$CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3 \quad (6)$$

$$CO(NH_2)_2 + 2H_2O + Ca^{2+} \rightarrow 2NH^{4+} + CaCO_3 \quad (7)$$

Various researchers studied isolation and application of urease enzyme producing organisms [32–34] and in the present study, five isolated strains having urease production capacity that were subjected to test were identified using the following procedures [31, 35]:

(i) Ingredients of 0.15 g of agar, 0.01 g of dextrose, 0.02 g of monopotassium phosphate, 0.01 g of peptone, 0.00012 g of phenol red, 0.05 g of sodium chloride, and 0.2 g of urea were dissolved in 100 ml distilled water.

(ii) The pH of the medium was adjusted to 6.7 at room temperature, followed by filtration and sterilization were carried out.

(iii) Agar was dissolved in 100 ml distilled water and autoclaved for 120 °C for 20 min and allowed to cool.

(iv) Both solutions obtained from steps (ii) and (iii) were mixed well and transferred to boiling 10-ml tubes.

(v) Isolated pure culture from petri dishes was inoculated in boiling tubes and kept in bacteriological incubator for 37.5 °C.

After 7 days, the boiling tubes were examined for change color, indicating that hydrolysis of urea to ammonia and carbon dioxide.

### 2.3.2 Calcium carbonate production

Microbial-induced calcium carbonate production is an eco-friendly and innovative approach to heal internal cracks that can increase the durability of structures [36–38]. In the present study, quantification of calcium carbonate production was ascertained based on the procedure documented from Alemu et al. [8]. In such a way, a nutrient broth was inoculated with 2% of urea and calcium chloride. Later, each bacterial strain was inoculated and kept in a shaker (120 rpm) for 7 days at 37.5 °C. After 7 days, deposited calcium carbonate was noted at the bottom of the conical flask as shown in Fig. 4. Using a Whatman filter paper, calcium carbonate precipitates were filtered and dried at 55 °C using an oven for 8 h. The weight of the calcium carbonate ($W_c$) produced was calculated from Eq. (8).

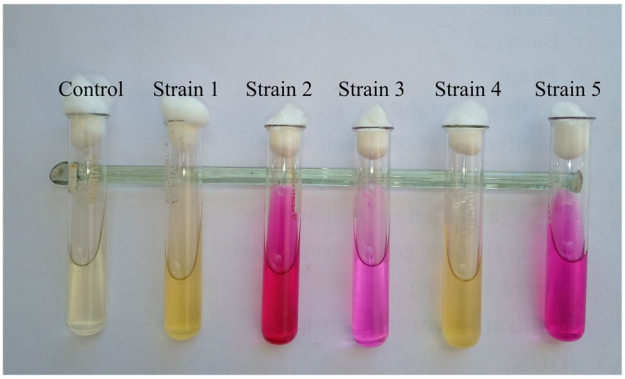


**Fig. 4** Urease enzyme production

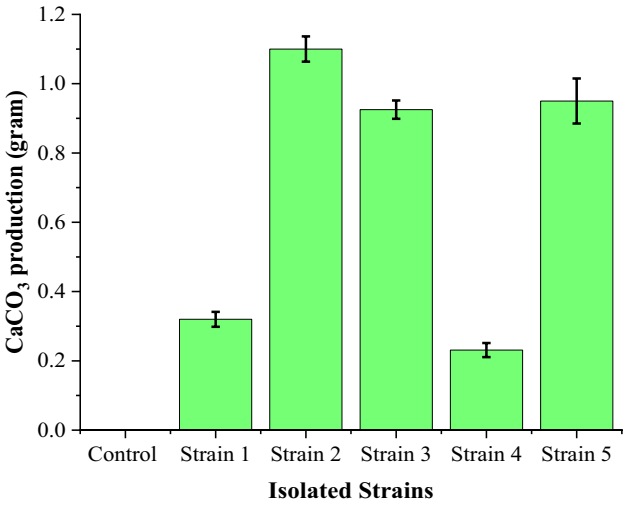


**Fig. 6** Yield value of calcium carbonate precipitation by different bacterial isolates

$$W_c = W_{fc} - W_f \tag{8}$$

where $W_{fc}$ is the weight of filter paper containing the calcium carbonate precipitant and $W_f$ is the weight of empty filter paper.

## 2.4 Concrete ingredients, healing solution details, and soil incubation details

The concrete ingredients were evaluated using mix calculations as per concrete mix proportioning guidelines, IS 10262:2009 [39]. In this respect, the volume of concrete ingredients used in this study contained cement, 435 kg/m$^3$; fine aggregate, 712 kg/m$^3$; coarse aggregate, 1108 kg/m$^3$; and water, 182 kg/m$^3$. A 5% (v/v) bacterial solution ($10^7$ cells/ml) was added directly to the concrete by replacing the volume quantity of designed mix water and well-mixed. Based on preliminary tests conducted with cell concentrations, it was found that $10^5$ cells/ml had high compressive strength while $10^7$ cells/ml showed high crack healing efficiency as reported by Krishna et al. [40]. Then, all the cubes (100 mm × 100 mm × 100 mm) were cast with bacterial concentration of $10^7$ cells/ml and cured in normal water for a period for 7 days. After curing, the cubes were pre-cracked using a compression testing machine of 3000 kN. The pre-cracked cubes were kept in incubation pit of 500 mm × 500 mm × 500 mm.

In this sense, the top 50-mm layer of soil was removed completely to avoid small herbs and dry leaves. The incubation pit of 4 Nos were allotted and the pre-cracked concrete cubes were placed in two layers (4 Nos each) as shown in Fig. 3(a, c). Big lumps in soil were broken and sieved to ensure uniform grain size distribution. The required quantity of soil sample was taken and tested for various properties. The pH value and natural water content of soil were found to be 5.1 and 32% respectively. Crack healing behavior of the cubes in soil incubation was carried out during (June to August), so that the crack healing mechanisms were correlated with water table of ground structures with regard to climatic conditions. In addition, the surrounding soil was provided with varying proportions of healing solution (bacterial and nutrient solution) as shown in Fig. 3(b). Nutrient solution was provided mixture of yeast extract (3 g/l), urea (5 g/l), and calcium lactate (5 g/l). The bacterial solution comprising of bacteria inoculated nutrient broth was kept in a shaker overnight. Variations in the healing solution and bacterial solutions were added in pit soil based on the existing natural water content. Using soil tensiometer, the available water content of pit soil was

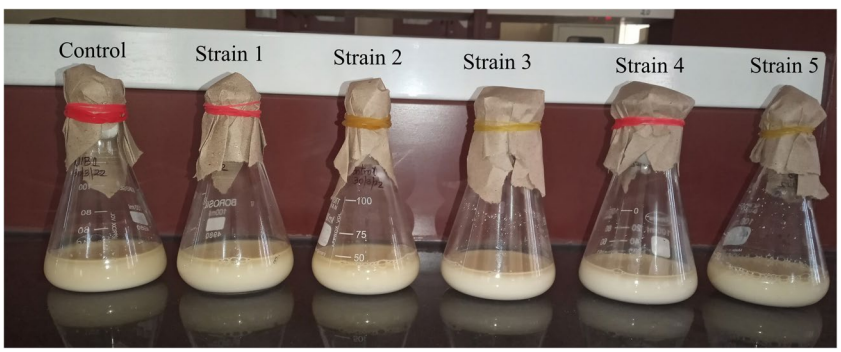


**Fig. 5** Precipitation of calcium carbonate produced by different bacterial isolates

maintained along with addition of bacteria and nutrient solution. Based on the quantity of soil excavated, bacterial and nutrient solutions were added in such a way that the natural water content can be remained unchanged. Addition of bacterial and nutrient solution in various pits is listed in Table 3. The incubation period was 120 days to resemble water fluctuations in the ground due to climatic conditions [18].

### 2.5 Ascertainment of crack healing

Crack healing was not ascertained periodically, as the activity disturbs soil around cubes and affects the strains in the precipitating calcium carbonate. The width of the pre-cracked cubes was in a range of 0.5 to 0.8 mm. The degree of crack healing was determined using the method documented by Sumathi et al. [15]. Percentage of crack healing can be defined as difference between the initial crack width and the observed crack width after the incubation period. It was deduced using Eq. (9).

$$\text{Percentage of crack healing} = \left[\left(C_i - C_o\right) \times 100\right]/C_o \quad (9)$$

where $C_i$ is width of the initial crack and $C_o$ is the width of crack observed after healing.

### 2.6 X-ray diffraction and SEM

The microstructure study on soil and white precipitate in the pre-cracked cube specimens was carried out using X-ray diffraction and scanning electron microscopy methods. X-ray diffraction spectroscopy analysis was carried out using peak values of the mineral compounds formed by bacteria during the 120 days of incubation. X-pert high score plus software (Version 5.2) was used to identify the mineral compounds formed during the healing cum incubation periods. Using the obtained score values, the minerals were matched with the reference codes from available data bases. Scanning electron microscopy (SEM) was undertaken to study the morphological components formed in the incubation soil and the pre-cracked cube specimen. A VEGA 3 TESCAN scanning electron microscope and Bruker D8 concentrate unit were used.

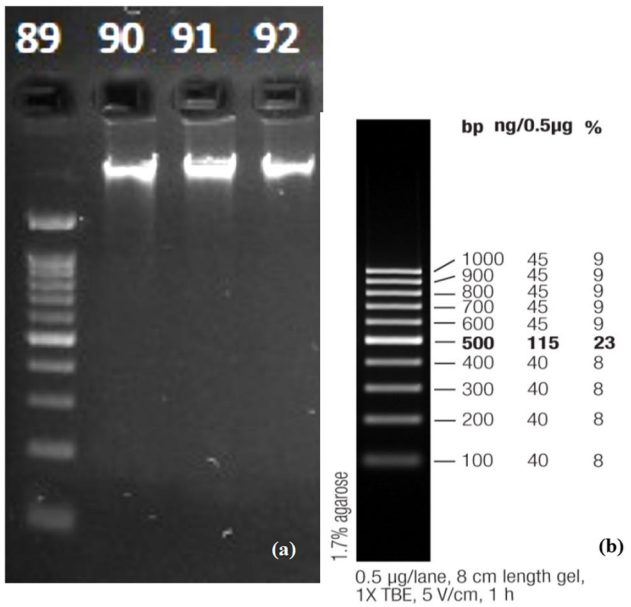


**Fig. 7** Gel-documentation image of isolates bacterial strains

### 2.7 Statistical analysis

In the present work, a two-way analysis of variance (ANOVA test) was used to analyze statistically data using the SPSS Statistical Package Program [41]. When there were substantial differences between the treatments, the Duncan multiple range test was used to compare their means [42]. All tests were ensured with a $p \leq 0.05$ level of significance. Means and standard error were used to represent the results.

## 3 Results and discussion

### 3.1 Gram staining test

In order to get basic characteristics of bacterial isolate, Gram staining technique was executed using the method documented by Tripathi et al. [43]. The principle involves the retention of crystal violet dye by the bacterial cell wall in a solvent of acetone and ethanol. Crystal violet dye was first used to stain the slides initially, followed by iodine which was used to fix the dye resulting in the formation of crystal violet iodine complex and decolorizer (solvent of acetone and ethanol). Finally, basic fuchsin stain was used to provide a pink color to gram-negative bacteria. From the result of gram staining, strains 1, 2, 4, and 5 were gram-positive due to high peptidoglycan content and strain 3 was gram-negative due to lipid content [44].

### 3.2 Urease enzyme production

Among the 5 isolated bacterial strains, strains coded as 2, 3, and 5 have been identified as capable of producing the urease enzyme, revealing that these strains had the ability to perform in concrete environment to biologically precipitate calcium carbonate in cracks. The strains coded as 1 and 4 failed to hydrolyze urea into ammonia and carbon due to their natural behavior as shown in Fig. 4. The findings were similar to the documented works by Mokhtar et al. and Durga et al. [13, 31].

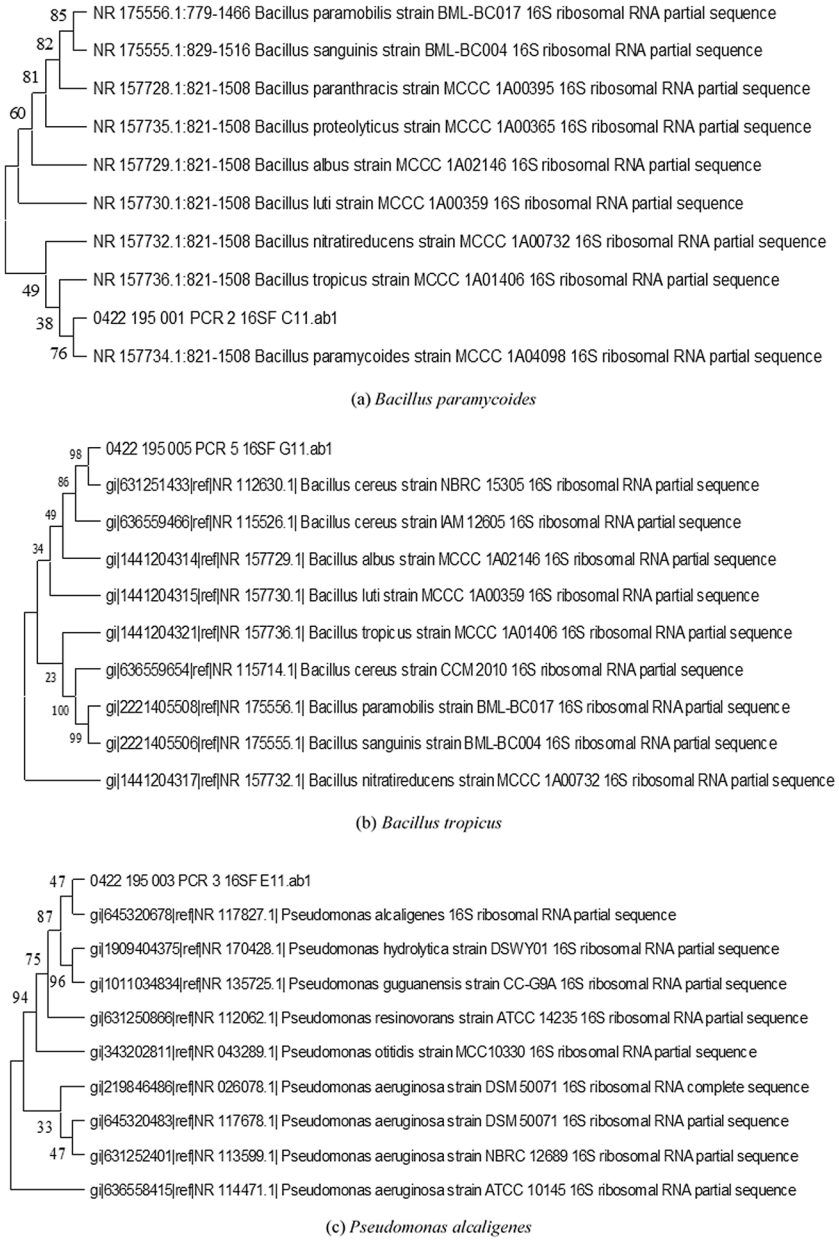


**Fig. 8** Phylogenetic relationship of isolated strains

### 3.3 Calcium carbonate production

After inoculating bacteria in a medium containing broth, urea, and calcium chloride, white precipitates were deposited at the base of the conical flasks as shown in Fig. 5. After 7 days, the precipitates were collected and weighed. It was found that the control strain had nil value of precipitation, strain 1 produced 0.32 g, strain 2 produced 1.10 g, strain 3 produced 0.925 g, strain 4 produced 0.231 g, and strain 5 produced 0.95 g. The results revealed that strains 2, 3, and 5 can be utilized in concrete crack healing applications due to their substantial ability for precipitating calcium carbonate. The test results of calcium carbonate precipitation are presented in Fig. 6. The results are supported by Mokhtar et al. [13].

### 3.4 16S rRNA sequencing

From the results from Sections 3.1 to 3.2, it was apparent that the isolate strains coded 2, 3, and 5 had showed better performance in producing the $CaCO_3$, indicating that they can be potentially utilized in concrete healing process and soil environments. In order to understand the species level information, the three strains, coded as 2, 3, and 5, were subjected to 16S rRNA sequencing technique.

In accordance with that, the DNA purification kit supplied by GeNei™ Bangalore, India, was used to isolate the DNA of each bacterial strain. Universal primers for strain2 was forward primer 5′-GGGAATGAGTGCTTAGTGTT-3′ and reverse primer 5′-CTTCCTCCCGGTTTCCCCTT-3′,

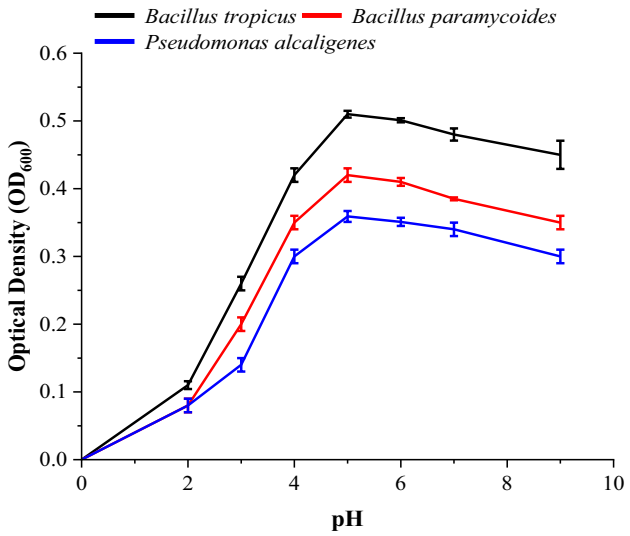


**Fig. 9** Bacteria growth for pH condition

for strain 3 was forward primer 5′-CGGATGTCGACTAGC CGTTG-3′ and reverse primer 5′-GGGGGGGGGGGGGGG GGG-3′, and for strain 5 was forward primer 5′-TGGAGG AGTGCTAAGTGTTA-3′ and reverse primer 5′-ATAATG TACGGAAATGGGAG-3′. A PCR thermal cycling was done using the method reported by Zhang et al. [6]. Agarose gel electrophoresis followed with gel documentation was used to amplify the DNA. The gel documentation image of isolates and bacterial stains is presented in Fig. 7.

Using genetic analyzer, the purified 16S rRNA of three different species was analyzed. With NCBI GenBank database, 16S rRNA gene sequence was carried out using BLAST (Basic Local Alignment Search Tool). Depending on the maximum similarity index, the top ten sequences of bacteria were aligned with CLUSTAL W software. A phylogenetic tree was constructed for each bacterial species, shown in Fig. 8a, b, and c using MEGA 11 software [45, 46]. The

**Table 4** Crack width and area of crack width observation of cubes

| Pit ID | Crack width after 7th day of pre-crack | Crack width after 120th day of pre-crack | Crack width pattern before healing | Crack width pattern after healing |
|---|---|---|---|---|
| BP0 |  0.52 mm |  0.34 mm |  |  |
| BP1 |  0.61 mm |  0.244 mm |  |  |
| BP2 |  0.7 mm |  0.238 mm |  |  |
| BP3 |  0.72 mm |  0.086 mm |  |  |

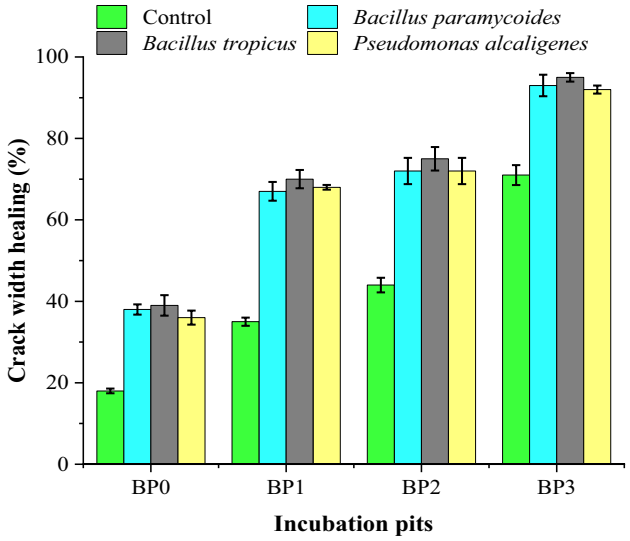


**Fig. 10** Crack healing percentage after 120 days of incubation

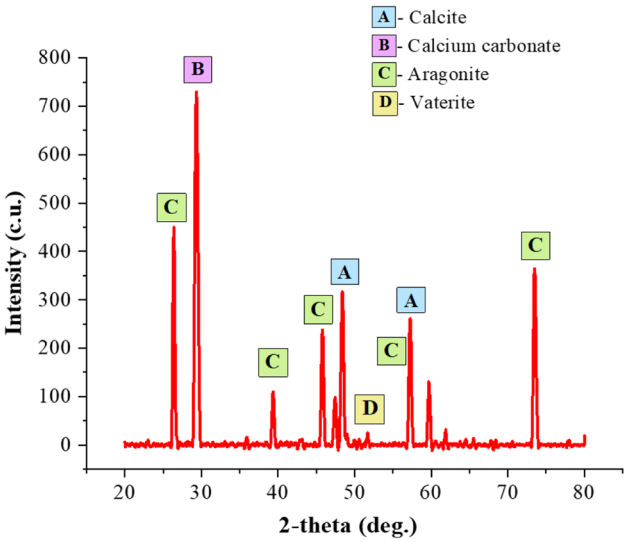


**Fig. 11** XRD analysis for white precipitate

**Table 5** Details of minerals analyzed for white precipitate in healed cube specimens

| Reference code | Score value | Mineral |
|---|---|---|
| PAN 98–001-2744 | 56 | Calcite |
| PAN 00–005-0586 | 48 | Calcium carbonate |
| PAN 98–011-0543 | 23 | Aragonite |
| PAN 98–010-9796 | 13 | Vaterite |

results indicated that strain 2 was identified as *Bacillus paramycoides*, strain 3 was identified as *Bacillus tropicus*, and strain 5 was identified as *Pseudomonas alcaligenes*. Further,

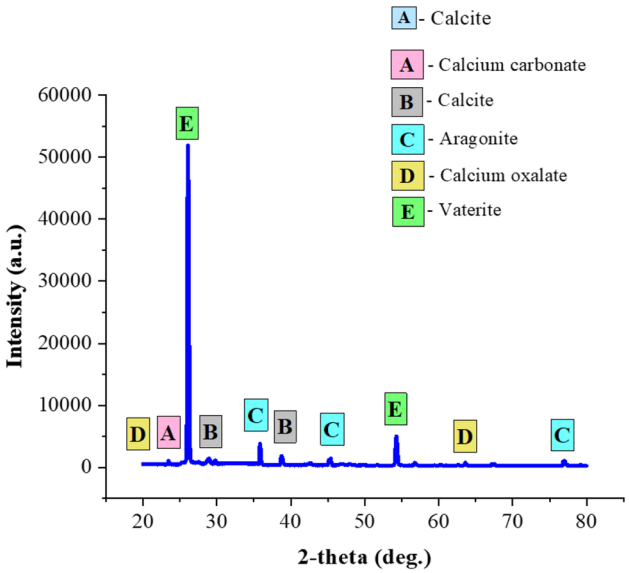


**Fig. 12** XRD analysis for soil added with bacteria and nutrient

**Table 6** Details of minerals analyzed for soil (bacteria and nutrient added)

| Reference code | Score value | Mineral |
|---|---|---|
| PAN 98–011-1012 | 38 | Calcium carbonate |
| PAN 98–002-1913 | 27 | Calcite |
| PAN 98–011-4646 | 21 | Aragonite |
| PAN 98–011-9941 | 17 | Calcium oxalate |
| PAN 98–010-9796 | 15 | Vaterite |

the sequences were deposited in GenBank (NCBI) and accession numbers ON860661, ON849099, and OP592221.

## 3.5 Effect of pH in bacterial growth

Effect of pH on the growth of isolated bacterial strain was further evaluated; since the soil sample was collected in different locations, they had different pH values (2 to 10). Hence, a nutrient medium was prepared and adjusted with different pH values from 2 to 10. All nutrient media with varying pH values were autoclaved for 121 °C; after cooling to attain room temperature, single bacterial colony from each bacterium was inoculated and kept in a shaker. Preliminary studies were carried on isolated bacteria to determine the maximum absorbance wavelength. For a wavelength range of 550–700, a maximum absorbance was observed for 600 nm for inoculum volume of 0.5 to 2%, and maximum cell density was found to be 1.5%. The above findings were well supported by Kiranmaye et al. [17]. Later optical density at 600 nm was used to construct growth curve for three bacterial species (1.5% inoculum). The obtained results are

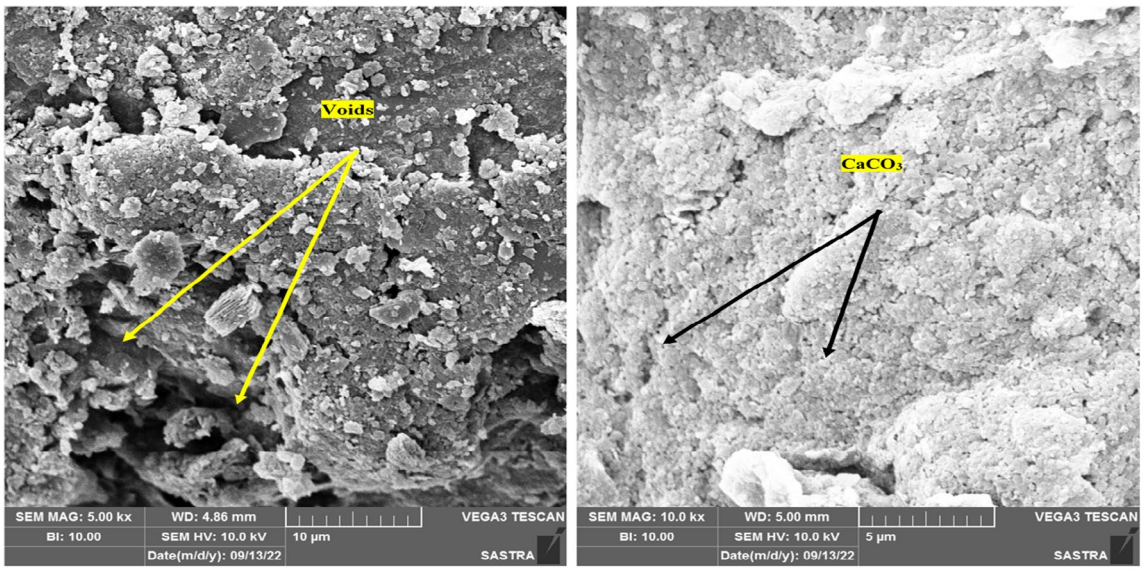


**Fig. 13** SEM images of different soil samples

presented in Fig. 9. Usually, when gram-positive bacteria are exposed to an acidic environment, they go through a number of protective mechanisms to counteract the damaging effects on their cell walls, such as pumping protons, producing ammonia, and changing the amount of lipid in the cell membrane [47]. The transport of protons to the cytoplasm in hydrophobic unionized form caused by the acidic environment lowers their intracellular pH [48]. Due to its increased diameter, the porins in the outer membrane of bacterial cell walls are unable to stop protons from moving [49]. Although the inner membrane of the cytoplasm appears to represent a primary barrier, non-ionized molecules enter and cause dysregulation of the periplasm [50]. Bacterial growth is finally slowed when their internal pH reaches their exterior pH [51]. Additionally, due to environment's rapid transition to an alkaline state and the resulting stress on bacteria, their viability and capacity for growth are both reduced [52]. The temperature in soil exposure environment, to which bacteria were added, was $18 \pm 2$ °C. To determine the exact growth potential of bacteria, all pH conditions were maintained at same temperature. Decrease in temperature slowed the splitting efficiency of bacteria as also bacterial growth [31] as seen in Fig. 9. In all acidic and basic environmental conditions, *B. tropicus* showed the highest growth rate compared to other isolates *B. paramycoides* and *P. alcaligenes*. Hence, it was apparent that the

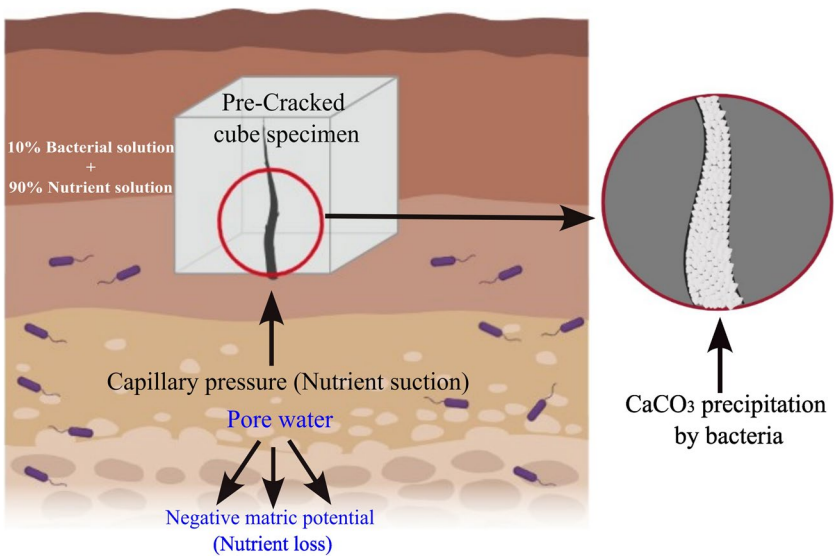


**Fig. 14** Supply of nutrients to bacteria through capillary pressure in crack healing mechanism

pH and growth temperature conditions played an important role in growth characteristics of bacteria.

### 3.6 Crack healing quantification

After pre-crack on the 7$^{th}$ day, one face on each cube was chosen for visual examination and the crack width is observed. Subsequently, the crack width of each cubes was noted after 120 days of soil incubation as shown in Table 3. Using a microscope (ZEISS SteREO Discovery. V12), the crack healing quantification was measured. It was inferred that bacterial concentration of $10^7$ cells/ml reacted better in calcite precipitation by consuming the nutrient source, calcium source, and nitrogen source from the yeast extract, calcium lactate, and urea while in other combinations exhibited lesser performance due to inadequate nutrient source and higher volume of cell concentrations. The area of crack width reduction was computed using the ImageJ software as shown in Table 4.

The results showed that the addition of healing solution (90% nutrient solution and 10% bacterial solution) during incubation on pit BP3 produced an average of about 88.05% healing rate after 120 days. BP0, BP1, and BP2 had showed an ascending order of healing percentages, 34.61%, 60%, and 66% respectively as can be seen in Fig. 10. In a study elsewhere, Esaker et al. [18] found that, at pH 4.5, crack healing efficiency of the bacteria-doped specimens under soil incubation was observed to be 43% lesser when compared to the neutral soil of pH 7. However, in the present study, report revealed that if the incubation soil was supplemented with appropriate nutrients with the use of *Bacillus tropicus* bacteria, maximum healing can be achieved up to 88.05%.

### 3.7 Microstructure study

#### 3.7.1 X-ray diffraction and SEM analysis

X-ray diffraction analysis of $CaCO_3$ precipitates obtained from the specimen's pit, BP3, showed a major elemental composition having descending score values of calcite, calcium carbonate, aragonite, and vaterite. The spectrum acquired is presented in Fig. 11. The obtained XRD peak elements were similar to previous studies [5–7, 37]. From the database available in the software, X'pert high score plus, the compounds were identified with their respective reference code as shown in Table 5. According to the results, the elemental composition of soil material–incorporated bacteria and healing solutions were found to be calcium carbonate, calcite, aragonite, calcium oxalate, and vaterite as shown in Fig. 12 with the database compounds listed in Table 6. The score value results obtained from the above two cases were quite different due to the process of chemical equilibrium which emitted $CO_2$ in void space during respiration process [47]. Hexagonal and spherical crystal shapes were obtained in the precipitates formed due to presence of nutrient solution variations. This crystallinity and crystal size decreased with an increase in the concentration of nutrient sources like calcium, urea, and yeast extract [47].

SEM images revealed the presence of voids in the soil samples without bacteria and nutrient addition; however, the samples with bacteria and nutrient addition showed void reduction due to the formation of calcium carbonate crystals as can be seen in Fig. 13a and b. The identified polymorphs of calcium carbonate compounds were similar to compounds identified from [18–21, 47]. These crystals remained unchanged even after 120 days which resembles the concentration effect of urea source for crystal formation underneath the soil.

Nutrient loss was found in the bacteria-added specimen due to larger crack widths through negative matric potential [19]. If soil was not added to the bacteria and nutrient solution, the cracks was unhealed. If the surrounding soil was supplemented with bacteria and nutrient solution, the nutrients required for the bacteria were absorbed through capillary pressure as described in Fig. 14.

## 4 Conclusion

The present study was aimed to investigate on bacterial precipitation of calcium carbonate for effective exploitation to heal in concrete cracks. The results showed that the increasing percentage of nutrient solution and bacterial solution in the soil surrounding the concrete specimens can improve the percentage of healing cracks. Bacteria performance on concrete crack healing did not depend on locations where they were isolated. Calcium precipitating bacteria could perform in any substructure location which should be provided with appropriate nutrient source. *Bacillus paramycoides*, *Bacillus tropicus*, and *Pseudomonas alcaligenes* had showed efficient performance on crack healing in soil exposures. These bacteria can induce calcium carbonate precipitation in soil environment in line with cracks in concrete. Compared to *Bacillus paramycoides* and *Pseudomonas alcaligenes*, *Bacillus tropicus* had explicated better crack healings under soil exposure conditions. Loss of nutrients through larger crack widths in cube specimen was compensated by nutrient supply in nearer areas through capillary pressure principle. Natural water content available in soil was as suitable environment to bacteria in crack closure activities. Organic content in existing soil did not affect the performance of bacteria during crack closure behaviors. Hence, it was hypothesized that these bacteria could be added in substructures and its surrounding soil through proper injection techniques. The percentage of healing ratio was found to be maximum for the 90% nutrient solution and 10% bacterial solution. From this result, it was clear that a lesser volume of bacterial solution

could heal a higher percentage of cracks with a higher volume of nutrient solution. The microstructure study resembled the formation of calcium carbonate mineral compounds in both concrete and soil. It was evident that this precipitate reflected in increasing durability in concrete structures. The determination of survival potentiality of bacteria (in added environment) for long periods has gained mere attention among earlier researchers. Crack healing percentages for small specimens were explored in this study and hence this study must be extended for large specimens in ground exposure conditions. In addition, future study can be focused on the above considerations along with techno-economics or the feasibility for large-scale implementations.

**Author contribution** Anbazhagan Rajesh: methodology, investigation, writing—original draft. Arunachalam Sumathi: conceptualization, supervision, writing—review and editing. Dharmalingam Gowdhaman: conceptualization, supervision. Sundramurthy Venkatesa Prabhu: methodology, writing—review and editing, validation.

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**Data availability** Not applicable.

## Declarations

**Ethical approval** Not applicable.

**Conflict of interest** The authors declare no competing interests.

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