Urease activity and deposition of calcium carbonate layers on a 16th century Mughal monument

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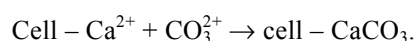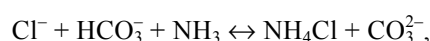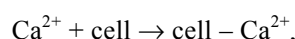National Museum Institute, Department of Conservation, Janpath, New Delhi 110 011, India

In the present study, bacterial populations capable of re-precipitating calcium carbonate were isolated from the exterior oozed-out calcite deposition of Salabat Khan Tomb, a 16th-century Mughal monument in western India. The deposits were analysed for identification of the materials that led to microbial induced calcite precipitation (MICP) on the surface of the monument. Quantification of MICP by XRD and visualization by SEM showed direct involvement of these isolates in the precipitation of calcium carbonate. FTIR spectra showed clear peaks for calcite along with a peak of methane probably due to dead bacterial cells. Preliminary examination, isolation and partial sequencing of 16S rRNA gene showed that the major microbial population participating in calcite precipitation is heterotrophic and includes mainly *Bacillus* sp., *Arthobacter* sp., *Agromyces indicus* and *Aquamicrobium* sp. In future, the process may be applied for the conservation of many limestone/marble monuments using MICP in India and elsewhere.

Keywords: Ancient monuments, bacterial populations, calcium carbonate, microbial-induced calcite precipitation.

ONE of the most relevant examples of mineralization that can be traced back to the Precambrian period is the precipitation of calcium carbonate. Both abiotic changes (physico-chemical factors) and biotic changes (microbial action) participate in the natural precipitation of calcium carbonate. The autotrophic and heterotrophic bacteria, including sulphur, photosynthetic and nitrogen-fixing bacteria which hydrolyses urea and cellulose participate in calcium precipitation. Under favourable conditions, most of these bacteria can produce calcite[1]. The mechanisms by which bacteria precipitate carbonates are still unclear. The precipitation of calcium carbonate can occur through several different pathways[2,3]. Calcium ion concentration, pH and the presence of nucleation sites control the chemical precipitation of calcium carbonate[4]. In microbial calcium carbonate precipitation, bacteria themselves behave as nucleation sites in which calcium ions bind to microbial cell surfaces[5]. When these surfaces become saturated with calcium ions, the ions begin to aggregate, thus initiating the first urease crystalline nuclei which then grow from these structures[6].

From the role of Ca^{2+} on urease activity, a ten-fold increase is observed in the presence of 30 μm Ca^{2+} than in the absence of Ca^{2+} for certain isolates[4]. The precipitation rate of $CaCO_3$ obeys the first or second order of kinetics which is, in general, a linear function of Ca^{2+} and CO^{3-} concentration products, where one of the reactants is in excess. Supersaturation of the solution is reached when the concentration of Ca^{2+} and CO_3^{2-} exceeds the solubility product leading to precipitation of calcium carbonate. Due to the enzymatic urea hydrolysis, it is possible that individual microorganisms produce ammonia to create an alkaline microenvironment around the cell. The high pH of the localized area does not result in an initial increase in pH of the entire medium, but brings about growth of $CaCO_3$ crystals around the cell. The possible biochemical reaction in urea–$CaCl_2$ medium can be summarized as

$$Ca^{2+} + cell \rightarrow cell - Ca^{2+},$$

$$Cl^- + HCO_3^- + NH_3 \leftrightarrow NH_4Cl + CO_3^{2-},$$

$$Cell - Ca^{2+} + CO_3^{2+} \rightarrow cell - CaCO_3.$$

An alkaline pH is the primary source through which the microbes promote precipitation of calcium carbonate[2,7]. Different effects of minerals on urease activity have been proposed[8] with some of them showing contradiction in results. With the addition of Ni^{2+} in the growth medium of *Escherichia coli*, an increase in the precipitation of calcium carbonate has been observed[9]. It is reported that $CaCO_3$ precipitation increases considerably by the addition of Ni^{2+} ions (5–100 μm). The role of Ni^{2+} on urease activity was also confirmed by some other researchers[10]. Previous studies have also demonstrated the effect of heterotrophic bacteria actinomycetes, on the formation of specific speleothems (mineral growths in caves, e.g. stalagmites)[11–16]. Bacteria precipitate extracellular calcium carbonate through a variety of processes[17–19].

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Figure 1. Map showing the location of the Salabat Khan Tomb, Ahmednagar, India.

The first mineralization occurs as a by-product of microbial metabolism that involves either autotrophic pathways which can deplete CO_2 locally or heterotrophic pathways which release it into the environment[2,20]. In these mechanisms, enzymatic hydrolysis of urea and the ammonification of amino acids can increase the pH which, if free Ca^{2+} is present, results in the precipitation of calcium carbonate. In the second mineralization due to ion exchange, carbonate nucleation takes place on the bacterial cell wall through the cell membrane by poorly understood mechanisms[20]. Mineralization may also occur through the adsorption of divalent cations into specific negatively charged functional groups on the cell wall[6]. Nevertheless, the cell acts as a nucleation site once carbonate has begun to precipitate on the cell surface. This stage is demanding in initiating mineral precipitation; consequently $CaCO_3$ precipitation may be purely inorganic.

The third possibility involves unique proteins present in the extracellular polymeric substances (EPS) produced by bacterial communities that can trap sediments and are often essential for direct deposition of microbial carbonate[21,22]. Formation of bacterial calcium carbonate through urea hydrolysis is known as bacterial calcite precipitation (BCP). It is pollution-free and a natural phenomenon. The major application of BCP is in the removal of contaminants from wastewater, protection and restoration of limestone monuments, formation of sacrificial patina on limestone and production of biological mortar, plugging of pores of oil reservoir, etc. Carbonate stones (for example, limestone and marble) are particular-

ly susceptible to weathering because of their composition and textural characteristics, leading to increased porosity and decrease in mechanical strength and cohesion[23]. For monument conservation, the modern trend is to preserve the original stone structure without any chemical coating and minimal human activity. In all cases of stone weathering, water transfer within porous stone is a common problem. With the aim to modify some of the stone surface characteristics, many conservation treatments have been applied in order to decrease susceptibility to decay. Mainly, water repellants are used on the stone surface to prevent the entry of water and other weathering agents. Indeed, water-repellant resins polymerize on porous stone resulting in film formation on stone surfaces. In some cases the resin polymerizes inside the pores, thus completely filling them up[24]. As preventing water intrusion into porous stone is crucial, it is also significant to maintain gaseous exchange between the stone and the environment[25]. Hence, human action should be restrained to limiting water penetration while allowing bidirectional gaseous transfer.

To meet these criteria, a bio-mineralization treatment as an alternative process was developed on the basis of the ability of bacteria to induce calcium carbonate precipitation[26]. The process to develop biological mortars and patina on limestone is now successful[3]. This method involves spraying bacteria on the entire surface of limestone and subsequently providing nutritional medium containing calcium and urea. By this process, a protective surface coating of $CaCO_3$ (several microns thickness) is formed. For the first time bio-coating was successfully
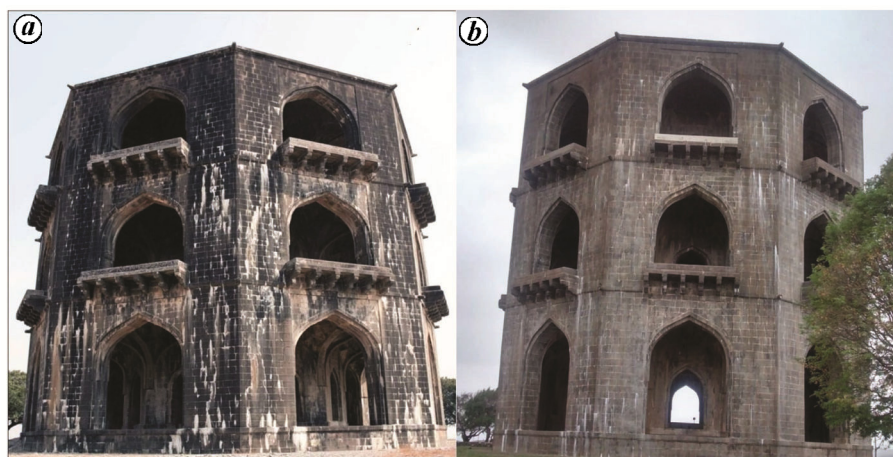
Figure 2. *a*, General view of the Salabat Khan Tomb. *b*, The tomb after removal of calcite concretions.
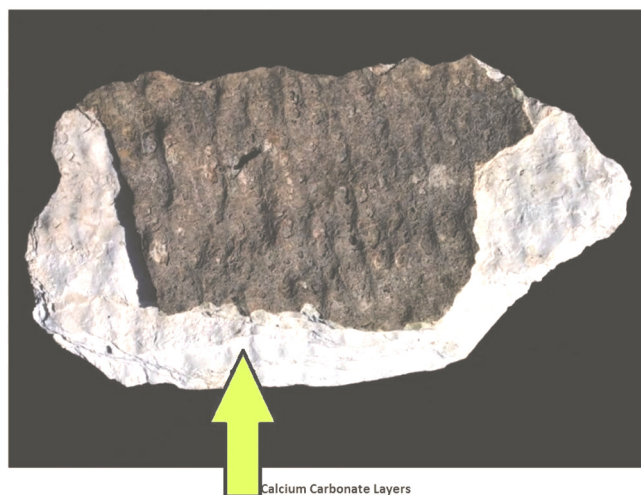

Figure 3. A large calcite concretion showing the stratified layers of deposition.

experimented on a surface area of 50 sq. m on the south-east tower of the Thouars Church, France[27]. Since then, this treatment is being applied on various buildings and houses in Paris and other parts of France. In India, many such crack remediation programmes have been success-fully completed through the bacterial process to reinforce strength and durability of concrete[28,29]. The technique has also been successfully used in the field of geotechnical engineering.

Although a geotechnical revolution is being witnessed with the help of bio-cementation, the process is not suitable in case of the 16th-century Mughal monument in the Deccan plateau of western India, viz. Salabat Khan Tomb, located about 125 km from the historic city of Aurangabad, Maharashtra, and lying between 182 and 199 N lat, 739 and 755 E long, situated partly in the Bhima basin and partly in the upper Godavari basin, the inter-fluves in between forming the extensive Ahmednagar pla-teau (Figure 1). Figure 2 *a* shows the general view of the

tomb. The Salabat Khan Tomb is situated at a hilltop on an average elevation of 900 m amsl. Constructed with black basalt, the foundation of the tomb has been laid on a circular path, but the monument is octagonal in shape with the height of tomb being 80 ft. High-speed wind lashes the monument during all seasons and the tomb is surrounded by windmills on the nearby hilltops all around.

The major conservation problem of this monument is the leaching of binding materials from within the constructed wall during rainy season when rainwater seeps through the roof of the tomb. These leached-out lime-based materials oozes out through the basalt stone joints and flow down to get deposited at the immediate surrounding stone surfaces all around the monument. However, the upper part of the monument shows scarce deposits of leached-out materials probably due to flow of water down with force. The deposited materials can be clearly marked on the outer stone surface of the monu-ment (Figure 2 *a*). A close inspection provides a clear view of various layers deposited during alternate rainy seasons (Figure 3). Here, bacterial activity results in hardening of deposits and cementing of the layers to make a hard mass, thus masking the aesthetic presenta-tion of the monument. At many points, the exterior sur-face of the deposits is contaminated due to deposition of dust, dirt and other accretionary materials from the surroundings. On account of hardening of calcite, physio-chemical methods have to be employed for the effective removal of deposits from the stone surface.

In the present study, calcite deposits were analysed for their chemical composition, petrological behaviour, and mechanical characteristics. Further by culturing microor-ganisms under controlled laboratory conditions, it is possible to define their ability to alter the chemistry of their microenvironment and produce biominerals. We used laboratory-based cultures to examine the extent to which heterotrophic calcifying bacteria are effective in

Table 1. Chemical composition (% weight) of the Interior lime and calcite deposits

Oxides	Sample 1 (interior lime core)	Sample 2 (calcite deposit)	Sample 3 (calcite deposit)	Sample 4 (calcite deposit)	Sample 5 (calcite deposit)
Na_2O	0.00	2.85	3.90	3.5	3.1
K_2O	0.00	1.02	0.57	0.89	0.74
MgO	8.65	0.76	1.22	1.04	0.5
CaO	75.83	87.28	88.07	87.87	88.16
Al_2O_3	8.73	0.00	0.65	0.35	0.14
SiO_2	2.46	0.89	0.55	0.72	0.65
Fe_2O_3	2.90	0.77	0.52	0.65	0.42
MnO	0.00	0.57	0.33	0.54	0.36
ZnO	0.00	0.65	0.30	0.74	0.62
SeO_3	0.00	1.41	0.00	1.30	0.85
CuO	1.43	1.10	0.92	0.82	1.23
P_2O_5	0.00	2.71	2.97	2.48	2.19

Table 2. Minerals present in the sample

Sample no.	Major mineral	Minor minerals
1	Calcite	Quartz, biotite
2	Calcite	Quartz, biotite
3	Calcite	Quartz, biotite
4	Calcite	Quartz, biotite

calcite deposition. Instrumental techniques such as FTIR, XRD and SEM were used for characterization of calcite. The present study outlines the role of bacteria in hardening the calcite deposits of Salabat Khan Tomb, which is aesthetically unsuitable for this monument. However, the technique can be applied successfully in the conservation of many limestone/marble monuments.

Materials and methods

Chemical characterization

As the monument is sound and structurally compact, it is not possible to obtain samples of the inner lime core for analysis without causing damage to the structure. Therefore, from a small aperture present on a damaged hole on the first floor, few milligrams of the sample was collected and subjected to chemical and petrological analysis. We could only collect one lime core sample for which analytical study was carried. Four samples from exterior calcite deposits collected from all sides of the monument were subjected to chemical and petrological analysis. Besides, four samples of exterior calcite deposits were also removed mechanically from all the sides and immediately packed in sterilized air container for carrying out detailed studies using FTIR, SEM, mechanical properties and microbial analysis in the laboratory.

X-ray fluorescence (Philips 1410, holland) set-up was used to determine the chemical composition of the samples. Sample 1 in this analysis represents the lime core

and samples 2–5, the deposited and hardened calcite patches on the exterior surfaces of the basaltic stone monument. Table 1 shows the results of the chemical analysis.

Petrological analysis

Samples 1–4 were also observed under a petrological microscope for their mineral content (Table 2). Calcite was the major mineral present in the sample while very small quantities of quartz and biotite were also seen. For petrological analysis, condense polarized light microscope (Carl Zeiss JENA POL) was used. The sample was also analysed by heating a known weight of the sample at 105°C, 400°C and 900°C to determine the water vapour, organic matter and carbon dioxide content.

Collection, isolation, crystallization and initial urea analysis using 16S rRNA gene sequencing

Sample collection: The samples for microbial analysis were collected from the monument using sterile swabs. The exterior flora was wiped by swabs grown in the laboratory. Air flora was collected by exposing a sterile medium plate in air.

The internal flora of calcium deposits was isolated using a sterile needle. The needle was inserted in the calcium deposits and then inoculated in the sterile broth medium.

The large fragments of calcium sample were broken in a laminar air-flow hood. The outer covering of the solution which was contaminated due to deposition of dust and dirt from the environment was carefully removed and inner part of the sample was suspended in soybean casein digest medium. Then the medium was incubated at room temperature for five days.

The typical colonies were maintained on soybean casein digest agar slants. These selected colonies were then

inoculated in soybean casein digest medium with calcium acetate. The flask was incubated at room temperature for five days and then observed for precipitation. Figure 4 shows turbidity in the flask after the aforesaid process.

After incubation, serial dilutions were made from 10^{-1} to 10^{-10} using 0.9% saline, and 0.1 ml of each dilution plated on precipitation medium containing 3 g nutrient broth, 20 g urea, 2.12 g $NaHCO_3$, 10 g NH_4Cl and 30 mM $CaCl_2 \cdot H_2O$. The plates were incubated at room temperature for five days. After incubation, the total viable count was also determined. The specific colonies were selected for microbial identification of bacterial isolates based on 16S rRNA gene sequencing. The typical colonies were maintained on the above-mentioned medium.

Isolation: The isolated strains were introduced into sterile conical flask containing 100 ml of nutrient broth followed by incubation at 37°C overnight and subjected to plating on nutrient agar plates with appropriate dilution. The colonies were assessed once in every 5 days with the help of a stereomicroscope and positive colonies were chosen based on the formation of visual crystals within 10 days. Positive colonies isolated was purified by repetitive dilution and plating (Figure 5 *a* and *b*). Four isolates, viz. S1–S4 were chosen and the above cultures were preserved on Luria agar slants till further use.

PCR amplification of 16S rRNA gene: A DNA template for PCR amplification from pure cultures was obtained by extracting total genomic DNA using Axy Prep Bacterial Genomic DNA Miniprep Kit, following the manual instructions, and its purity and quantification were accomplished by Nanodrop spectrophotometer (Thermo, USA). The PCR master mixture contained each primer at a concentration of 500 nM, each deoxynucleotide

triphosphate at a concentration of 200 µM, 15 mM $MgCl_2$, 10 µl of thermophilic DNA polymerase (sigma), 400 ng/µl of bovine serum albumin (Boehringer) and addition of DNAase and RNAase free sterilized water making a final volume of 100 µl. Next, 1 µl DNA template was added to 24 µl of master mixture. The 16S rRNA gene with universal 16S rRNA PCR forward primer (5′-AGAGTTTGATCTGGCTCAG-3′) and reverse primer (5′-AAGGAGGTGTCCACC-3′) was used for amplification. PCR reaction was performed in the thermal cycler (Bio-rad) using the following conditions: initial denaturation of 5 min at 94°C, followed by 35 cycles consisting of 30 s at 94°C (denaturation), 20 s at 58°C (annealing) and 1 min 30 s at 72°C (extension) and final extension of 15 min at 72°C (Table 3).

DNA sequencing and sequence analysis: The PCR products were analysed by 1.2% agarose gel electrophoresis; amplified products in the gel were cut by clean scalpel, purified using Quigen quick PCR purification kit and DNA was sequenced (Xcleris, India). BLAST
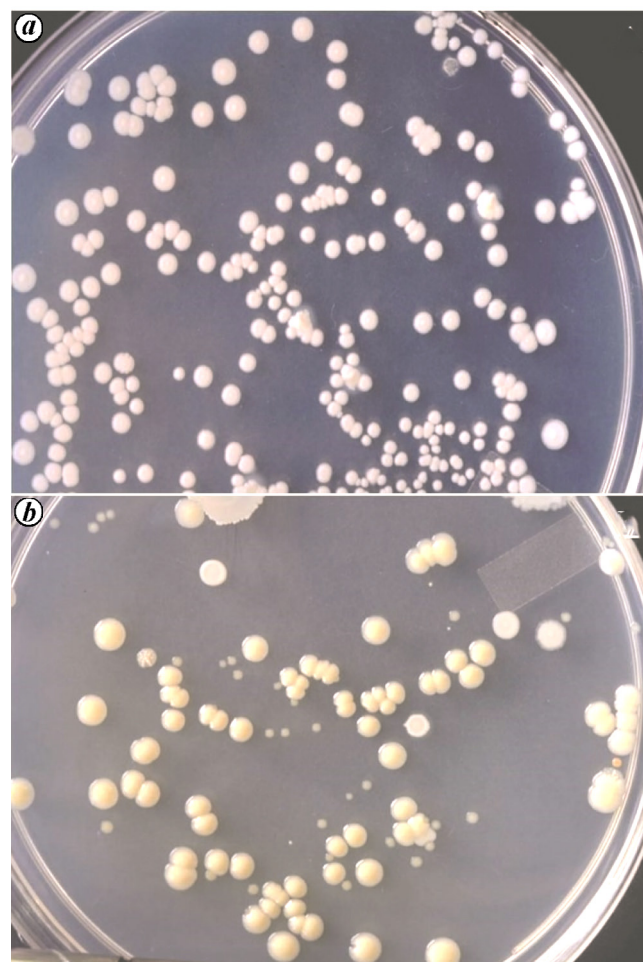
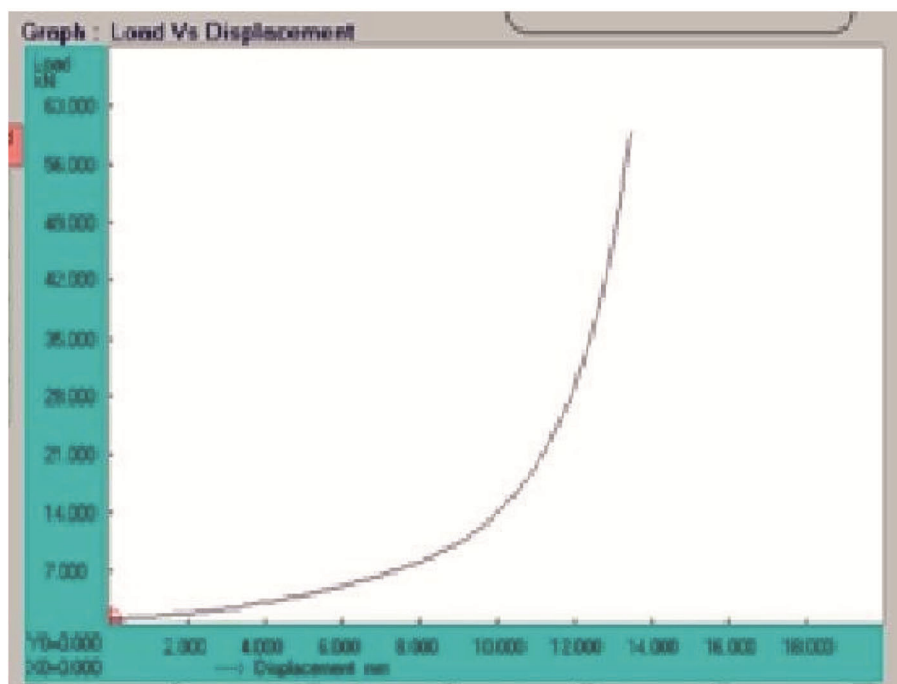**Figure 5 *a* and *b*.** Bacterial colonies from calcite samples of the monument.

Figure 4. Sample of calcite incubated in nutrient broth.

Figure 6. Strength gauge measurement of calcite sample.

Table 3. Identification of bacterial isolates

Isolates	Bacteria
S1	*Bacillus* sp.
S2	*Aquamicrobium* sp.
S3	*Agromyces indicus*
S4	*Arthobacter* sp.

algorithm was used in the analysis of DNA sequences and homology.

Mechanical properties

Mechanical properties of the leached and hardened calcite were measured with a universal testing machine (UTE-100). For determination of comprehensive strength, two hardened calcite samples were selected. For the first test a calcite sample of 78,000 mm^2 cross-section area was selected to determine comprehensive strength (Figure 6).

XRD measurement

X-ray diffraction analysis is commonly used to determine the reticular plane and crystalline structure of samples. The XRD spectrum recorded was in the 2θ region from 5° to 120° with peaks characteristics of CaCO$_3$ which can be attributed to JCPDS file 040637 (Figure 7). The crystal system was hexagonal, rhombo-centred with space group of R3C9 (167) and cell parameters of a = 5.35 and c = 16.80. XRD spectrum was recorded using a Phillips 2404, Holland X-ray diffraction machine with a graphite monochromator and CuKα radiation.

SEM and FTIR analysis

Morphology and size of the crystals and microorganism were analysed by SEM at different magnifications. The SEM photomicrograph of the bulk plaster (Figure 8) was obtained with the help of SEM (FEI ESEM Quant 200, USA) at a range of magnifications to identify the components of deposits. FTIR spectra analysis of calcite deposits was also carried out for their characterization. For this, a small quantity of calcite deposit was mixed with KBr; the pellet was freshly prepared and used. The FTIR spectra were obtained in the regime 4000–550 cm^{-1} using Agilent 600 series FTIR spectroscopy equipped with nitrogen-cooled MCT detector. The spectra were obtained from different areas of the sample (Figure 9).

Results

Table 1 lists the major chemical oxides identified in the samples. For this study, three analyses were carried for each of the samples and average of the data was taken for consideration. From the chemical analysis data of the original lime core (sample 1), it was observed that the mortar used in the construction of the Salabat Khan Tomb
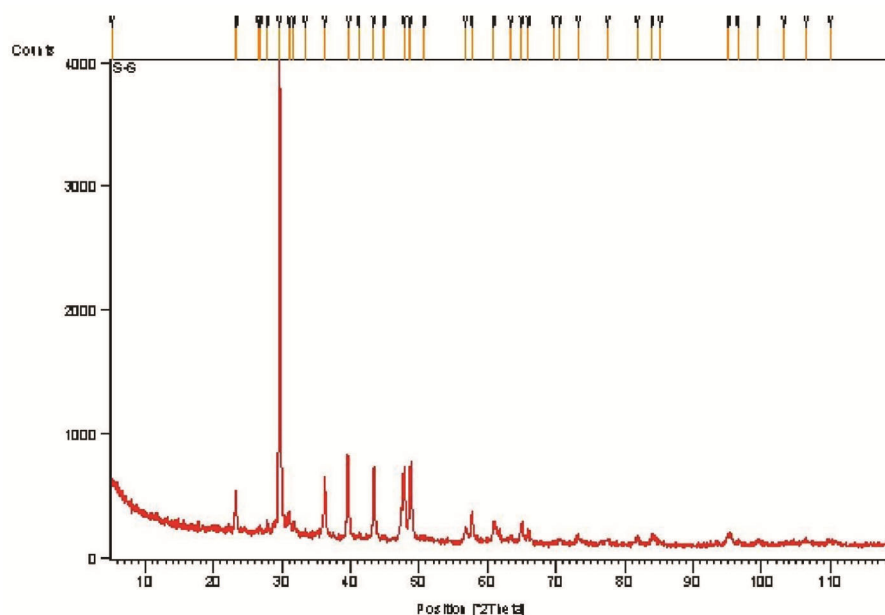
Figure 7. X-ray diffraction spectrum of calcite sample.

was a dolomitic lime (MgO 8.65%) and that the mortar was of poor quality. Besides, addition of aggregate in lime to provide strength and durability to the mortar was just negligible (SiO_2 – 2.4%). From the chemical composition data of sample 1, it can be deduced that almost pure dolomitic lime with its impurities has been used in the construction of the Salabat Khan Tomb. The addition of less aggregate in the mortar works has also been confirmed by SEM images of sample 1. Phenolphthalein gives colour when mixed in the lime solution, indicating that some of the limes still not carbonate and are in the form of hydroxides. As the lime mortar has not sufficiently carbonated, there is a probability of its leaching when rainwater seeps through the roof of the structure, as in the case of this monument. During rainy season, dissolved lime oozes out either on the fresh stone surface or on the previously deposited leached-out calcite surface, which can be well observed on the monument surface. It was also observed that magnesium salts being more soluble preferably leached out from the parent mortar. However, magnesium and calcium displacement reactions come into play here resulting a decrease in magnesium content in the leached-out materials and simultaneously proportionate increase in the calcium ions in the dried deposits. Such types of reaction are common in soil science/ groundwater survey. In the deposited material there was increase of phosphate due to bacterial dead cells. It must be noted that increased Ca^{2+} ions and phosphate cause higher precipitation of calcite due to microbial activity. Traces of silica, iron, zinc and copper observed in the sample are due to exterior contamination of dirt and dust from air.

Table 2 shows results of petrological analysis of the samples. It can be observed that the deposits mainly consist of calcite with minor minerals of quartz and biotite. The four isolated potential organisms were identified by 16S rRNA gene sequencing. The 16S rRNA region was amplified as a single amplicon with 16S primer set. The homology of the 16S rRNA sequence was matched to the NCBI database. Depending on the percentage of matches and total mismatches, the strains were identified (Table 3).

The load versus displacement curve (Figure 6) indicates that the calcite particles compress in a relatively linear manner with increase in load at the initial stage and then inflection points appear in the region 2–8 mm, where significant changes occur in displacement indicating that the particles can no longer withstand the load and suddenly deform by a large amount. With an increase in load up to 60.150 kN, the material becomes brittle at a maximum displacement of 13.5 mm. Calcite has a comprehensive strength of 0.001 kN/mm^2. Figure 6 shows comprehensive strength graphs for calcite. In the second measurement, 24,012 mm^2 cross-sectional area was considered for comprehensive strength determination. For this analysis, maximum force of 17.850 kN was applied. The sample showed a comprehensive strength of 0.001 kN/mm^2 at this instant as well. This indicates that comprehensive strength throughout the length and width of the leached-out calcite is the same, irrespective of the area considered for analysis. A continuous rise in the graph can be seen even after failure of the material, as calcium has a natural tendency to agglomerate on fracturing under compression.

The XRD results showed that the bacteria mainly precipitated calcite as maximum number of calcite peaks were observed (Figure 7). XRD analysis also showed that the carbonate formation from which the microorganism
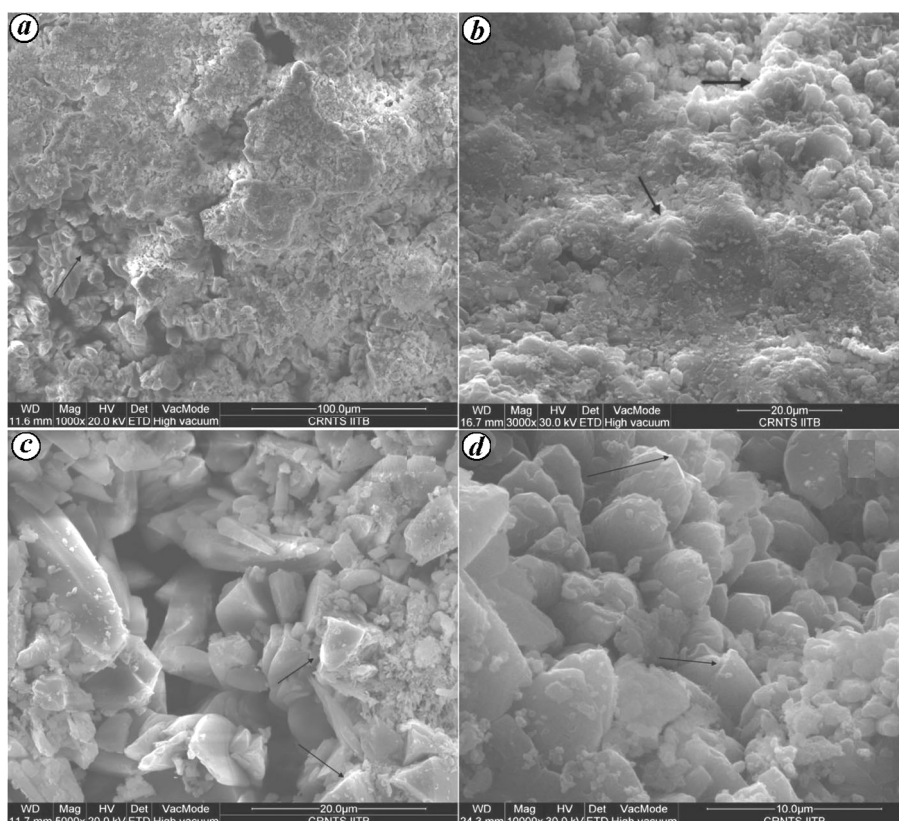
Figure 8 *a–d*. SEM images of calcite sample at 1000×, 1000×, 5000× and 10000× magnification.

was isolated was from pure calcite. Bacteria served as nucleation sites during mineralization process. This was confirmed by the presence of crystalline calcium carbonate associated with bacterial colonization.

SEM photomicrographs of calcite deposit are shown in Figure 8 *a–d* for different magnifications. From the SEM image of sample 1 (Figure 8 *a*), it is observed that the binding mortar used is almost pure lime with little aggregate. The photomicrograph also shows tangled calcite needles giving a rather rough surface. The pore size of the mortar sample is uniform but at some points, few fine cracks have developed. It is observed from SEM images of samples 2 and 3 (Figure 8 *b–d*) at different magnifications that after bio-deposition, the surface has strongly modified and formed a less rough surface producing smooth calcite layers. SEM analysis of microbial samples shows distinct rhombohedral-shaped calcite crystals with rounded bacterial spores. Ovoid bacterial bodies (2 µm long and 0.5 µm wide) due to precipitation of bacterial membrane (especially carbonate mineralization) have created a relief on the smooth surface. Moreover, crystal growth is observed after bio-deposition along with numerous cracks. The presence of calcite in the form of calcium carbonate can be confirmed with high amount of calcium in all the bacterial samples. While examining the calcite crystals using SEM, it was observed that virtually all the crystals included pits and depressions that matched

the structure of precipitating bacteria both in terms of size and morphology. These cracks are common features of biofilms observed by SEM. In case of FTIR spectra (Figure 9), generally, the 1409 and the 872 cm^{-1} peaks are due to –OH bending vibration and –OH out-of-plane bending vibration respectively. In the white deposit analysed, carbonate peaks were observed at 1400 and 872 cm^{-1}. In the FTIR spectra, small peak was also observed at 711 cm^{-1}, which can be attributed to the methylene (–CH$_2$–) rocking vibration. If some microorganisms are present, methylene gas may evolve, and the small peak denotes the presence of microorganism in calcite.

Discussion

Bio-cementation or bio-grout as an *in situ* calcium carbonate precipitation is preferable to chemical cemented materials in reducing porosity, permeability of the material, and resistance to weathering. Conservation material cemented chemically is subjected to weathering that leads to increase in porosity, thus altering its mechanical behaviour. In geotechnical engineering, chemical cementation or chemical grout is a process through which voids in sandstone are mostly filled. In the presence of excess calcium ions, in bio-cementation process, carbonates are released by ureolytic bacteria
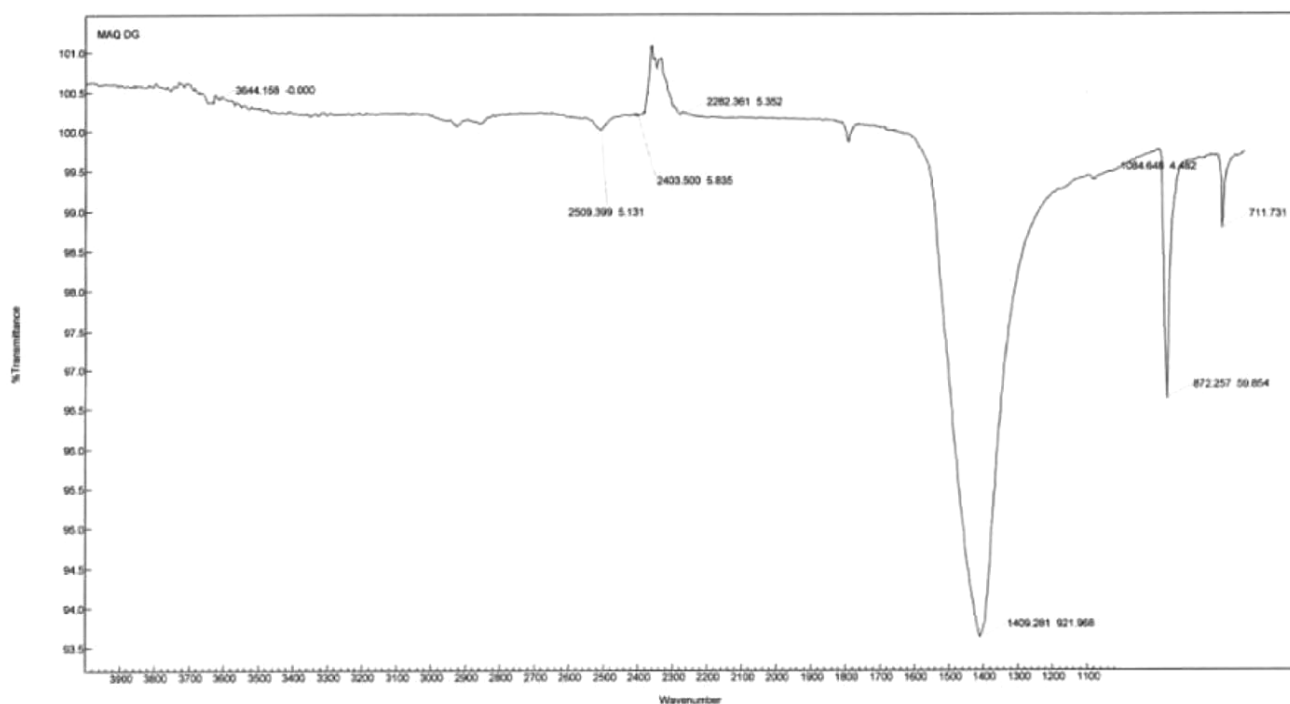
Figure 9. FTIR spectrum of calcite sample.

through urea hydrolysis to form *in situ* calcite. Calcite, aragonite and vaterite are the three crystals formed by nucleation (same chemical formula but different structure). The most stable form of $CaCO_3$ with single rhombohedral shape is considered to be calcite. Under suitable conditions, calcite can be of commercial use and can result in soil solidification. An innovative technique of remediating cracked structural element has now been advanced by engaging selective microbial plugging process in which calcium carbonate precipitation is promoted by microbial metabolic activities. Research is presently being carried out for the development of calcite precipitation using bacteria and to explore its commercial use. From the various instrumental analyses and microbial tests carried out on the calcite deposits of the Salabat Khan Tomb, the role of bacteria in hardening the deposits on the monument surface can be well visualized.

The isolates were well characterized at the genus level by almost complete 16S rRNA gene sequencing. As the monument is lashed by fierce winds with an average wind speed of about 20–25 mph, the deposits also dry quickly. In the summer season when the ambient temperature around the monument is around 42–45°C, water evaporation from the interior of deposits takes place rapidly and this in turn causes its further hardening. This is one of the reasons for less water content in the deposited material during the analysis. This has caused cementing of calcite and further difficulty in its removal from the stone surface of the tomb during cleaning.

Therefore, the naturally occurring process of calcite precipitation on the basaltic stone surface of the Salabat Khan Tomb is not desirable. The removal of whitish patches is necessary for aesthetic presentation of the monument. Recently, structural conservation measures in the form of water tightening on the roof of the monument were carried out to stop ingress of water into the interior walls. The work was carried out using lime aggregate by filling up all the open joints and damaged parts of the old roof of the monument. It is hoped that after this conservation measure water ingress to the monument will not occur. At this stage, it is also essential to remove calcite deposited in patches from the exterior surface. Accordingly, its removal by physico-chemical process was extensively carried out with great care and precaution. Diluted acetic acid solution (3%–5%) was used at first to loosen the calcite deposits. All necessary precautionary measures were taken during the process. It was observed that at some points the repeated deposits had made the surface 4–5 cm thick and at other point the thickness observed was less than 1–2 mm. During the process of loosening the deposits by acetic acid, time of reaction was adjusted according to thickness of the deposits. After this treatment, the loosened calcite was slowly removed with a soft brush and nebulized water. The removed calcite deposits with bacterial cells within can also find many commercial uses, such as purification of wastewater, culturing of bacteria or hardening beneath the building. However, calcite precipitation through microbial process

on the stone surface of limestone/marble monuments can be of immense value in their preservation.

Conclusion

Although bio-cementation is a desirable natural process with immense commercial applications, the same is unsuitable in case of the Salabat Khan Tomb. However, the removed calcite deposits how can be unleashed in the field of geotechnical revolution by bio-cementation and can be used commercially. The process can also be utilized in the protection and conservation of many marble/limestone
monuments.

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