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# *Bhargavaea beijingensis* a promising tool for bio-cementation, soil improvement, and mercury removal

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**Microbially Induced Calcite Precipitation (MICP) has emerged as a promising technique for bio-cementation, soil improvement, and heavy metal remediation. This study explores the potential of *Bhargavaea beijingensis*, a urease-producing bacterium, for these applications. Six ureolytic bacteria were isolated from calcareous bricks mine soil and screened for urease and calcite production. *B. beijingensis* exhibited the highest urease activity and calcite precipitation. Urease activity, calcite precipitation, sand solidification, heavy metal removal efficiency, and compressive strength were evaluated. It showed significant heavy metal removal efficiency, particularly highest for $HgCl_2$. Mortar blocks treated with *B. beijingensis* or its crude enzyme exhibited improved compressive strength, suggesting its potential for bio-cementation. Crack remediation tests demonstrated successful crack healing in mortar blocks using the bacterium or its enzyme. This study identifies *B. beijingensis* as a novel and promising MICP agent with potential applications in bio-cementation, soil improvement, and heavy metal remediation. Hence, *B. beijingensis* diversified abilities prove superior performance compared to commonly used strains like *Bacillus subtilis* and *Shewanella putrefaciens* in bio-cementation applications. Its high urease activity, calcite precipitation, and heavy metal removal abilities make it a valuable candidate for sustainable and eco-friendly solutions in various fields.**

**Keywords** *Bhargavaea beijingensis*, Bio-cementation, Calcite precipitation, Heavy metal removal, MICP, Soil improvement, Urease activity

Microbially Induced Calcite Precipitation (MICP) can be used to solve real-world problems by cementing soil, producing new biomaterials, production of biocement, reducing ammonia emission, improving soil strength, and reducing permeability [1–3]. MICP can also be used to study the pore structure of rocks, which is important for the evaluation and exploitation of oil and gas reservoirs [4]. In order to ensure the safe storage of $CO_2$, MICP technology can also be used to seal leakage paths in geological formations [5]. Moreover, MICP can enhance the static and dynamic characteristics of geomaterials, improving their bearing capacity and resistance to liquefaction of construction materials. In addition, there are various problem in traditional cement production presents significant environmental challenges due to high $CO_2$ emissions, energy consumption, and limited resource dependence. Whereas bio-cement offers a sustainable solution by capturing $CO_2$, utilizing less energy, and incorporating renewable or recycled materials. While bio-cement technology is still under development, ongoing research and advancements hold great promise for the future of environmentally friendly construction. These applications of MICP have practical significance for engineering applications, environmental friendliness, and the study of fluid seepage in porous media. As a result, microorganisms play a significant role in the formation of calcite and have an enough ability to accomplish it through physiological and metabolic processes [6].

Likewise, *Bacillus pasteurii*, also called *Sporosarcina pasteurii*, is frequently utilised for MICP due to its strong ureolytic activity [7]. One of the benefits of utilising *B. pasteurii* for MICP is that it can consistently create calcium carbonate crystals, which may be utilised to enhance the physico-mechanical characteristics of soil and consolidate mineral particles [8,9]. It has been demonstrated that *B. pasteurii* can sustain cell viability and MICP potential even after being stored at low temperatures for an extended amount of time [10]. Furthermore, *B. pasteurii*

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has been shown to be effective in bioconsolidation as evidenced by the rapid precipitation of calcium carbonate on its cell surface [11]. However, there are drawbacks to employing *B. pasteurii* for MICP as well. Natural soils contain native bacteria that may compete with *B. pasteurii* for nutrients, slowing the MICP process. Moreover, *B. pasteurii's* culture in a complicated medium may provide moderate biomass concentrations, which restricts the range of technological applications for it. *S. pasteurii* is an organism with a broad variety of growth needs that is generally flexible and adaptable. Because of this, it may flourish in a variety of settings and contribute significantly to a range of ecological processes [7,12].

Despite that potential, *B. beijingensis* is a moderately halophilic bacterium within the *Methylococcaceae* family. It is also facultatively methylotrophic and alkaliphilic. This bacterium has a unique combination of features that allow it to thrive in environments with high levels of salinity, alkalinity, and methanol availability. Compared to *S. pasteurii*, *B. beijingensis* is a mesophile with an ideal growing temperature range of 30–37 °C. The organism *B. beijingensis* is able to withstand temperatures between 15 °C and 42 °C. It is facultative anaerobic, but grows more quickly in the presence of oxygen. It is alkaliphilic, flourishing in a pH range of 7.5 to 10.0, with the optimal pH range being 8.0 to 9.0. The bacterium has an ideal salinity range of 1–3% NaCl and is mildly halophilic, but can tolerate a range of 0.5-5% NaCl. *B. beijingensis* may use methanol as a major carbon source when available since it is a facultative methylotroph. However other carbon sources may also be used, such organic acids including lactate and acetate and carbohydrates as fructose and glucose. Its preferred source of nitrogen is ammonium ions. It may, however, also absorb nitrate and other substances that contain nitrogen in some situations [13]. *B. beijingensis* may grow in both bright and dark conditions since it cannot be photo-sensitive. All things considered, *B. beijingensis* is an amazing creature with special adaptations to hostile conditions. Understanding the distinct physical and biochemical requirements of this organism is essential for researching its ecology, possible uses, and ideal growth conditions.

*S. pasteurii* is known for its high urease activity, which allows it to hydrolyze urea and use the released ammonia as nitrogen [14]. This contributes significantly to nitrogen metabolism and makes it well suited to urea-rich environments such as soils and wastewater [15]. *B. beijingensis* does not have significant urease activity and does not rely only on the breakdown of urea for its growth but also capable to carbonic anhydrase activity and breakdown of bicarbonate. This is in contrast to S. *pasteurii's* reliance on its ureolytic abilities [16]. Overall, the key metabolic differences between *S. pasteurii* and *B. beijingensis* lie in their carbon and nitrogen source preferences, energy metabolism pathways, and the presence of urease activity. These differences allow them to thrive in different ecological niches and utilize different available resources for growth.

*Sporosarcina pasteurii*, when exposed to modest amounts of zinc, manganese, and copper, exhibits a moderate tolerance to these metals. However, it remains susceptible to heavy metals such as cadmium, lead, and mercury, even at low concentrations. Notably, *S. pasteurii* does not actively accumulate metals. It lacks specialized processes for concentrating metals, although it can passively absorb some of them due to environmental influences [17]. The bacterium's restricted efflux pump activity and general stress response mechanisms likely contribute to its modest metal tolerance. Importantly, the absence of specific transport or chelation mechanisms is suggested by the lack of active metal accumulation.

The remarkable resistance of *B. beijingensis* to elevated quantities of heavy metals, including chromium, cobalt, zinc, and nickel, is well-known. This tolerance, which enables *B. beijingensis* thrive in metal-contaminated environments, can be attributed to particular mechanisms such efflux pumps and metal chelation. It has the ability to rapidly and persistently collect certain metals, including zinc, cobalt, and chromium. Because of this capability, *B. beijingensis* might be used to recover and concentrate metal-contaminated materials from habitats, a process known as bioremediation [18,19]. Specific absorption and intracellular sequestration processes involving metal-binding proteins or compartmentalization are necessary for hyperaccumulation [20]. Overall, *B. beijingensis* shows substantial resistance to heavy metals and active hyperaccumulation, while *S. pasteurii* shows limited accumulation and moderate metal tolerance. Their disparate capacities are a reflection of how differently they have adapted to various environmental stresses. Therefore, *B. beijingensis*' remarkable metal tolerance and accumulation potential make it a viable organism for bioremediation applications.

A substantial contribution to MICP production was proposed by certain molecular pathways of the organisms. The *ureABC* gene encodes the three urease subunits that are necessary for the release of carbonate ions, which are the building blocks of calcite precipitation, and for the hydrolysis of urea. An auxiliary protein involved in urease synthesis and stability is encoded by the *ureD* gene [21]. The two forms of carbonic anhydrase enzymes expressed by the *CA5A* and *CA5B* genes, as described by Holmes [22], play essential roles in ammonia detoxification and glucose metabolism. These enzymes catalyze the hydration of carbon dioxide and are involved in the conversion of dissolved $CO_2$ to bicarbonate ions, providing a carbonate source for the synthesis of calcite. The *CA* gene from *Bacillus mucilaginosus*, *CA4*, has been shown to promote carbonate formation and the capture of $CO_2$, leading to the synthesis of $CaCO_3$ crystals, as demonstrated by Zheng et al. [23]. Moreover, proteins involved in surface adhesion and biofilm development are encoded by the *tasA/sipW* genes, which may have an impact on calcite nucleation and crystal development [24]. A complex polysaccharide biosynthesis process is encoded by the *epsA-O* operon, and the EPS may have an impact on surface adherence and crystal form [25].

Certain regulatory genes, including *ureR*/*ureG* genes, control how the urease operon is expressed and reacts to environmental signals like pH and nitrogen availability [26]. A regulator of carbonic anhydrase activity that is encoded by the *cph2* gene may have an impact on the pace at which calcite precipitates [27]. Some of the patrial as *pks* genes that are engaged in pathways of polyketide synthase that could be involved in the synthesis of organic molecules that affect the development of calcite crystals. Overall, according to Elmi et al. [28] it was found that *Bhargavaea cecembensis* exhibited a high level of urease activity and produced a large amount of calcium carbonate. This strain showed high levels of tolerance to different conditions of temperature, pH, and salinity. On the other hand, *S. pasteurii*, another ureolytic bacterium commonly used in MICP, was found to compete poorly with natural bacteria and decreased in abundance after sequential stimulation treatments. Therefore, it can be

inferred that *Bhargavaea* spp. may have a competitive advantage over *S. pasteurii* in MICP due to its ability to tolerate extreme conditions and maintain high urease activity [29].

The aim of the research is to (i) Evaluate the potential of *B. beijingensis*, a urease-producing bacterium, for MICP applications and compare with other ureolytic bacteria, (ii) Characterize the key properties relevant to MICP, such as urease activity, calcite precipitation efficiency, and sand solidification ability and heavy metal removal efficiency, (iii) Validate the potential of *B. beijingensis* as a novel and sustainable MICP agent for various environmental and engineering applications. Overall, the research explores the possible of *B. beijingensis* as a promising tool for MICP-based solutions in bio-cementation, soil improvement, and heavy metal remediation.

## Materials and methods

### Isolation and screening of ureolytic bacteria

Ureolytic bacterial strains were screened and isolated from the soil samples collected systematically from the calcareous bricks mine located near Junagadh, Gujarat, India, ensuring the maintenance of sterility by employing polypropylene bags. The enrichment process for urease-producing bacteria commenced by inoculating one gram of soil into 100 mL nutrient broth containing 2% urea. The inoculated samples were incubated at 37 ℃ for 120 h under shaking (150 rpm) conditions. Bacterial enumeration was performed using the serial dilution technique on nutrient agar plates, with the isolated bacteria subsequently maintained on Nutrient agar plates as per the methodology established by Sharma et al. [30]. Further refinement of isolates involved their transfer to modified Christensen's Urea base medium, a urease-selective medium containing 5 g NaCl, 2 g monopotassium phosphate, 1 g glucose, 0.2 g peptone, 0.012 g phenol red, and 20 g urea (filter sterilized) per Liter. The medium was adjusted to a pH of 6.8 before autoclaving. This selective medium enabled the assessment of urease production by observing a distinctive change in color to pinkish-red. This alteration indicated an increase in pH, confirming the active production of the urease enzyme by the isolated bacteria, as established by Montaño-Salazar et al. [31]. This stringent screening process ensures the identification of potent ureolytic bacteria for subsequent stages of the research.

### Calcite precipitation screening of urease-producing isolates

In order to assess the potential of urease-producing isolates for calcite precipitation, a systematic screening was conducted using both solid and liquid media.

*Solid media screening: YE-Urea agar medium*

Urease-producing isolates were subjected to calcite precipitation screening on YE-Urea agar medium composed of yeast extract (1 g $L^{-1}$), NaCl (5 g $L^{-1}$), urea (20 g $L^{-1}$), and $CaCl_2$ (10 g $L^{-1}$) (Himedia, Mumbai, India). To initiate this process, the optical density (OD) of overnight bacterial strains was adjusted to 1.0 at a wavelength of 600 nm. Subsequently, a 50 µl aliquot of the bacterial culture was inoculated onto the surface of YE-Urea agar medium plates. After an incubation period of 1–2 h at room temperature, the plates were left undisturbed and then placed in a 37 °C incubator for a duration of 7 days. The emergence of a distinctive white powdery halo zone around the inoculated area was indicative of calcite precipitation. This visually identified zone was meticulously scraped off for further analysis, conforming to the methodology outlined by Shaheen et al. [32].

*Liquid media screening: YE-Urea broth medium*

Concurrently, urease-producing isolates underwent calcite precipitation screening in YE-urea broth medium, which comprised yeast extract (1 g $L^{-1}$), NaCl (5 g $L^{-1}$), urea (20 g $L^{-1}$), and $CaCl_2$ (10 g $L^{-1}$) (Himedia, Mumbai, India). Prior to autoclaving, the pH of the media was adjusted to 6.5 using 1 N HCl, excluding urea and $CaCl_2$. Post-autoclaving, filter-sterilized urea and $CaCl_2$ were aseptically introduced into the medium. Inoculated flasks were then incubated at room temperature for 7 days. The presence of white precipitates at the bottom of the flasks indicated the successful formation of calcite, following the methodology proposed by Dikshit et al. [33].

The confirmation of precipitated calcite was initially performed through a chemical method. Hydrochloric acid was introduced to the dried powder of precipitates, and the resultant effervescence was observed as Hydrochloric acid broke down into calcium chloride, releasing $CO_2$. Subsequently, dried powder of precipitates was subjected to 1 N hydrochloric acid (HCl), confirming calcite dissolution, as per the rigorous approach outlined by Shaheen et al. [32]. This comprehensive screening methodology ensures the robust identification and confirmation of urease-producing isolates with the capacity for calcite precipitation, forming a critical foundation for subsequent research phases.

### Determination of urease activity

To quantify urease activity, a modified phenol-hypochlorite assay was employed, ensuring precision and reliability in the assessment. Ammonium chloride ($5 \times 10^{-4}$ M stock solution) served as the standard for calibration.

The procedure involved the addition of 200 µL of culture filtrates to a mixture comprising 1800 µl of urease buffer (1 mM EDTA, 50 mM HEPES and 20 g $L^{-1}$ filtered urea). The controls were also performed, as negative control (without urease) and positive controls (standard sample of ammonia with known urease activity). This concoction was then incubated at 30 ℃ for 10 min. Following the initial incubation, phenol nitroprusside and sodium hypochlorite (500 µl each) were added to the mixture, and a subsequent incubation at 30 ℃ for 20 min ensued. The optical density of the resulting solution was measured at 630 nm.

The determination of urease activity was based on the principle that one unit of urease is defined as the amount of enzyme capable of hydrolysing 1 µmole of urea per minute [34]. Regular monitoring of changes in pH and urease enzyme activity was conducted on a daily basis for a comprehensive understanding and subsequent analysis. This meticulous method ensures accurate and reproducible quantification of urease activity, forming a crucial aspect of the study's methodology.

## Estimation of precipitated calcite

The quantification of calcite precipitation by JCP-5 was undertaken by inoculating the strain in the YE-Urea medium, followed by incubation at 37 ℃ for a duration of 5 days, in accordance with the methodology established by Peng and Liu [35].

Subsequent to the incubation period, the content of the flask underwent filtration using pre-weighed Whatman No. 1 filter paper with a diameter of 125 mm. The filter paper, laden with the precipitates, was then dried at 50 ℃ for 48 h. After the drying process, the post-weight of the filter paper was measured to determine the yield of precipitated $CaCO_3$ crystals [36]. The quantification of precipitates was calculated using the formula:

$$\text{Percent Yield of Calcite} = \frac{\text{Actual Yield}}{\text{Theoretical Yield}} \times 100\% \quad (1)$$

Where, the theoretical yield is the amount of calcite estimated to form after a calcium chloride (limiting reactant) is completely consumed. The actual yield is the quantity of calcite that is actually obtained. This systematic approach provided a reliable means to measure the percentage of calcite precipitated by JCP-5. The utilization of precise measurements and standardized procedures ensures the accuracy and reproducibility of the results, contributing to the robustness of the study's methodology.

## Characterization and confirmation of $CaCO_3$ using FTIR

The identification and confirmation of calcite precipitation were accomplished through infrared spectroscopic analysis utilizing an FTIR spectrometer (IRSpirit-X, Shimadzu). This analytical technique provided valuable insights into the composition and structure of the produced calcium carbonate during the experimental procedures.

For the FTIR analysis, 1 mg of precipitate (dry weight) was meticulously mixed and ground with 100 mg of KBr. As a reference, pure calcium carbonate was employed as a standard to facilitate the identification of $CaCO_3$ minerals. The FTIR spectra, ranging from 4500 to 500 $cm^{-1}$, were then acquired to scrutinize the characteristics of the precipitation induced by the JCP-5 isolate, adhering to the established methodologies of Achal and Pan [37] and Šovljanski et al. [38].

This methodological approach not only ensured the precise identification of the calcium carbonate minerals but also provided a comprehensive understanding of the chemical composition and structural features of the precipitated calcite. The incorporation of a standardized reference allowed for a robust comparison, enhancing the reliability and interpretability of the FTIR spectra obtained.

## SEM-EDX analysis

Scanning electron microscopy (SEM) (EVO-18, Zeiss) stands as a powerful technique utilizing high-energy electron beams to generate diverse signals on specimen surfaces, revealing crucial information about morphology, chemical composition, and material orientation. In our research, SEM-EDX (Amitek) analysis was instrumental in comprehensively examining the morphology and composition of calcium carbonate precipitates, guided by the methodology outlined by Dikshit et al. [33].

The precipitates for SEM-EDX analysis were obtained from a 100 mL urea medium containing the following components (g $L^{-1}$): 1 yeast extract, 5 NaCl, 20 urea, and 1% $CaCl_2$. The medium was inoculated with the JCP-5 isolate, initially possessing an OD (600 nm) of 1.00, and subsequently incubated at 37 ℃ in a rotary shaker set at 150 rpm for a duration of 10 days. Following incubation, the precipitates were meticulously washed three times with distilled water, and the resulting pellet was subjected to oven-drying at 50 ℃ for 72 h in preparation for SEM-EDX analysis, aligning with the methodology established by Rautela and Rawat [39].

This comprehensive approach allowed for detailed insights into the morphology and elemental composition of the calcium carbonate precipitates, contributing to a more nuanced understanding of the structural aspects of the formed material. The stringent washing and drying procedures ensured the removal of any extraneous substances, enhancing the accuracy and reliability of the SEM-EDX analysis results.

## Sand solidification test for MICP

To evaluate the sand solidification capabilities of the isolated strain, a small-scale sand syringe solidification test was conducted. The isolated strain was initially cultured in Nutrient broth to ensure optimal growth and metabolic activity. Subsequently, a 50 mL syringe, measuring 11 cm in height and 3 cm in diameter, was employed for the test. Dried and autoclaved Red and Black River sand, totalling 65 g, were loaded into the syringe. In a sequential process, 15 mL of the bacterial culture solution and 20 mL of the consolidation solution (g/L) (composed of 3 Nutrient broth, 30 Urea, 55.5 $CaCl_2$, 2.12 $NaHCO_3$, and 10 $NH_4Cl$) were injected into the syringe. After a 2-hour interval, the syringe was flushed, leaving approximately 2 mL of the solution near the sand surface.

The consolidation solution injection and drainage process were repeated daily for a period of 21 days. Throughout this period, pH values and $Ca^{2+}$ concentrations from the effluent were measured at 3-day intervals using the EDTA titrimetric method. This comprehensive methodology, adapted from Al Imran et al. [40] and Gowthaman et al. [41], provided a systematic approach to assess the sand solidification potential of the isolated strain through MICP. Regular monitoring of pH and $Ca^{2+}$ concentrations facilitated a thorough understanding of the solidification process dynamics throughout the experimental duration (Fig. 1).

## Heavy metal removal experiments

Efficiency in the removal of heavy metals, including Ni, Co, Mn, Hg, Zn, and Ba, was systematically evaluated in this study. The heavy metal stock solutions, prepared at a concentration of 1 M using $NiCl_2$, $CoCl_2$, $MnCl_2$, $HgCl_2$, $ZnCl_2$, and $BaCl_2$, were further diluted with distilled water before application. The experimental setup

**Figure 1**. Setup for sand solidification test.

for heavy metal removal mirrored the methodology employed for $CaCO_3$ precipitation, where the isolates were introduced into YE-Urea medium broth as outlined by Qiao et al. [42].

Following a 48-hour incubation period, a bacterial crude enzyme suspension (30 mL) was added to heavy metal solutions (30 mL) with varying concentrations ranging from 100 to 500 mM, adhering to the protocols established by Jalilvand et al. [43] and Qiao et al. [42]. The presence of precipitated carbonates was initially confirmed through a chemical method similar to that utilized for $CaCO_3$ detection and formed precipitates were filtered through the preweighted filter paper, allowed it to dry and post weighted the filter paper and calculated the heavy metal removal efficiency as explained by Shaheen et al. [32].

To quantify heavy metal carbonate precipitates, a solution mixture of the crude enzyme and heavy metal solution underwent filtration using pre-weighed Whatman filter paper, following the same procedure employed for $CaCO_3$ estimation [36]. This rigorous and standardized approach ensures the accurate assessment of heavy metal removal efficiency, contributing to the robustness of the experimental design and subsequent analysis in this research study.

$$\text{Percent Yield of Removal efficiency} = \frac{\text{Actual Yield}}{\text{Theoretical Yield}} \times 100\% \quad (2)$$

Where, the theoretical yield is the amount of precipitated heavy metals estimated to form after a limiting reactant ($NiCl_2$, $CoCl_2$, $MnCl_2$, $HgCl_2$, $ZnCl_2$, and $BaCl_2$) is completely consumed. The actual yield is the quantity of precipitated heavy metals that is actually obtained.

## Compressive strength testing

The study on the influence of bio-cementation on mortar involved the meticulous preparation of cubes using a mixture of natural river sand and cement in a 3:1 ratio (w/w). Adhering to IS 4031–1988 standards outlined by Sharma et al. [30], cube molds with dimensions of 70.6 mm were employed. Compressive strength testing was executed through three distinctive approaches.

In the first approach, denoted as BC + CBE, sand and cement were thoroughly mixed, and a grown culture of *B. beijingensis* was added with an optical density ($OD_{600}$) of 1.0. This mixture was further supplemented with the inclusion of the crude enzyme produced by the bacterial culture.

The second approach, referred to as OBC, involved the addition of the bacterial culture alone to the mixture of cement and sand. Finally, the third approach, OBCE, solely introduced the bacterial crude enzyme into the cement and sand mixture [44].

Cubes with a width of 70.6 mm were cast and compacted in the designated molds. Following demolding, all specimens were cured in an appropriate consolidation solution at room temperature. Compression testing was then conducted at intervals of 3, 7, and 28 days using compression strength testing machine (Heico – compression testing machine capacity 3000kN). To ensure robust results, all experiments were performed in triplicate. This comprehensive methodology enabled a thorough exploration of the distinct contributions of bacterial culture, crude enzyme, and their combined effect on the compressive strength of the bio-cemented mortar cubes.

## Crack remediation test

In this investigation on crack remediation, cracks were intentionally induced in the blocks by incorporating a piece of cardboard within the mold before the addition of mortar material. Following the formation of the mortar blocks, they were subjected to a curing period of 7 days in water. Subsequent to this curing phase, the crack remediation test was conducted in two sets.

In the first set, the bacterial enzyme (urease) produced by *B. beijingensis* was added alongside the consolidation solution. Conversely, in the second set, the bacterial culture of *B. beijingensis*, in conjunction with the consolidation solution, was introduced. For a duration of 10 days, 1 ml of bacterial cell suspension and 2 ml of consolidation solution were systematically applied onto the surface of the cracks three times each day. In the set involving the bacterial enzyme, 1 ml of bacterial cell suspension was replaced with the bacterial crude enzyme.

Control samples were treated solely with the consolidation solution, excluding any addition of bacterial cell suspension or bacterial enzyme, in line with the methodology established by Intarasoontron et al. [45]. To assess the healing performance of the specimens, the healing ratio was calculated by comparing the cracked area before and after the healing process using Eq. (3). This comprehensive crack remediation test allowed for a detailed examination of the efficacy of bacterial culture and enzyme in promoting healing and closure of induced cracks in the mortar blocks.

$$\text{Remediation ratio } (\%) = \frac{\text{Healed crack area (mm)}}{\text{Initial crack area (mm)}} \times 100\% \tag{3}$$

# Results and discussion

## Isolation and characterization of MICP bacterial strain

*Isolation and screening*

In the initial phase of our study, isolates were meticulously screened on a modified Christensen's Urea base medium, and subsequently, they were maintained on Nutrient agar plates. The hydrolysis of urea by bacteria during the incubation period led to the production of ammonia, impacting the pH. The alkaline conditions induced a distinctive pink coloration in the presence of the phenol red indicator, thus confirming the activity of urease enzyme, as elucidated by Sharma et al. [30]. From this screening process, six urease-producing bacterial strains (JCP-4, JCP-5, JCP-13, JCP-15, JCP-22, and JCP-23) were identified based on the intensity of the pink colour observed in Christensen's Urea base medium. These strains were further purified through repetitive transfers onto Nutrient agar plates, preparing them for subsequent investigations.

Urease, a pivotal enzyme in the microbial hydrolysis of urea, plays a significant role in the production of ammonia and carbonate. This process is crucial for the formation of MICP, as highlighted by Wang et al. [46]. All six urease-producing isolates were subjected to scrutiny for calcite precipitation, exhibiting growth and calcite production both on plates and in YE-urea broth medium. The calcifying bacterial strains manifested halos and crystal formation around the bacterial inoculation on plates, and white precipitates at the bottom of flasks in the YE-urea broth medium confirmed positive results for calcite formation. This confirmation was further validated through the addition of 1 N Hydrochloric acid, resulting in effervescence and observed dissolution of precipitates.

Among the isolates, JCP-5 demonstrated the highest urease activity, correlating with a substantial production of calcite. Consequently, JCP-5 was selected for further in-depth analysis. The confirmation of calcite precipitation was also achieved through FTIR spectroscopy (mentioned in the Sect. 3.5 (Fig. 6)). Following a comparative assessment of calcium carbonate production among the six strains, JCP-5, exhibiting robust capabilities, was singled out for subsequent exploration. This led to an investigation into the relationship between urease activity and the production of precipitated calcite by the bacteria, forming a critical aspect of our study.

*Sequencing and identification of bacterial strain*

The isolated strain (JCP-5) was molecularly identified as *B. beijingensis* based on its 16 S rRNA gene sequences. The nucleotide BLAST analysis revealed that the isolate belongs to the phylum Firmicutes and the family *Planococcaceae*. Phylogenetic analyses of the 16 S rRNA sequence demonstrated a reasonable degree of correlation with the morphological classification schemes of species within the genus. The sequence was submitted to the NCBI with the accession number OP120916.1. Phylogenetic analysis was performed using the neighbor-joining method via MEGAx software. The phylogenetic tree of the isolated *B. beijingensis* is depicted in Fig. 2.

## Urease enzyme activity

To assess urease enzyme activity, we employed the modified Phenol-hypochlorite assay method, a reliable technique for detecting the production of ammonia. *B. beijingensis* was introduced into the YE-Urea medium and incubated in a rotary shaker for 7 days at room temperature. Daily monitoring of changes in pH and enzyme activity provided valuable insights into the kinetics of urease production.

Comparing our findings to the work of Sharma et al. [30], which highlighted *Sporosarcina pasteurii* as a reference, our research with *B. beijingensis* revealed noteworthy outcomes. *S. pasteurii* demonstrated maximum urease production in YE-urea medium, reaching up to 366 U $mL^{-1}$ at 120 h [30]. In our investigation, *B. beijingensis* exhibited remarkable urease activity, reporting a maximum of 135.93 IU $mL^{-1}$, and a corresponding pH of 9.34 after 120 h of incubation (Figs. 3 and 4). The enzyme activity is comparatively lower than the *S. pasteurii*. The observed lower activity could be attributed to the sensitivity of enzyme at acidic and alkaline conditions [47]. Interestingly, *B. beijingensis* exhibited significantly higher compression strength against calcite compared to

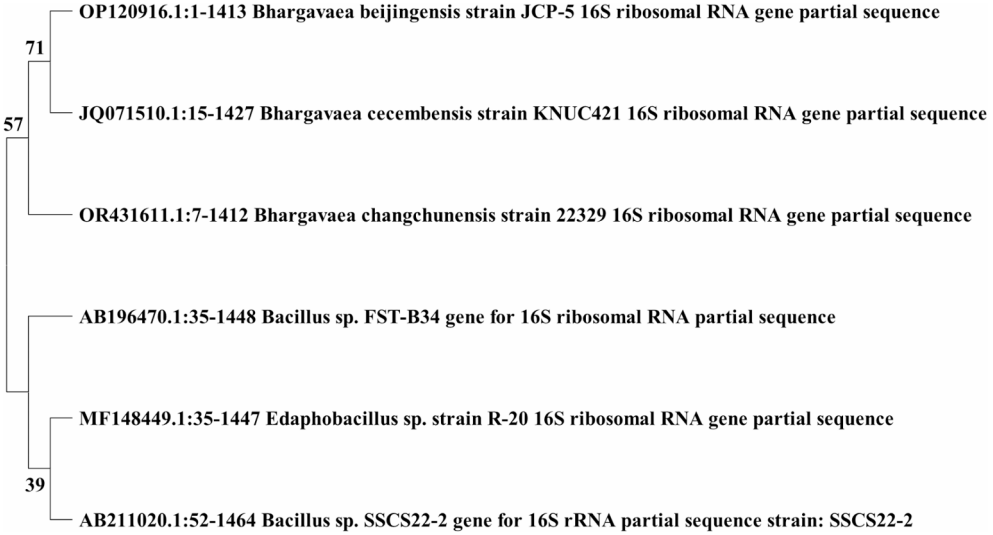


**Figure 2**. Neighbor-joining tree based on bacterial 16S rRNA gene sequence data from different isolates of this study along with sequences available in the NCBI GenBank database. Numerical values indicate bootstrap percentile from 1000 replicates.

other strains. The observed correlation between pH and enzyme activity further strengthens the robustness of our results.

These findings not only underscore the potency of *B. beijingensis* in urease production but also provide a valuable contribution to understanding the kinetics of urease activity in comparison to established microbial references. The correlation between pH and enzyme activity serves as a crucial indicator of the dynamic interplay within the microbial system, enhancing our comprehension of urease-related processes.

## Estimation of precipitated calcite

To ascertain the extent of calcite formation induced by *B. beijingensis*, daily collection of precipitates was conducted through filtration, followed by meticulous weighing. Notably, the observed trend revealed a consistent increase in the amount of calcite precipitation throughout the incubation period, directly linked to the urease enzyme production pathway. Strikingly, the highest accumulation of calcite occurred on the 5th day of incubation, reaching an impressive percentage of 98.88% (Fig. 4).

This dynamic pattern of calcite precipitation underscores the efficiency and sustained activity of the urease enzyme pathway catalysed by *B. beijingensis*. The peak observed at the 5th day indicates an optimal period for calcite production, potentially offering valuable insights for future applications and process optimization. This mechanism confirms the MICP mediated Biocement production. The approach opens new avenues for the construction industries and civil engineering as it can be used for the production of Biocement, Bioconcrete, Biomortar, Durable and self-healing construction material. The results not only validate the prowess of *B. beijingensis* in mediating calcite precipitation but also contribute to the temporal understanding of the urease-

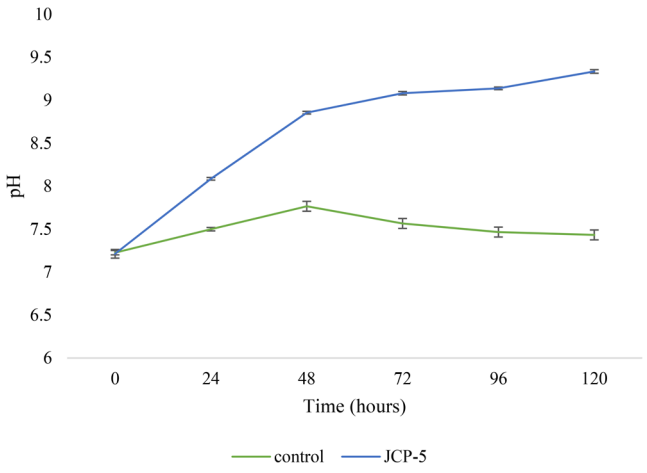


**Figure 3**. pH profile of *Bhargavaea beijingensis* in the YE-Urea medium (Error bars show SD).

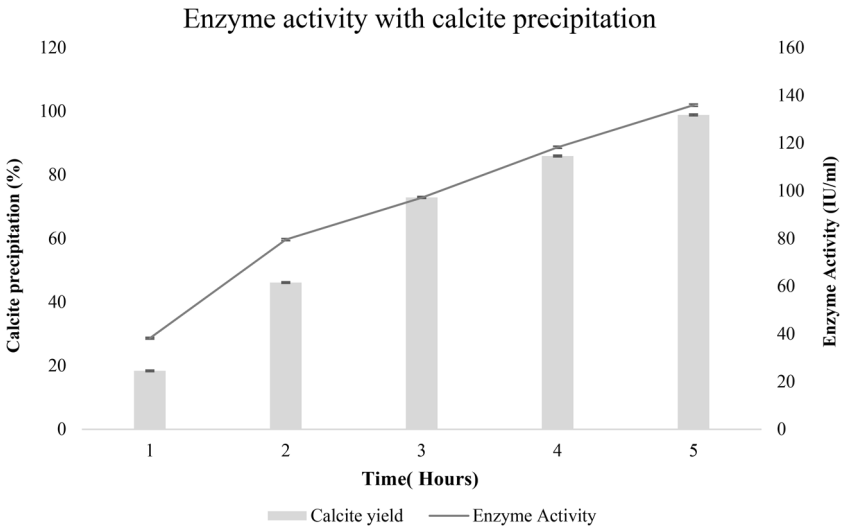


**Figure 4**. Urease enzyme activity and estimation of calcite precipitation by *Bhargavaea beijingensis* in YE-Urea medium (Error bars show SD).

driven calcification process [35]. This can help in developing targeted strategies to control or enhance calcite precipitation.

## Sand solidification test for MICP

The temporal variations in $Ca^{2+}$ concentration and pH effluent, illustrated in Fig. 5, unveil compelling insights into the urease-producing isolate *B. beijingensis* and its proficiency in MICP. The continuous increase in pH and simultaneous decrease in $Ca^{2+}$ ion concentration signifies the robust capacity of *B. beijingensis* to orchestrate MICP. Interestingly, the red sand substrate outperformed the black sand substrate. This discrepancy can be attributed to the finer particle size of red sand, which enhances its ability to retain bacterial cells for an extended period, preventing their flush-out from the syringe compared to the larger particles present in black sand. As the red sand was small particulate sand that had less pore space as compare to black sand. Thus, red sand consolidated well using MICP mechanism than the black sand on the basis of their size and pore space.

This result confirms that the sand solidification occurs in the syringe using MICP technology. This approach can open new ways to control soil erosion occurs through wind and water because MICP treated soil forms crust on the surface and that reduce the risk of soil erosion. Wang et al. [48] described the use of MICP technology to reduce the wind erosion rate of sandy soil. The results showed that the wind erosion rate upto 10.23% was observed for the untreated sandy soil, but MICP treated soil for more than three times, the wind erosion rate dropped and found below 0.4% for sandy soil. Therefore, solving these problems using the MICP technology can be the direction of future research to control soil erosion.

## FTIR analysis for the detection of calcite

FTIR spectroscopy is used to analyse the composition of calcite formation during the process of biomineralization. FTIR spectroscopy emerges as a pivotal tool in unravelling the composition of precipitates generated by the *B. beijingensis* bacterial isolate. As depicted in Fig. 6, the FTIR spectra of calcium carbonate (calcite) precipitates in YE-Urea medium supplemented with calcium chloride revealed distinct bands at 713, 874, and 1794 $cm^{-1}$, showcasing a remarkable alignment with the standard spectrum of $CaCO_3$. The bands within the range of 3,600 to 3,200 $cm^{-1}$ indicated intricate interactions involving hydroxyl and amine groups. This underscores the utility of FTIR as an effective method for the early identification of calcium carbonate precipitated by bacteria in a liquid medium. Predominantly, the mineral formed by the *B. beijingensis* bacterial isolate was identified as calcite, aligning with findings from previous studies [37].

As per the Achal and Pan [37], *Bacillus* sp. CR2 formed calcite as the predominant mineral as the relative intensity of the bands at 873 $cm^{-1}$ and 1,800 to 1,550 $cm^{-1}$, the sharper bands were observed in the media containing calcium chloride and calcium nitrate and that confirms the capacity of the such calcium sources to promote the precipitation of calcite. Thus, the formation of sharp band at the 874 $cm^{-1}$ majorly confirms the calcite formation.

## Element analysis of calcite by scanning electron microscopy

The optical microscopy initially revealed the presence of white crystals, affirming the calcium carbonate precipitation by *B. beijingensis*. Subsequently, SEM-EDX examination provided a comprehensive confirmation of calcium contents and silica materials. The EDX spectra demonstrated the unmistakable presence of calcium content, corroborating the precipitation of $CaCO_3$. Further quantitative analysis revealed the composition, with calcium oxide, silica oxide, and calcium elements (wall stone) constituting 21.10%, 56.66%, and, 21.24%, respectively (Table 1).

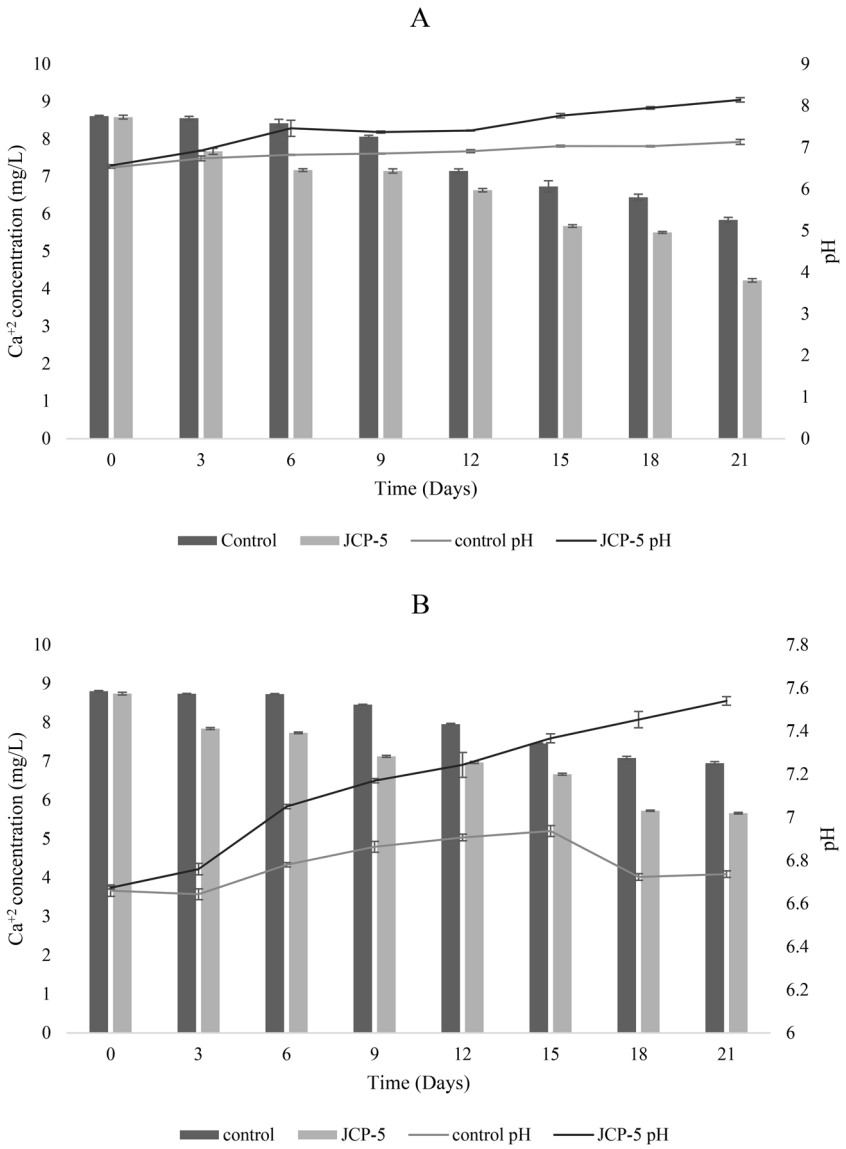


**Figure 5**. Changes in pH and $Ca^{+2}$ concentration during the sand solidification test; (**A**) For red river sand (**B**) For black river sand (Error bars show SD).

These findings elucidate the multifaceted contributions of these elements to the calcite formation in the sample. The result clearly explained that the *B. beijingensis* produced calcite and that plays the major role in the biocement production and crack remediation. Additionally, as per the result silica composition present in the precipitates helps in the sand consolidation mechanism and harden the soil upper layer so that reduces the soil erosion problem. The results not only underscore the organism's capability to produce calcite under controlled conditions but also shed light on the intricate interplay of elements in the MICP process, as illustrated in Figs. 7 and 8.

## Heavy metal removal efficiency

To quantify the formation of heavy metals carbonate by *B. beijingensis*, precipitates were meticulously filtered and weighed. Remarkably, the urease enzyme production pathway exhibited an additional capability in precipitating heavy metals, as confirmed by the introduction of 1 N Hydrochloric acid, resulting in effervescence and dissolved precipitates. $HgCl_2$ demonstrated the highest precipitation among the tested heavy metals (Table 2). Using $CaCl_2$ as a positive control and heavy metal solutions without bacterial urease enzyme as negative controls, the heavy metal removal efficiency showed an increasing trend with the rise in heavy metal concentration. At 500 mM concentrations of mercury, cobalt, zinc, nickel, manganese, and barium, the maximum heavy metal removal efficiency found was 97.68%, 93.62%, 91.19%, 88.98%, 78.90%, and 76.93%, respectively. The observed heavy metal removal efficiency of *B. beijingensis* followed the order: Hg > Co > Zn > Ni > Mn > Ba (Fig. 9), substantiating the promising potential of MICP in heavy metal bioremediation processes.

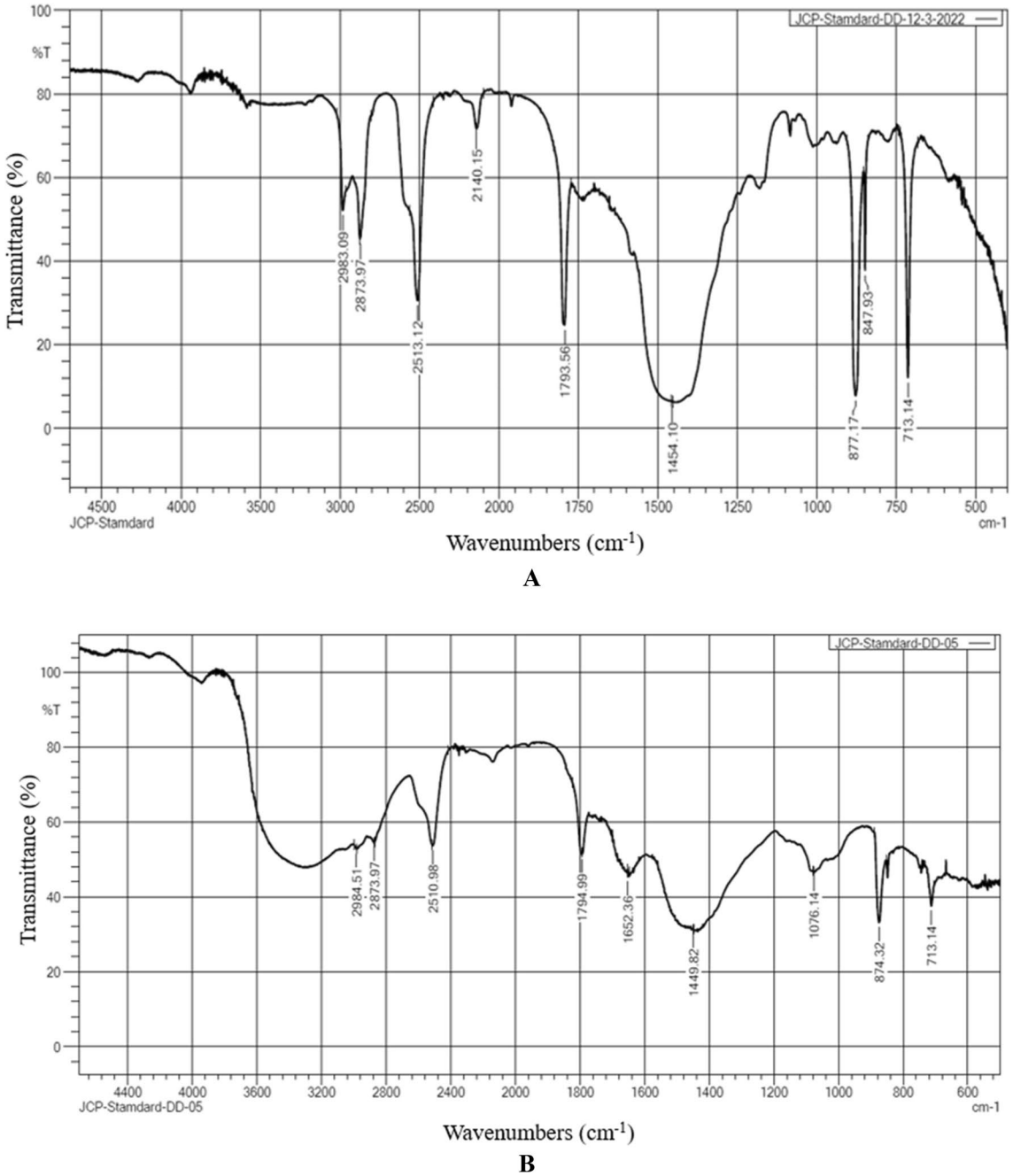


**Figure 6**. FTIR analysis confirming calcium carbonate precipitation induced by Bacterial isolate *Bhargavaea beijingensis* in the media Containing calcium chloride. (**A**)-Standard Calcium carbonate (**B**)- Calcite produced by *Bhargavaea beijingensis*.

| Element | Weight% | Atomic% |
|---|---|---|
| C | 21.10 | 29.93 |
| O | 56.66 | 60.35 |
| Na | 0.70 | 0.52 |
| P | 0.30 | 0.17 |
| Ca | 21.24 | 9.03 |
| Totals | 100.00 | |

**Table 1**. Element presents in the sample.

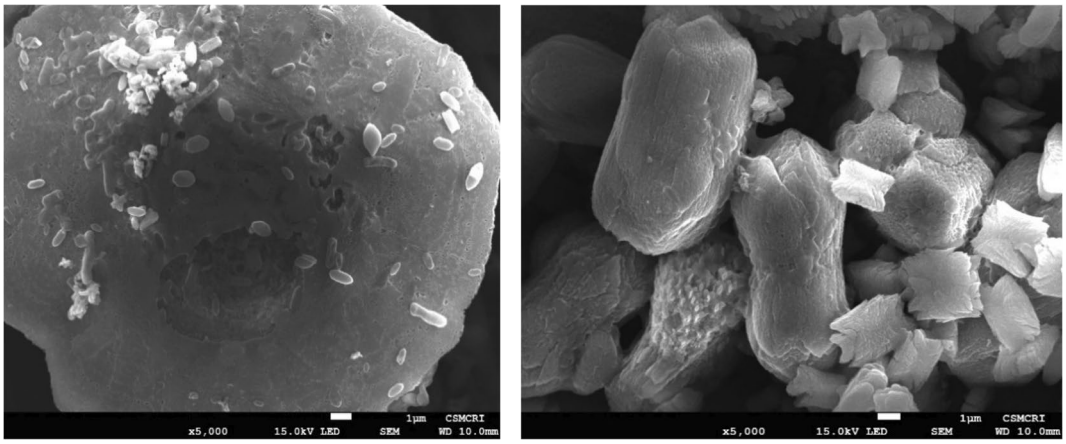


**Figure 7**. Precipitation of calcium carbonate confirmed by Scanning electron microscopy.

Jalilvand et al. [43] explained that the *S. pasteurii* produced higher amounts of metal carbonates. However, the use of selected bacteria in bioremediation of contaminated sites may be more effective due to the stability of these bacteria in high concentrations of Pb, Zn, and Cd. *Stenotrophomonas rhizophila* (A323) and *Variovorax boronicumulans* (C113) produced the highest carbonate minerals of heavy metals. *S. rhizophila* removed 96.25%, 71.3%, and 63.91% of Pb, Cd, and Zn, respectively, after 72 h of incubation. Similarly, *V. boronicumulans* removed 95.93% of Pb, 73.45% of Cd, and 73.81% of Zn after having the similar incubation time.

In our research the selected bacteria *B. beijingensis* has the potential to remove many heavy metals within very short time. The study on the enzyme mediated heavy metal removal was very limited but, in our research, we found that the highest removal of mercury along with other heavy metals as well which opens new path for the enzyme mediated bioremediation of heavy metal. The enzyme makes them a more environmentally friendly approach to mercury removal. Therefore, the enzymes can potentially be used for in-situ remediation, meaning they can be applied directly to contaminated sites like soil. This eliminates the need for expensive and disruptive excavation and transportation of contaminated materials. It's important to note that enzyme-mediated removal is still an emerging field.

## Compressive strength testing

The compressive strength had increased for the mortar cubes that contained microbial cells irrespective of the media used to grow the cells compared to control (Fig. 10). The compressive strength was obtained for three different approaches using the bacterial isolate *B. beijingensis* at the intervals of 3,7- and 28-days incubation. The mortar cubes OBC that contained bacterial cell culture suspension with the consolidation solution showed around 33.48% improvement in compressive strength at 28 days (29.58 N/mm$^2$) with respect to control (22.16 N/mm$^2$), the mortar cubes OBCE that contained crude bacterial enzyme solution with the consolidation solution showed around 41.47% improvement in compressive strength at 28 days (31.35 N/mm$^2$) with respect to control (22.16 N/mm$^2$) while the BC + CBE that contained bacterial cell culture suspension and crude bacterial enzyme solution showed highest compressive strength around 86.46% improvement in compressive strength at 28 days (41.32 N/mm$^2$) with respect to control (22.16 N/mm$^2$). The deposition of CaCO3 on the bacterial cell surfaces and inside the pores of the cement-sand matrix, which plugs the holes in the mortar, is probably responsible for the improvement in compressive strength. Salmasi and Mostofinejad et al. [49] also demonstrated that the addition of *Shewanella* species improved the compressive strength of cement mortar. Because certain hydrolase group of bacteria can influence the precipitation of calcium carbonate minerals, which help in fill the pores

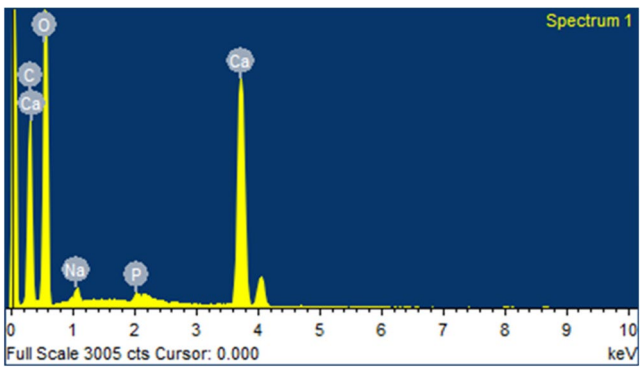


**Figure 8**. The element present in the sample was confirmed by SEM-EDX spectra.

| Concentration of Heavy metals | Heavy metal removal efficiency (%) | | | | | |
|---|---|---|---|---|---|---|
| | Mercury | Cobalt | Zinc | Nickel | Manganese | Barium |
| 100mM | 10.74 ± 0.83 | 8.67 ± 0.32 | 7.39 ± 0.20 | 7.97 ± 0.26 | 7.41 ± 0.16 | 6.80 ± 0.26 |
| 200mM | 21.48 ± 0.15 | 16.84 ± 0.52 | 17.39 ± 0.50 | 15.11 ± 0.37 | 15.63 ± 0.51 | 19.07 ± 0.27 |
| 300mM | 40.98 ± 0.21 | 33.49 ± 0.26 | 29.45 ± 0.43 | 29.38 ± 0.41 | 42.88 ± 0.25 | 39.67 ± 0.23 |
| 400mM | 71.94 ± 0.18 | 67.79 ± 0.84 | 65.65 ± 0.45 | 47.00 ± 0.35 | 54.65 ± 0.23 | 60.36 ± 0.28 |
| 500mM | 97.68 ± 0.41 | 93.62 ± 0.93 | 91.19 ± 0.51 | 88.98 ± 0.43 | 78.90 ± 0.15 | 76.93 ± 0.36 |

**Table 2**. Heavy metal removal efficiency for the different concentration of different heavy metals.

and strengthen the material. Hence, *B. beijingensis* has the same effect, but the prior research with *Shewanella* provides a strong foundation for investigation.

The mortar cubes OBCE showed around 5.92% improvement in compressive strength at 3 days with respect to OBC mortar cubes, Mortar cubes BC + CBE showed around 10.53% improvement in compressive strength at 3 days with respect to OBCE mortar cubes, while the mortar cubes BC + CBE showed around 16.45% improvement in compressive strength at 3 days with respect to OBC mortar cubes. The improvement in the compressive strength at 7 days for the mortar cubes are as; OBCE cubes showed 3.85% improvement in compressive strength with respect to OBC cubes, BC + CBE cubes showed 16.63% improvement in compressive strength with respect to OBCE cubes, while cubes BC + CBE showed 20.48% improvement in compressive strength with respect to OBC cubes. The improvement in the compressive strength at 28 days for the mortar cubes are as; OBCE cubes showed 7.99% improvement in compressive strength with respect to OBC cubes, BC + CBE cubes showed 44.99% improvement in compressive strength with respect to OBCE cubes, while cubes BC + CBE showed 52.98% improvement in compressive strength with respect to OBC cubes.

This explains the behaviour of the increased compressive strength in cement mortar cubes prepared with microbial cells and microbial enzyme at the age of 28 days. *B. beijingensis* significantly improved the compressive strength of cement mortar cubes. Therefore, it was determined that the consolidation of the pores inside the cement mortar cubes with microbiologically induced calcium carbonate precipitate is the main reason of the rise in compressive strengths. Addition of *B. beijingensis* has a beneficial impact on the compressive strength of cement mortar. Certainly, *B. beijingensis* not only offers a nucleation site for calcite precipitation, but it also produces an environment that is alkaline, which promotes the formation of calcite.

## Crack remediation test

Visible crack remediation was successfully achieved, with images capturing the progressive healing and gradual reduction in crack width presented in Table 3. Notably, the set treated with bacterial crude enzyme exhibited faster healing (7 days) compared to the set with bacterial cell suspension (10 days). The direct application of bacterial enzyme, alongside the consolidation solution, proved to be more effective in achieving complete healing within a shorter timeframe. The images post-healing revealed micro-cracks sealed with white precipitates, indicative of calcium carbonate formation. This successful crack remediation underscores the potential of *B. beijingensis* in enhancing the durability and integrity of cement mortar structures (Fig. 11).

The results in this study indicate that the crack closing method seems to have more efficiency in the remediation of crack width compared to the case of self-healing method, which heals the crack from inside. Wang et al. [50] described that concrete made from bacteria spores showed a maximum repair rate of 80% at the

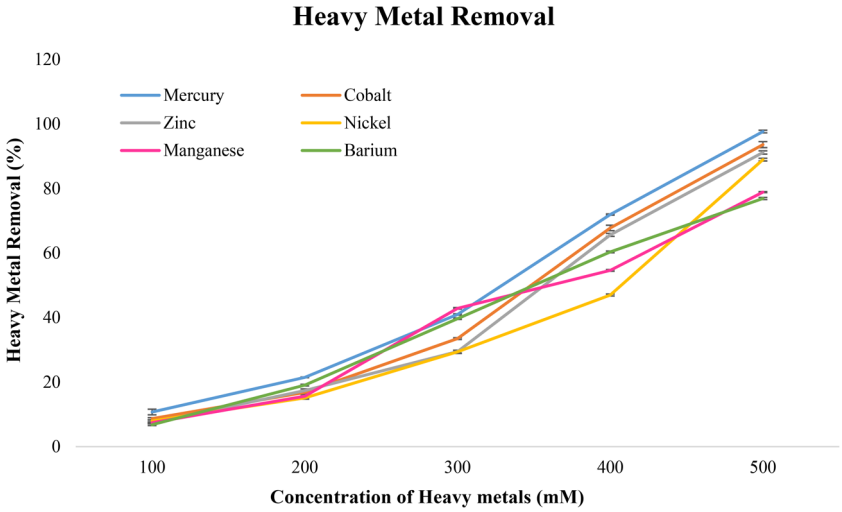


**Figure 9**. Heavy metals removal efficiency of urease produced by *Bhargavaea beijingensis* (Error bars show SD).

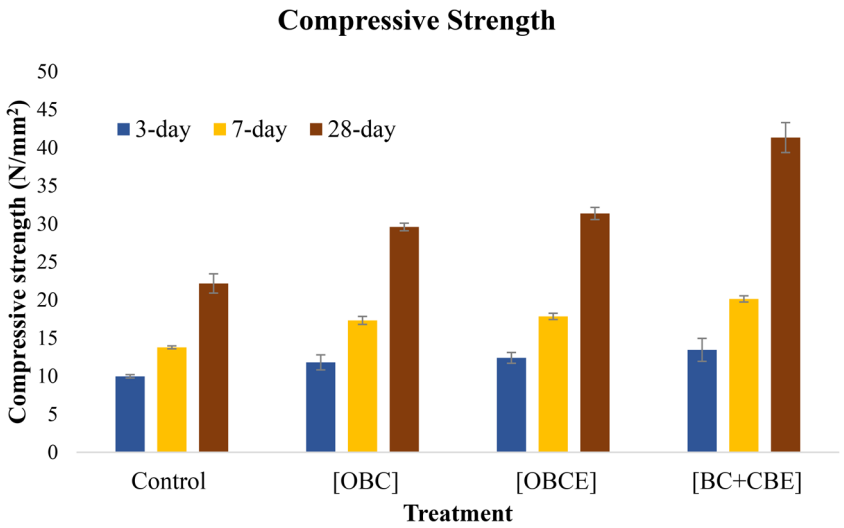


**Figure 10**. Compressive strength measurement of the cement blocks prepared using *Bhargavaea beijingensis* (Error bars show SD).

completion of 28 days. Also, Luo et al. [51] confirmed that the crack healing ratio extended from 50 to 70% for the average crack width of 0.3–0.5 mm even after 20 days of treatment. It is possible to claim that the localized repairing, crack closing, repairing agent, leading to achieve a high rate of crack healing.

Jongvivatsakul et al. [52] explained the remediation of crack on mortar surface was up to 84% when the mortar was treated for 14 days. After that, the percentage of crack healing increases insignificantly to 85% at the end of 20 days. A high concentration of the repair agent may be obtained through localized repair, or crack sealing, which would result in a high rate of crack healing.

In our research we have used two approaches; one is bacterial cell suspension and other is using crude enzyme. In that the crude enzyme remediated crack faster as compare to bacterial cell suspension and this will open the novel method of crack remediation faster using the crude enzyme produced by the bacterial isolate *B. beijingensis*.

## Conclusion

The study effectively isolated and identified a bacterial strain, *B. beijingensis*, that produces urease, from soil samples taken from a mine containing calcareous bricks. This isolate exhibited its capacity for MICP over a range of tests. *B. beijingensis* demonstrated a significant level of urease activity and the ability as MICP to precipitate calcite. The presence of calcite in the precipitates was confirmed using FTIR and SEM-EDX. This provides more evidence of the MICP potential of *B. beijingensis*. The sand solidification test provided evidence of the efficacy of *B. beijingensis* in compacting sand particles. This indicates the possible use of this material in the process of soil stabilization. In the investigations on heavy metal removal, the bacterial isolate showed efficient precipitation of many heavy metals. This underscores the capacity of *B. beijingensis* in the process of bioremediation for the contaminated locations. The compressive strength testing shown a notable enhancement in the mortar cubes treated with *B. beijingensis* and its enzyme. This demonstrates its capacity for bio-cementation in construction applications. In summary, this research showcases the considerable capabilities of *B. beijingensis* as a bacterium that can be used for MICP. This bacterium has the potential to be applied in several fields such as bio-cementation, heavy metal removal, and soil stabilization. Additional investigation is required to enhance the utilization of this bacteria and its enzymes for extensive commercial purposes. This research unveils the

| | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Bacterial cell suspension + consolidation solution | | | | Crude bacterial enzyme solution + consolidation solution | | | |
| | Crack width (mm) | | | | Crack width (mm) | | | |
| Days | Before | After | Healed area | Healing ratio (%) | Before | After | Healed area | Healing ratio (%) |
| 0 day | 3.2 | 3.2 | 0 | 0% | 3.2 | 3.2 | 0 | 0% |
| 2nd day | 3.2 | 2.9 | 0.3 | 9.37% | 3.2 | 2.4 | 0.8 | 25% |
| 4th day | 2.9 | 2.4 | 0.5 | 17.24% | 2.4 | 0.3 | 2.1 | 87.5% |
| 7th day | 2.4 | 1.1 | 1.3 | 54.16% | 0.3 | 0 | 0.3 | 100% |
| 10th day | 1.1 | 0 | 1.1 | 100% | - | - | - | - |

**Table 3**. Crack width measurements of mortar specimens.


**Figure 11**. Crack remediation experiment.

remarkable potential of *B. beijingensis* as a novel and sustainable MICP agent, opening exciting possibilities for environmental remediation, infrastructure development, and soil health improvement. Further research and development efforts are warranted to unlock its full potential and advance the field of bio-based solutions for a cleaner and more sustainable future.

## Data availability

The 16s rRNA sequencing data used in this study are available in the NCBI archive by the accession numbers of OP120916.1. All other datasets generated during and/or analysed during the current study are available from the corresponding authors upon reasonable request.

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## Author contributions

M. G. wrote the main manuscript text and did the complete research work.D.D. provided the resources for the work, supervised the research and B.J. and R.P reviewed the manuscript S.V. provided instrumental and laboratiry facilities for the research work.

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## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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