Biomineralization and High Calcite Formation by *Bacillus*: Implications for Fiber Bio-Concrete Applications

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Abstract

Concrete is essential in construction, yet its susceptibility to cracking is a major concern. This study explores biomineralization through Microbially Induced Calcite Precipitation (MICP) as an eco-friendly self-repairing solution that uses microbes to generate calcite for filling cracks. The research aimed to identify high calcite-producing *Bacillus* strains and optimize conditions for calcite formation to minimize cracking and maintenance requirements. Soil samples from Pakistan were used to isolate *Bacillus* strains via the ureolytic pathway. The process involved bacterial isolation, calcite production screening, and confirming ureolytic activity. Six urease-producing strains were identified, with *Bacillus mojavensis* and *Bacillus pasteurii* demonstrating the highest precipitation rates 27.39% at pH 7.5 and 35.90% at pH 8, respectively. *Bacillus pasteurii* showed notable resilience in harsh conditions, while *Bacillus mojavensis* improved concrete strength by 20% and *Bacillus* pasteurii by over 50%. Ureolytic bacteria enhances the autonomous healing, durability, and sustainability of concrete by effective calcium carbonate formation.

Keywords: *Bacillus*; MICP; Bio-Calcite; Mineralization; Concrete auto-healing.

1. Introduction

Concrete is essential in modern constructions because of its strength and versatility [1]. However, It tends to crack during shrinkage and hardening, which accelerates the deterioration of the material as well as the corrosion of any steel reinforcements [2]. While there are new advancements in crack prevention, solving these issues in large-scale constructions is a Herculean task [3]. Nature-inspired innovative solutions include autonomous healing concrete that uses chemical agents embedded in fibers or microcapsules that release when cracks form from nature-inspired autonomous healing mechanisms, though issues of timing and durability remain [6-8]. Autonomous healing concrete technology uses the bacteria notorious for producing calcium carbonate crystals during metabolism which strengthens the concrete while needing little infrastructure to implement the healing mechanisms [4]. Improved mechanical strength and healing effectiveness is obtained with the addition of natural fibers like hemp or jute, and encapsulated bacteria [5]. With this method, micro-cracks are healed, and porosity and strength are increased [6], while corrosion is mitigated through bacterial activity [7]. *Bacillus* bacteria are especially effective due to their resilience in alkaline and humid environments, ensuring lasting autonomous healing properties in concrete [8].

Bio-mineralization, driven by microbial activity, leads to the precipitation of over 60 minerals, predominantly calcium carbonate ($CaCO_3$) [9]. This process generally occurs extracellularly, form-

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ing inorganic crystals, although intracellular mineralization is also possible [10, 11]. While primarily inorganic, the minerals may contain trace organic compounds that affect their formation [12]. Bio-mineralization encompasses three mechanisms: biologically controlled, biologically influenced, and biologically induced, with microbially induced calcium carbonate precipitation (MICP) being particularly notable [13, 14]. MICP is used in environmental remediation and construction, where microbes release metabolic byproducts like CO_3^{2-} that react with Ca^{2+} ions to precipitate $CaCO_3$ [26]. *Bacillus pasteurii* enhances this process through ureolysis, which raises pH and creates nucleation sites for calcite deposition [15, 16]. While studying biological ammonia production via ureolysis has its difficulties, it presents promising opportunities to look into other methods [17]. Achieving efficient $CaCO_3$ precipitation and optimization of MICP additionally requires understanding how microbes interact with their environment [18]. The *Bacillus* genus is known to efficiently induce calcite formation, as bacterial cell walls serve as nucleation centers [19]. In addition, the calcium carbonate polymorphism influenced by the fundamental bacterial mineral constituents illustrates the intricate nature of microbial mineralization [20].

Microbially Induced Calcite Precipitation (MICP) is an innovative biotechnological advancement with applications in farming, healthcare, environmental sustainability, and engineering, thus promoting sustainability in these sectors [21]. In agriculture, MICP enhances crop yield and improves soil erosion while better nutrient retention and soil health [22]. It aids in food safety by acting as a biosensor for environmental contaminants. In healthcare, MICP supports tissue regeneration by bio-fabricating bone and dental substitutes [23]. Moreover, MICP aids in bio-mineralization of carbon dioxide, thus capturing carbon in a sustainable manner and combating global warming [24]. In construction, MICP is added to fiber bioconcrete to increase its mechanical properties and water resistance, self-healing, and crack mitigation therefore strengthening the structure [25]. Studies show that MICP bacterial-based fiber reinforcement achieves 85% healing in pre-cracked specimens within a week and supports eco-friendly construction practices with a lower environmental footprint [26]. MICP has over 90% precipitation efficiency while *Bacillus* species account for 70% of the calcite formation, thus strengthening sustainability and resilience.

This research explores non-pathogenic, high calcite-forming *Bacillus* bacteria for Microbial Induced Calcite Precipitation (MICP) of concrete cracking in order to provide an environmentally safe and sustainable path. The objective is to establish and define ureolytic *Bacillus* isolates with dominant calcite-forming potential to increase the concrete's resilience. The project aims to validate *Bacillus*-induced calcite precipitation through the development of fiber-reinforced bio-concrete, integrating microbial solutions into construction practices to reduce reliance on harmful chemicals. Key tasks include characterizing selected *Bacillus* strains, assessing their calcite precipitation potential, and analyzing their performance in various concrete compositions to optimize durability and sustainability.

2. Materials and Methods

2.1. Bacterial Preparation, Identification, and Growth Assessment

Four bacterial strains were isolated from 1-gram soil samples from Bahria Town, Phase 8, Rawalpindi, Pakistan, using a urea agar-based medium with phenol red indicator to detect urease production. Fuchsia-colored colonies were purified by the streak plate method on nutrient agar under sterile conditions and stored at −80°C in a glycerin solution. For strain identification, 16S rRNA gene sequencing was performed using the 27F/1492R primers on an ABI 3730XL DNA Analyzer, and a phylogenetic tree was constructed using MEGA 11 with ClustalW alignment and the Neighbor-Joining method with the Kimura-2-parameter model, followed by bootstrap verification with 1000 replicates for accuracy. Growth optimization involved supplementing the medium with 1M urea and 0.5M $CaCl_2$, assessing bacterial growth initially and after 24 hours. The MICP activity, vital for calcite precipitation, was evaluated by incubating bacteria in a reaction solution with urea and calcium chloride, using gravimetric analysis (dry weight measurement) and EDTA titration for quantification. Additionally, bacterial tolerance to varying pH (7–14) and NaCl concentrations (0–5%) was tested using nutrient broth with pH adjustments made using NaOH/HCl buffers, with growth monitored at OD600 every 6 hours over a 48-hour period.

2.2. Selection, Preparation, and Testing of Fiber Bio-Concrete Samples

Natural fibers, including sisal, coir, bamboo, flax, banana, hemp, and jute, are noted for enhancing concrete strength. Jute was chosen for this study due to its high tensile strength, durability, and water absorption capacity, making it suitable for improving concrete performance. Nine concrete cylinders (4x8 inches) were prepared by mixing Ordinary Portland Cement (OPC) with a water-to-cement ratio of 0.45, fine and coarse aggregates, fiber, bacteria, and chemicals, then molded and cured for 28 days. Jute fibers were added at 0.5% of the cement composition and were surface-treated using an alkali treatment (5% NaOH) to enhance bonding with the concrete matrix. The fibers were randomly dispersed throughout the mix to ensure uniform reinforcement. After curing, compressive and tensile strength tests were conducted using a Universal Testing Machine (UTM) at a loading rate of 0.5 MPa/s, following ASTM C39 and ASTM C496 standards, with failure loads recorded for analysis. Each test was conducted with three replicates per condition to ensure statistical reliability. Crystal formation on concrete surfaces was documented using a digital camera to understand mineral deposition. Additionally, bacterial calcium carbonate precipitates were analyzed by X-ray diffraction (XRD) with a scanning range of 5°–80° 2θ and a step size of 0.02° to assess mineral composition, phase purity, and structure.

3. Results

3.1. Isolation and Characterization of Urease-Producing Bacillus Strains for Microbially Induced Calcite Precipitation

Two bacterial strains isolated from soil samples in Rawalpindi, Pakistan, were screened for urease production, with strains 68, and T1 exhibiting positive results (Fig. 1). Strain T1 demonstrated the fastest urease activity, producing visible results within an hour, while the others took 24 hours. Phylogenetic analysis classified these ureolytic strains as members of the strain 68 as B. mojavensis, and strain T1 as B. pasteurii. In growth optimization studies, T1 displayed the highest optical density (OD) after 24 hours, indicating robust growth. For Microbially Induced Calcite Precipitation (MICP) efficiency, strain 68 led in calcite production, achieving 35.90% precipitation at pH 6.5, followed by T1 with 27.39% at pH 7. Salt and alkali resistance testing showed that strain 68 was highly resilient, maintaining significant growth at 5% NaCl and up to pH 11 (Table 1), highlighting its suitability for applications requiring durability in extreme conditions.

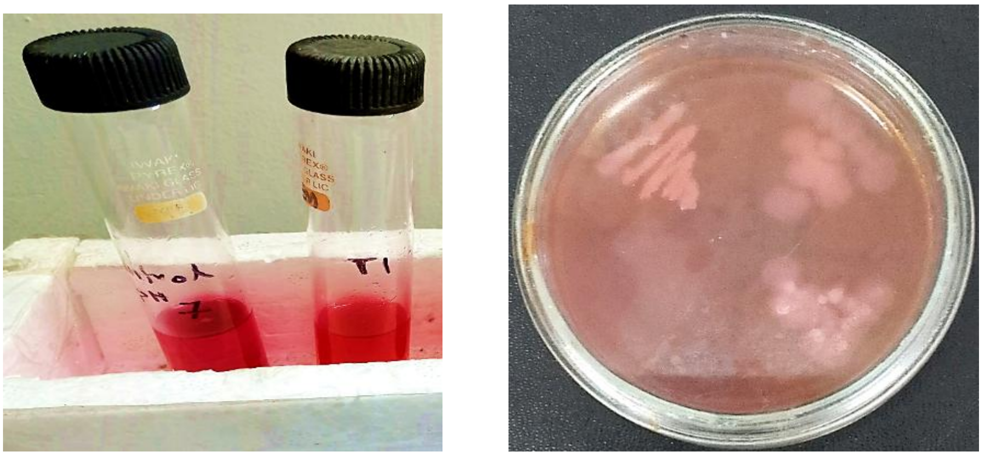

Figure 1. Urease Activity and Bacterial Purification of Strain 68 and T1.

Table 1. Tests performed on Isolated Strains.

Parameter	Strain 68	Strain T1
Urease Positivity	+	+
Reaction Time	**24 hrs.**	**2 hrs.**
Identification	*Bacillus mojavensis*	*Bacillus pasteurii*
Optical Density (OD) at 0 hrs	0.007	0.015
Optical Density (OD) at 24 hrs	0.432	1.014
Calcite Weight (g) without filter	0.546	0.482
Percentage without filter (%)	0.546%	0.482%
Calcite Weight (g) with filter	0.196	0.132
Percentage with filter (%)	27.39%	35.90%
Salt Tolerance (OD600 at 0% NaCl)	0.820	0.862
Salt Tolerance (OD600 at 1% NaCl)	0.842	0.724
Salt Tolerance (OD600 at 3% NaCl)	0.464	0.461
Salt Tolerance (OD600 at 5% NaCl)	0.431	0.352
pH Tolerance (OD600 at pH 7)	0.166	0.162
pH Tolerance (OD600 at pH 14)	0.047	0.049

3.2. Outcomes of Fiber Bio-Concrete Cylinder Evaluation

The evaluation of the nine concrete cylinders, each with a diameter of 100 mm and a length of 200 mm, revealed significant improvements in both compressive and tensile strengths, as well as in the characteristics of calcium carbonate crystal growth and precipitation. The concrete mix was formulated with a ratio of 1:2:4:0.6 (C:S:A), consisting of 4.6 kg of cement, 9.6 kg of sand, 19.5 kg of aggregates (0.75-inch size), 2764.6 ml of water, and 0.5% of jute fiber, combined with a 100 ml bacterial solution (107 CFU/ml of *Bacillus* strains 68 and T1), 110 g of urea, and 100 g of calcium chloride. The average compressive strength for the Control samples was recorded at 5.0885 MPa, which increased to 7.0265 MPa for sample set 68, and peaked at 12.3275 MPa for the T1 samples. Similarly, the split tensile strength improved from 3.323 MPa in the Control group to 6.514 MPa in the T1 samples, with corresponding increases in load-bearing capacities. Crystal growth analysis indicated a range of calcium carbonate crystals, with larger crystals associated with higher strength values.

4. Conclusions

This study focused on isolating, identifying, and evaluating urease-producing *Bacillus* strains from soil samples in Rawalpindi, Pakistan, highlighting their ability to precipitate calcium carbonate and their resilience in salt and alkaline conditions for bioconstruction applications. Key findings include:

- Two urease-producing *Bacillus* strains were isolated, *Bacillus mojavensis* and *Bacillus pasteurii* achieving the highest calcite precipitation rates of 35.90% and 27.39%, respectively, at optimal pH levels of 6.5 and 7.0. Strain T1 (*B. pasteurii*) exhibited rapid urease activity, showing results within 2 hours, while other strains took 24 hours. Strain T1 maintained significant growth at salt concentrations of up to 5% NaCl, demonstrating strong environmental adaptability.

- Concrete cylinders incorporating strain T1 (*B. pasteurii*) achieved a compressive strength of 12.33 MPa and a split tensile strength of 6.51 MPa, representing increases of 142% and 96% over control samples, which had strengths of 5.09 MPa and 3.32 MPa, respectively.

Bacillus pasteurii's high urease activity and reliable calcite production position it as an excellent candidate for large-scale microbial-induced calcite precipitation applications, bolstering concrete durability and sustainability. This research advocates for incorporating *Bacillus* strains into bioconstruction practices to enhance environmental resilience and mechanical properties.

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