

Bioconsolidation on Stone Cultural Heritage Surfaces by Bacteria

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Abstract

Stone-based cultural heritage undergoes progressive deterioration due to natural weathering, pollution, salt crystallization, moisture fluctuations, and biodeterioration. Traditional chemical consolidants often show limited compatibility, aesthetic changes, pore blockages, internal cracking, and irreversible alterations. These limitations have led to increasing interest in bacterial bioconsolidation based on the Microbiologically Induced Carbonate Precipitation (MICP) approach. Such bacteria being either indigenous or non-native species had been reported to precipitate calcium carbonate mineralogically similar to the original stone substrate. This enables the internal consolidation, crack sealing, reduced porosity, and mechanical strength enhancement on the treated surface. As had been observed in multiple studies and in situ applications worldwide, these carbonatogenic phenomena had been reported to produce stable calcium carbonate polymorphs, with enhanced cohesion and durability and reduced water permeability. This review focuses on recent advances in bacterial bioconsolidation, underlying mechanisms, compares its performance with conventional consolidants, and identifies future directions for research and application in cultural heritage conservation.

Keywords: Bioconsolidation, Stone surfaces, cultural heritage, Carbonatogenic bacteria, Calcium carbonate precipitation, Microbiologically Induced Carbonate Precipitation (MICP)

1. INTRODUCTION

Stone surfaces in cultural heritage such as monuments, painted murals, sculptures, and archaeological artefacts are continuously threatened by natural and anthropogenic factors. Stone-based artworks and architectural elements are particularly vulnerable, as prolonged exposure to weathering, pollution, microbial activity, and natural aging results in progressive structural weakening. Carbonate stones such as limestone and marble, widely employed in historic monuments across Europe and South Asia, frequently exhibit dissolution of the crystal matrix, increased porosity, internal cracking, and reduced mechanical resistance. These deterioration processes are further intensified in outdoor settings, where cycles of moisture, salt crystallization, and climatic fluctuations accelerate mineral loss, often leading to irreversible damage. Among these agents, biodeterioration is especially critical, as high humidity, temperature variations, restricted ventilation, and elevated pollutant loads promote the

proliferation of microorganisms such as bacteria, fungi, algae, and lichens - that actively contribute to surface decay (Soffritti et al., 2019).

Conservation efforts have traditionally relied on synthetic or inorganic consolidants intended to enhance cohesion, restore weathered surfaces, and reduce water absorption while maintaining vapour permeability (Spairani-Berrio et al., 2023). Commercial consolidants such as potassium aluminate, sodium and potassium silicates, and magnesium-, zinc-, or aluminium-fluorosilicates have been widely used; however, their interaction with mineral substrates often results in increased porosity, reduced mechanical strength, colour changes, and the formation of superficial microlayers that may accelerate long-term deterioration. Other widely used treatments, including alkoxy silanes, nano-silica, and ethyl silicate, can improve certain mechanical properties but frequently occlude pores or generate internal gel-like layers prone to cracking, detachment, or incompatibility with highly porous or clay-rich substrates. Other organic polymer-based consolidants likewise show limitations, as their chemical mismatch with stone matrices often results in irreversible alterations such as yellowing, darkening, and long-term instability (Spairani-Berrio et al., 2023).

The limitations of chemical consolidants have highlighted the need for sustainable and substrate-friendly alternatives. This shift has led to growing interest in microbial bioconsolidation, a biologically driven approach that precipitates calcium carbonate and offers high compatibility with minimal intervention (Rinaldi, 2025; Spairani et al., 2021). Bacteria provide several advantages over chemical treatments. Their microscopic size enables penetration into fine pores and fissures, allowing internal consolidation rather than forming superficial layers that may crack or detach (De Muynck et al., 2010). They can also tolerate fluctuations in environmental and nutrient parameters, and can be selected from indigenous communities, enhancing ecological safety (Jroundi et al., 2017). Collectively, these characteristics justify the use of bacteria as eco-friendly, sustainable, compatible and minimally invasive agents in cultural heritage consolidation. This review focuses on bacterial bioconsolidation as a compatible approach for stone cultural heritage surfaces.

2. Mechanism of bioconsolidation in cultural heritage

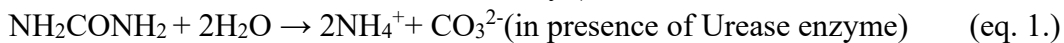
Bacteria used in the bioconsolidation of cultural heritage, particularly stone monuments, sculptures, and wall paintings, possess specific biochemical and metabolic properties that enable them to precipitate stable calcium carbonate (Jroundi et al., 2021). Because the CaCO_3 produced closely resembles the natural mineral components of stone, these microorganisms are especially effective for strengthening weakened substrates. Microbiologically Induced Carbonate Precipitation (MICP) or carbonatogenesis is the principal mechanism underlying bioconsolidation. This technique mimics natural geological processes to stabilize and strengthen weathered, porous materials (Marvasi et al., 2020; Perito & Mastromei, 2011).

The first essential stage in MICP is bacterial adherence to the surface of cultural heritage materials. Bacterial adherence is critical for creating a coherent calcite layer that strengthens weathered, porous surfaces without changing their porosity or colour (Castanier et al., 2000; Jroundi et al., 2021). This stage includes: (i) transport to the mineral surface, (ii) initial adhesion, (iii) attachment via extracellular polymeric substances (EPS) or fibril formation, (iv) colonization of bacterial cells (i.e., biofilm formation) (Rodriguez-Navarro et al., 2012).

The second stage involves the metabolic mechanisms responsible for precipitation of calcium carbonate, which may occur through active or passive pathways. These mechanisms directly or indirectly contribute

to the mineral consolidation of cultural heritage surfaces. Active mechanism (bio precipitation) involves direct metabolic processes carried out by living bacteria. The bacteria actively consume nutrient broth (often including calcium sources) to catalyze the reaction. Active MICP is characterized by enzymatic mediation, intentional alkalisation, and rapid, controlled CaCO₃ formation. Some of pathways include:

- **Ureolysis (Urea Hydrolysis):** This is the most common active pathway for MICP. The ureolytic bacteria possessing negatively charged cell surfaces and urease activity can hydrolyze urea (from cementation solution) to generate carbonate and ammonium ions (eq. 1.), thereby facilitating the binding of calcium ions to the cell surface (eq. 2.) and CaCO₃ precipitation (eq. 3.) (De Muynck et al., 2010). The cementation solution applied on stone surface contains urea, and acetate or chloride salts of calcium, as stoichiometric reactants for providing nitrogen and calcium for MICP reactions. The concentration of cementation solution critically influences the MICP reaction, the Ca²⁺ concentration influences urease activity (Luan et al., 2026; Bicer et al., 2026; Helmi et al., 2016b).



- **Metabolic by-products:** Processes such as denitrification and nitrate reduction also raise alkalinity and facilitate carbonate mineral formation.
- **Use of Indigenous or Introduced Bacteria:** Active bioconsolidation treatments may involve indigenous (autochthonous) bacteria or introduced (allochthonous) strains, depending on ecological considerations.

In contrast, passive mechanism depends on the non-metabolic properties of microbial cells and their residues. Here, bacteria do not actively change the environment; rather, their cell walls, extracellular polymeric substances (EPS), dead cells, and biofilm residues provide high surface area sites that promote CaCO₃ nucleation. Passive precipitation involves slower geochemical transformations such as ammonification and sulfate reduction of organic matter in the presence of calcium, leading indirectly to the formation of carbonate ions, and further resulting in calcium carbonate formation (eq. 2.) (De Muynck et al., 2010). MICP is mainly governed by pH, concentration of calcium ions, dissolved inorganic carbon, and availability of nucleation sites. Based on these factors, precipitation pathways include sulfate reduction, photosynthesis, methane oxidation, ammonification, denitrification, sulfate reduction, and ureolysis (Ortega-Villamagua et al., 2020).



Supersaturation occurs when carbonate ions react with calcium salts from cementation solution on the nucleation site (eq. 2.). Because passive mechanisms lack strong metabolic alkalisation, CaCO₃ formation is slower and more diffusion-controlled. MICP reactions directly precipitate calcium carbonate mineralization (eq. 3.) within the pore network to enhance structural integrity without chemical alteration or damage to stone substrate (Luan et al., 2026).

The third stage consists of CaCO₃ nucleation on bacterial cells. Dead or live bacterial cells act as nucleation sites where Ca²⁺ and CO₃²⁻ accumulate and deposit to form CaCO₃ crystals directly on the bacterial cell wall. Depending on the cell surface properties of bacteria, especially proteins and extracellular polymeric substances (EPS), the morphology and mineralogy of CaCO₃ may vary, including amorphous calcium carbonate, calcium carbonate monohydrate, calcium carbonate hexahydrate, vaterite (hexagonal), aragonite (needle-like structure), or calcite (rhombohedral) (Ortega-Villamagua et al., 2020).

The final stage leads to consolidation. The formation of CaCO₃ contributes to sealing micro-cracks, preventing water ingress into the stone matrix, reducing porosity, and enhancing mechanical strength. This biological healing process, driven by carbonatogenic bacteria, also mimics natural stone formation mechanisms and results in a mineral product that closely resembles the stone substrate (Marvasi et al., 2020).

MICP also serves as an eco-friendly alternative to the traditional consolidants (see Table1).

Table 1: Advantages of bioconsolidation approach over conventional methods

Characteristics	Conventional Materials	Bioconsolidation
Material compatibility	Often low	High (CaCO ₃ matches stone)
Aesthetic alteration	Common	Minimal
Environmental impact	High (Synthetic material used)	Low (Eco-friendly)
Reversibility	Limited	More reversible
Depth penetration	Variable, often poor	Better (in situ bioconsolidation)
Risk of cracking	High	Low
Durability	Moderate	High due to natural calcite growth
Cost	Variable	Low (for long term)

3. Bacterial Species for Cultural Heritage Bioconsolidation

A wide range of bacteria—including *Bacillus*, *Lysinibacillus*, *Sporosarcina*, *Myxococcusxanthus*, *Pseudomonas*, *Pantoea*, *Cupriavidus*, and *Acinetobacter* sp. have been observed to induce CaCO₃ precipitation in cultural heritage (mentioned in Table 2).

Table 2: Applications of bacterial bioconsolidation in Cultural Heritage

Cultural Heritage Substrate	Bioconsolidation Approach	Major Outcomes	Reference
Weathered bricks of Han Dynasty Dingtao Royal Mausoleum, China	<i>Sporosarcinapasteurii</i> (DSMZ 33 / ATCC 11859); one-phase low-pH MICP; cementation solution (urea + calcium acetate)	Unconfined compression strength improved by 5–6 times; CaCO ₃ (calcite + vaterite) filled pores and bonded cracks; bio-slurry effective for fracture repair	Luan et al., 2026
Lime mortar for heritage structures (in laboratory)	<i>Bacillus halodurans</i> (live & dead cells at varying concentrations)	Live cells initially reduced carbonation (7–14 days) but by day 28, 10 ⁹ cells/mL increased carbonation depth and compressive strength due to biomineralization and crack self-healing; dead cells showed weaker effects.	Srivastava et al., 2026
Marble surfaces (in laboratory)	<i>Bacillus subtilis</i> ATCC 6633 (ureolytic); live and dead cells; CaCl ₂ and	Live cells produced stable calcite/aragonite, dead cells + calcium acetate favoured vaterite; biocalcification sealed micro-	Bicer et al., 2026

	Ca(CH ₃ COO) ₂ ; CO ₂ pre-treatment	cracks and enhanced surface healing; CO ₂ pre treatment improved nucleation and calcification uniformity.	
Stone wall cracks, Kaifeng Zhouqiao Sites, China	Recombinant Bacillus subtilis expressing B. pasteurii urease gene cluster (Strain B) and native B. pasteurii (Strain A)	Gene-optimized strain showed 44.6% higher urease activity and 2× calcite production; optimized cementation ratios; repaired 2–4 cm cracks; strengthened compressive & flexural properties. Demonstrated denser, more stable CaCO ₃ and superior MICP-based stone crack repair.	Zhang et al., 2026
Calcarenite, Travertine, Marble	Bioconsolidation via carbonatogenic Lysinibacillus fusiformis 3.20	Highly effective consolidation in Calcarenite, and Travertine, with substantial CaCO ₃ precipitation. Least effective in marble due to its low porosity and limited pore connectivity. Efficiency strongly governed by pore size, shape, connectivity, and overall porosity. Calcarenite showed the greatest penetration depth. Mechanical properties (stiffness, strength) improve with reduced effective porosity, while shear resistance remains largely unchanged.	Benedetti et al., 2025
Acid-damaged Dungri marble (Makrana)	Ureolytic Bacillus paramycoides MD6	MICP filled pits caused by acid-induced granular disintegration; formed uniform CaCO ₃ coating restoring lost mass; weight gain increased with treatment duration; demonstrated effectiveness even under harsh acidic conditions; improved mechanical integrity and surface aesthetics.	Sidhu et al., 2025a
Limestone samples from Chicanná archaeological site, Campeche, Mexico	MICP treatment using ureolytic, carbonatogenic indigenous strain Rothia halotolerans TM1B-475; comparison with M-3P medium and Ca(OH) ₂	Produced CaCO ₃ crystals confirmed as aragonite. Consistent reduction in detachment (peeling test) from long term up to 150 days. Surface hardness lacked a clear pattern, yet bacterial treatment showed the best final hardness improvement. Demonstrates feasibility of MICP consolidation under tropical environmental conditions.	Ortega-Morales et al., 2025
Acid-damaged Dungri marble (Makrana)	Synechocystis pevalekii BDHKU 35101 (photoautotrophic) used for MICP treatment applied on the artificial	Demonstrated rhombohedral calcite and spherical vaterite crystals on treated surfaces. Aesthetic properties (smoothness, gloss, texture) significantly improved.	Sidhu et al., 2025b

	acidic damaged surface		
Stone surfaces (in laboratory)	Sterile M-3P nutritional solution applied by spraying twice daily for 7 days; maintained at 20–30°C protected from sunlight	Improved surface cohesion, reduced water permeability, increased hydrophobicity; maintained vapour permeability and original colour; no aesthetic alteration.	Spairani-Berrio et al., 2023
Ancient Maya carved volcanic tuff stone at Copán, Honduras	Sterile nutritional solution (M-3P medium) applied to selectively stimulate indigenous bacteria	M-3P stimulation produced CaCO ₃ biocement that bonded well with original matrix, strengthening fragile areas. Bacterial EPS imparted hydrophobicity, reducing clay swelling and moisture-related stress.	Elert et al., 2021
Carbonate stones	M3P / MYXOSTONE nutritional solution to activate indigenous microbiota	Produced 1–1.5 µm natural CaCO ₃ coating (calcite/vaterite), well-adhered and penetrating pores; increased mechanical strength without color or porosity changes.	Spairani et al., 2021
Fresco mural paintings (plaster, mortar layers)	Bacillus licheniformis biomineralization using urea-based media as a urease source	Successful CaCO ₃ precipitation on fresco layers; improved cohesion of stratified surfaces; demonstrated applicability to complex artistic materials.	Helmi et al., 2016a
Degraded limestone, plaster, and decayed wall-painting samples	Immersion of deteriorated samples in sterile nutrient media inoculated with Bacillus sphaericus	Demonstrated effective consolidation of non-sterilized deteriorated wall-painting samples under controlled treatment conditions. Formation of calcite as the major mineral observed.	Helmi et al., 2016b
Globigerina limestone, Malta	Bacillus subtilis as high-urease biocalcifying agent for MICP	Demonstrated consolidation of deteriorated limestone; significant surface strengthening via CaCO ₃ precipitation.	Micallef et al., 2016
Marble	Urease-active Cupriavidus metallidurans ACA-DC 4073	Demonstrated strong CaCO ₃ biomineralization capacity; identified as a promising candidate for marble bioconsolidation.	Daskalakis et al., 2014
Historical gypsum plasters	Activation of native carbonatogenic bacteria via nutrient application	Superior consolidation performance compared to conventional chemical treatments; effective binding of plaster grains.	Jroundi et al., 2014
Limestone; in situ test at Angera Church	Dead Bacillus subtilis cells & Bacterial Cell Wall Fraction (BCF)	Induced crystal formation; reduced water absorption up to 16.7%; modest cohesion improvement; demonstrated passive biomineralization potential.	Perito et al., 2014
Calcarenite	(1) Inoculation of	Activation of native microbiota more	Jroundi et

stone	carbonatogenic bacteria (e.g., <i>Myxococcus xanthus</i>) (2) Activation of native bacteria using M3P	effective; consolidation enhanced with certain strains (e.g., <i>A. crystallopoietes</i>); identified optimal bacterial candidates based on CaCO ₃ production.	al., 2012
Calcitic substrates (marble, limestone), silicate substrates (glass, sandstone)	M-3P medium with <i>Myxococcus xanthus</i> , <i>Brevundimonas diminuta</i> , or native communities	Demonstrated mineralogy-dependent CaCO ₃ precipitation; both inoculated and native bacterial communities formed stable mineral coatings.	Rodríguez-Navarro et al., 2012
Calcarenite stone blocks, San Jerónimo Monastery, Spain	Nutritional solution with/without inoculation of <i>Myxococcus xanthus</i>	Treatment stimulated native heterotrophic, carbonatogenic bacteria; produced new calcite/vaterite cement; effective in situ consolidation of porous limestone.	Jroundi et al., 2010

4. Limitations and Future Prospects of Bacterial Bioconsolidation

Despite its growing recognition as a sustainable conservation strategy, bacterial bioconsolidation faces several limitations that currently restrict its widespread application. A major constraint lies in the sensitivity of biomineralization to environmental factors: temperature, humidity, pH, luminosity, and stone porosity, which makes it challenging to achieve consistent and uniform consolidation across varying stone surfaces. Treatments based on non-native bacterial cultures may introduce ecological uncertainties, as introduced strains may struggle to survive, compete with native populations, or unintentionally alter the existing microbial balance. In addition to these biological and environmental challenges, biochemical side-effects can complicate field applications. For instance, production of ammonia during urea hydrolysis can cause stone discoloration and environmental concerns, and the resulting ammonium may further oxidize into nitric acid through denitrifying bacteria (Dhami et al., 2014). Such issues highlight the importance of carefully choosing metabolic pathways and treatment conditions to avoid introducing new deterioration risks. Furthermore, while many studies report promising short-term improvements, long-term durability, water retention behaviour, and the stability of precipitated minerals under fluctuating climatic conditions remain insufficiently documented.

Looking ahead, the future prospects of bacterial bioconsolidation are remarkably strong, driven by advances in geomicrobiology, molecular monitoring tools, and site-specific treatment design. A particularly promising direction is the increasing emphasis on activating indigenous carbonatogenic bacteria rather than introducing foreign strains. Using native microbiota offers several advantages: these bacteria are already ecologically adapted to the stone's mineralogy, climate, and microenvironment; they often inhabit niches directly involved in natural carbonate cycling; and their activity integrates seamlessly into existing microbial communities, minimizing ecological disturbance. As been observed in multiple researches, selectively stimulating these indigenous bacteria with tailored nutrient formulations can promote CaCO₃ biocementation that is highly compatible with the original stone matrix, improving adhesion and reducing risks of biological imbalance. Future developments may refine such nutrient-

activation strategies for greater control over crystal polymorphs, distribution patterns, and precipitation rates. Moreover, interdisciplinary research integrating microbiology, conservation science, materials engineering, and environmental modelling can enhance the predictability of treatment outcomes under diverse climatic conditions. In the long term, the adoption of indigenous bacteria-based bioconsolidation may evolve into a sustainable, self-regulating conservation method capable of providing durable, material compatible reinforcement for a wide variety of heritage substrates.

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