



# Bacterial Calcium Carbonate Mineralization *in situ* Strategies for Conservation of Stone Artworks: From Cell Components to Microbial Community

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Calcareous stones have been widely used in artworks and buildings by almost all human cultures. Now, more than ever, the increased environmental pollution and global warming are threatening the stone cultural heritage. Weathering due to physical, chemical and biological factors results in monumental calcareous stone deterioration. These agents induce a progressive dissolution of the mineral matrix, increase porosity, and lead to structural weakening. Bacterial Calcium Carbonate Mineralization is a widespread naturally occurring process which in the last decades was proposed as an environmentally friendly tool to protect monumental and ornamental calcareous stones. The advantage of this treatment is that it mimics the natural process responsible for stone formation, producing a mineral product similar to the stone substrate. This mini review highlights the milestones of the biomineralization approaches with focus on *in situ* stone artworks protection. The strategies explored to date are based on three main approaches: (i) the use of allochthonous and (ii) autochthonous alive cells that, due to the bacterial metabolism, foster biomineralization; (iii) the cell-free approach which uses fractionated cellular components inducing biomineralization. We discuss the challenging aspects of all these techniques, focusing on *in situ* applications and suggesting perspectives based on recent advances.

**Keywords:** calcite biomineralization, biodeposition, bioremediation, stone conservation, stone microbiota, cultural heritage

## INTRODUCTION

Bacterial Calcium Carbonate Mineralization (BCCM) is a widespread natural process of many bacterial taxonomic groups in different environments, ranging from microscopic crystals to large geological formations (Boquet et al., 1973; Ehrlich, 2002; Zavarzin, 2002; Dupraz et al., 2009; Perito and Mastromei, 2011).

According to Hammes and Verstraete (2002), BCCM is regulated by four key factors: calcium concentration, concentration of dissolved inorganic carbon (DIC), pH, and the availability of nucleation sites. Bacteria can foster an alkaline environment and increase DIC through different autotrophic and heterotrophic metabolic pathways (Castanier et al., 1999; Dhami et al., 2014;

Zhu and Dittrich, 2016). If calcium ions and nucleation sites are available in the environment, BCCM then occurs.

Bacterial surfaces such as cell walls or esopolymeric substances (EPS), due to their metal binding properties, serve as nucleation sites and constitute particularly favorable templates for heterogeneous nucleation and crystal growth (Fortin et al., 1997; Douglas and Beveridge, 1998). The EPS act as matrix templates influencing CaCO<sub>3</sub> crystal morphology, polymorphism, spatial position and growth (Braissant et al., 2003; Tourney and Ngwenya, 2009; Ercole et al., 2012; Oppenheimer-Shaanan et al., 2016). CaCO<sub>3</sub> crystals usually grow on bacterial cell surfaces (Rivadeneira et al., 1998; Castanier et al., 1999). The polymorph produced (mainly calcite, aragonite and vaterite) depends both on environmental conditions and bacterial strains (Ben Omar et al., 1997; Rivadeneira et al., 1998; Brennan et al., 2004).

During the last decades, BCCM application was proposed as an environmentally friendly tool for conservation and reinforcement of monumental and ornamental calcareous stones (Oriol et al., 1993). Weathering by physical, chemical and biological factors increases the porosity and dissolution of the mineral matrix thus progressively weakening the structure (Tiano et al., 1999). Organic products used to reduce monument deterioration present several drawbacks related to incompatibility with the stone, while inorganic consolidants show poor performance (De Muynck et al., 2010). The advantage of a BCCM-mediated treatment is that it mimics the natural process responsible for stone formation, producing a mineral product similar to the stone substrate. The aim is dual: to provide a coherent CaCO<sub>3</sub> layer on the surface of deteriorated stone, protecting against the intake of water or chemicals, and to consolidate the inner, weakened structure. In literature a number of comprehensive reviews are available about biodeposition of CaCO<sub>3</sub> on stone and building materials, highlighting mechanisms, limitations, challenges, and perspectives of this technology (De Muynck et al., 2010; Dhami et al., 2014; Anbu et al., 2016; Nazel, 2016; Zhu and Dittrich, 2016; Castro-Alonso et al., 2019). In this mini review, we fill a literature gap, by focusing on current BCCM technologies for *in situ* cultural stone conservation. We highlight the typology of interventions and recent improvements of *in situ* applications and provide viewpoints based on recent advances.

## BCCM-Based Approaches for Cultural Stone Conservation

### Living Cells, Single Selected Bacterial Strain

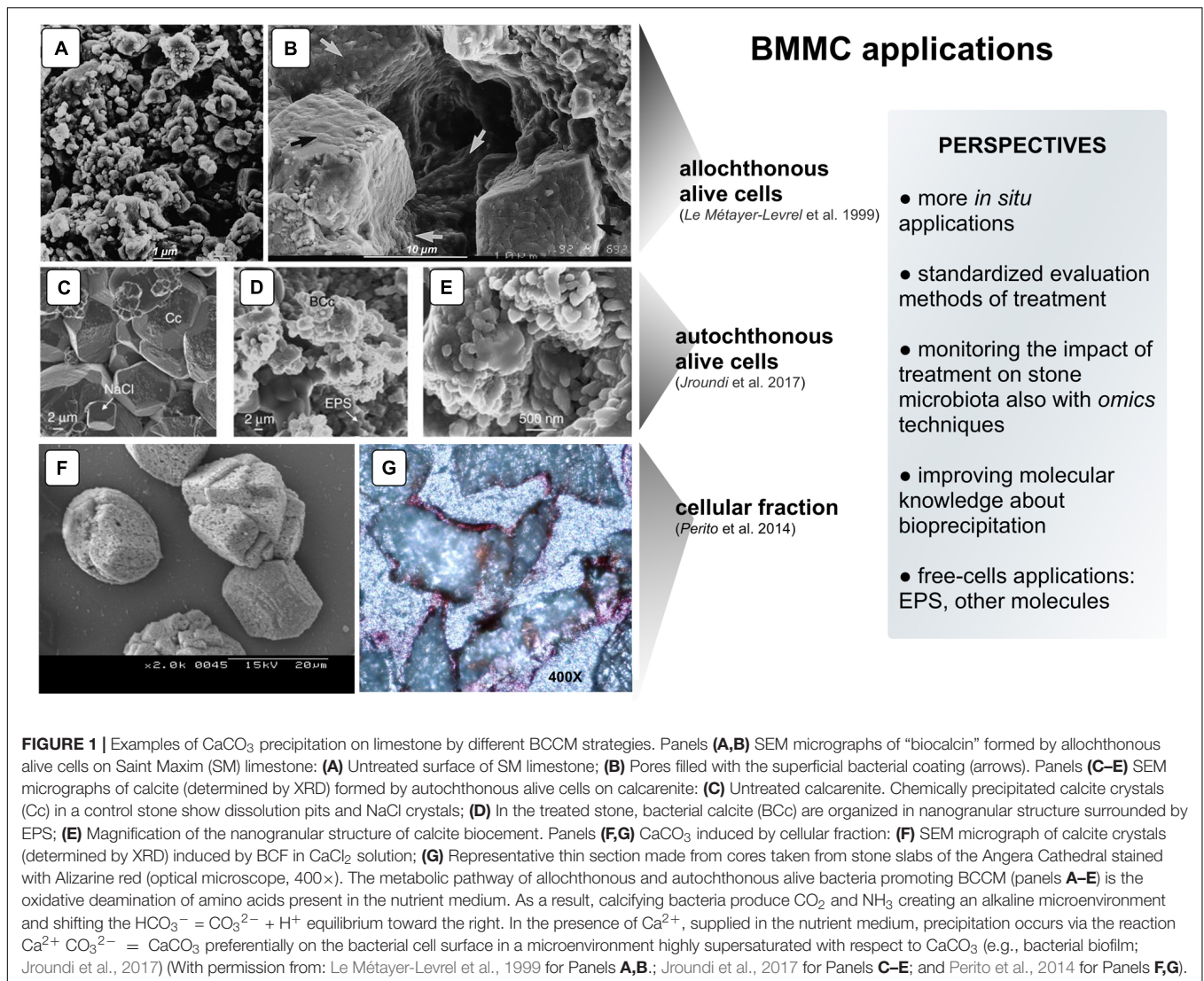
The application of BCCM for cultural heritage conservation was proposed by a pioneer French group that developed the so-called *Calcite Bioconcept* technology, covered by a now expired patent (Adolphe et al., 1990). This methodology was based on the application of cultures of selected bio-calcifying strains by spraying them on the stone surface and then feeding them by applications of a nutrient medium. The result was the formation of a new calcareous coating layer called *biocalcin*.

This few μm thick layer was coherent to stone and made of encrusted bacterial bodies mixed with CaCO<sub>3</sub> (Figures 1A,B). A preliminary screening of bacteria isolated from natural carbonate environments allowed the selection of a *Bacillus cereus* strain exhibiting the highest precipitation performance via the ammonification of amino acids (Table 1; Castanier et al., 2000). After testing it on limestone specimens, the technology was transferred to *in situ* applications (Le Métayer-Levrel et al., 1999). The first application was made in 1993, testing an area of 50 m<sup>2</sup> of the tower of the Saint Médard Church in Thouars. Evaluation of the treatment was carried out 6 months and 1 year after the application (Table 1). The treatment had no influence on the color or other aesthetical features and the water absorption rate was up to five times less. Following this approach, a number of façades of French historic and private buildings were treated by the Calcite Bioconcept Company (Castanier et al., 2000; Anne et al., 2010; De Muynck et al., 2010). No scientific reports can be found about these treatments. At the same time, several groups have worked to improve this system by isolating and testing different microorganisms, exploring different metabolic pathways and application conditions mainly in laboratory settings, showing, in many cases, similar results (reviewed by Nazel, 2016).

Over the last 20 years, a Spanish group of Granada made efforts to further develop this technology. They promoted the use of *Mixococcus xanthus*, a Gram-negative, non-pathogenic soil bacterium, to overcome drawbacks of previous treatments: the thin layer of the new formed bio-cement, the possible formation of endospores, and uncontrolled biofilm by *Bacillus* clogging stone pores. In an *in vitro* model, sterilized calcarenite slabs were immersed in a liquid medium containing *M. xanthus* and nutrients activating the ammonification of amino acids (Rodriguez-Navarro et al., 2003). Newly formed coherent carbonate cement of calcite grains was deposited into the pores without plugging them to a depth ≥500 μm. No myxospore formation was found in the tested culture media.

### Living Cells, Microbial Community of Stone

A further step in the development of this technology proposed by Jimenez-Lopez et al. (2007) was bio-precipitation fostered by the microbial community inhabiting the stone. The advantage was that it supported the autochthonous CaCO<sub>3</sub> producing-bacteria without introducing exogenous microorganisms. Initially, quarry porous limestone slabs were immersed in a M-3P nutritive buffered solution with/without *M. xanthus* (Jimenez-Lopez et al., 2008). Treated stones showed newly precipitated CaCO<sub>3</sub> overgrowth without pore plugging and, accordingly, weight increase, regardless of the presence or absence of *M. xanthus*. In comparison to sterilized slabs used as controls, the treated slabs maintained their original pore size distribution and were more resistant to mechanical stress. The M-3P medium, stimulating heterotrophic carbonatogenic bacteria via the ammonification of amino acids (Table 1), was patented (González-Muñoz et al., 2008).



The M-3P treatment was then tested *in situ*, with and without *M. xanthus*, on selected areas of decayed calcarenite stone of three historic buildings in Granada: San Jeronimo Monastery, Hospital Real and Royal Chapel (Jroundi et al., 2010; Rodriguez-Navarro et al., 2015). The evaluation included both the technical efficacy and, for the first time, the monitoring of the bacterial community of the decayed stone by culture-dependent and independent techniques (Table 1). Medium/long-term efficacy and detrimental side-effects were monitored up to 4 years after treatments (Rodriguez-Navarro et al., 2015). In all the three cases, the newly formed  $\text{CaCO}_3$  (mostly calcite) created a cement that consolidated the deteriorated calcarenite with a significant surface strengthening neither plugging pores nor causing aesthetical changes. The efficacy of the treatment *in situ* was independent of the presence of *M. xanthus*. The carbonatogenic bacterial population initially increased after treatment applications, but over time reached values close to those observed before treatment.

In those cases where the stone microbiota was altered and/or suppressed (e.g., application of biocides), the same authors proposed a bioconsolidation treatment with carbonatogenic bacteria selected from calcareous stones as inoculants (Jroundi et al., 2012). Bacteria were isolated from altered calcarenite stone slabs by the application of M-3P medium, then precipitating bacteria belonging to Actinobacteria, Gamma-proteobacteria and Firmicutes were selected and single strains were tested for bio-consolidation capability *in vitro*, with and without *M. xanthus*. They found that *Acinetobacter* spp. strains were the most appropriate candidate bacteria.

To test the self-inoculation biotreatment *in situ*, an indigenous community was recovered by cultivation from salt damaged carbonate stone in a historic building (San Jeronimo Monastery), activated via M-3P, and applied back onto the same stone (Jroundi et al., 2017). Firmicutes was the dominant phylum in the inoculum (~79%). Test evaluation methods are reported in Table 1. The effective consolidation was due to the formation of

**TABLE 1** | Features of *in situ* stone treatments of the three BCCM-based approaches. Details of the methodology of treatment, as found in the cited literature, are reported in the notes.

BCCM-based approach	Bacterium/a (concentration) or medium	Mechanisms driving precipitation	Monumental Site-kind of limestone	Testing area (m <sup>2</sup> )	Evaluation of treatment <sup>1</sup>				References	
					Parameter	Method	Effect on stone	Monitoring Time		
Selected viable strain	<sup>a</sup> <i>Bacillus cereus</i> (Not found)	Deamination of amino acids and nucleation sites	Saint Médard Church- Tuffeau (Thouars, France)	50	Water absorption	Water pipe	Biodeposition	6 and 12 months after treatment	<i>Le Métayer-Levrel et al., 1999; Castanier et al., 2000</i>	
Community inhabiting stone	<sup>b</sup> M 3-P Nutrient medium	Deamination of amino acids and nucleation sites	San Jeronimo Monastery-calcarenite, Royal Hospital-calcarenite, Royal Chapel-calcarenite (Granada, Spain)	From 0.15 to 2.0 if flat surfaces	Colour	Colorimetry	Safety			<i>Jroundi et al., 2010; Rodriguez-Navarro et al., 2015</i>
					New calcite microtexture	XRD SEM/EDS	Biodeposition			
					Surface cohesion	Peeling tape test	Reinforcement	Before and up to 4 years after treatment		
Community inhabiting stone	<sup>c</sup> Self-inoculation, indigenous bacterial community (~8.2 × 10 <sup>8</sup> CFU ml <sup>-1</sup> )	Deamination of amino acids and nucleation sites	San Jeronimo Monastery-calcarenite (Granada, Spain)	Not found	Colour	Spectrophotometry	Safety			<i>Jroundi et al., 2017</i>
					Stone microbiota	Culture-dependent (Total title, selected groups). Culture-independent (total DNA, 16S rDNA amplification, DGGE and clone libraries)	Safety			
					New calcite	SEM/XRD	Biodeposition			
					Porosity and pore size distribution	Porosimetry (mercury intrusion)	Biodeposition	Before and 5, 12, and 24 months after treatment		
Cell Components	<sup>d</sup> BCF from <i>B. subtilis</i> (8.5 g l <sup>-1</sup> )	Nucleation sites	Angera Church-Angera (Angera, Italy)	0.29	Surface cohesion	Peeling tape	Reinforcement			<i>Perito et al., 2014</i>
					Cohesion profiles	DRMS <sup>2</sup>	Reinforcement			
					Colour	Spectrophotometry	Safety			
					Stone microbiota	Cultivation (total number, selected groups)	Safety			
					Water absorption	Contact sponge	Biodeposition	Before and 4 months after treatment		

(Continued)

TABLE 1 | Continued

BCCM-based approach	Bacterium/a (concentration) or medium	Mechanisms driving precipitation	Monumental Site-kind of limestone	Testing area (m <sup>2</sup> )	Evaluation of treatment <sup>1</sup>				References
					Parameter	Method	Effect on stone	Monitoring Time	
					New CaCO <sub>3</sub> Cohesion profiles Colour	Alizarine red staining DRMS <sup>2</sup> Colorimetry	Biodeposition Reinforcement Safety		

<sup>1</sup> The parameter assesses the effect of the treatment on: biodeposition, safety (condition of being protected from aesthetical, structural, or microbiological changes), and reinforcement. <sup>2</sup> Drilling Resistance Measurement System. <sup>a</sup> The treatment is generally described in Le Métayer-Léveil et al. (1999). It consists of first spraying the entire surface to be protected with a suitable bacterial suspension culture. Afterward, the deposited culture is fed every 24–48 hours, usually for the next four days, with a suitable medium. <sup>b</sup> At the San Jeronimo Monastery and the Royal Hospital, the M-3P treatment was compared with the M. xanthus treatment. The treatments were applied by spray, twice a day, for 6 days (total volume applied 1–1.5 ml cm<sup>-2</sup>) on flat surfaces with similar wideness of calcarenite stone blocks. Treatment with M. xanthus involved two initial applications with inoculated M-3P (~10<sup>9</sup> cells/ml), followed by successive applications with sterile M-3P solution. At the Royal Chapel, only the M-3P treatment was performed on elements of the crest with carved surfaces. In all cases, the treated area remained covered with an aluminum/plastic (bubble wrap) foil up to two/ three days after treatment, to avoid the direct effect of sunlight and to minimize evaporation (Rodríguez-Navarro et al., 2015). <sup>c</sup> The self-inoculation treatment was compared with the M. xanthus and the sterile M-3P treatments. All three treatments were applied by spraying on adjacent stone blocks with similar exposure and decay levels. Approximately 0.125 ml cm<sup>-2</sup> were used for each application (twice a day for 6 days). In the M. xanthus and the self-inoculation treatments, the stone was treated on the first day with the bacterial culture and then with only the sterile M-3P nutritive solution. The treated areas were covered with an aluminum/plastic foil up to 3 days after treatment (Jroundi et al., 2017). <sup>d</sup> The field test was performed on the main façade of the Church of Angera. BCF in Super C solution and REF (only Super C) were sprayed on the treated surface in selected areas of 0.29 m<sup>2</sup> and 0.28 m<sup>2</sup>, respectively, for a total application of 1 l m<sup>-2</sup> solution for each spray application. Spray application steps: the first day BCF solution at concentration of 8.5 g l<sup>-1</sup> in Super C and REF were sprayed, the second day 0.032 g l<sup>-1</sup> BCF in SuperC plus nanoparticles and REF plus nanoparticles were sprayed, while the third day only SuperC plus nanoparticles solution was sprayed on the selected areas (Perito et al., 2014).

an abundant and exceptionally strong hybrid cement consisting of nanostructured CaCO<sub>3</sub> and bacterial EPS covering the substrate (Figures 1C–E). After 5 months, the viable titer of culturable microbiota increased and then after 24 months dropped back to about pre-treatment values.

## Cell Components

An Italian team of Florence investigated and assessed CaCO<sub>3</sub> mineralization on stone induced by a bacteria-mediated system in absence of viable cells (Perito et al., 2014). This investigation used the *Bacillus subtilis* strain 168 to identify bacterial structures or molecules inducing precipitation. The precipitation capability of bacterial dead cells was tested in a CaCl<sub>2</sub> solution as calcium source and with the sublimation of ammonium carbonate for alkalization. Dead cells were able to promote calcite formation, then cell fractions were tested and a bacterial cell fraction (BCF) containing the cell wall induced CaCO<sub>3</sub> formation (Figure 1F). Interestingly, the system was specific in generating crystal polymorphisms, since only calcite was found by X-ray diffraction.

Apparently, dead cells as well as BCF acted as crystallization nuclei in liquid medium. This hypothesis is supported by the capacity of cell walls to uptake cations such as Ca<sup>2+</sup>, as previously demonstrated for isolated *B. subtilis* walls (Beveridge and Murray, 1980), and fostering heterogeneous nucleation (Fortin et al., 1997). According to Dupraz et al. (2009), this process can be referred to biologically influenced mineralization.

BCF was stored as easy-to use lyophilized preparations, maintained a long-lasting activity and showed heat resistance. BCF treatment was tested on slab stones and then *in situ* on selected areas of the main façade of the Angera Cathedral, a 6th century monumental site in Italy (Perito et al., 2014). Lyophilized BCF was dissolved in a CaCl<sub>2</sub> solution, then sprayed on stone surface with a supersaturated calcium bicarbonate Ca(HCO<sub>3</sub>)<sub>2</sub> solution (Super C solution) for supplying calcium ions and CO<sub>2</sub>. The solution was supplemented with calcite nanoparticles to maintain supersaturation in the pore and increase calcium ions. Field evaluation tests after treatment showed that BCF treated areas had negligible color changes (Table 1). New crystals formed inside stone pores (Figure 1G) and, accordingly, there was a significant decrease in water absorption (up to 6.8%). The cohesion profiles were significantly increased in the first 3 mm (if compared with the control area treated with Super C alone). These results show that this application has potential, even if the authors concluded that further testing was needed to fully assess the treatment conditions for *in situ* applications.

## Perspectives: From Cell Components to the Microbial Community

BCCM biotechnology could be an ecological alternative to chemical treatments due to the low environmental impact and the production of a layer of CaCO<sub>3</sub> compatible with and coherent to the stone. A common point of improvement for the BCCM technologies is the consolidation performance, not yet comparable to that of synthetic polymers. The appropriate selection of stone types before application is important because pore structure affects penetration depth and treatment

performance (De Muynck et al., 2011). Nanomechanical properties of CaCO<sub>3</sub> polymorphs can also be improved by a better understanding of the bio-geo-chemical processes governing the formation of biominerals with high mechanical performance in natural environments (Dhami et al., 2018).

While the literature shows the potential in the laboratory of different bacterial applications to promote CaCO<sub>3</sub> mineralization, very few attempts have been made to test the technology *in situ* (Table 1). Based on these few studies, some companies have developed biomineralization products for cultural heritage by using cultures of selected strains (Amonit, France<sup>1</sup>) or media stimulating stone microbiota (KBYO Biological, Spain<sup>2</sup>). Nevertheless, the translation of other promising results obtained *in vitro* into practical bioremediation applications on heritage stone *in situ* remains the challenge for the immediate future (Webster and May, 2006); as examples, the well documented biocalcite production by bacterial urease or carbonic anhydrase (Castro-Alonso et al., 2019). Scaling up will be needed in order to develop this technology (Figure 1, box perspectives). *In situ* applications always have additional problems when compared with the *in vitro* conditions, especially concerning heterogeneity and conservation state of the stone, delivery systems, outdoor or indoor environmental conditions, type of feasible evaluation tests and the value of the artwork. For this reason, preliminary *in situ* small-scale testing should adopt treatment conditions mimicking those to the follow in larger-scale applications.

Another general comment concerns the heterogeneity of the treatment evaluation tests (Table 1). Although the choice of the monitoring methods sometimes depends on the experimental set-up, evaluating methods must be rapidly standardized for comparing results and for metadata analyses. Standard methods should consider the effectiveness of the treatment in terms of both consolidation and safety of stone (impact on structural and aesthetical features as well as on resident microbiota).

Living bacteria require the application of nutrient media on the stone. The possibility of undesirable side-effects on stone is controversial and it needs to be carefully evaluated (González-Muñoz, 2008; Nazel, 2016). The metabolic pathway activated *in situ* is the oxidative deamination of amino acids (Table 1), which increases the alkalinity by production of ammonia (Castanier et al., 1999; Lee and Park, 2019). The convenience of obtaining byproducts as ammonia and using spore-forming bacteria as *Bacillus* on stone has been recently discussed (Dhami et al., 2014; Zhu and Dittrich, 2016). More generally, promotion of undesired microbial growth can produce mineral changes or appearance of stained patches on stone, as found by Tianio et al. (1999). Such drawbacks may be encountered both in case of activating allochthonous or autochthonous strains. While aesthetical changes can be easily evaluated, growth of unwanted microorganisms and/or changes in the autochthonous community structure affecting the original ecological niche is harder to analyze. Microbes can strongly contribute to stone deterioration (Pinna, 2017) and the application of new

biotechnologies by conservators requires knowledge about the risk factors, in particular on the long-term effects (Webster and May, 2006; De Muynck et al., 2010). In this respect, the work about the long-term monitoring of stone microbiota carried by Etenauer et al. (2011) and Jroundi et al. (2017) is remarkable. However, knowledge about microbial communities inhabiting heritage stone mainly comes from cultivation studies (Scheerer et al., 2009). Microbial communities of stone were only recently investigated using Next Generation Sequencing and omics techniques (Perito and Cavalieri, 2018; Marvasi et al., 2019). The latest studies suggest that natural community structure detected by metagenomics is quite different from that of enriched communities cultivated from calcareous stone in precipitating media where Firmicutes are dominant (Dhami et al., 2018; Li et al., 2018).

Meta-omics techniques as a whole (metagenomics, metatranscriptomics and metabolomics) will promote a further step to improving BCCM technology, because they provide a wider view of the microbial community structure, fluctuations and metabolic potential (Marvasi et al., 2019). In regard to the cultivation bias (Hardoim et al., 2014), omics technologies will provide a better understanding of the stone microbial community structure to allow treatment monitoring as well as the identification of the community components with biomineralization potential. Chimienti et al. (2016) used metagenomics to identify the presence of microorganisms known as carbonatogenic (i.e., *Arthrobacter*) within the overall microbial community from stone slabs of a medieval church. Zanardini et al. (2019) reconstructed the carbon, nitrogen and sulfur cycles and their biodeterioration potential within the prokaryotic community of decayed sandstone of a medieval castle by 16S rRNA and functional gene analyses. Using a similar approach, the carbonatogenic potential of metabolic pathways linked to these biogeochemical cycles could be inferred. On the other hand, cultivation is more valuable than ever in the omics era (Gutleben et al., 2018) because it is needed to confirm the predicted carbonatogenic ability of stone populations as well as for other applications. But then again, meta-omics techniques can also provide useful information to improve cultivation strategies for the isolation of potential calcinogenic bacterial populations from calcareous environments.

The cell-free approach offers several advantages: the cellular components act as mineral nucleation and growth sites in the absence of nutrients, components smaller than cells penetrate more in depth into pores and microcracks, interventions on the chemical environment governing precipitation are easier (Hammes and Verstraete, 2002). Alkaline buffering or different supersaturated calcium solutions should be further developed and compared to that used by Perito et al. (2014). However, the preparation of the BCF product is more complex compared to alive cellular strategies but could have as target calcareous objects where minimum change in their chemistry is required (Perito et al., 2014). A cell-free approach has not been explored further.

Very little is still known about the molecular basis of the calcium biomineralization process (Perito and Mastromei, 2011). *B. subtilis* laboratory strain 168 was used to identify cellular fractions as well as genes and molecules with key roles in inducing

<sup>1</sup>[http://www.amonit.fr/fr/calcite\\_\\_1](http://www.amonit.fr/fr/calcite__1)

<sup>2</sup><http://kbyobiological.com/en/>

precipitation (Barabesi et al., 2007), as found for mollusks (Falini et al., 1996). Characterization of *B. subtilis* mutants impaired in CaCO<sub>3</sub> precipitation suggested a link between biomineralization, redox reactions of fatty acid metabolism, changes in phospholipids membrane composition and surface properties (Barabesi et al., 2007; Marvasi et al., 2010, 2016; Frandi et al., 2011; Perito et al., 2018a). In *Lysinibacillus*, CaCO<sub>3</sub> precipitation can modify membrane rigidity by upregulating the branched chain fatty acid synthesis (Lee and Park, 2019). We speculate that intervention on these metabolic switches could help in the search for bacterial molecules fostering precipitation and, at the same time, improving precipitation performance by bacteria.

On the other hand, it is well known that bacterial macromolecules, like the EPS, act as matrices which promote mineralization and are trapped in the growing calcite (Decho, 2010; Marvasi et al., 2012; Perito et al., 2018b). According to Jroundi et al. (2017), the hybrid cement due to the incorporation of organisms and EPS within the nanostructured CaCO<sub>3</sub> in the self-inoculation biotreatment was responsible for the high

consolidation effectiveness. Further studies are needed in order to identify and test different EPS or to design bacteria-based biomimetic matrices promoting calcite growth on stone. This would represent a further advancement of the cell-free technology since it would reduce the complexity of organic matter to apply, increasing its penetration inside stone.

Concluding, in our opinion all the different approaches explored in this mini review are worth further development for *in situ* applications, even if two of them are already available on the market. Fascinating challenges for the future include advances in exploitation of bacterial pathways, cell components and single (macro)molecules.

## AUTHOR CONTRIBUTIONS

BP provided the general concept. BP and MM wrote the manuscript. MM, GM, and BP revised and approved the manuscript. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer CO declared a past collaboration with one of the authors BP to the handling Editor.

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