Biomineralization induced by Myxobacteria

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Myxococcus xanthus is a Gram negative, non-pathogenic, common soil bacterium that belongs to the δ-subdivision of the Proteobacteria. According to the data, it is demonstrated that, depending on the chemistry of the culture media, M. xanthus is able to induce the formation of phosphates (struvite, schertelite, newberyte), carbonates (calcite, Mg-calcite, vaterite) and sulfates (barite, taylorite). This remarkable capacity for biomineralization of M. xanthus has important implications. On one hand, the fact that M. xanthus is able to induce the precipitation of vaterite, it may shed light to understand the processes involved in the precipitation and stabilization of this rare polymorph of calcium carbonate. Moreover, bacterial vaterites show differences when compared to those inorganically produced that may be used as biomarkers with the goal of determining the bacterial/inorganic origin of natural samples with implications in identifying bacterial activity on Earth and elsewhere. Finally, biomineralization induced by M. xanthus is of singular interest when applied to the protection and conservation of ornamental stone, in particular to the treatment of porous limestone.

Keywords Myxococcus xanthus; bacterial biomineralization

1. Introduction

Organisms which have the capability of precipitating minerals are present in all the major groups, from Bacteria to Chordata. However, concerning the way in which these biominerals are formed, a marked difference exists among the different taxonomic groups [1]. The foremost group able to induce the precipitation of minerals is the animals. The second group is bacteria, then vascular plants and finally fungus and protozoa [2]. The most numerous biominerals are phosphates, followed by the oxides and carbonates. Roughly 25% of the biominerals are hydrated and 25% are amorphous [1].

Bacteria are considered as agents that disperse, fractionate or concentrate material. As concentration agents, they can: 1) accumulate inorganic material via processes such as intracellular deposition, adsorption, and fixation at cellular level and 2) precipitation of insoluble extra or intracellular compounds. Bacteria are able to induce the precipitation of minerals, either by highly controlled biomineralization processes like the formation of magnetite by magnetotactic bacteria [3], or by inducing the precipitation of minerals through processes that involve little control over the biomineralization (so called “biologically induced biomineralization”), for instance, by changing the chemistry of the environment as a result of the bacterial metabolic activity. In reference to the first type of biomineralization, i.e. the so called “biologically controlled biomineralization”, the magnetotactic bacteria exercise exquisite control over the biomineralization of the magnetosome mineral phase. Although a great deal of this control is likely to be at the gene level, local environmental conditions also appear to have a role in the regulation of the biomineralization processes involved in the magnetosome biosynthesis. Elucidation of the biomineralization processes involved in the magnetosome formation could yield valuable information in the production of ordered arrays of new inorganic, electronic, optical and magnetic materials at the nanometric scale.

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On the other hand, there are a considerable number of microorganisms able to induce the extracellular precipitation of a wide range of minerals by the so called “biologically induced biomineralization”. Microorganisms have a geochemical activity which is responsible to a great extent for the deposition of minerals throughout the history of the Earth. Their central role over a wide range of mineralization processes has already been established. On one hand, bacteria can change the chemistry of the environment where they grow as a result of their metabolic activity. Therefore, they can create supersaturated conditions with respect to a particular mineral phase thus inducing the precipitation of such a phase. The processes that result in the precipitation of the different biominerals are very different, due to the great variety of bacterial metabolic pathways. On the other hand, as it has been mentioned by numerous authors, bacteria may contribute to mineral precipitation not only actively, but also passively, by serving, both the cell, cell structures and the cell debries, as nucleation sites for mineral deposition [4, 5, 6, 7]. In this regard, bacteria can induce heterogeneous nucleation of minerals by providing not only a surface that lower the energy barriers for mineral precipitation, but also a stereochemical arrangement of the mineral components. Many studies place special emphasis on the role of microbial extracellular substances (EPS). These exopolymers and the biofilms are commonly dominated by negatively charged polysaccharides [8] and can contribute to mineral precipitation in numerous ways: 1) by trapping positively charged cations in the negatively charged sites of the EPS and serving as a template for the nucleation of crystals [9] and/or 2) by trapping detrital seed crystals which act as nuclei for heterogeneous mineral precipitation [5].

Regarding the biominerals precipitated by bacteria, there is a wide spectrum of bacteria able to induce the precipitation of carbonates [10, 11, 12, 13, 14], oxides [15, 16], sulphates [17, 18] and phosphates [19, 20]. The biosedimentary structures known as stromatolites provide another example of biomineralization linked to bacteria [1, 21]. These structures can be found in fossil records and still form under natural conditions, as in western Australia.

Because biomineralization is such a wide phenomenon, there is an increasing interest to understand the mechanisms involved in such process, since they can provide unique information regarding, for instance, the origin and evolution of life in Earth and elsewhere in the solar system, bacterial interactions with the environment, environmental conditions of sediments of all ages and the recognition of the bacterial role of different human pathologies. Also, biomineralization has a high potential of being used in a wide range of technical applications, including for instance, nanotechnology, implants and restoration [11, 22].

2. Myxobacteria

Myxobacteria are Gram-negative soil-sliding bacteria. Myxobacteria cells are long and thin rods with a length of 3-12 µm and diameter of 0.7-1.2 µm [23, 24] (Fig. 1a). They have been isolated from different types of habitats, although they are especially abundant in soils that are rich in organic material such as cultivated soils and rotting wood. Myxobacteria show several striking characteristics that make them very different from the rest of the δ subdivision of the Proteobacteria. Firstly, they present a characteristic social behaviour and a peculiar life cycle. In addition, due to the high amount of enzymes produced, Myxobacteria play a key role in the degradation of the organic material, constituting an important group in soil ecology.

Myxobacteria live forming large communities known as swarm and feed by degrading a large variety of macromolecules such as proteins, starch, lipids, nucleic acids and even cellulose because they produce an extraordinary battery of extracellular hydrolytic substances. In fact, they can use not only macromolecules, but also entire cells such as E. coli, yeasts or other different species of Myxobacteria.

Moreover, Myxobacteria have developed different adaptative mechanisms to survive and compete in their habitats. When they live in a medium with enough nutrient supply they follow a vegetative life cycle, during which each cell elongates and finally divides by binary fission to originate two daughter cells. However, when nutrient supply is depleted, cells initiate a developmental cycle, which is very unique among the prokaryotes, although it shows several similarities with the developmental cycle of certain eukaryotes, such as Dictyostelium. Under these conditions of poor nutrient supply and when cell
density is high enough, cells inside a swarm move to certain points in where they aggregate and originate multicellular structures known as fruiting bodies that can be observed with the naked eye (Fig. 1b). Inside the fruiting bodies cells differentiate and originate myxospore which are dormant stages and are resistant to several stress conditions such as desiccation, UV irradiation and sonication. Fruiting bodies appear as colored masses since myxobacterial cells contain carotenoids. In some genera, such as *Myxococcus*, fruiting bodies appear as mounds, while in others such as *Stigmatella* or *Chondromyces*, they are more sophisticated and consist of a stalk and several sporangioles.

![Fig 1 a) Scanning electron microscopy (SEM) photomicrograph of vegetative cells of *Myxococcus xanthus*. Bar represents 2 µm. b) SEM photomicrograph of a fruiting body of *M. xanthus*. Bar represents 10 µm.](image)

Finally, two other significant characteristics of Myxobacteria are their moving by sliding on solid substrate and, secondly, their abundant production of extracellular polysaccharide.

In this bacterial group, *Myxococcus xanthus* presents a pronounced autolysis at the end of the exponential phase when grown in liquid culture and produces an abundance of extracellular polysaccharide.

### 3. Mineral production by *Myxococcus xanthus*

There are above sixty biogenic minerals. Among them, about twenty can be induced by bacteria. According to our data, the precipitation of more than sixteen minerals has been induced by different species of the genus *Myxococcus*. This obviously implies a substantial ability to biomineralize. Moreover, there are minerals, like barite, whose induction by bacteria has only been reported by our research group when working with *M. xanthus*. Focussing in one specie of *Myxococcus*, it has been demonstrated in numerous experiments that, depending on the chemistry of the culture media, *M. xanthus* is able to induce the precipitation of phosphates (struvite, schertelite, newberyite: [7, 17, 25, 26, 27, 28, 29, 30]), carbonates (calcite, Mg-calcite, vaterite: [12, 22, 31, 32]) and sulfates (barite, taylorite: [33]).

#### 3.1 Production of phosphates

Phosphates belong to the wide range of minerals produced by bacteria [34, 35], struvite (MgNH₄PO₄·6H₂O) being one of them. The production of struvite by bacteria deserves special attention by microbiologists. Not only can it be formed by a large variety of strains in laboratory conditions but for the clinical implications of the struvite mineralization [36, 37]. In natural environments, struvite has been found in association with various organic materials in decomposition as in guano deposits, manures, sediments rich in organic remains, in basaltic caves and marshlands. The bacterial production of struvite as a consequence of the pH increase resulting from the ammonium release produced by the metabolism of nitrogenous compounds and the combination of such ammonium ions with phosphate and magnesium present in the environment was described by [38, 39, 40].

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The production of struvite by two species of myxobacteria: *Myxococcus coralloides* and *M. xanthus* was extensively studied by our research group [7, 17, 25, 26, 27, 28, 29, 30]. Bacterial metabolic activity provides the physico-chemical conditions for the crystallization of struvite. The pH increases due to the release of ammonium resulting from the metabolism of the nitrogen compounds present in the culture media, thus reaching the necessary alkaline environment for the precipitation of struvite. Those studies demonstrate the following conclusions: 1) there is a wide diversity of struvite crystal morphologies (Figs. 2a and 2b) depending on the physico-chemical conditions of the culture media and, to a certain extent, on the specie that was being used, 2) there are different preferential types of morphologies, habits and crystalline grouping linked to different physicochemical conditions, 3) the efficiency of the production of struvite varied with the age of the cells-culture, 4) although the production of struvite by these bacteria seems to be a consequence of their metabolism, the results show that changes on the chemistry of the culture media alone were not enough to produce struvite, but it was also necessary the real physical presence of the bacteria and/or cellular debris, 5) moreover, the data also demonstrated that the production of struvite was linked to the autolysis of *M. xanthus* cultures and 6) that dead cells, disrupted cells and membrane fractions of this microorganism induced struvite crystallization. These findings indicate that bacterial cells (or certain parts of the cells) can act as heterogeneous nuclei for crystallization, meaning that the charged points of the external side of the cellular structure reduce the metastability field for struvite when appropriate supersaturated solutions are used.

![Fig. 2](image)

*Fig. 2*  a) and b) SEM photomicrographs of struvite crystals induced by *M. xanthus* in different culture media. Bars represent 200 µm.

*M. xanthus* cells are also able to precipitate Uranium as meta-autunite, a mineral phase of uranyl phosphate, as has been demonstrated [17]. In this work the authors used U LIII-edge EXAFS spectroscopy, TEM, and EDX analyses in a combination with microbiological methods to elucidate the interaction mechanisms between *M. xanthus* cells and U(VI) at different pH values. Results showed that there are significant differences in the structural parameters of the U complexes formed by this bacterium at pH 2 and 4.5. At very low acidic pH of 2, the cells accumulated U(VI) as organic phosphate-metal complexes. At pH 4.5, however, the cells of this bacterium precipitated U(VI) as meta-autunite-like phase. TEM analyses demonstrated that the uranium accumulates were located mainly at the cell surface, and within the extracellular polysaccharides characteristic of this bacterium (Figs. 3a and 3b). The Autunite is a major source of naturally occurring secondary uranium ore and is known to persist under oxidizing conditions on a geological time scale [18].The precipitation of uranium as mineral phases may lead to more stable U(VI) sequestration that may be suitable for remediation purposes. These observations, combined with the very high uranium accumulation capability of the studied bacterial cells indicate that *M. xanthus* may significantly influence the fate of uranium in soil environments where these bacterial species are mainly found [17].
3.2 Production of carbonates

Bacterially induced or mediated carbonate mineralization is thought to be important in a range of processes including atmospheric CO₂ budgeting, carbonate sediment and rock formation, biogeochemical cycling of elements, metal-contaminated groundwater bioremediation, and conservation of ornamental stone [41].

It is known that a number of processes are involved in bacterially induced calcium carbonate precipitation, including non-methylotrophic metanogenesis, photosynthesis, ammonification, denitrification and sulphate reduction [11, 42]. In fact, it has been observed that the number of bacteria capable of producing calcium carbonate is considerably high, among others: sulphate-reducing bacteria and cyanobacteria [10], 

Calcite and aragonite, the two most stable polymorphs of calcium carbonate, are the most common microbial carbonates [41]. Interestingly, microbial biscuits of vaterite, the rare metastable CaCO₃ polymorph, have been found in a lake [43], while in vitro bacterially mediated vaterite precipitation has also been reported [i.e., 22, 44, 45, 46, 47, 48]. These occurrences suggest that bacterial vaterite precipitation is more common than previously thought. Moreover, elucidating how bacterial vaterite mineralization occurs may have far reaching implications: it may lead to a better understanding of microbial carbonate mineralization, and help identify biosignatures both on Earth and elsewhere. Furthermore, it may shed light on the “calcium carbonate polymorphism problem”, which is one of the biggest challenges in the field of biomineralization.

It has been demonstrated that *M. xanthus* has a remarkable ability to induce the precipitation of carbonates, mainly calcite, vaterite and Mg-calcite [12, 22, 31, 32]. *M. xanthus* induces carbonate precipitation by changing the supersaturation conditions with respect to the particular carbonate phase. *M. xanthus* increases the alkalinity of the culture media as a consequence of the release of CO₂ and NH₃ resulting from its metabolic activity. Extracellular ammonia release raises pH values and therefore CO₃^− concentration, according to these equilibria:

\[
\text{NH}_3(g) + H_2O \rightleftharpoons \text{NH}_4^+(aq) + \text{OH}^-(aq) \quad (1)
\]

\[
\text{CO}_2(g) + H_2O(l) \rightleftharpoons \text{H}_2\text{CO}_3(aq) \rightleftharpoons \text{HCO}_3^-(aq) + \text{H}^+(aq) \rightleftharpoons \text{CO}_3^{2-}(aq) + 2\text{H}^+(aq) \quad (2)
\]

Precipitation of a calcium carbonate phase occurs when a sufficient supersaturation is reached with respect to this phase:
\[
\text{Ca}^{2+\text{(aq)}} + \text{CO}_3^{2-\text{(aq)}} \rightleftharpoons \text{CaCO}_3\text{(s)}
\] (3)

Calcite frequently precipitates from culture media that contain calcium and which have a starting supersaturation value relatively low. Figs. 4a and 4b show calcite crystals induced by \textit{M. xanthus} in M3-P culture medium (1% Bacto Casitone, 1% Ca(CH\textsubscript{2}COO)\textsubscript{2}·4H\textsubscript{2}O, 0.2% K\textsubscript{2}CO\textsubscript{3}·1/2H\textsubscript{2}O in a 10mM phosphate buffer, pH 8; [22]).

Fig. 4 a) and b) SEM photomicrographs of calcite crystals induced by \textit{M. xanthus} in M3-P culture medium. Bars represent 200\,\mu m.

Very interesting is the ability that \textit{M. xanthus} has to induce the precipitation of vaterite, the most unstable polymorph of calcium carbonate. Vaterite has been found in calcareous sediments, metamorphic rocks and drilling muds, portland cement, and in mortars of the Florence Cathedral. It has also been reported vaterite presence in Pesyanoe meteorite, although it was unclear whether it was terrestrial or antecedent in origin. Vaterite is sometimes found in hard tissues of some marine organisms, egg-shells of gastropods, birds, vertebrate otoconia, crustacean statoliths and fish otoliths (references for these occurrences in [32]). Despite such occurrences, some controversy exists regarding whether vaterite can precipitate inorganically from natural waters. As mentioned above, most natural occurrences of vaterite are associated with biogenic activity. This is the case for abnormally calcified tissues, including regenerated damaged gastropod shells [49], and human pathological concretions such as gallstones [50], pancreatic stones, and cloggings in human heart valves [51].

Vaterite typically precipitates in a spherical shape when grown in the laboratory from highly supersaturated and moderately alkaline solutions. It has a hexagonal structure and it is unstable under practically all known conditions [52, 53]. The latter is caused by the higher solubility and lower density of vaterite as compared to those of calcite and aragonite. In aqueous solution vaterite rapidly transforms into one of the latter phases. Nonetheless, it appears that for reasons yet unknown vaterite is more stable than previously thought.

\textit{M. xanthus} is able to precipitate vaterite in culture media containing calcium and which have relatively higher supersaturation conditions, as it is the M-3 (1% Bacto Casitone, 1% Ca(CH\textsubscript{2}COO)\textsubscript{2}·4H\textsubscript{2}O, 0.2% K\textsubscript{2}CO\textsubscript{3}·1/2H\textsubscript{2}O, in distilled water, pH 8; [28]) and CC (0.3 wt % Bacto Casitone, 0.4 wt % Ca(CH\textsubscript{2}COO)\textsubscript{2}·4H\textsubscript{2}O, 0.1 wt % CaCl\textsubscript{2} 0.3% NaHCO\textsubscript{3}, 0.1 wt % yeast extract in distilled water, pH 8; [54]) culture media. The vaterite induced by \textit{M. xanthus} shows a spheroidal and lentils-like morphology (Figs. 5a and 5b). Bacterial production of CO\textsubscript{2} and NH\textsubscript{3} and the transformation of the NH\textsubscript{3} to NH\textsubscript{4}\textsuperscript{+} and OH\textsuperscript{−}, thus increasing solution pH and carbonate alkalinity, set the physicochemical conditions (high supersaturation) leading to vaterite precipitation in the microenvironment around cells, and directly onto the surface of bacterial cells. In the latter case, fossilization of bacteria also occurred. Vaterite crystals formed by aggregation of oriented nanocrystals with c-axes normal to the bacterial cell wall, or to the core of the spherulite when bacterium was not encapsulated. While preferred orientation of vaterite c-
axes appears to be determined by electrostatic affinity (ionotropic effect) between vaterite crystal (0001) planes and the negatively-charged functional groups of organic molecules on the bacterium cell-wall or on EPS. Analysis of the changes in the culture media chemistry as well as high resolution transmission electron microscopy (HRTEM) observations point to polymorph selection by physicochemical (kinetic) factors (high supersaturation) and stabilization by organics, both connected with bacterial activity. The latter is in agreement with inorganic precipitation of vaterite induced by NH\textsubscript{3} and CO\textsubscript{2} addition in the protein-rich sterile culture media. The results obtained by [32] as well as recent studies on vaterite precipitation in the presence of different types of bacteria suggest that bacterially mediated vaterite precipitation is not strain-specific.

Fig. 5 a) and b) SEM photomicrographs of vaterite crystals induced by M. xanthus in M3-P culture medium. Bar represent 5 µm. Vaterite displays spherical morphology as well as lentils-like.

Moreover, when M. xanthus grows in culture media that contain calcium and magnesium, is able to induce the precipitation of magnesium calcite [31] (Fig. 6a). In these conditions it was observed that the mineralization occurred firstly on the edge of the M. xanthus colonies, implying that it is precisely there where supersaturation condition to respect a Mg-calcite are firstly reached. Interestingly it was observed that with time, Mg-calcite precipitation occurred in concentric rings from the edge to the center of the colony (Fig. 6b). This was explained as a result of a series of local fluctuations in supersaturation as a consequence of, on one hand, the diffusion of the released CO\textsubscript{2} and NH\textsubscript{3} by bacterial activity from the zones of higher density of microbial growth to the culture media and, on the other hand, the diffusion of ions in the opposite direction forced by the consumption of nutrients by bacterium. Therefore, the counter diffusion of microbial metabolites and culture ions produce spatio-temporal gradients of concentration that at certain moments and at certain points raise the supersaturation conditions to allow the precipitation of the specific mineral phase. In each ring, a variety of morphologies of Mg-calcite was observed altogether, since saturation conditions were not static over time. This result is of particular interest when compared to inorganic experiments, where only a type of morphology is observed in a certain zone in a gradient of supersaturation, where this saturation is kept fixed over time.
Finally, *M. xanthus* calcified itself when it grows in a culture medium containing calcium (Figs. 7a and 7b), as demonstrated by the findings of bacterial cells surrounded by nanometer-sized carbonate crystals. Some vaterite spheroids encapsulated bacterial cells (Fig. 7b). Nevertheless, it appears that the presence of bacterial cells is not a prerequisite for the formation of the vaterite spheroids, although bacterial activity does seem to be a prerequisite for vaterite formation.

### 3.3 Production of barite by *Myxococcus xanthus*

The mechanism by which barite (BaSO$_4$) precipitates in undersaturated seawater is one aspect of the Ba biogeochemical cycle that still remains unknown. Considerable research has focused on this mineral phase over the last 2 decades, since it is known to be a reliable indicator for variations in marine biological productivity. Several authors have proposed that the precipitation of barite could be biologically mediated, however there was not experimental evidence that support this hypothesis. Barite precipitation by living organisms (protozoa) has been demonstrated in lacustrine freshwater environments. In marine
environments, intracellular barite crystals have also been found in vacuoles of unicellular *Exanthemachrysis gayraliae* and in *Xenophyophorea* (references in [33]). However, these organisms do not appear to account for the abundance of barite crystals in the water column, and the living organisms which directly precipitate barite had not yet been identified in seawater. Bacterial precipitation of barite under laboratory conditions was demonstrated for the first time by our research group [33]. The bacterium *M. xanthus* was cultivated in a solid medium with a diluted solution of barium chloride. Crystallization occurred as a result of the presence of live bacterium and the bacterial metabolic activity. A phosphorous-rich amorphous phase preceded the more crystalline barite formation. These results indicate the involvement of bacteria in the barium biogeochemical cycle, which is closely related to the carbon cycle. Bacterially induced barite precipitation suggests that, in marine environments, bacteria may enhance barite production by providing nucleation sites and by favouring crystal growth. This is, however, only an initial approach for future investigation regarding the role of bacteria in the Ba biogeochemical cycle. Further research will be required in order to determine the exact role of bacteria in marine barite precipitation.

4. Applications

4.1 Biomarkers

Very interesting and important research in Geomicrobiology, carried out at the present, is focused on the finding of biosignatures in minerals with the end of recognizing bacterial/inorganic origin of natural samples. Valuable information regarding the bacterial/inorganic origin of natural vaterites on Earth and elsewhere may be related to the complex inner structure and ultrastructure of the biotic vaterite spheroids obtained by [32]; i.e., degree of crystal orientation, amount of organic matter, presence of various shells, and hollow core-shell structure. However, a purely morphological (size and shape) analysis of such vaterite structures can not be used as an unambiguous biosignature since similar (spherulitic) morphologies are obtained in both bacterially induced and inorganic vaterite. Nonetheless, the presence of organics appears to be a prerequisite for vaterite formation and stabilization. Results from [32] suggest that vaterite precipitation occurs at a very high supersaturation resulting from bacterial activity or, in the absence of bacterium, from the addition of NH$_3$ and CO$_2$(gas). At such a high supersaturation 3D nucleation of vaterite nanoparticles occurs in the microenvironment surroundings of the bacterial cells. Oriented aggregation of nanoparticles then occurs and leads to the incorporation of organics into vaterite structures. Incorporation of organics within vaterite leads to highly insoluble structures and contributes to vaterite stabilization. Considering that bacteria have been related to human pathological calcification, vaterite being found in human gallstones and aortic valves, results from [32] may help determine whether such vaterite pathological concretions are of bacterial origin, which is critical to the design of adequate medical treatments.

4.2 Consolidation of ornamental stone

The progressive deterioration of the built and sculptural heritage represents a problem that considerable resources have attempted to address and manage. There are numerous treatments based on the application of a consolidating agent to the substrate that result in the organic and/or inorganic precipitation of new cement within the porous system of deteriorated ornamental stone. However, the effect of such treatments have not been as encouraging as expected because of the compositional and textural complexities encountered. Among these complexities are the long-term incompatibility between the substrate and the new cement used for consolidation, and the plugging of pores in the treated material induced by the new cement. The observed incompatibility of most organic consolidants has prompted the search for more compatible (inorganic) and effective conservation treatments in recent years.
Among such new conservation treatments, there is a new promising procedure to consolidate ornamental stone that makes use of bacterially induced mineralization [11, 22]. Some studies [11, 55] tested the effectiveness of *Bacillus cereus* when used to protect stone and found that this bacterium was able to induce extracellular precipitation of calcium carbonate on decayed limestones. This calcium carbonate was compatible with the substrate and significantly reduced the water sorptivity of the treated stone. However, the layer of the new cement induced by *B. cereus* was very thin, of only a few microns. Another treatment was proposed by our research group [22] who tested a bacterial conservation method based on the use of *M. xanthus*. This method appears to be more effective than that based on the use of *Bacillus*. The remarkable capability for biomineralization of *M. xanthus* is of particular importance because, by creating a new cement that is compatible with the substrate, *M. xanthus* may thus be used to consolidate a wide spectrum of materials. Furthermore, it displays gliding motility and, being that the latter is linked to interfaces, *M. xanthus* could be able to colonize not only the surface of the stone, but also more deeply into the stone pores, thus enabling the bacterially induced calcium carbonate to root in the porous system increasing its consolidation efficiency.

The ability of *M. xanthus* to induce calcium carbonate precipitation on sterilized slabs of porous limestone was also tested and it was found that: i) a coherent carbonate cement of 10 to 50 µm in thickness coated the treated stones; ii) the new cement was compatible with the substrate; and iii) this cement was rooted down to a depth of ~1 mm while at the same time stone porosity remained nearly unaltered. The newly formed bacterial cement was more resistant to mechanical stress, i.e. more consolidated, than the substrate. Moreover, the consolidation of the treated stone is enhanced when a *M. xanthus*-inoculated culture medium is applied to a stone whose microbial community is not eliminated [56]. This result is very promising, given the fact that porous limestone is the most common material used as ornamental stone in the Mediterranean Basin.

This line of research opens a new and promising future based on the development of a method to activate, among the microbial community of the stone, those bacteria able to induce the precipitation of calcium carbonate while avoiding the growth of those microorganisms, also belonging to the microbial community of the stone, whose growth could be potentially dangerous for the protection and/or conservation of the stone being treated.

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