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THE ROLE OF BACTERIAL EXOPOLYSACCHARIDES IN MICROBIOLOGICAL CALCIFICATION

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Abstract: Microbiological calcification is a biologically mediated process in which microorganisms promote the precipitation of calcium carbonate ($CaCO_3$) through their metabolic activities and interactions with their surrounding environment. Among the microbial factors involved, bacterial exopolysaccharides (EPS) play an important role in regulating mineral nucleation, crystal growth, and calcium carbonate deposition. These extracellular polymers contain functional groups capable of binding calcium ions, providing nucleation sites, and creating localized microenvironments favorable for biomineralization. Furthermore, EPS contribute to bacterial adhesion, biofilm formation, and the stabilization of mineral structures. Understanding the relationship between EPS production and microbiological calcification is essential for developing sustainable biotechnological applications, including self-healing concrete, soil stabilization, and the remediation of porous materials. This review summarizes the principal mechanisms through which bacterial EPS influence calcium carbonate precipitation and discusses their potential applications and current research challenges.

Keywords: bacterial exopolysaccharides, microbiological calcification, calcium carbonate, biomineralization, microbial-induced calcium carbonate precipitation, extracellular polymeric substances.

1. Introduction

Microbiological calcification is an important form of biologically mediated mineral formation in which microorganisms facilitate the precipitation of calcium carbonate under suitable environmental conditions. This process occurs in natural ecosystems, including soils, sediments, saline environments, and aquatic habitats, and contributes to carbonate cycling, sediment lithification, and the formation of mineral structures. Microbial-induced calcium carbonate precipitation (MICP) has attracted considerable scientific interest because of its potential applications in environmental biotechnology, geotechnical engineering, and sustainable construction materials [1].

The formation of calcium carbonate is influenced by several interconnected factors, including microbial metabolism, calcium ion availability, pH, dissolved inorganic carbon, and the physicochemical characteristics of microbial cell surfaces. In addition to these factors, bacterial extracellular polymeric substances, particularly exopolysaccharides (EPS), can substantially influence the initiation and development of mineral precipitation. EPS are high-molecular-weight

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polymers secreted by microorganisms and commonly consist of polysaccharides, proteins, extracellular DNA, and other organic compounds. Their chemical composition and structural properties determine their interactions with mineral ions and growing crystals [2].

Although bacterial metabolic activity is a major driver of MICP, EPS provide an additional mechanism through which microorganisms regulate mineral nucleation, crystal morphology, and the spatial distribution of calcium carbonate. Consequently, understanding EPS-mediated biomineralization is important for optimizing microbial calcification processes and developing efficient biological mineralization technologies [3].

2. Mechanisms of EPS-Mediated Microbiological Calcification

2.1. Calcium binding and mineral nucleation

One of the principal functions of bacterial EPS in microbiological calcification is their ability to bind calcium ions (Ca^{2+}). Many extracellular polysaccharides contain negatively charged functional groups, including carboxyl, hydroxyl, and, in some cases, sulfate and phosphate groups (fig. 1). These functional groups can interact with calcium ions through electrostatic interactions and coordination processes, resulting in localized calcium accumulation within the extracellular matrix [4].

The accumulation of calcium ions creates favorable conditions for the initial stages of mineral nucleation when sufficient carbonate ions are available. As the local solution becomes supersaturated with respect to calcium carbonate, nucleation and subsequent crystal growth may occur on EPS-associated sites. The extracellular matrix therefore acts as a potential template for mineral deposition, facilitating the formation of mineral structures in close association with bacterial cells and biofilms [5].

However, EPS do not invariably promote precipitation. Depending on their concentration, molecular structure, and degree of ionization, they may also bind calcium strongly enough to inhibit crystal nucleation or delay crystal growth (fig. 2).

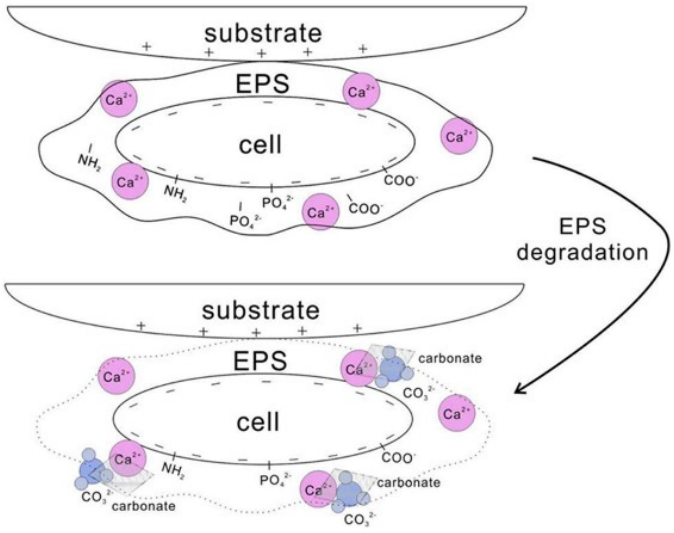


Figure 1. Schematic representation of calcium binding by bacterial extracellular polymeric substances (EPS) and subsequent calcium carbonate precipitation associated with EPS degradation

Thus, the effect of EPS on calcification depends on the balance between calcium sequestration, nucleation-site availability, and solution supersaturation [6].

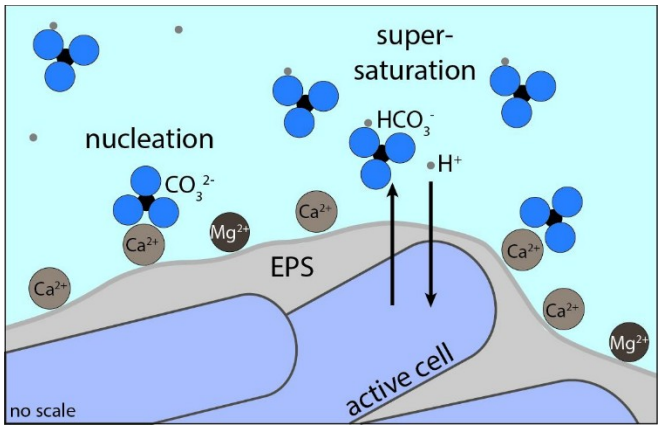


Figure 2. Schematic representation of EPS-associated calcium carbonate nucleation under supersaturated conditions, illustrating the interactions of calcium and carbonate species with the bacterial extracellular matrix

2.2. Regulation of crystal growth and morphology

In addition to facilitating nucleation, EPS influence the growth and morphology of calcium carbonate crystals. Their polymeric structure provides a three-dimensional matrix in which mineral particles can accumulate and interact. EPS may adsorb onto specific crystal surfaces, modifying crystal growth rates, particle aggregation, and the development of different calcium carbonate morphologies [6, 7].

Calcium carbonate can occur in several mineral forms, including calcite, aragonite, and vaterite. The resulting polymorph depends on multiple factors, including temperature, pH, calcium and carbonate concentrations, and the composition of the surrounding organic matrix. EPS may influence these processes through selective ion binding, surface adsorption, and spatial confinement of mineral growth [2, 6, 8].

The extracellular matrix can also contribute to the formation of composite structures consisting of organic polymers and inorganic minerals. Such structures may exhibit improved cohesion and stability compared with mineral particles formed in the absence of an organic matrix. Nevertheless, the precise effects of individual EPS components on calcium carbonate polymorphism remain dependent on the microorganism and the experimental conditions [6, 9].

2.3. Interaction between EPS production and bacterial metabolism

EPS-mediated calcification is closely connected with bacterial physiological activity. In ureolytic bacteria, the hydrolysis of urea generates ammonia and dissolved inorganic carbon, generally increasing the pH and favoring carbonate availability. In the presence of sufficient calcium ions, these changes can promote calcium carbonate precipitation [10, 11].

EPS complement this metabolic process by providing calcium-binding sites and a matrix for mineral nucleation. They may also facilitate bacterial attachment to surfaces and biofilm development, creating localized microenvironments in which mineral precipitation occurs. However, the contribution of EPS must be distinguished from the effects of urease activity, pH changes, and carbonate generation because these factors can independently influence the quantity and characteristics of the resulting mineral [10, 12].

The relationship between EPS production and calcium carbonate precipitation is therefore not necessarily linear (fig. 3).

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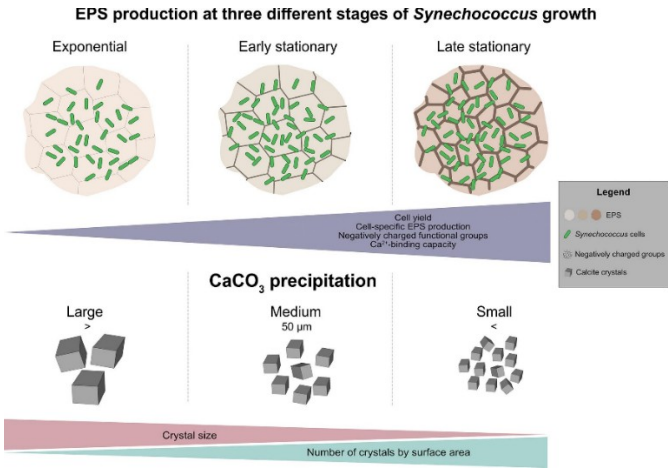


Figure 3. Schematic representation of growth-phase-dependent EPS production and its potential influence on calcium carbonate precipitation and crystal size

Moderate EPS production may enhance nucleation and mineral retention, whereas excessive polymer accumulation may restrict ion diffusion or alter calcium availability. Determining the optimal balance between bacterial metabolism and EPS synthesis is consequently an important consideration in the development of efficient biomineralization systems [2, 13].

3. Biotechnological Applications of EPS-Mediated Calcification

The ability of bacterial EPS to facilitate mineral formation has important implications for several biotechnological applications. In construction engineering, MICP is being investigated as a means of producing bio-based materials, sealing cracks, and reducing the permeability of porous structures (fig. 4). The diagram illustrates the effects of nutrient availability and seawater Mg^{2+}/Ca^{2+} ratios on mineral precipitation and polymorph formation. Bacterial urease activity promotes urea hydrolysis, generating carbonate species that react with calcium ions to form calcium carbonate minerals. Extracellular polymeric substances (EPS) may contribute to bacterial attachment, ion retention, and mineral nucleation, supporting the deposition of minerals within concrete cracks. EPS can support bacterial attachment, retain mineral particles, and contribute to the cohesion of calcium carbonate deposits. When combined with appropriate bacterial strains and mineralization conditions, these properties may improve the effectiveness of biological crack repair and soil consolidation [14, 15].

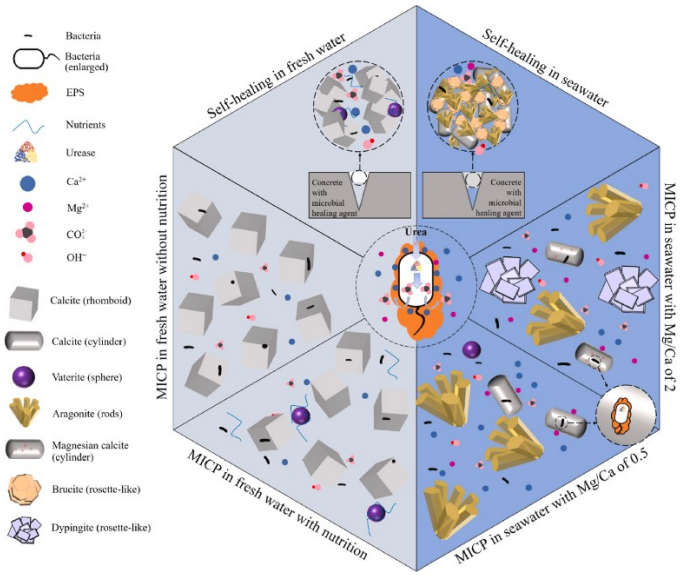


Figure 4. Schematic representation of microbially induced calcium carbonate precipitation (MICP) and its applications in concrete self-healing under different environmental conditions

In environmental applications, EPS-mediated calcification may contribute to the stabilization of soil particles, the reduction of pore connectivity, and the immobilization of selected contaminants through mineral precipitation or adsorption. The potential for these processes to improve soil structure is particularly relevant in environments affected by erosion, salinity, or degradation. However, their effectiveness depends on soil chemistry, microbial survival, mineralization rates, and the long-term stability of the precipitated minerals [16-19].

EPS-producing bacteria also represent promising candidates for developing sustainable biomineralization technologies. For example, strains of *Bacillus* and other mineral-forming bacteria may combine urease activity with extracellular matrix production, supporting calcium carbonate deposition under controlled conditions. Nevertheless, the suitability of individual strains must be established experimentally by evaluating EPS composition, urease activity, calcium carbonate yield, crystal morphology, and the mechanical properties of the resulting materials [15, 17, 18, 20].

4. Conclusions and Future Perspectives

Bacterial exopolysaccharides are important structural and biochemical components of microbiologically induced calcium carbonate precipitation. Their principal contributions include calcium ion binding, provision of potential nucleation sites, regulation of crystal growth, and stabilization of mineral deposits within microbial extracellular matrices. Through interactions with bacterial metabolism and environmental conditions, EPS can influence both the efficiency and the characteristics of biological calcification.

Despite substantial progress in understanding microbial biomineralization, further research is required to clarify the relationships between EPS composition, polymer concentration, urease activity, and calcium carbonate production. Integrated investigations combining quantitative EPS analysis, mineralogical characterization using X-ray diffraction (XRD), morphological examination using scanning electron microscopy (SEM), and elemental analysis using energy-dispersive X-ray spectroscopy (EDS) would provide a more comprehensive understanding of these interactions.

Overall, elucidating the mechanisms of EPS-mediated calcification may facilitate the development of environmentally sustainable technologies for construction, soil stabilization, and environmental remediation. In particular, investigating bacterial strains isolated from extreme ecosystems may reveal novel combinations of mineralization activity and extracellular polymer production that are valuable for applications under challenging environmental conditions.

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