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EXOPOLYMERIC SUBSTANCES INVOLVED IN CALCIUM CARBONATE BIOMINERALIZATION AND THEIR USE TO PRESERVE AND RESTORE STONE MONUMENTS

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Abstract

Many different bacterial species precipitate carbonate in alkaline environments rich in Ca^{2+} ions. Numerous authors have described the capability of bacteria to precipitate carbonate in both natural habitats and laboratory culture.
Bacterially induced carbonate mineralization has recently been proposed as an environmentally friendly method to protect decayed ornamental carbonate stone. This new conservation method mimics what nature has been doing for eons, since many carbonate rocks have been cemented by microbe-induced calcium carbonate precipitation.
Biocalcification by bacteria is an emerging restoration technique that is still being developed, and requires further research. However, the production of acid substances, the development of colored spots by microbial metabolism or bacterial survival within the carbonate crystal could have serious implications in restoration techniques. It has already been demonstrated that uncontrolled bacterial growth can damage stone. To over-come these problems, development of stone treatment without viable cells seems a better biotechnological tool.
This study highlights the role of specific outer bacterial structures, such as glycocalix, in calcium carbonate crystallization in *vitro*. We described the formation of calcite crystals by extracellular polymeric materials (EPM), such as exopolysaccharides (EPS) and capsular polysaccharides (CPS) isolated from *Bacillus firmus* and *Nocardia calcarea.* EPM were isolated from calcifying bacteria grown on synthetic medium-in the presence or in the absence of calcium ions-and their effect on calcite precipitation was assessed. SEM observations and EDS analysis showed that CPS and EPS fractions were involved in calcium carbonate precipitation, not only serving as nucleation sites but also through a direct role in crystals formation. The utilization of different synthetic media, with and without addition of calcium ions, influenced the EPS/CPS, biofilm production and protein profile of EPM. Proteins of CPS fractions isolated from *Nocardia calcarea* with a molecular mass between 25 and 70 kDa were overexpressed when calcium ions were present in the medium. SEM observations showed that some CPS overexpressed proteins were involved in calcium carbonate precipitation. The discovery that outer structures such as exopolysaccharides (EPS) and capsular polysaccharides (CPS) and /or proteins isolated from bacterial strains are able to mediate $CaCO_3$ precipitation *in vitro,* shows new perspectives in their biothecnological applications such as stone treatment without viable cells.