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The effect of bacterial surfaces on the precipitation of calcite induced by bacterial ureolysis

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Introduction

The solid phase capture and co-precipitation of contaminants in calcite precipitates induced by the enzymatic hydrolysis of urea has been demonstrated to be a potential bioremediation strategy for aquifers contaminated with divalent metals and radionuclides [1]. Calcite precipitates from experiments demonstrate ureolytic bacteria buried within growing calcite crystals (Figure 1). However, it is unclear whether calcite precipitation occurs on bacterial surfaces, or from a combination of homogeneous nucleation in the bulk solution, as well as in alkaline microenvironments around the bacteria. A suite of experiments were performed in order to investigate the influence of bacterial surfaces on calcite precipitation by bacterial ureolysis.

Methods and Results

Duplicate experiments were performed at 25ºC over 8 days in microcosms containing an artificial groundwater (AGW) and 25 mM urea. Microcosms were inoculated with the ureolytic bacteria, *Bacillus pasteurii* ATCC 11859. Bacteria in the microcosm were contained within a cellulose dialysis membrane, resulting in bacteria inclusive and bacteria free AGW solution. Urea hydrolysis by *B. pasteurii* resulted in the production of ammonium and dissolved inorganic carbon, and an increase in pH in the whole AGW solution, which lead to the precipitation of calcite crystals in both the bacteria inclusive and bacteria free zone of the AGW. Differences in the morphology and crystal size distribution of the resulting precipitates in the bacteria inclusive and bacteria free zones of the AGW suggest bacteria surfaces directly impacted upon the precipitation process.

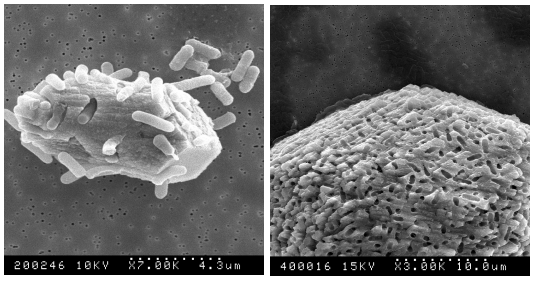

Figure 1. Calcite precipitates by bacterial ureolysis.

References

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2.7.P02

Isolation of a 35 kDa protein from the organic matrix of seashell of *Haliotis fulgens*

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The abalone seashell is an acellular bioceramic containing organic and inorganic phases that are sequentially assembled during the development of mollusk. The seashell usually is constituted by calcium carbonates. The more abundant isoforms are aragonite and calcite; these minerals are spatially separated in distinct parts of the shell. Previous studies have demonstrated that the proteins that involve the organic matrix of the shell participate in the nucleation, growth and formation of minerals. The organic matrix of the nacre of Haliotis fulges was analyzed using reversed-phase HPLC, electrophoresis and immunological characterization. During the analysis of the soluble matrix we observed a complex protein mixture, with proteins of an ample rank of molecular weight. By means of reversed-phase HPLC we separated several fractions of proteins from the seashell extract. With electrophoresis we observed that the most abundant proteins have approximated weights of 17 and 35 kDa. The column fraction containing 17 and 35 kDa proteins was further resolved by SDS-PAGE to separate the two proteins. Gel pieces containing 35 kDa was excised and the protein electroeluted. The 35 kDa protein was used to immunize rabbits for polyclonal antibody production. Once purified, the antibody, was used to purify more 35kDa protein by affinity column. The 35 kDa protein was used for *in vitro* tests of calcite crystalization.