

# Ca-Carbonate Precipitation by Ureolysis Microorganisms from Rhodoliths in Marine Sediments and Speleothems in a Limestone Cave

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Microbially induced calcium carbonate precipitation (MICP) generally refers to a process in which the urease secreted by microbes hydrolyzes urea to ammonium and carbon dioxide, thus causing an increase in the surrounding pH which promotes calcium carbonate ( $\text{CaCO}_3$ ) precipitation in calcium-rich environments [1]. The objective of this study was to investigate the mineralogical characteristics of calcium carbonate precipitated by ureolysis microorganisms cultured in various environments. The two types of carbonate-forming microorganisms (CFMs), mixed cultures hydrolyzing urea, were enriched from rhodoliths in marine sediments and speleothems in a limestone cave. The experiments using a CFM, *Sporosarcina pasteurii*, was used for comparison. All microbes were cultured aerobically in D-1 growth media including urea. To investigate the effect of microbial growth states on calcium carbonate precipitation, calcium ion (Ca-acetate) was injected into the media before and after microbial growth, and into the cell-free EPS solution. XRD and SEM-EDS analyses were used for mineralogical characterization of the precipitated calcium carbonates.

Calcium carbonates precipitated under all experimental conditions. The most rapid precipitation of calcium carbonates occurred in the cell-free EPS solution while forming calcite (size = 5–15  $\mu\text{m}$ ). Both calcite and vaterite were formed under the experimental conditions containing microbial cells. When the injected concentrations of Ca-acetate were varied from 0 to 0.5 M, the highest amounts of calcite (22.8 g/L) were produced when 0.3 M Ca-acetate was injected. Therefore, carbonate-forming microorganisms that decompose urea are inhabited in various natural environments, and microbial growth and the presence of EPS affected the calcium carbonate formation rate and types of carbonate minerals. The amounts of calcium carbonate could be optimized by controlling the concentration of calcium ion. Moreover, the use of cell-free EPS solution is expected to be applicable to calcium carbonate precipitation in an environment where microbial growth is unfavorable.

[1] Mitchell et al. (2010) *Environ. Sci. Technol.* **44**, 5178-5185.