

## An early-branching microbialite cyanobacterium forms intracellular carbonates

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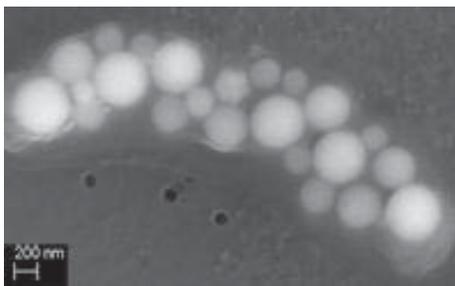
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### Abstract

The evolution of Earth environments has been deeply linked with that of Cyanobacteria for billions of years. Cyanobacteria have impacted major geochemical cycles (C, N & O) through Earth history. They have played a major role in the carbon cycle by converting CO<sub>2</sub> into organic carbon and carbonates and by enriching the atmosphere in oxygen. They have been looked for in the geological record in the form of fossil encrusted cells based on the assumption that cyanobacterial calcification is always an extracellular process. Here, we report the discovery of a cyanobacterium found in microbialites from Lake Alchichica (Mexico) [1,2] that forms intracellular carbonate phase inclusions, revealing an unexplored pathway for calcification. Electron diffraction shows that these phases are amorphous although XANES nanospectroscopy reveals local ordering consistent with the structure of benstonite, a Mg-Ca-Sr-Ba carbonate. Phylogenetic analyses place this cyanobacterium within the deeply divergent order Gloeobacterales. Accordingly, we tentatively name it *Candidatus* Gloeomargarita lithophora. This discovery opens the possibility that ancestral calcifying cyanobacteria may have biomineralized carbonates intracellularly, and thus are not prone to encrustation in extracellular precipitates. This lack of encrustation provides an alternative explanation for the absence of cyanobacterial microfossils in the oldest fossil stromatolites and opens questions about the evolution of calcification in cyanobacteria.



**Figure:** SEM picture (secondary electron mode) of a cell of *Ca. G. lithophora*. Intracellular carbonate inclusions filling the cell are visible.

[1] Couradeau *et al.*, (2011) *PLoS ONE* **6**(12): e28767

[2] Couradeau *et al.*, *Science*, under review

## Diminished S isotope fractionation accompanies internal sulfate accumulation by sulfate-reducing bacteria

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Microbial sulfate reduction (MSR), one of the earliest metabolisms to appear on Earth, is a process by which some prokaryotes use sulfate as an electron donor to obtain energy for metabolic processes. BSR results in the fractionation of sulfur isotopes, with the reactant sulfate enriched in <sup>34</sup>S, and the product sulfide depleted in <sup>34</sup>S. As studies have shown that MSR in low sulfate conditions results in small S isotope fractionations, limited variability in S isotope fractionation in ancient biogenic sulfides has been interpreted to indicate sulfate-poor conditions in early oceans.

We conducted closed-system experiments using two pure strains of acid-tolerant sulfate-reducing bacteria (M1, optimum pH 4; and GBSRB4.2, pH 4.2) in non-sulfate-limiting conditions. We identified two distinct fractionation regimes during the growth cycle: 1) low (<5‰) fractionation in the beginning lag phase, when sulfate reduction and growth are negligible, and 2) higher fractionation (≈11-24‰) during the exponential phase, when cell growth and sulfate reduction are occurring rapidly. The low fractionation during the lag phase apparently results from initial sulfate uptake by the cells, which leads to internal sulfate accumulation prior to the initiation of sulfate reduction.

The small S isotope fractionations that we have identified are consistent with rare experiments that have been designed to specifically investigate sulfate uptake during MSR. Isotopic calibration of the first link in the MSR processing chain contributes to our understanding of the multi-step process of MSR and may help in developing a mechanistic interpretation of S isotope compositions in modern and ancient biogenic sulfides.