**Effect of culture pH on cyanobacterial EPS composition:
Implications for carbonate precipitation**

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Cyanobacteria are cosmopolitan pelagic or benthic phototrophs that are responsible for a third of the annual global aquatic carbon production. Their photosynthetic activity consumes dissolved inorganic carbon, which could increase pH, thus favoring CaCO3 precipitation. In addition to fixing CO2, cyanobacteria are also main producers of exopolymeric substances (EPS). Some EPS contain acidic functional groups (*i.e.,* -COO-) that bind cations, including Ca2+, and provide nucleation sites for carbonate mineral precipitation. Therefore, EPS properties, such as the acidity and polymer density, are important in the precipitation process and may affect the carbonate mineralogy. The picoplankton species *Synechococcus*, one of the most abundant cyanobacterial species, plays an important role in global carbon cycling. The growth and EPS production of *Synechococcus* are affected by the local environmental conditions, including pH. The pH may not only impact the growth of this organism, but also the quality and quantity of EPS it produces, and thus have multiple effects on carbonate mineral production.

In this study, different size fractions of EPS were extracted from the freshwater cyanobacterium *Synechococcus elongatus* PCC 7942. This strain was grown in both buffered and unbuffered medium with the initial pH set at 7.5. The final pH of the medium was 7.5 for buffered and averaged 10.5 for unbuffered growth. High (> 10 kDa) and low (3-10 kDa) molecular weight EPS fractions were obtained, and their major properties were determined. Our analyses revealed that EPS extracted from unbuffered growth medium is more acidic and contains more protein than EPS from buffered medium. Calcium carbonate inhibition and forced precipitation assays were carried out in both EPS fractions. A higher quantity of smaller crystals precipitated in EPS obtained from unbuffered grown cells (protein-rich EPS). However, the total amount of carbonate that precipitated appeared higher in EPS from growth in buffered medium. Calcite formed at all EPS concentrations (1-64 µg/ml) tested, but vaterite was only produced at relatively low concentrations of EPS (1-8 µg/ml).

We conclude that the environmental pH controls the carbonate mineral precipitation indirectly and directly. Indirectly, pH affects the cyanobacterial metabolism. As a consequence, the EPS produced by *Synechococcus* grown in unbuffered medium (pH = 10.5) has a higher protein content, which increases the acidity and the Ca2+ binding capacity of the polymeric matrix. Directly, elevated pH creates favorable physicochemical conditions (*i.e.*, alkaline conditions) for carbonate precipitation and increases the deprotonation of functional groups and thus cation binding capacity of EPS. In sum, the cosmopolitan lifestyle of *Synechococcus* spp.,in combination with the findings of this study shed new light on carbonate mineral precipitation during whiting events on a global scale, through geologic time.