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Invited research article

Impact of the cyanobacterium *Gloeomargarita lithophora* on the geochemical cycles of Sr and Ba

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ABSTRACT

The cyanobacterium *Gloeomargarita lithophora* forms intracellular amorphous calcium carbonates and has been recently shown to accumulate Ca, Sr and Ba at a high level with a preference for Ba > Sr > Ca. This is of interest to the design of bioremediation strategies for Sr and/or Ba pollutions in aquatic systems. However, this preference has so far been evidenced at high dissolved Sr and Ba concentrations only, i.e. conditions rarely encountered in the environment. Here, we followed the uptake of alkaline earth metals (Mg, Ca, Sr and Ba) by *G. lithophora* in alkaline solutions with more environmentally-relevant, low Sr and Ba concentrations. Two settings were analyzed: 1) batch cultures in which alkaline earth metals were added only at the beginning; 2) cultures supplemented continuously with fragments of alkaline earth element-containing carbonate microbialites. The growth of *G. lithophora* was higher in the presence of microbialites, suggesting that some limiting elements, possibly P and/or Ca, were supplied by their dissolution. In the two settings, the concentration of dissolved Ca was in the micromolar range, while it was more than three orders of magnitude lower for Ba and Sr. In cultures with microbialite fragments, these low Sr and Ba concentrations resulted from an input by aragonite dissolution balanced by high Sr and Ba uptake by the cyanobacterial cells with high affinity. Thereby, the continuous supply of alkaline earth metals by microbialite dissolution increased the Sr and Ba content of the cyanobacterial intracellular carbonates over time. *G. lithophora* incorporated preferentially Ba and Sr over Ca even at very low Ba and Sr concentrations and despite the presence of Ca at a much higher concentration. Overall, this shows the capability of *G. lithophora* to impact the geochemical cycles of Sr and Ba by buffering dissolved Sr/Ca and Ba/Ca ratios at low levels.

1. Introduction

Strontium and barium have been traditionally considered as alkaline earth elements that are non-essential for life (Loewen et al., 2016). Yet, their content ratios to Ca in some biominerals have been used as proxies for paleotemperature for Sr/Ca (e.g., Gagnon et al., 2013) and changes in seawater Ba/Ca due to variations e.g., in the terrestrial runoff and/or the decomposition of Ba-rich biogenic particles in the deep ocean (e.g., Allen et al., 2016). Sr/Ca and Ba/Ca ratios in organisms have also been suggested to provide an ecological proxy in some cases, these ratios tending to decrease with higher trophic levels, a process called "biopurification" (Peek and Clementz, 2012). However, in contrast with this biopurification, some organisms sequester Sr and/or Ba preferentially to Ca, i.e. increase their Sr/Ca and/or Ba/Ca content relatively to their environment (Peek and Clementz, 2012). This is the case for organisms forming intracellular barite (Ba-sulfate) and/or celestite (Sr-sulfate), such as the marine and freshwater algae *Closterium moniliferum* (Brook et al., 1988; Krejci et al., 2011) and *Spirogyra* (Kreger and Boeré, 1969), some protozoans, such as acantharian (Bernstein et al., 1992, 1998), *Loxodes* (Finlay et al., 1983; Hemmersbach et al., 1999; McGrath et al., 1989), *Sphaerozoum punctatum* (Hughes et al., 1989) and *Xenophyophore* (Gooday and Nott, 1982; Swinbanks and Shirayama, 1986). This selective uptake of Sr and Ba may be used to remediate Sr or Ba pollutions in the environment (Cam et al., 2016). It may also impact the geochemical cycles of Sr and/or Ba (Griffith and Paytan, 2012). Indeed, these organisms have a Ba content significantly higher than sea water concentrations (Fisher et al., 1991), and therefore constitute a reactive geochemical reservoir of Ba,

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where barite can precipitate, feeding the export of Ba from the water column to the sedimentary reservoir (e.g., Bernstein et al., 1998). Griffith and Paytan (2012) reviewed four modes of barite formation in the ocean: marine or pelagic barite, hydrothermal barite, cold seeps barite and diagenetic barite. The role of microorganisms in the formation of marine or pelagic barite has been clearly pointed out and the sediment content of pelagic barite has been classically used to reconstruct past variations in ocean biological productivity (Paytan and Griffith, 2007). However, there is still some debate about the relative importance of intracellular biomineralization vs. mediation of precipitation by extracellular nucleation on cell surfaces in the formation of these biological Ba-phases. Moreover, mechanisms involved in the concentration of Ba and Sr in the biomass remain poorly understood.

Krejci et al. (2011) suggested that the bioaccumulation of Sr and Ba in the desmid *Closterium moniliferum* was not due to a selective intracellular transport of Sr and Ba but resulted from partitioning of Ca, Sr and Ba by the precipitation of highly insoluble (Sr, Ba)-sulfates. While this might be true for these organisms, bioaccumulation of Sr and Ba clearly results from different processes in others. For example, the freshwater unicellular alga, *Tetraselmis cordiformis* and the freshwater cyanobacterium *Gloeomargarita lithophora* were recently shown to accumulate Sr and Ba preferentially to Ca in association with the formation of intracellular amorphous calcium carbonates (ACC) but not sulfates (Couradeau et al., 2012; Martignier et al., 2017). Such a bioaccumulation, not related to the formation of intracellular sulfates, may be quantitatively important: *G. lithophora* and related species have been shown to have a widespread geographic and environmental distribution and to have adapted to a wide range of temperatures (Ragon et al., 2014). These environments are exclusively terrestrial and include karsts in France, hypersaline mats in Spain, hot springs in Algeria, central Tibet and Yellowstone (Turner et al., 1999; Lau et al., 2009; Amarouche-Yala et al., 2014; Ragon et al., 2014); *Tetraselmis cordiformis* forms Sr- and/or Ba-rich intracellular carbonates in one of the largest lakes in Western Europe: the meso-oligotrophic Lake Leman (Martignier et al., 2017). Cam et al. (2015) showed that the partitioning of Ca vs Sr and Ba observed in *G. lithophora* cells was not due to the ACC precipitation process but to some transport mechanism operating at the cell wall. These cyanobacteria accumulated Ba first, then Sr and finally Ca in a culture medium amended with 250 μM of Ca and 50 or 250 μM of Sr and Ba (Cam et al., 2016). However, the initial Sr and Ba concentrations used by these experiments were substantially higher than what is usually found in the environment, i.e., between 0.01 and 18 μM in surface freshwater and 0.032–114 μM in seawater (Chowdhury and Blust, 2011; Peek and Clementz, 2012). The concentrations of dissolved Sr and Ba in Lake Alchichica, where *G. lithophora* was first observed with Sr- and Ba-containing ACC inclusions, were in the nanomolar range (Couradeau et al., 2012). Whether the observed selective accumulation of Sr and Ba still occurs at such low concentrations with a continuous supply of alkaline earth elements remains to be proved.

Here, we cultured *G. lithophora* in the presence of solid carbonates which provided a continuous supply of Sr and Ba at nanomolar concentrations as well as in the absence of such a continuous supply of Sr and Ba. Variations in the concentrations of dissolved Mg, Ca, Sr and Ba were measured over time. In parallel, the distribution of alkaline earth metals in cells was assessed by electron microscopy. This allowed 1) determination of Sr and Ba affinity of cells and 2) study the impact of these cyanobacteria on the cycles of Sr and Ba in these experimental geochemical systems.

2. Material and methods

2.1. Cultures

Gloeomargarita lithophora strain C7 has been previously cultured in BG-11 as described by Moreira et al. (2017). In this medium, cells grow up to a cell density of ~10^8 cells/mL, under shaking at 30 °C and with continuous light exposure. In order to test the uptake of Sr, Ba, Ca and Mg by *G. lithophora* under more environmentally-relevant conditions, the strain was inoculated in a solution, called aquarium water thereafter, equilibrated beforehand during several weeks with microbialite fragments collected in Lake Alchichica (Couradeau et al., 2012). These microbialite fragments were composed of hydromagnesite ($Mg_5(CO_3)_4(OH)_2.4H_2O$) and aragonite ($CaCO_3$) as determined by x-ray diffraction (Couradeau et al., 2012). Chemical analyses of the microbialite fragments by ICP-AES showed that 1 g of microbialite contained 1.28 μmol of Ba, 9.87 μmol of Sr, 7.4 mmol of Mg and 2.3 mmol of Ca. Aquarium water was filtered at 0.22 μm before inoculation. These batch cultures were conducted with day/night cycle (12:12 h) during one month at 20 °C. We could not measure the microbialite mass loss due to dissolution during the experiments, since we could not sort cells from microbialites fragments. However, as an indication, the dissolution of 1‰ of the microbialite fragments would have supplied 261 μmol of Ca, 1.46 nmol of Ba and 11.2 nmol of Sr to the 70 mL of solution in the experiments.

In order to test the possibility that microbialites act as a continuous supply of alkaline earth elements (Mg, Ca, Sr and Ba) and to determine how this affects cell growth and uptake, *G. lithophora* was also cultured in filtered aquarium water supplemented with sterile microbialite fragments. Microbialites were first washed with Tris buffer (50 mM, pH 8.5) and dried at 120 °C during 1 h to inactivate biofilms. Then, fragments of similar mass (1.1 g) were placed in each Erlenmeyer flask. They were further sterilized by autoclaving at 120 °C and then dried at 120 °C during 1 h. Sterile microbialites were soaked in 70 mL of filtered aquarium water for 80 h before inoculation to buffer the solution. Non-inoculated, abiotic controls supplemented, or not, with sterilized microbialites, were also studied. Evaporation was regularly compensated by addition of sterile milli-Q water before solution analyses for each condition. Planktonic cell growth was determined by measuring the optical density at 730 nm. Measurements were performed on single samples so we could not assess their precision. However, the instrumental precision on OD measurements was 0.005. One OD unit corresponded to 9.10^7 cells/mL for *G. lithophora*.

2.2. Scanning transmission electron microscopy (STEM) and energy dispersive x-ray spectroscopy (EDXS)

Two milliliters of cultures were centrifuged at 5000*g* for 10 min. The cell pellets were washed three times with milli-Q water and used for STEM analyses. Washing was necessary to avoid the precipitation of salts upon drying. This sample preparation procedure has been used and tested repeatedly in past studies on cyanobacteria forming intracellular carbonates (Benzerara et al., 2014; Li et al., 2016). Although it may induce some alterations of the morphology of the cells (e.g., some collapse), it allows the preservation of intracellular $CaCO_3$ inclusions on the contrary to procedures using chemical fixatives (Li et al., 2016). In the present study, only STEM analyses of $CaCO_3$ inclusions are discussed, not cell morphological features.

After washing, two microliters of the suspensions were deposed on Formvar™ carbon coated copper grids and dried at room temperature. Cells collected at several time points (21, 88, 255, 351, 534 and 831 h) were observed using a JEOL 2100F equipped with field emission gun and operating at 200 kV. STEM images were acquired in the high angle annular dark field (HAADF) mode. Semi-quantitative analyses of energy dispersive x-ray spectrometry (EDXS) spectra were processed using the JEOL Analysis Station software following the procedure by Li et al. (2016). This was based on the use of K factors. The background noise in the EDXS spectrum was subtracted out and the atomic percentages were determined after a Gaussian fit of selected elemental peaks and a calculation of the area under each peak.