MINERAL PRECIPITATION BY *IDIOMARINA LOIHIENSIS* AND *MYXOCOCCUS XANTHUS*: IMPLICATIONS FOR THE BIOGEOCHEMICAL CYCLES OF CARBON AND BARIUM IN THE MEDITERRANEAN

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Abstract

Microbial processes played a major role in mineral precipitation throughout the Earth's history. Such processes have been widely discussed for the last few decades with regard to carbonate precipitation. Other than microbial precipitation of carbonates, in natural environments is also of great interest the potential role of microbes in the precipitation of marine barite. Regarding carbonates, our experiments show how certain isolated bacteria from the Alboran Sea surface waters are able to precipitate different Mg-rich carbonates at modern marine seawater salinity. Ongoing work also focuses on bacterial precipitation of marine barite and its role in the biogeochemical cycle of barium and in the record of this element in Mediterranean paleoenvironments.

Keywords: Bacteria, Mineralogy, Alboran Sea, Sediments

Introduction

Microbial processes have played an important role mediating carbonate production throughout the Earth's history [e.g., 1]. In particular, precipitation of microbial dolomite has been the focus of extensive research [e.g., 2], being this mineral is of key importance for understanding biogeochemical processes and cycles in past and modern environments. Thus, understanding of the microbiogeochemical processes involved in the precipitation of Mg-rich carbonates is essential to further understand the dolomite production in ancient environments. In this context, recent experiments have revealed that Ca-Mg kutnahorite, a mineral with a dolomite-ordered structure, can also be precipitated by bacteria [3]. As for dolomite, precipitation of Ca-Mg kutnahorite has been mostly obtained in hypersaline bacterial culture experiments. However, our recent work has demonstrated that marine bacteria were also able to precipitate this mineral at modern marine seawater salinity [4]. In addition to the investigation of microbial processes in relation to carbonates and the carbon cycle, the investigation of the biogeochemical cycle of Ba is also of major importance since both cycles are closely related and Ba is considered and excellent proxy of past marine biological productivity. The mechanism by which barite precipitates in undersaturated seawater still remains unknown. Considerable research has focused on this element over the last decades, but living organisms that directly precipitate barite have not yet been identified in seawater. Nevertheless, a reasonable proposal would seem to be that bacteria could play a role in this process. As an initial approach to this hypothesis we carried out an experiment using *Myxococcus xanthus*, an abundant and ubiquitous heterotrophic soil bacterium, and it was demonstrated that this microorganism induced barite precipitation [5]. As myxobacteria are recognized mainly as soil bacteria, ongoing work is being focused on the role of marine bacteria in sulfate precipitation.

Materials and methods

For carbonate precipitation experiments, a bacterial strain, MAH1, has been isolated from a seawater sample collected from the Alboran sea surface waters. It has been characterized using polyphasic taxonomy as Gram-negative, growing between 2-43 ºC, heterotrophic, aerobic and required NaCl for growth. Results of phylogenetic analyses evidenced that the strain belongs to *Idiomarina loihiensis* species. For biomineralization experiments, the solid medium used was the marine medium (MM) (% w/v, yeast extract 0.5%, triptone 1%, purified agar-agar 2% in seawater from Alboran Sea, pH 7.6). For sulfate precipitation experiments, *M. xanthus* cells were inoculated on a solid culture medium with Ba (CM-Ba) (0.4% yeast extract, 2 mM $BaCl2 \cdot 2H2O$, 2% purified Difco agar-agar in distilled water). To recover the precipitates formed, a needle was used for the largest crystals, and the small ones were recovered by melting the solid medium in a microwave oven. After this, crystals were washed with distilled water to eliminate culture medium remains and cell debris. Precipitate composition and morphologies were studied by Scanning Electron Microscopy (LEO Carl Zeiss GEMINI-1530, coupled with energy-dispersive X-ray microanalysis). Precipitate mineralogy was determined by X-ray diffraction using a Bruker D8 Advance diffractometer.

Results and dicussion

The MAH1 produced Ca-Mg kutnahorite and struvite at seawater salinity conditions. In this case, the amino acid metabolism resulted in a release of ammonia and CO_2. Such release increased the pH and $CO_3{}^{2-}$ concentration of the culture medium, creating an alkaline environment where saturation is reached and thus carbonate precipitation occurred. It has also been suggested that specific attributes of certain bacteria promote calcium carbonate formation [6]. Precipitation of carbonates may thus be related to heterogeneous nucleation on negatively charged bacterial superficial structures. Furthermore, the nature of the organic matrix determines which ion is preferentially adsorbed and, consequently, which mineral phase is formed. Bacterial carbonate precipitation could therefore be strain specific. In this sense, a significant characteristic of the *Idiomarina* genus is its uniquely high content in odd-iso-branched fatty acids, suggesting that this particular membrane characteristic could induce Ca-Mg kutnahorite production. In fact, this production is not related to the medium composition since other bacteria also cultivated in the MM medium did not produce this mineral. In this regard, the precipitation of Mg-Ca kutnahorite by the investigated *Idiomarina* strains strengthens the hypothesis that the precipitation of a carbonate with a dolomite-type-ordered structure also occurs in marine environments at standard salinity [4]. In the case of sulfate precipitation by *M. xanthus*, crystal growth evolves from spheres to aggregates in which barite crystals become visible. The initial phase is a P-rich precursor phase, which suggests that phosphoryl and carboxyl groups in the structural polymers of the cell wall outer membrane may be sorbent constituents, which play an important role in the precipitation process [5]. These results were the first to indicate that barite precipitates in bacterial cultures, and the results support the hypothesis that the origin of this mineral may be bacterially mediated. Such precipitation suggests that in marine environments, bacteria may enhance barite production by providing nucleation sites and by producing crystal growth. This is, however, only an initial approach for future investigation regarding the role of bacteria in the Ba biogeochemical cycle.

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