Precipitation of Minerals by 22 Species of Moderately Halophilic Bacteria in Artificial Marine Salts Media: Influence of Salt Concentration

M.A. Rivadeneyra\textsuperscript{a}, R. Delgado\textsuperscript{b}, J. Párraga\textsuperscript{b}, A. Ramos-Cormenzana\textsuperscript{a}, G. Delgado\textsuperscript{b*}

\textsuperscript{a}Department of Microbiology, \textsuperscript{b}Department of Applied Geology, Faculty of Pharmacy, University of Granada, 18071 Granada, España

\text{e-mail} gdelgado@ugr.es

Received 28 July 2005
Revised version 16 February 2006

ABSTRACT. Precipitation of minerals was shown by 22 species of moderately halophilic bacteria in both solid and liquid artificial marine salts media at different concentration and different \text{Mg}^{2+}-to-\text{Ca}^{2+} ratio. Precipitation of minerals was observed for all the bacteria used. When salt concentration increased, the quantity and the size of bioliths decreased, the time required for precipitation being increased. The precipitated minerals were calcite, magnesian calcite, aragonite, dolomite, monohydrocalcite, hydromagnesite and struvite in variable proportions, depending on the bacterial species, the salinity and the physical state of the medium; the Mg content of the magnesian calcite also varied according to the same parameters. The precipitated minerals do not correspond exactly to those which could be precipitated inorganically according to the saturation indices. Scanning electron microscopy showed that the formation of the bioliths is initiated by grouping of calcified cells and that the dominant final morphologies were spherulitic with fibrous radiated interiors. It was demonstrated that moderately halophilic bacteria play an active role in the precipitation of carbonates and we hypothesize about this process of biomineralization.

Abbreviation

\text{SI} \quad \text{saturation index}

Bacterial activity is related to the precipitation (biomineralization) of a wide variety of authigenic minerals including carbonates, oxides, phosphates, sulfides and silicates (Ferris 2000). Boquet \textit{et al.} (1973) described calcite production by 210 soil bacteria isolated on a solid medium with added \text{Ca}^{2+}, concluding that carbonate precipitation is a generalized phenomenon among bacteria. Knorre and Krumbein (2000) found that bacteria from freshwater, marine and hypersaline environments precipitated carbonates when cultivated in artificial laboratory media, the cyanobacteria being most active in this biomineralization process although heterotrophic bacteria were also involved.

Lowenstan and Weiner (1989) considered bacterial precipitation of carbonates and other types of minerals to be a process of induced biomineralization since the process of mineral formation takes place in an open medium without the participation of specific macromolecules. Thus, in these processes the composition and ionic concentration of the medium have a great influence on mineral precipitation. Moderately halophilic bacteria can grow in a wide range of salt contents (Kushner and Kamekura 1988) and are thus an eminently suitable group for the study of the influence of these parameters on biomineralization processes. This will further our understanding of the involvement of these bacteria in mineral deposition in saline habitats. Some species of moderately halophilic bacteria are capable of inducing carbonate precipitation, although with differences between them, principally in the mineralogy of the precipitates (Ferrer \textit{et al.} 1988; Rivadeneyra \textit{et al.} 1994, 1998, 2004). An in-depth study will increase our knowledge of the precipitation mechanism or mechanisms involved and of the role of bacteria in carbonate precipitation in nature.

We investigated here (1) the precipitation of minerals in artificial marine salts media by 22 species of moderately halophilic bacteria for which mineral precipitation has not previously been described and demonstrated the influence of salt concentration and type of medium (solid or liquid) on the mineralogy and morphology of the crystals precipitated, (2) the formation process of the biominerals precipitated by the species \textit{Halomonas aquamarina}, \textit{Salibacillus salixigen} and \textit{Pseudomonas halophila}, and (3) in order to deter-
mine the role of bacteria in precipitation, the mineral saturation indices in the ionic solutions used to prepare the culture media, using the geochemical program PHREEQC (Parkhurst and Appelo 1999).

We believe that this study will provide valuable information regarding the role of bacteria in mineral precipitation in their natural habitats.

MATERIALS AND METHODS

Microorganisms. Twenty-two bacterial strains of moderately halophilic bacteria were used, viz.

15 species of Halomonas:
- *H. aquamarina* ATCC 14400
- *H. canadiensis* ATCC 43984
- *H. cupida* ATCC 27124
- *H. elongata* ATCC 33173
- *H. halmopila* ATCC 19717
- *H. halodenitrificans* ATCC 13511
- *H. halodurans* ATCC 29686
- *H. marina* ATCC 25374,

2 species of Marinomonas:
- *M. communis* DSM 5604 and *M. vaga* ATCC 27119,


Culture media. Modified MH medium (in %, M/V) was used: yeast extract 1, proteose-peptone 0.5, glucose 0.1, supplemented with a balanced mixture of sea salts (Subow 1931) to a final concentration of 2.5, 7.5, 15 and 20 % (M/V). The medium was amended with 0.4 % calcium acetate and the pH adjusted to 7.2 with 1 mol/L KOH. To obtain solid media, 20 g/L bacto-agar was added.

Crystal formation. The 22 strains were inoculated onto solid media and into 500-mL flasks containing 150 mL of liquid culture medium, at 2.5, 7.5, 15 and 20 % salt concentration, and incubated at 32 °C for 40 d. Experiments were done in triplicate.

In solid media the plates were examined periodically for the presence of crystals by optical microscopy; the crystals formed after 40 d were removed by cutting out agar blocks and placing them in a boiling water bath until the agar dissolved; the supernatant was decanted and the sediments resuspended and washed in distilled water until the crystals were free of impurities.

In liquid media the flasks were periodically observed for the presence of crystals; they were collected after 40 d, transferred to distilled water and washed free of impurities. The crystals collected were air-dried at 37 °C.

Controls consisted of uninoculated culture media, and media inoculated with autoclaved bacterial cells (cell concentration ~10^7/mL) were included.

Geochemical study. The activity of dissolved species and the degree of saturation in the solutions assayed were determined using the geochemical computer program PHREEQC version 2 (Parkhurst and Appelo 1999). The results are presented in terms of SI for each predicted mineral. SI is defined as

\[
SI = \log (IAP/K_{sp}),
\]

where IAP is the ion activity product of the dissolved mineral constituents in a solubility product \((K_{sp})\) for the mineral. Thus, \(SI > 0\) implies supersaturation with respect to the mineral, whereas \(SI < 0\) means undersaturation.

Determination of nitrogen and phosphorus. Total nitrogen in the culture media was determined by Kjeldhal method, total phosphorus was determined colorimetrically in the nitrogen digests, generating the phosphomolybdate complex (Page et al. 1982).

Analysis of crystals. The purified crystals were examined by X-ray diffraction (powder diagram) in a Siemens D5005D diffractometer with CuKα radiation with a graphite monochromator. Preparation of the samples involved a hand pulverizing in an agate mortar before analysis. The mineral phases were identified in accordance with *JCPDS* and *ASTM* (1981) criteria. Fisher quartz was added to the samples as a standard to calibrate the diffractometer. Semiquantitative analysis of mineral species was performed following the intensity factors methods (Klug and Alexander 1976). The distance between crystallographic planes 104 \((d_{104})\) was calculated by the position of the diffraction peak corresponding to these planes; \(d_{104}\) was used to determine the approximate proportions of Mg\(^{2+}\) in the formula of magnesian calcite (Goldsmith et al. 1961).