Microbial Dolomite Precipitation under Aerobic Conditions: Results from Brejo do

Espinho Lagoon (Brazil) and Culture Experiments

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ABSTRACT

Microbially mediated high Mg-calcite and dolomite precipitation occurs under oxic conditions in Brejo do Espinho lagoon, Brazil, within the upper 5 cm below the sediment/water interface. With burial to < 25 cm in the sediment sequence, early diagenesis associated with sulfate reducing bacterial activity transforms the mixed carbonate mineralogy to 100% dolomite, as the pore water becomes undersaturated with respect to calcite, while remaining supersaturated with respect to dolomite. Laboratory culture experiments using moderately halophilic aerobic bacteria (*Virgibacillus marismortui* and *Marinobacter sp.*) isolated from the uppermost part of the microbial mat in Brejo do Espinho succinctly demonstrate that microbially mediated dolomite precipitation can occur under ambient Earth's surface conditions in the presence of oxygen. These results add an additional metabolic process, aerobic respiration, to bacterial sulfate reduction and methanogenesis, which have previously been identified with dolomite formation. Furthermore, the formation of carbonate minerals with spherulitic structures in both the natural environment and laboratory culture experiments points to microbial involvement, as recognized in numerous other modern environments and ancient systems. This study suggests that previously recognized modern dolomite-forming environments, such as the supratidal areas of Andros Island, Bahamas with Recent dolomite crusts, should be revisited to evaluate the importance of aerobic respiration in dolomite precipitation.

Keywords Moderately halophilic aerobic bacteria, microbial dolomite, hypersaline environment, Brejo do Espinho, Brazil, spherulites

INTRODUCTION

Dolomite is a common carbonate mineral in sedimentary rocks throughout the geological record, especially in Precambrian carbonate rocks where it is abundant and often found associated with microbial structures, e.g. stromatolites, but it is rarely found precipitating in modern environments. In the 1960's, the discovery of dolomite forming in specific environments, such as beneath the Abu Dhabi sabkhas, U.A.E. and in the ephemeral lakes of the South Australian Coorong Lagoon, provided new insights into the physico-chemical controls on the process. Apropos to the theme of this special publication, Robert N. Ginsburg and his colleagues contributed to this renaissance in dolomite research in the 1960's with their discovery of modern dolomite precipitation in extensive supratidal crusts on Andros Island, Bahamas (Shinn et al., 1965; 1969). Although these modern environments presented the possibility to study the geochemical parameters promoting dolomite precipitation, attempts at laboratory experiments based on these actualistic studies proved to be of limited or no success in precipitating dolomite under Earth's surface conditions (Land, 1985, 1998). Thus, the rarity of modern environments relative to the rock record and the lack of success with experimental studies led to a continuing enigma, often called the Dolomite Problem.

In recent years, however, a new approach using microbial experiments has provided fundamentally new data to understand the mechanisms that may be involved in dolomite precipitation under Earth's surface conditions (Vasconcelos et al., 1995; Warthmann et al., 2000) and has inspired the development of the microbial dolomite model based on a study of a modern dolomite-forming hypersaline coastal lagoon, Lagoa Vermelha, Brazil (Vasconcelos and McKenzie, 1997). In specific modern hypersaline environments, sulfate-reducing bacteria apparently induce dolomite precipitation (Vasconcelos and McKenzie, 1997; van Lith et al., 2002, 2003; Wright and Wacey, 2005). These studies demonstrate the importance of anaerobic processes in carbonate mineral formation, but many modern geological settings, such as the

Andros Island, Bahamas supratidal environment, indicate that aerobic microbial processes may also play an important role in dolomite precipitation.

A diverse microbial population is generally involved in carbonate precipitation in natural environments, including photoautotrophic cyanobacteria and bacteria producing ammonia from the degradation of nitrogen-rich organic matter (Thompson and Ferris, 1990; Rivadeneyra et al. 2000). Rivadeneyra and collaborators (1993, 2004) have demonstrated that halophilic aerobic bacteria mediate primary precipitation of dolomite associated with calcite and high-magnesium (Mg) calcite and, sometimes, monohydrocalcite. These results indicate that heterotrophic microorganisms, using molecular oxygen as an electron acceptor, may be able to precipitate dolomite at low temperatures and may be important players in modern environments, as well as in the geologic past.

Riding (2000) has suggested that morphology (microfabric) studies can provide insight on biomineralization processes, as well as on the environmental conditions where dolomite precipitates. This combined information can be useful for the identification of microbial fossils and/or precipitates, such as biomarkers in carbonate sedimentary rocks (Buczynski and Chafetz, 1991; Allen et al., 2000; Reid et al., 2000; Riding, 2000). Regardless of bacterial type (autotrophic or heterotrophic; marine or non-marine), carbonate morphologies mediated by microorganisms are distinguishable from inorganic precipitation (Krumbein, 1979; Buczynski and Chafetz, 1991; Chafetz and Buczynski, 1992; Knorre and Krumbein, 2000; Braissant et al., 2003).

This study focuses on the role of aerobic bacteria in dolomite formation. A comparative analysis of modern dolomite formation in Brejo do Espinho (Brazil), with generally oxic conditions at the sediment/water interface, and dolomite precipitation in laboratory experiments, using two moderately halophilic aerobic bacteria isolated from Brejo do Espinho, links microbial

metabolism with the natural observed conditions, such as pH, salinity, oxygen and carbonate mineralogy. In addition, we evaluate the role of these two previously undescribed bacterial strains in dolomite precipitation and postulate on the mechanism for microbially mediated dolomite formation under aerobic conditions.

GEOLOGICAL SETTING

Brejo do Espinho is a very shallow (< 0.5 m) hypersaline coastal lagoon located east of Lagoa Vermelha about 100 km east of Rio de Janeiro city (Brazil) (Fig. 1). Situated within a Pleistocene dune system, the hydrology of Brejo do Espinho is influenced by seepage through the sands dunes from the hypersaline lagoon Lagoa Aruarama on the continental side and seawater from the Atlantic Ocean. The climatic conditions in this area are peculiar due to the occurrence of an upwelling zone offshore that contributes to the semiarid climate characteristic for the region. These special conditions lead to a strong inter-annual variability of the regional climate, whereby evaporation exceeds precipitation (Barbière, 1985).

Brejo do Espinho lagoon water has a typical seawater Mg/Ca molar ratio of ~ 5 , indicating a seawater origin modified by evaporation and dilution processes (van Lith et al., 2002; Moreira et al., 2004). Brejo do Espinho sediment contains nearly stoichiometric dolomite, which appears to precipitate predominantly in periods of the year with the highest salinities (van Lith et al., 2002). Thick, multi-coloured microbial mats develop on the surface of Brejo do Espinho during flooded periods, which are controlled by two factors, surface run-off during the wet season versus influx of seawater through the dunes during the dry season. The microbial mat exhibits a stratification of mat-forming microbial communities, which is visible as different coloured layers (Fig. 2). For this study, we isolated microorganisms from the uppermost layers where oxygen tends to persist.

METHODS

Culture medium

The culture medium (D-1) used in our laboratory experiments has the following composition (% w/v): 1% yeast extract; 0.5% proteose peptone; 0.1% glucose; and 3.5 % NaCl. This medium was supplied with calcium and magnesium acetate to adjust the Mg/Ca molar ratio to 7.4. To obtain a semi-solid medium, 20 g/l Bacto-Agar (Difco) was added. After the pH was adjusted to 7.0 with 0.1 M KOH, the medium was sterilized at 121°C for 20 minutes.

Microorganisms

The bacterial strains used for this study were isolated from the uppermost part of the microbial mat in Brejo do Espinho lagoon and are designated Strain BE1 and BE2. These strains are heterotrophic, obligate aerobic bacteria. In order to obtain pure cultures of these single strains, dilution series from the isolated samples taken from the microbial mat were inoculated onto Petri dishes containing the culture medium described above and incubated aerobically at 30°C. The Petri dishes were examined periodically to determine if the colonies were able to induce mineral precipitation.

The colonies forming a visible concentric corona of carbonate minerals were isolated. These pure strains were selected for phylogenetic 16S Ribosomal Deoxyribonucleic Acid (rDNA) analysis (Institut für medizinische & molekulare Diagnostic AG, Zürich). The obtained sequences (ranging from 794 to 802 base pairs) were compared with the National Center for Biotechnology Information (NCBI) database. The results from the approximate phylogenetic affiliation revealed that the closest relative of strain BE1 is *Virgibacillus marismortui* (NCBI, accession number AJ009793) with 99.8% homology, and strain BE2 has a 100% homology to

Marinobacter sp. NT N31. Strain BE1 and BE2 are hereafter designated as *V. marismortui* and *Marinobacter sp.*, respectively.

V. marismortui (Heyrman et al., 2003) was first isolated in 1999 from the Dead Sea (Arahal et al., 1999), and was originally described as *Bacillus marismortui*. *Marinobacter sp.* NT N31 was first isolated from deep-sea sediment below a microbial mat in 3708 m water depth (Okamoto and Naganuma, unpublished).

Study of mineral formation

The ability of *V. marismortui* and *Marinobacter sp.* cells to induce carbonate precipitation was tested using the prepared semi-solid medium D-1 because bacterial carbonates are commonly found in natural gel-like mucilaginous structures (Chafetz and Buzcynsky, 1992), such as microbial mats or biofilms. Biofilms comprise bacteria immersed in a matrix of extracellular polymeric substances (EPS) (Riding, 2000; Decho, 2000). The studied bacteria were inoculated in triplicate onto the surface of semi-solid media. Control experiments, consisting of non-inoculated culture media or media inoculated with autoclaved bacterial cells, were run in parallel.

Recovering of minerals from plates

The Petri dishes were sealed with parafilm to avoid water evaporation and incubated aerobically at 30°C for 30 days. In order to detect the presence of precipitates during incubation, Petri dishes were observed once a day with light microscopy (20x). After the incubation period was completed, precipitates were recovered by scraping the colony from the agar surface. Subsequently, they were washed several times with distilled water to eliminate the nutritive solution, remaining agar and cellular debris and dried at 37 °C. Microscopic observation demonstrated that this treatment does not alter the morphology of the crystals.

pH measurements were performed at the end of the growth and carbonate formation experiments. pH-indicator paper (Merck Spezial-Indikatorpapier) was directly applied to the semi-solid surface.

X-ray diffraction analysis

The purified minerals formed in the laboratory culture experiments by the two different bacteria strains and the carbonate sediment sampled at 2.5 and 21 cm depths in the Brejo do Espinho lagoon were powdered and analyzed for their mineralogy on a Scintag XDS 2000 X-ray diffractometer (XRD). The samples were scanned by continuous scan at 1°/min from 5 to 70° with Cu-K α radiation. From the d_{104} of the diffraction spectra, the Mg/Ca ratio of the carbonate minerals was calculated after Lumsden (1979).

Scanning electron microscope analysis

The minerals formed in the laboratory experiments and the Brejo do Espinho sediment were prepared for scanning electron microscopic (SEM) observation. In order to preserve the biologic structure as well as possible, the following treatment was applied to the uppermost sediment sample in the laboratory: samples were fixed with 2.5 % glutaraldehyde in 0.2 M Na-cacodylate buffer for 90 min at 4 °C. Subsequently, the samples were washed twice with distilled water, 30 % ethanol, dehydrated in acetone, and finally critical-point dried in liquid CO₂. Samples were

analyzed by a field emission SEM (Leo 1530, 143 eV resolution, LEO Electron Microscopy LTD, Germany).

Stable carbon and oxygen isotopes

The carbon and oxygen stable isotopic compositions of powdered bulk carbonate sediment samples were analyzed. Samples were dissolved using an on-line common acid bath attached to a VG PRISM mass spectrometer. The reaction time was set at 10 minutes. The isotope data are reported in the standard δ notation relative to the international standard PDB (Craig 1953, 1957). The analytical precision of the mass spectrometer is ± 0.1 ‰ for $\delta^{13}\text{C}$, and ± 0.2 ‰ for $\delta^{18}\text{O}$. The isotopic composition of the water was determined by equilibration with CO_2 through the use of an automated ISOPREP 18 equilibration device coupled to a FISIONS-OPTIMA mass spectrometer. Analytical reproducibility of $\delta^{18}\text{O}$ water is ± 0.06 ‰. The oxygen isotopic results are reported in the conventional per mil notation with respect to Vienna standard mean ocean water (VSMOW). All $\delta^{18}\text{O}$ values have been corrected for dolomite – phosphoric acid fractionation at 90°C using the fractionation factor of 1.0093 (Rosenbaum and Sheppard, 1986). Conversions between $\delta^{18}\text{O}_{\text{PDB}}$ and $\delta^{18}\text{O}_{\text{SMOW}}$ were calculated by using the equation in Coplen et al. (1983). The sediment and pore water isotope data are listed in Table 1.

Total organic carbon and sediment porewater pH

Sediment samples were analyzed using UIC, Inc. coulometer system. The amount of total organic carbon (TOC) in the samples was obtained as the difference between the measured inorganic carbon and the total carbon. The % TOC data are given in Table 1. Standard pH-microelectrodes (Methrom) were used in the field to measure the pH of the sediment porewater.

RESULTS

Description of the sediment and mineralogy

The 25 cm sediment core, taken from the center of Brejo do Espinho lagoon, contains fine laminations below 10 cm with less distinct laminations above (Fig. 3A). The carbonate mineral composition of the sediments includes some calcite but predominantly comprises a mixture of high-Mg calcite and dolomite, in varying proportions (Fig. 3B). There is a down-core trend in the relative ratio of high-Mg calcite to dolomite with the latter increasing to 100% at 21 cm. XRD analysis of the sediment samples from 5 and 21 cm indicates that the dolomite is nearly stoichiometric with approximately 47 to 52 mol % Mg, respectively, whereas the high-Mg calcite from 5 cm contains 25 mol % Mg (Figs. 4A,B). The increase in % dolomite with depth is probably related to early diagenetic processes (Vasconcelos & McKenzie, 1997). The primary carbonate precipitate in the uppermost sediments of Brejo do Espinho contains high concentrations of high-Mg calcite, which are susceptible to early diagenetic alteration to dolomite with burial. The sediments are organic carbon-rich but show a steadily decreasing trend in %TOC from 12.5% at the surface to 2.5% in the level of 100% dolomite (Table 1).

Stable isotope data

The carbon and oxygen isotopic compositions of the bulk sediment remain basically unchanged until 18 cm depth (Fig. 3C; Table 1). As the percentage of dolomite increases to 100% at 20-23 cm depth, the $\delta^{18}\text{O}$ values increase slightly as a new isotopic equilibrium with the pore water is obtained, whereas the $\delta^{13}\text{C}$ values become relatively much more negative over the same interval.

The large change in $\delta^{13}\text{C}$ indicates isotopic reequilibration with the input of dissolved carbonate ions derived from organic matter as a result of microbial activity.

Using the $\delta^{18}\text{O}$ paleotemperature equation calibrated for dolomite (Vasconcelos et al., 2005), we can calculate the diagenetic formation temperature of the dolomite from 20-23 cm depth. With an average $\delta^{18}\text{O}_{\text{SMOW}}$ of 2.31 ‰ for the pore water and $\delta^{18}\text{O}_{\text{PDB}}$ of 3.14 ‰ for the dolomite, the calculated temperature for the early diagenesis is 21°C, which corresponds well to an average value observed with field measurements at this depth below the sediment/water interface.

Scanning electron microscopy of sediment

SEM imaging, it is possible to detect microbial colonies associated with the sedimentary carbonates in Brejo do Espinho. Figure 5 shows detailed views of the sediment sampled at 5 cm depth, which indicate the presence of cells within a matrix of nanocrystals (Fig. 5A) and coccoide colonies shown typically embedded in EPS (Fig. 5B). Figure 6 is an SEM photomicrograph of the well-crystallized rosettes comprising nanocrystals found in the 100 % dolomite sediment from 21 cm depth. These SEM images support the hypothesis that microbes are associated with primary dolomite precipitation in the sediment near the sediment/water interface and the reequilibration of the crystals with early diagenesis at shallow depths. This ageing process was also observed in the nearby Lagoa Vermelha sediment, where it occurs under reducing conditions in the presence of sulfate reducing bacteria (Vasconcelos and McKenzie, 1997).

Aerobic culture experiments

Laboratory culture experiments were performed using the two heterotrophic microorganisms, *V. marismortui* and *Marinobacter sp.* (strains BE1 and BE2, respectively), isolated from the uppermost part of the microbial mat in Brejo do Espinho. Under low temperature (30°C), hypersaline conditions in the presence of oxygen, it was possible to precipitate dolomite in semi-solid cultures. A significant rise in pH occurred in the growing cultures from the original pH 7.0 of the D-1 medium up to ~ pH 7.9. In the control experiments using non-inoculated medium, neither mineral precipitation nor changes in pH were detected.

Figure 7A gives an overview of the biologically mediated crystals formed in aerobic culture experiments with *V. marismortui*. All of the observed crystals have the same spherical morphology but with variable diameters. The larger spheres (diameter $\approx 200\ \mu\text{m}$) are hydromagnesite (hydrated magnesium carbonate) and the smaller spheres (diameter $\approx 10\ \mu\text{m}$) are dolomite (Fig. 7B). At higher magnification, the dolomite spheres formed in the aerobic culture experiments with *Marinobacter sp.* are composed of multitudinous nanocrystals of dolomite, and the mineral micro-spheres and dumbbells are enveloped in a biofilm or EPS (Figs. 7C and D, respectively). These spherical and dumbbell morphologies are typical for microbially induced carbonates (Buczynski and Chafetz, 1991; Knorre and Krumbein, 2000; Warthmann et al., 2000).

The co-presence of spheroidal dolomite and hydromagnesite in our experiments suggests that spheroidal morphology and hydromagnesite both may be indicators for microbial dolomite. Indeed, the same co-precipitation with similar morphology has been found in other modern environments, such as Lake Walyungup in South Australia (Rosen and Mcnamara, 1998; Coshell et al., 1998). These results link bacterial precipitation of dolomite and hydromagnesite, both of which are found in natural habitats (Renaut, 1990; Warren, 1990; Russell et al., 1999; Last and Ginn, 2005).

DISCUSSION

Mechanism of carbonate precipitation

The two heterotrophic microorganisms, *V. marismortui* and *Marinobacter sp.*, which were isolated from microbial mats growing in Brejo do Espinho lagoon, mediate the precipitation of dolomite under oxic conditions in laboratory experiments. Although our experimental results were produced outside of the complex microbial community structure found in the Brejo do Espinho sediments, we postulate that the experimental process may be similar to the one inducing dolomite precipitation in the uppermost sediment layers of Brejo do Espinho, as observed at 5 cm depth. A mechanism for carbonate precipitation has been previously proposed for both natural environments (Ehrlich, 2002) and aerobic culture experiments (Rivadeneyra et al., 1998; 2004). *Bacillus sp.* and *Marinobacter sp.* can metabolize nitrogenated organic material (proteins, amino acids, nucleic acids) releasing CO₂ and NH₄⁺, which lead to the observed increase of pH (and alkalinity). However, by using proteinaceous substrate, as the “peptone” medium used in this study, there is more acid produced by CO₂ than base by ammonia, according to the following equation, showing, as an example, the degradation of the amino acid serine: C₃O₃H₇N + 2.5 O₂ → 3 HCO₃⁻ + 2 H₂O + 1 NH₄⁺ + 2 H⁺

To clarify the observed carbonate precipitation, we simulated the expected pH change using the PHREEQC program, provided by the US Geological Survey (USGS) including the parameters pH, alkalinity, CO₂, NH₄⁺, Mg²⁺ and Ca²⁺. The calculated pH change by microbial degradation of 10 mM amino acids indicates that a decrease from pH 7.0 to 6.3 would occur with aerobic respiration. Considering that bicarbonate is already present in the natural sediment, which would buffer the pH, the oxidation of biomass would decrease the pH to a lesser extent than the calculated value. However, to explain the observed increase of pH in the laboratory

culture experiments from 7 to ~7.9, a massive outgassing of CO₂ must have occurred (probably more than 70 % of the produced CO₂). In the culture medium, the calculated saturation index of dolomite is +1.7 and calcite +0.39. In the microenvironment adjacent to the bacterium, the saturation may be even higher, inducing a local supersaturation with subsequent precipitation of carbonate. In the presence of Ca²⁺ and Mg²⁺, the bicarbonate ion will precipitate as Ca- and Mg-carbonates, such as dolomite CaMg(CO₃)₂ and hydromagnesite Mg₅(CO₃)₄(OH)₂·4(H₂O), as observed in the aerobic culture experiments. Similar conditions may exist in the dolomitic sediment of Brejo do Espinho, which contains abundant organic matter (mean value of TOC = 6.2 %) and has a pH close to 9.

Carbonate morphologies

Bacterial metabolism and calcium-magnesium carbonate precipitation lead to spatial-temporal changes in pH and ion concentration. These biochemical factors influence the morphology of the carbonate minerals, as well as the organic film covering minerals (Fig. 7C) and extracellular organic matter attached to the mineral (Fig. 7D). The extracellular organic matter appears to play an important role in determining the morphology as indicated by a number of *in vitro* studies, which used different marine and freshwater bacterium (Castanier et al., 1999; Fujita et al., 2000; Warren et al., 2001). Similar morphologic features, such as spheres and dumbbells, have been found in the geological record (Chafetz, 1986; Pedley, 1992; Folk, 1993; Kazmierczak and Altermann, 2002). Interestingly, rod-like carbonate features were found in Martian meteorite ALH84001 and have been presumed to be of bacterial origin (McKay et al., 1996, 1997).

Van Lith et al. (2003) and Rivadeneyra et al. (2004) discussed the fact that bacteria have the capacity to adsorb ions, mainly Ca²⁺ and Mg²⁺, on their cell envelope, creating a microenvironment which induces the precipitation of minerals. Each bacterium has particular

activities and cell envelope characteristics that create its own chemical microenvironments leading to the precipitation of different minerals, which would not form in the absence of bacterial activity. Dolomite crystals formed in the aerobic culture experiments and in Brejo do Espinho lagoon show similar morphology, particularly the internal structure of multitudinous nanocrystals constructing macroscale spheroidal structures (Figs. 6, 7).

Spherulites in the geologic record as an evidence for microbial involvement

Numerous examples of spheroidal dolomite, similar to our microbial dolomite, have been reported in the literature. Modern spheroidal dolomite was reported from the Coorong lakes, South Australia (von der Borch and Jones, 1976) and in ancient rocks from Kuwait, ranging in age from Eocene to Quaternary, which are characterized by unique spheroidal dolomite cement (Guanatilaka et al., 1987; Guanatilaka, 1989). Nielsen et al. (1997) proposed that spheroidal dolomite in the karstified top of a Dinantian dolomite sequence, in Eastern Belgium, was bacterially induced or mediated precipitates. Furthermore, Mansfield (1980) reported urolitic biogenic dolomite with spheroidal structures. Mastandrea et al. (2006) found nano-scale spheroids associated with microbial primary dolomite within a microbiolitic facies in Late Triassic carbonate platforms of southern Italy. Lee and Golubic (1999) interpreted spherulitic structures in Mesoproterozoic Gaoyuzhuang Formation, China (1.2-1.5 Ga), as being microbial in origin. Buczynski and Chafetz (1991) suggested that “sheaf-of-wheat” or dumbbell morphologies are unique to bacterially induced precipitates and can be used to identify such precipitates in the rock record. Apparently, carbonate spherulites are the final stage of dumbbell growth (Buczynski and Chafetz, 1991; Warthmann et al., 2000). However, previous inorganic experiments result in the precipitation of spherical morphology (Malone et al. 1996; Golden et al., 2001), which implies that the morphology alone is not necessarily indicative of a biological

process. In this study, the association of microbes with mineral precipitation in both the laboratory experiments and environmental samples does, however, support the biological involvement associated with the spherical morphology.

Spherulite internal structure

In general, spherulites are composed of fibrous radiate crystals, an observation consistent with spherulite surface features shown in Figure 7D. Apparently, the presence of polymeric organic molecules can promote the spherulitic growth of calcium carbonate by effectively minimizing the surface area (Yu et al., 2002; Coelfen and Qi, 2001). In contrast, the present work shows that the presence of *V. marismortui* and *Marinobacter* sp. cells are important in mediating calcium-magnesium carbonate (dolomite) spherulitic growth under oxic Earth's surface conditions. In addition, our results imply that the agar gel medium, which is often used in biomineralization experiments (Buczynski and Chafetz, 1991; Chafetz and Buczynski, 1992), plays a crucial role in the development of these specific morphologies and carbonates (e.g. by providing the organic polymeric compounds, as mentioned above).

Whereas the spheroidal and dumbbell shapes serve as evidence for bacterially induced carbonate precipitation, the organic molecules originally in the gel medium or produced by the bacteria appear to be crucial for the development of these morphologies. In other words, the gel-like culture medium D-1 simulates biofilms and/or microbial mats and provides an organic matrix in which to experimentally study biomineralization. The control experiments consisting of non-inoculated culture media do not produce carbonate precipitates and, thus, emphasize the role of these bacteria in mineral precipitation. Indeed, microbes can provide nucleation sites and may actively contribute to the alkalization of the medium.

Dolomite formation in Brejo do Espinho lagoon

Our laboratory culture experiments demonstrate that the two strains isolated from Brejo do Espinho (*V. marismortui* and *Marinobacter sp.*) can mediate the precipitation of dolomite under aerobic, low-temperature conditions within a solid organic substrate. In natural systems, the process of dolomite precipitation actually occurs, however, within a more complex microbial community. Based on our laboratory culture experiments and studies of both Brejo do Espinho and Lago Vermelha, we propose that microbially mediated dolomite precipitation occurs in a series of changing microenvironments with a combination of metabolic processes acting separately or in conjunction, as follows: (1) In the dry season, high-Mg calcite and dolomite precipitation is mediated by aerobic bacteria in the uppermost oxic sediment near the sediment/water interface and is subsequently buried to depths where early diagenetic processes occur under anoxic conditions, (2) Additionally, in the wet season, anoxygenic photosynthetic sulfur bacteria mediate the precipitation of Ca-Mg carbonates directly below the sediment/water interface, as illustrated in the microbial mat forming in the neighboring hypersaline lagoon, Lagoa Vermelha (Vasconcelos et al. 2006), (3) With burial below the level of oxygen diffusion, early diagenetic processes driven by bacterial sulfate-reducing activity occur under anoxic conditions leading to an ageing process where high Mg calcite and Ca dolomite (20 -40% Mg), which are metastable with respect to stoichiometric dolomite, would be expected to become stoichiometric dolomite (Malone et al., 1994). The occurrence of 100% dolomite concretions at shallow depths of < 25 cm, together with XRD and SEM studies (Fig. 7), in both Brejo do Espinho and Lagoa Vermelha (Vasconcelos and McKenzie, 1997), provide evidence for this process. And, (4) as hypothesized by Moreira et al. (2004), the bacterial sulfate reduction is complemented by *in situ* sulfide oxidation which results in pore waters being undersaturated with respect to (magnesium) calcite while remaining supersaturated with respect to dolomite. In the

natural environment, aerobic and anaerobic carbonate-forming processes may alternate in seasonal driven cycles. In summary, dolomite formation in the Brejo do Espinho lagoon undoubtedly occurs in a dynamic environment, strongly influenced by seasonal changes (wet vs dry, oxic vs anoxic), resulting in different microbial processes being involved in the formation of dolomite.

CONCLUSIONS

Our study of natural samples from Brejo do Espinho lagoon and laboratory experiments using discrete, moderately halophilic aerobic bacteria isolated from the uppermost oxic sediments provides new evidence to expand our understanding of the microbial processes associated with carbonate mineral formation. We have demonstrated in the laboratory that isolated aerobic strains (*V. marismortui* and *Marinobacter sp.*) are able to mediate dolomite precipitation in a synthetic hypersaline medium in a manner consistent with dolomite formation under ambient Earth's surface conditions in the presence of oxygen. Thus, a new metabolic process, aerobic respiration at the water-sediment interface, can now be included in the list of microbial factors that contribute to the precipitation of dolomite, in addition to bacterial sulfate reduction (Vasconcelos et al., 1995; Warthmann et al., 2000) and methanogenesis (Roberts et al., 2004). The proposed mechanism is based on a metabolically mediated pH shift and associated increase in alkalinity with precipitation occurring within an EPS matrix.

The similarity of crystal habit (shape) between the experimentally and naturally produced dolomite is strong evidence for the involvement of microorganisms in the precipitation processes in the latter. The morphologies of the bacterial precipitates may have important implications for the recognition and interpretation of carbonate sediments and microcrystalline cements in marine carbonates. Furthermore, the crystal habit is obviously not specific to a particular bacterial genus

and is also common to bacterial Mg-calcite, calcite and aragonite precipitates. The recognition of bacterial carbonate morphology will contribute substantially to our understanding of recent carbonate formation and to the identification of past bacterial activity in the rock record.

Finally, the demonstration that moderately halophilic aerobic bacteria can induce dolomite precipitation suggests that this process may be active in other modern carbonate-forming environments. For example, the Recent dolomitic crusts, studied by Robert N. Ginsburg and his colleagues, are reported to occur at or near the surface in an area covering hundreds of square kilometers on Andros Island, Bahamas (Shinn et al., 1965; 1969). Revisiting this supratidal environment to test for the activity of aerobic bacteria mediating dolomite precipitation in this important cementation process would surely add a new perspective to Bahamian carbonate sedimentology.

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FIGURE CAPTIONS

Fig. 1. Satellite image showing Brejo do Espinho (22°56'08 S, 42°14'18 W) located between the Atlantic coast and Lagoa Araruama in the State of Rio de Janeiro, Brazil. Image courtesy of Earth Sciences and Image Analysis Laboratory, NASA Johnson Space Center.

Fig 2. Brejo do Espinho microbial mat with characteristic colored layers. Green corresponds to cyanobacteria, pink presumably to purple sulfur bacteria, dark brown to zone of anaerobic metabolism and white to carbonate biominerals.

Fig. 3. (A) Photograph of Brejo do Espinho short core. A layer of 100% dolomite is present between 20 and 23 cm, based on the XRD carbonate mineral analysis of samples as shown in (B), which indicates a marked change from predominantly high-Mg calcite above 20 cm to predominantly dolomite below. C) Stable isotope compositions of bulk carbonate samples and associated pore water show that the $\delta^{18}\text{O}$ value of the 100% dolomite samples below 20 cm

becomes more positive, while the $\delta^{13}\text{C}$ trends to relatively negative values, indicative of microbial activity.

Fig. 4. X-ray diffractograms of Brejo do Espinho samples from (A) 5 cm depth showing a mixture of high-Mg calcite and dolomite and (B) 21 cm depth showing 100% dolomite. X-ray diffractograms of biogenic crystals formed by (C) *V. marismortui* and (D) *Marinobacter sp.* (Note: D = dolomite, HMC = high magnesium calcite and HM = hydromagnesite.)

Fig. 5. SEM photomicrographs of Brejo do Espinho sediment taken from 5 cm in short core. (A) Close-up of the fine-grained sediment showing the presence of cells (marked with arrows). (B) Coccoide bacterial cells embedded in extracellular organic matter.

Fig. 6. SEM photomicrograph of the 100% dolomite sediment from 21 cm depth in Brejo do Espinho showing an early diagenetic transition from spheroidal clusters of nanocrystals to rhombohedra with well-defined crystal faces.

Fig. 7. Spherical carbonate minerals formed in pure culture experiments. (A) Large hydromagnesite spheres (200-300 μm) and smaller dolomite spherulites (5-25 μm), formed in *Bacillus marismortui* culture. (B) Detailed view of the dolomitic spherulites on the surface of a hydromagnesite sphere. (C) Dolomite, with spheroidal morphology covered by organic film (arrow), formed in *Marinobacter sp.* culture. (D) Small nucleating dumbbell, with well-defined crystallized-surface texture, attached to extracellular organic matter (arrow), formed in *Marinobacter sp.* culture.

Fig. 1

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Fig. 7

Table 1. Mineralogic & geochemical data for Brejo do Espinho sediment and pore water.

Depth (cm)	High-Mg-calcite (%)	Dolomite (%)	TOC (%)	$\delta^{13}\text{C}_{\text{PDB}}$ (‰)	$\delta^{18}\text{O}_{\text{PDB}}$ (‰)	$\delta^{18}\text{O}_{\text{SMOW}}$ (‰)
0	84	16	12.50	-3.40	0.59	1.62
2	90	10	11.49	-2.76	0.99	1.78
3	79	21	11.55	-2.23	1.68	2.00
4	80	20	11.35	-2.35	1.64	2.4
5	79	21	9.75	-2.14	1.97	2.63
6	69	31	9.80	-1.99	1.95	2.70
7	75	25	7.75	-2.16	1.86	2.85
8	70	30	7.05	-2.30	2.06	2.85
9	62	38	5.35	-2.71	2.16	2.40
10	58	42	5.30	-2.66	2.06	2.24
11	55	45	4.71	-2.78	2.04	2.55
12	63	37	5.72	-2.70	2.08	2.63
13	67	33	5.73	-2.15	2.04	2.70
14	71	29	4.82	-2.33	2.00	2.89
15	67	33	4.70	-2.09	1.96	3.17
16	69	31	4.15	-2.42	2.06	3.11
17	65	35	3.69	-2.22	2.08	2.60
18	59	41	3.76	-2.67	2.25	2.20
19	44	56	3.43	-4.04	2.67	2.10
20	31	69	2.65	-5.74	2.97	1.78
21	0	100	2.55	-10.16	3.49	1.30
22	0	100	2.45	-9.71	3.62	1.50
23	0	100	2.35	-9.10	3.79	1.62