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Dynamic controls on accretion and lithification of modern gypsum-dominated thrombolites, Los Roques, Venezuela

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1. Introduction

Microbialites are accretionary organosedimentary structures that form as a result of a benthic microbial community trapping and binding detrital sediment and/or forming the locus of mineral precipitation (Burne and Moore, 1987). Accordingly, stromatolites (Kalkowsky, 1908) and thrombolites (Aitken, 1967) are distinct types of microbialites characterized, respectively by laminated and mesoclotted internal fabrics (Kennard and James, 1986; Riding, 2011). Both stromatolites and thrombolites typically develop in shallow subaqueous settings, such as intertidal flats and hypersaline lagoons (Jørgensen et al., 2010). Morphologically, fossil gypsum microbialites may occur as domes, columns or planar structures (Rouchy and Monty, 2000). Texturally, they are more commonly distinguished by their dominant internal thrombolitic fabric (Riding, 2008), however, gypsum, as primary mineralogy, could be replaced early during diagenesis by carbonates (Vogel et al., 2010, and references therein). Hence, a more complete

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understanding of the mechanisms by which modern subaqueous gypsum-dominated thrombolites form today would be useful to understand their ancient diagenetically modified analogs; thereby the problem of the significance of dubious pseudomorphic features in the highly fragmented stromatolitic record could be addressed (see Riding, 2008, p. 77–78, for details).

The bacterial role in gypsum precipitation, as well as the effects that early gypsum precipitation may have in microbialite fabric development has yet to be fully explored, mostly because gypsum is rarely found in modern microbialites (Kobluk and Crawford, 1990; Roughy and Monty, 2000). By using transmitted and reflected light, scanning electron microscopy (SEM), electron probe microanalysis (EPMA) and laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS), we describe here the biogeochemical and mineralogical processes occurring in an alkaline and hypersaline lagoon in the Archipelago Los Roques, Venezuela. In the shallower areas (to a depth of ~1 m) complex photosynthetic microbial mats are associated with thrombolites. The mineralogy and texture of the decimeter- to meter-scale thrombolites from Los Roques differ from most modern occurrences because of the abundance of gypsum, which is being pseudomorphically replaced by aragonite; while the lithification of the structures is mostly due to micritization of aragonite pseudomorphs after gypsum.

The relationships between internal microfabrics, primary and secondary mineralogies, and the distribution of biochemical signatures have not been systematically studied in modern thrombolites. Specifically, we were interested in documenting: (1) the affinity of extracellular polysaccharide (EPS)-rich organic matrices for certain dissolved metals which may serve as "biological proxies" (Fazio et al., 1982; Decho, 1990; Decho et al., 2005; Perry et al., 2005; Braissant et al., 2007; Ortega-Morales et al., 2007; Flemming and Wingender, 2010); and (2) if the degradation of EPS trapped during accretion produces complex physicochemical conditions and influences cation fluxes and internal diffusion pathways towards active sites of reactive carbonate precipitation (e.g., Kamber et al., 2004; Braissant et al., 2009; Decho, 2010). Importantly, if unique trace metal trends are preserved in these modern thrombolites, it might then be possible to identify whether biological processes similarly led to the accretion of ancient thrombolites, and for that matter, stromatolites exhibiting heterogeneous internal fabrics.

2. Geological and environmental settings

The Archipelago comprising Los Roques National Park is located in the southern Caribbean Sea, approximately 130 km offshore of the Venezuelan mainland. The archipelago has 42 low-laying islands and a large number of sand banks distributed in an isolated, oval-shaped rimmed shelf (Fig. 1), approximately 1250 km² in extent. The inner shelf consists of a composite lagoon which has maximum and average water depths of 50 m and 5 m, respectively (Amend, 1992). The composite lagoon is partially enclosed by coral reefs, which are better developed along the eastern and southern margins of the archipelago (Fig. 1). The reef grades to sand banks that form supratidal islands and a modern sandbank; thereby the evaporatively concentrated water is constantly fed by seepage seawater reflux (i.e. Babel, 2007). The lagoon has maximum and average depths of 1.6 and 0.5 m, respectively. The water body is density stratified and its hypolimnion consists of poorly oxygenated to sulfidic brine (Fig. 2). The average temperature of the shallow margins of the lagoon is 34 °C.

Sedimentation at the lagoon margins consists of whitish, finely crystalline crusts formed by evaporation. These crusts, which can be up to 3 cm thick, are polymineradic and contain halite, gypsum and aragonite, thus when dry they are easily erodible by ablation or can be partially dissolved by sporadic showers. Subaqueously, lithified domal-shaped thrombolites, with sizes ranging from less than 10 cm in diameter to larger coalesced structures up to 4 m large, develop along the shallow margins in a zone where water depths range from tens of centimeters down to about 1 m (Fig. 3A, B). The narrow actively-growing thrombolite zone is subparallel to the shore, where most of the subaqueous sedimentary substrate has been colonized by complex microbial mats up to 2 cm thick (Figs. 3C–F, 5). The actively growing thrombolite zone laterally gives way basinwards within a few tens of meters to a thick deposit, consisting on an un laminated organic-rich gelatinous mud that supports the growth of millimeter-scale gypsum granules (Fig. 3A), here referred as gypsumoids (after Vogel et al., 2009). With increasing deep, the authigenic sediment becomes less organic-rich and the gypsumoids form a coarser chicken wire-like depositional fabric (Fig. 3G).

3. Methods

3.1. Collection of samples

3.1.1. Sediment samples

Two thrombolites (~10 cm×15 cm) were randomly sampled by hand from the shallow lagoon (0.15 to 0.45 m). A thick microbial mat coating from one of the specimens was collected and its different layers (Fig. 3A) were carefully separated with tweezers and sampled. In addition, the organic sediments in the deepest part of the lagoon (Fig. 3G) were retrieved using a PVC dredging device and sampled. In order to preserve the microorganisms for scanning and transmission electron microscopy all the organic samples were placed in sterile 0.5 ml Eppendorf PCR polypropylene tubes containing filtered lagoon water and aqueous glutaraldehyde to a final concentration of 2% v/v.

3.1.2. Water samples

Water samples were collected directly from the lagoon using a 60-ml sterile syringe. The syringe was then fitted with a 0.2 µm micro pore filter through which water was expelled into metal-free polycarbonate screw cap sample bottles. Filtered water samples were collected in duplicate, with one being treated with analytical grade nitric acid (to a final concentration of 10% v/v) for cation analysis and the other left unacidified for anion analysis. Overlaying water temperatures and pH values were measured immediately upon collection using a Ross (Orion) combination pH electrode. Total alkalinity was measured in situ by using acid titration.

3.2. Biogeochemical profile

A rough estimation of the non-static biogeochemical zoning of the microbial mat overlaying the thrombolites (e.g. Revsbech et al., 1983) was obtained by microsensing the unthi lified part of the specimen shown in Fig. 3E, which was growing with an overlaying water depth of around 10 cm (Fig. 3D). O₂ and H₂S microsensors (UniSense A/S, Denmark, 50 µm, response time, t₉₀, of ~0.3 s), connected to a picocameter (UniSense), were simultaneously inserted in the mat via a micromanipulator mounted on a heavy solid stand. Measurements were performed at ambient temperature of ~31 °C in 100 µm increments of vertical depth and recorded by using the computer.
software Profix (UniSense A/S, Denmark). Prior to the measurements, the O$_2$ microsensor was linearly calibrated by using picoamperes readings in the O$_2$-saturated overlying water and in ascorbic acid (0.1 M, for 0% O$_2$ saturation). The calibration of the H$_2$S microsensor was performed using a sulfide standard with an ionic strength of 0.5 M buffered to the pH of the overlying water (~9.1).

3.3. Aqueous geochemistry

3.3.1. SO$_4^{2-}$ and Cl$^-$ ions chemistry

Quantitative analyses of SO$_4^{2-}$ and Cl$^-$ anions were performed using a Dionex DX600 Ion Chromatograph (IC) at the University of Alberta. Samples were diluted as required before analysis to reduce salinity to operational values adequate for the analytical machine.

3.3.2. Major and trace metal concentrations

For determination of major and trace metal compositions of the Los Roques lagoon water, the samples were digested with HNO$_3$ (8 N) and analyzed using a Perkin-Elmer Elan6000 quadrupole ICP-MS coupled to a New Wave UP-213 laser ablation system at the University of Alberta. The ICP-MS instrument parameters were as follows: RF power 1200 W, peak hopping acquisition, 50 ms dwell time. Quantitative results were obtained via calibration of relative element sensitivities against a solution containing 10 ppm of Br, In, and Sc as internal standards. Relative standard deviations (2σ level) were between 3% for Na and Fe, 0.35% for Al and Zn, and between 0.005 and 0.06% for most analyzed elements. Detection limits were between 0.006 ppb (e.g., Mn and U) and 0.75 ppb (e.g., Fe).

3.3.3. Speciation modeling

The degree of saturation of dissolved mineral species in the lagoon water was determined using the geochemical speciation program PHREEQC (Parkhurst and Appelo, 1999). Theoretical speciation results are presented in terms of the saturation index (SI) for predicted minerals, where SI is defined by $SI = \log\left(\text{IAP}/K_{sp}\right)$, with IAP and $K_{sp}$ being the ion activity product of the dissolved constituents and solubility product for the minerals considered, respectively.
3.4. Solid phase mineralogical/geochemical analyses

3.4.1. Major element geochemistry

We systematically analyzed various levels from the two thrombolite samples for major element geochemistry by electron microprobe analysis (EPMA) after the samples were mounted in epoxy. Wavelength dispersive analyses were conducted using a JEOL 8900 Superprobe (15 kV accelerating voltage; 10 μm beam diameter, and 15 nA current and 30 s counting time). The following mineral and synthetic crystals were used for calibration: F-93(Mg), diopside (Ca, Si), willemite (Mn), Hem639 (Fe), magnesite (Mg), barite (SO4) and pyrope (Al2O3). Analytical precision was monitored through triplicate analyses at each spot area. Data reduction and concentration determinations were obtained using the GLITTER® (New Wave Research) laser ablation software. In addition, ~20 mg of authigenic carbonate phases present in the microbial mat coating the same specimen (40 laser spots).

Deviation of between 0.01 and 0.5% (2σ) was determined for the basal, middle, and uppermost crusts of the specimens. These results indicate that the carbonate content and major element compositions are reliable. The detection limits are indicated where appropriate. Trace elements (except for elements like, B, Yb, Lu) were determined by electron microprobe analysis for the unknowns (Jarosewich, 2002). The spot size was 30 μm in diameter, with the averages of least three laser spots reported. Concentrations below the detection limits are indicated where appropriate. Trace elements were determined for the basal, middle, and uppermost crusts of the specimens (for a total of 94 laser spots), and in the authigenic carbonate phases present in the microbial mat coating the same specimen (40 laser spots).

Data reduction and concentration determinations were obtained using the GLITTER® (New Wave Research) laser ablation software. Repeated analysis (n = 6) of the standard yielded relative standard deviations of between 0.01 and 0.5% (2σ level) and detection limits between 0.02 ppm (e.g., Zn, Cd, Cu) and 0.06 ppm (e.g., Mg, Sc, Sr) for most elements (except for elements like, B, Yb, Lu).

3.4.2. Trace element geochemistry

The samples analyzed by EPMA were also analyzed for trace element composition by ICP-MS. Quantitative results were obtained via the calibration of relative element sensitivities against the NIST 612 standard with analyses being normalized to [Ca], previously determined by electron microprobe analysis for the unknowns (Jarosewich, 2002). The spot size was 30 μm in diameter, with the averages of least three laser spots reported. Concentrations below the detection limits are indicated where appropriate. Trace elements were determined for the basal, middle, and uppermost crusts of the specimens (for a total of 94 laser spots), and in the authigenic carbonate phases present in the microbial mat coating the same specimen (40 laser spots).

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3.4.3. Stable C- and O-isotope analyses

In order to evaluate isotopic shifts within the thrombolites, four calcified crusts occurring at different depths within the selected specimens were sub-sampled in duplicate. In addition, ~20 mg of authigenic carbonate grains were carefully picked from freeze-dried specimens were sub-sampled in duplicate. In addition, ~20 mg of authigenic carbonate grains were carefully picked from freeze-dried specimens were sub-sampled in duplicate. In addition, ~20 mg of authigenic carbonate grains were carefully picked from freeze-dried specimens were sub-sampled in duplicate. In addition, ~20 mg of authigenic carbonate grains were carefully picked from freeze-dried specimens were sub-sampled in duplicate. In addition, ~20 mg of authigenic carbonate grains were carefully picked from freeze-dried specimens. The resulting blocks of embedded biofilm were sectioned with a diamond knife mounted in an ultramicrotome (Reichert Ultracut E). Ultrathin sections for morphological studies were cut at 50 to 70 nm and mounted on formvar-covered TEM grids (Marivac Canada) with, and without, counterstaining. Sections of biofilm were observed and images captured in transmission mode using a Philips/FEI (Morgani) Transmission Electron Microscope with CCD camera (TEM-CCD) at an accelerating voltage of 80 kV at the Department of Biology, University of Alberta.

Prior to analysis, the subsamples were treated for 48 h with H2O2 (30%) to remove residual organic matter, rinsed three times using ultrapure water, and dried overnight in a vacuum oven at 30 °C. Stable C-isotope analyses of carbonates were performed by immersing whole-rock powders in 100% phosphoric acid while under vacuum (McCrea, 1950) and analyzing the released CO2 on a Finnegan MAT 252 mass spectrometer at the University of Alberta. The data are reported in δ-notation with respect to V-PDB for carbon and oxygen (Craig, 1957, 1961).

3.4.4. Scanning electron microscopy (SEM)

Thin sections and grain mounts were sputter coated with gold. SEM observations were performed at the University of Alberta on a JEOL JSM-6301FXV instrument, which has an attached Norvar energy-dispersive spectrometer system (PGT). Images were taken at 5 kV, while EDX analyses were done at 20 kV; a constant working distance of 15 mm was used throughout our SEM analyses. The textural relationships between the exopolymeric matrices and their authigenic phases were visualized by using both low-temperature (Cryo-SEM) and the critical point drying methods.

3.5. Biological SEM and TEM

Millimeter-size samples were rapidly frozen by immersion in liquid nitrogen (−180 °C), then introduced in an Emitek K1250 cryochamber where they were fractured at −180 °C, superficially sublimated at −90 °C, gold coated at −180 °C, and then introduced in the refrigerated column of the JEOL 6301F at −180 °C to conduct high-resolution examination of internal biofilm structure and mineral/ microbe textural features.

In addition to the techniques describe above, the correlative TEM preparatory scheme of Liss et al. (1996) was used to analyze individual portions of the microbial mat that coated the microbialite specimens under examination. Samples were embedded in standard low-viscosity epoxy resin, and subsequently polymerized (Spurr, 1969). The resulting blocks of embedded biofilm were sectioned with a diamond knife mounted in an ultramicrotome (Reichert Ultracut E). Ultrathin sections for morphological studies were cut at 50 to 70 nm and mounted on formvar-covered TEM grids (Marivac Canada) with, and without, counterstaining. Sections of biofilm were observed and images captured in transmission mode using a Philips/FEI (Morgani) Transmission Electron Microscope with CCD camera (TEM-CCD) at an accelerating voltage of 80 kV at the Department of Biology, University of Alberta.
4. Results

4.1. Lagoon water chemistry

At the time of the field work (February 2009), measured pH values of the surface waters of Laguna Pirata varied between 8.7 and 9.3. The water temperature fluctuated between 28 and 35 °C and the average salinity was approximately 90 ‰, reaching up to 140 ‰ in the adjacent sabkha (Fig. 3D). In terms of major ion chemistry the Mg/Ca molar ratio was found to be 6.1, showing that the lagoonal surface waters were relatively enriched in Mg as compared with Los Roques seawater (Mg/Ca = 5.6). Other relevant chemical features of the lagoon water are summarized in Table 1. Laguna Pirata waters are also slightly enriched in Co, Fe, Ni, Mn with regards to mean seawater; conservative with respect to Ti, Cu, Cr, Mo, U and V, and depleted in Zn, Zr, Ti, Nb and Sn (Fig. 4).

The hypersaline superficial waters are oversaturated with respect to dolomite (SI = 3.8), calcite (SI = 1.4), and aragonite (SI = 1.28), whereas gypsum, the most abundant mineral phase in Laguna Pirata, is slightly undersaturated (SI = −0.29). Undersaturation of the surface waters with respect to gypsum may be due to temperature, oxygenation and mixing (e.g., Last and Schweyen, 1983) or its high natural alkalinity (e.g., Thompson and Ferris, 1990).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ca²⁺ (mmol)</th>
<th>Mg²⁺ (mmol)</th>
<th>Sr²⁺ (mmol)</th>
<th>Salinity (%)</th>
<th>SO₄²⁻ (mmol)</th>
<th>Total alkalinity (mM/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laguna Pirata evaporated seawater</td>
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<td>108.9</td>
<td>97.12</td>
<td>90</td>
<td>66.8</td>
<td>169</td>
</tr>
<tr>
<td>Seawater at Los Roques</td>
<td>10.1</td>
<td>52.2</td>
<td>96.1</td>
<td>40</td>
<td>31.6</td>
<td>134</td>
</tr>
</tbody>
</table>
4.2. Geobiology of the microbial mats

4.2.1. The thrombolite-constructing mat

Microbial mats growing on the thrombolites reach up to 2 cm in overall thickness (Fig. 5A). The uppermost photosynthetic layer consists of a 4 mm thick yellowish to green zone that is dominated by filamentous cyanobacteria (e.g., Oscillatoria sp.) and algae (mostly unidentified diatoms) (Fig. 6). During daylight hours, these oxygenic photosynthesizers account for an increase in the partial pressure of oxygen at the water/mat interface from 0.25 to 0.95 atm. From 4 to 10 mm there is a zone of steep opposing gradients of oxygen (from above) and sulfide produced by sulfate reducing bacteria. 

Below the uppermost layer, a green layer extends from 4 to about 10 mm deep within the mat. It contains a high diversity of organisms, ranging from filamentous cyanobacteria, including a sulfide tolerant Spirulina-like cyanobacterium to clump-forming non-identifiable diatoms (e.g., Oscillatoria sp.) and algae (mostly unidentified diatoms) (Fig. 6). During daylight hours, these oxygenic photosynthesizers account for an increase in the partial pressure of oxygen at the water/mat interface from 0.45 to 1.05 atm.

At depths >10–12 mm, the microbial mat becomes increasingly sulfidic as evidenced by peaks in dissolved HS^- (Fig. 5B). At night, the top of the sulfidic zone shifts upwards. Fine scale microscopy of this layer reveals that it is made up mostly of rod-shaped bacteria encapsulated by EPS. Morphologically the cells resemble some of those occurring in thick EPS in the overlying layer (cf. Fig. 7C and G).

Storm-derived sediment grains are randomly dispersed within the lagoon’s microbial mat. The grains are mostly subangular in shape and consist of millimeter-scale fragments of the calcareous algae Halimeda sp.; other storm-transported bioclasts, such as ooids and shell fragments, are also present. The allochems are frequently affected by microborings (Fig. 8), which could represent biogeochemical dissolution produced by endolithic microbial communities (i.e., those between and upon mineral grains) (e.g., Kobluk and Risk, 1977; Reid and Macintyre, 2000). Microprobe analysis indicates that Mg-calcite may be formed as an internal microcrystalline precipitates within the microbore cavities; it also occurs as part of a thin microcrystalline cement often encrusting primary grains coated by EPS microfilms (Fig. 8).

As for the inorganic constituents of the thrombolite-constructing mat, calcium carbonate accumulation consists dominantly of crystal clusters (15–25 μm in diameter), which forms small peloids up to a few hundred micrometers in diameter (Fig. 9). Mineralogically, the authigenic precipitates consist of: (1) micrometer-scale aragonite needles usually forming peloidal aggregates of up to 400 μm in diameter (Fig. 9A, B); (2) ellipsoidal aggregates formed by blocky calcite crystals (Fig. 9C); (3) spheroidal forms of Mg-calcite, with grain sizes ranging from 2 μm up to 10 μm and which may, or may not, be calcified bacterial cells and their EPS; and (4) spherical Ca-phosphate particles up to 20 μm in diameter which are encrusted by calcified Mg-rich precipitates (Fig. 9E, F).

4.2.2. The gelatinous gypsooid-supporting matrix

In contrast to its shallower counterpart, the gelatinous organic matrix forming in the deepest lagoon (water depth >1.0 m) lacks a
multicolored mat and consists of a non-lithifying, purplish slime of about 30–40 cm thick. The organic matrix material at the hypolimnion is dominated by coccoid bacteria (~2–4 μm in diameter) that resemble those of the Chromatiaceae family (e.g., *Thiocapsa* sp.) (Figs. 6F, 7H) and generally exhibit intracellular S⁰ mineralization (Fig. 6H, I). The cells occur singly or in semispheroidal cell pairs, forming irregularly to ordered EPS-enclosed colonies (2–8, or more cells). Bacterial cells exhibiting similar morphologies were also observed in the suboxic layer of the thrombolite-constructing mat (Fig. 7D), but there they occur in lower population densities (Fig. 6F).

Authigenic gypsum is present at the bottom of the deepest lagoon where it forms centimeter-scale aggregates or gypsooids. The gypsooids are comprised of individual crystals with sizes ranging from 50 to 400 μm (Fig. 10A, B). Petrographical analyses of the gypsooids show that individual crystallites develop mostly as equant-shaped and distorted forms (Fig. 10). In addition, delicate gypsum rosettes were observed in the intracrystalline spaces of the distorted gypsooids. These polycrystalline aggregates were commonly observed coated by EPS, suggesting that their formation is associated with the presence of polysaccharide-rich organic matrix as has been previously demonstrated elsewhere (e.g., Cody and Cody, 1988).

Aragonite is subordinate in abundance to gypsum; when observed, aragonite grows rarely as delicate needles embedded in cracks parallel to the (010) cleavage of the host Ca-sulfate or as peloids exhibiting a

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**Fig. 6.** Delicate microbial features from the biofilm associated with the microbialites of the Laguna Pirata. (A) Autofluorescence in the upper layer of the microbial mat shown in Fig. 4A. Photosynthetic bacteria (red autofluorescence excitation) within an EPS matrix (blue autofluorescence). (B) and (C) Filamentous bacteria (in their amorphous exopolymERIC sheath). (D) Middle layer of the thrombolite-constructing mat showing high microbial diversity, spirochetes and other unidentified filamentous bacteria, presumably sulfate tolerant, are evident. (E) Shows abundant coccoid bacteria associated to a filamentous community. The arrow shows a micrometer-scale mineralized spheroid shown in detail in Fig. 9F; bottom layer at the shallow lagoon’s mat. (F) Bacterial community from the deepest thick-mat, these rod-shaped bacteria usually form aggregates within a continuous sheath (arrow).
radiating arrangement (Fig. 10C, F). A centimeter-scale accumulation of gypsooids sets apart a less-cohesive layer at the top of the sediment column from a relatively more coherent layer at the base (Fig. 3G). The sediment substrate consists of organic-rich sediment exhibiting a chicken wire fabric (Fig. 3G).

4.3. Geobiology of the fossil structures

4.3.1. Mesostructural features

The thrombolites occur as isolated domes arranged sub-parallel to the lagoon shoreline. They range in size from a few centimeters...
Gypsum accounts for more than 40–55% of the bulk mineralogy. Individual gypsum crystals (c-axis ranging from 200 to 450 μm) are present as mosaics of: (1) equant forms, with well developed {111} prisms; (2) lenticular forms; and (3) distorted tabular habits (Fig. 11). Individual crystals within the gypsum mosaics exhibit randomly oriented optical axes (Figs. 10, 11). Bulk X-ray diffraction analyses (not shown) indicate that the mineral assemblage accompanying gypsum consists of 20–35% aragonite, 15–20% calcite, and 5% halite. In thin section, 5 to 20% of the calcium carbonate is present as a finely crystalline cement that accumulates in the porous spaces (i.e. Reid et al., 1990), and about 15 to 25% occurs as pseudomorphic aragonite after gypsum. As observed by composite electron S and Ca content of S0 increase from less than 1% to up to 5% of the local bulk and in areas having a relatively higher pseudomorphic aragonite the biotite of 20 μm in diameter, probably representing mineralized bacteria-like remains, can be distinguished (Fig. 11B). As determined by laser ablation, strontium in gypsum crystals (n = 23) approaches concentrations of 995 ± 153 ppm. No inclusion treatment or corrections for celestite were conducted, but only crystals previously identified as gypsum in the microprobe were analyzed by LA-ICP-MS. The Sr content of aragonite replacing gypsum (n = 11) averaged 4,763 ± 1,460 ppm.

4.3.2. Microstructural features

Gypsum pseudomorphs. Aragonite, as verified by X-ray diffraction, occurs as a pervasive replacement phase that often mimics the crystalline pattern of gypsum, i.e., it is a pseudomorph (Fig. 9D). Gypsum replacement occurs along crystal boundaries and cleavage/fracture planes. The interface between gypsum and aragonite commonly occurs nearly orthogonal to the dissolution fronts along gypsum crystals (Fig. 9F). Elemental maps show that pseudomorphic crystals are frequently associated with traces of elemental sulfur (Fig. 13).

As determined by laser ablation, strontium in gypsum crystals (n = 23) approaches concentrations of 995 ± 153 ppm. No inclusion treatment or corrections for celestite were conducted, but only crystals previously identified as gypsum in the microprobe were analyzed by LA-ICP-MS. The Sr content of aragonite replacing gypsum (n = 11) averaged 4,763 ± 1,460 ppm.

Microcrystalline cements. Backscattered electron images and elemental maps indicate that the cements consist dominantly of Mg-rich micrite with scattered microsparite crystals (approximately 5 μm in size). Microspar crystals form an irregular interlocked mosaic with micritic cements (i.e., Reid et al., 1990) and they often exhibit fuzzy grain boundaries with partially replaced gypsum. The microcrystalline cements form patches in which isolated filamentous and coccolid shapes, of approximately 2 μm in diameter, probably representing mineralized bacteria-like remains, can be distinguished (Fig. 11B).

Although the porosity is typically high, within the crudely laminated zones porosity may decrease to nearly 3% as the fenestral features are progressively infilled by microcrystalline cements. In such
zones a fine regular micrometer-scale lamination, largely defined by its relative abundance of magnesium, develops to form stromatolitic fabrics that are locally distributed (Figs. 9A, F; 12). By contrast, in gypsum-dominated zones the microcrystalline peloidal cements are more abundant, thus these zones show a clotted thrombolitic fabric (Fig. 9B).

4.4. Carbon and oxygen stable isotopes composition

The oxygen- and carbon-isotopic data for the thrombolites are shown in Table 2. The $\delta^{18}O$ ratios fall between $-1.1$ and $-0.1$. The $\delta^{13}C$ ratios range from $-8.3$ to $-2.7$. In specimens with two types of carbonates, there exists (1) an isotopically lighter carbonate ($\delta^{13}C = -8.3$ to $-5.3\%$; $\delta^{18}O = -0.1$ to $+1.1\%$) replacing gypsum, and (2) a Mg-enriched and isotopically heavier dissolution-enhanced cement ($\delta^{13}C = -2.7$ to $-3.2\%$; $\delta^{18}O = -0.2$ to $-0.1\%$). The carbon isotope ratios also partially correlate ($R_s = 0.525, p = 0.01$) with the MgCO$_3$ content (weight %). Furthermore, the Mg- and trace metal-contents of these phases seem to correlate with the degradation states of microbial biomass trapped during accretion and areas exhibiting Mg enrichments typically show a significant decrease in the relative abundance of $S^0$.

4.4.1. Carbon isotopes

Inorganic carbon. Fig. 14 shows the carbon isotopic composition from the lithified and un lithified parts of a thrombolite specimen. A comparison between the average $\delta^{13}C_{\text{inorg}}$ values from the un lithified
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mat (δ13C = −6.1 ± 1.0‰) and the cements stabilizing the structure (−5.4 ± 2.5‰, Table 2), shows that the heavier values occur on the initial accretionary surfaces at the base of the lithified thrombolite. On the other hand, δ13C values of subsamples taken at the center of the specimen (−7.8 ± 0.2‰) are about −2.8‰ lighter than those at the thrombolite’s interface, the last in turn are slightly heavier than that of the authigenic carbonates precipitated in the mat (Fig. 14). These values suggest that towards the center there is an additional input of isotopically light carbon to the local pore-water reservoir, probably associated with heterotrophic decomposition of organic material trapped during accretion.

For comparison, Table 2 shows the isotopic ratios obtained for dispersed Halimeda sp. flakes fragments trapped by the microbial mat in the shallow lagoon. The 13C of the Halimeda sp. bioclasts averaged +2.1 ± 0.25‰. The 13C content of calcareous algae usually reflects significant metabolic effects and thus may exhibit a wide range of δ13C values (Lee and Carpenter, 2001); this variability, however, was not observed in the carbonates cements stabilizing the thrombolite.

**Organic carbon.** The organic carbon isotopic composition (δ13Corg) of the purple layer (Fig. 5) averaged a value of −15.6 ± 0.9‰ (V-PDB). Accordingly, the magnitude of the isotopic shift during organic to inorganic carbon fixation within the specimen under examination, Δδ13Corg-Inorg, is estimated in about 10‰. Such fractionation factor is consistent with the preferential removal of 12C by the microbial biomass.

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**Fig. 10.** Biological SEM of the organic sediment supporting gypsum crystals. Cryo-SEM (A, B, D) and critical point dried (C, E, F) samples showing morphologies of gypsum crystals embedded in the organic slime from the deepest part of Pirata Lagoon. (A) General view of the EPS network. Note subhedral gypsum crystals held in the mucous matrix. (B) Loosely packed subhedral gypsum within the EPS network. Note EPS strands crossing the porous space. (C) Gypsum crystal surface exhibiting a replacement feature composed of randomly oriented bladed aragonite (lower part of the photomicrograph). (D) A subhedral lenticular gypsum, the prisms faces {110}, {010}, and {111} are present. (E) Gypsum rosettes crystallized within the fibrous spaces of the crystal aggregates shown in “D”. The individual “petals” of these rosettes are lenticular crystals with minor twinning. (F) A plate-like aggregate with minor twin formation. These crystal aggregates are arranged sub-parallel to {103}, the face {111} is also present. The arrow shows fibrous interstitial aragonite.

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4.4.2. Oxygen isotopes

The mean $\delta^{18}O_{\text{PDB}}$ (0.4±0.14‰) of carbonates from the thrombolite are comparable with the values observed in the authigenic carbonates from the living mat, −0.9±0.25‰ (Table 2, Fig. 14). Such values contrast with the mean $\delta^{18}O_{\text{PDB}}$ of the trapped *Halimeda* sp. flakes, +2.2±0.13‰. The narrow range of $\delta^{18}O$ values in *Halimeda* sp., which is known to be relatively stable (Lee and Carpenter, 2001), reflects values close to the predicted for equilibrium with Los Roques’s seawater and is considered to represent the baseline for the $\delta^{18}O$ value of the bioclast material precipitated in equilibrium with seawater from the unrestricted setting, as such trapping seems to contribute marginally to microbialite accretion.

From the temperature range measured in situ (28 to 35 °C), and by using the equation of Friedman and O’Neil (1977) with a fractionation factor calculated as described in Faure (1991, p. 351), the lagoon water from which carbonates precipitated yields $\delta^{18}O_w$ values between +1.8‰ and +3.8 (SMOW). The oxygen isotopic values (V-PDB) of the samples are consistent with carbonate phases precipitated in isotopic equilibrium with the lagoon’s partially evaporated seawater in a relatively humid setting.

Fig. 11. Replacement of gypsum by carbonate. Microcrystalline cements can be found infilling inter- and intragranular spaces. Standard (A–C), backscattered electron (D) and SEM (E, F) petrography. (A) Base of the thrombolite, the image shows a subhedral, corroded, gypsum crystal (Gy) draped over by concentric micrite/microspar laminae. (B) Micrite/microsparite aggregates around corroded and partially dissolved gypsum crystals. Dark areas dominantly represent accumulation of organic material. (C) Irregular fenestral void enclosed by clotted-laminated micrite/microspar cements. (D) Calcification of gypsum leads to the formation of pseudomorphs. In the pseudomorphs, calcite replacement mimics the [001] crystallographic planes of gypsum although calcification appears to preferentially occur perpendicular to [010] cleavage of gypsum, preserving the (010) cleavage surface. (E) Poikilotopic gypsum enclosed by Mg-enriched cement. (F) Solution fronts on gypsum crystals often show aragonite crystals sub-perpendicularly orientated to the gypsum surfaces.
Fig. 12. BSE and composite X-ray maps (S, Mg and Ca) of different zones of the thrombolite specimen examined. (A–B) approximately 2 cm from the top of the structure; (C–D) approximately 7 cm from the top of the structure. (E–F) at the laminar basal part (~12 cm from the top) of the structure. Note S²⁻ and Mg²⁺ accumulations in B and D and the relative abundance of Mg-rich calcites towards the base (F).
4.5. Trace elements

Table 3 shows the enrichment factors of some minor and trace metals incorporated into carbonates from one lithified thrombolite specimen as compared with the authigenic carbonates from the associated microbial mat. There are striking similarities between the trace metal patterns of the carbonates providing structural integrity to the thrombolites and the trends exhibited by the authigenic fraction precipitated at the mat (Fig. 15), suggesting a common mechanism of trace element incorporation operating in both lithified and non-lithified zones of these microbialites.

Carbonates within the lithified structures are slightly enriched (up to 2×) in Li, Na, V, Cr, Fe, and Pb. Enrichment factors are higher for V, Co, Sb, U (2× to 4×), while Cu and Mo are concentrated between 7× and 12×. Ni exhibits a conservative behavior and its concentration in the authigenic carbonates within the mat match those values observed in the thrombolites. A high-resolution minor and trace element analysis of the thrombolite specimen is shown in Fig. 16. An increase in redox-sensitive elements approximately 6–8 cm below the mat/thrombolite interface is indicative of the local occurrence of more strongly reducing redox microenvironments as reported elsewhere (e.g., Tribovillard et al., 2006), and accounts for the maximum oxygen diffusion zone within the porous fabric.

5. Discussion

As discussed below, the dynamics of phototrophic and heterotrophic bacteria, together with local environmental factors such as the availability of accommodation space and hydrodynamics of the depositional settings, exert a strong control over the growth, mineralogy and fabric of the thrombolites in Laguna Pirata (i.e., Des Marais, 2003). The carbon isotopic signatures, as well as the minor and trace element distribution, within these structures may have relevance for better interpreting the mechanisms by which ancient thrombolites may have formed.

5.1. Accretion model and mineral paragenesis

The accretion of microbialites, in general, is a process sensitive to external or internal influences (e.g., Feldmann and McKenzie, 1998; Andres and Reid, 2006; Planavsky and Ginsburg, 2009), and even small changes in the local environment can yield notable ecological effects, including the reorganization of the entire microbial mat system (i.e., Gerdes et al., 2000; Des Marais, 2003). In this regard, the thrombolites in Laguna Pirata likely reflect a period of gradual lagoon water level decline which results in slow accretion, mostly linked to authigenic carbonate accumulation in the mats, that forms stromatolitic fabrics. As the structures reach the maximum water level, accommodation decreases and vertical accretion is substituted by lateral accretion forming the coalesced meter-scale thrombolites described herein.

Concomitant with mineral authigenesis, the motile photosynthetic microorganisms comprising the mat migrate vertically in order to...
access zones of optimal light conditions (Buczynski and Chafetz, 1993; Grotzinger and Knoll, 1999; Des Marais, 2003). As primary producers migrate upwards and away from the lithified substrate, the pore-water composition in the space formed between the detached photosynthetic mat and the thrombolite surface then becomes temporarily controlled by the activity of anoxygenic sulfide oxidizers. Their metabolic activity induces physicochemical conditions that lead to fast gypsum growth and further cementation that favor the development of the mosascale thrombolitic fabric. Because the gypsum exhibits distorted and equant forms, it is thought that the precipitation and growth of the primary sulfate crystals occurred under the influence of organic EPS matrices (e.g., Cody and Cody, 1988; Cody and Cody, 1991; Vogel et al., 2010).

As the EPS trapped within the gypsum crystals are partially degraded by endolithic sulfate reducers, which facilitates an increase in pore-water pH (Slaughter and Hill, 1991), the precipitation of gypsum is progressively inhibited because the alkalinisation reactions decrease the saturation state of gypsum (Sonnenfeld, 1984; Cody and Cody, 1988; Thompson and Ferris, 1990; Cody and Cody, 1991; Doğanp et al., 2004; Vogel et al., 2009, 2010). Concomitantly, these conditions lead to the early replacement of gypsum by aragonite (Sonnenfeld, 1984; Thompson and Ferris, 1990; Cody and Cody, 1991; Amjad, 1996; Vogel et al., 2010) as the high magnesium concentrations in the pore-water exert an inhibitory effect over precipitation of calcite (Peckman et al., 1999):

\[
\text{CaSO}_4 \cdot \text{H}_2\text{O} + 2(\text{CH}_2\text{O}) + 2\text{OH}^- \rightarrow \text{HS}^- + \text{CaCO}_3(\text{aragonite}) + \text{HCO}_3^- + 3\text{H}_2\text{O} \quad (1)
\]

Local extracellular accumulations of S\(^0\) seems to be associated to pseudomorphic gypsum replacement, suggesting that the replacement process involves the coupled activity of sulfate reducing bacteria (SRB) and sulfide oxidizing bacteria (SOB) (Kah et al., 2001; Visscher...
and Stolz, 2005; Fernández-Díaz et al., 2009; Vogel et al., 2009). Dissolved sulfide from reaction 1 may partially diffuse upwards and become chemically oxidized by O₂, which was formed within the cyanobacterial layers of the mat:

\[ 2\text{HS}^- + 0.5\text{O}_2 \rightarrow \text{Zn}^2+ + \text{H}_2\text{O} \]  \hspace{1cm} (2)

Reaction 2 leads to the characteristic elemental sulfur lamina that are often observed underlying the cyanobacteria and overlying the purple anoxygenic photosynthesizers (see van den Ende and van Gemerden, 1993; van Gemerden, 1993; Stefess et al., 1996 for details) (Fig. 17).

In addition, by forming dissolved sulfide and contributing to the formation of ammonia, the activity of SRB drives important changes in pore-water alkalinity, pH and metal activities within the thrombolite itself (Slaughter and Hill, 1991; Dupraz and Visscher, 2005; Wright and Wacey, 2005; Decho, 2010). Increased alkalinity leads to an increase in the activity of the Mg and carbonate ions (Berner, 1975) which co-precipitate with dissolved Ca to form Mg-enriched calcite phases that form mostly as microcrystalline cements, but may locally replace the metastable aragonite precursors (Fig. 17). Importantly, the early diagenetic microcrystalline cements provide structural coherence to the porous structures. A potential outcome from the ongoing diagenetic process is the total occlusion of the porous spaces by secondary carbonate cements and the complete calcification of primary sulfates. This may result in mesoclots separated by patches of mudstone and sparry cements, which, as discussed in the next section, have bioactive metal enrichment trends that correlate well with the degradation states of the organic matter trapped during accretion.

Interestingly, the thrombolites do not occur in the deep portion of the lagoon (water column > ~1 m) where sedimentation consists of chicken-wire gypsum nodules growing in a organic matrix that lacks carbonate cementation. In this zone, tidally-controlled seepage influx and scarce light penetration lead to stratification of the water column, which in turn prevents the development of photosynthetic microbial mats in the deepest part of the basin. As a consequence, the cascade of catalytic reactions controlling the accretion and lithification of sediments in the shallow lagoon realm do not take place, and the H₂S produced in the bottom sediment can be oxidized to elemental sulfur and sulfate (Canfield et al., 1993; van Gemerden, 1993; Kaufman et al., 1996; Visscher and Stolz, 2005) that rapidly react with excess Ca²⁺ ions in solution leading to bottom water gypsum supersaturation.

5.2. Chemical signatures

The degradation of the EPS releases to the pore-waters a number of redox sensitive bioactive elements that were previously sorbed to the functional groups of the trapped microbial biomass (e.g., V, Cr, Mo, Co, etc.; Hunter et al., 1998). Upon release, some of these metals form hydrated complexes with the reactive calcite surfaces and become incorporated into growing carbonate cements throughout a variety of mechanism enhanced by increased alkalinities (see Brand, 1994 for details). This process can lead to localized enrichment trends
that are consistent with the availability and degradation states of the organic matter within the structures (Fig. 16).

Similarly, the carbon and oxygen isotopic composition of the carbonate phases within the thrombolites reflect the timing of precipitation and the interplay of organic and inorganic carbon reservoirs; early-diagenetic carbonate cements (average $\delta^{13}C_{\text{PDB}} \approx -2\%$) are close to equilibrium with respect to inorganic carbon, whereas deeper in the thrombolites, gypsum-replacing carbonates are relatively enriched in $^{12}C$ (average $\delta^{13}C_{\text{PDB}} \approx -5\%$), likely incorporating carbon released by SRB heterotrophy of organic matter (Fig. 14). The uniformity of the $\delta^{18}O$ values, as compared with allochemical grains in the basin (aeolian-transported Halimeda sp. flakes, $\delta^{18}O_{\text{SMOW}} = -3.0\%$, Fig. 14), suggests that authigenic microbially-induced precipitation exceeds allochemical trapping and neomorphism.

The lack of correlation between carbon and oxygen isotopes (Fig. 14) suggests that the dissolution/lithification cycles leading to stabilization of these structures occurs entirely under continuous hypersaline conditions. Accordingly, the influence of major storm events, which would result in correlative $C$ and $O$ isotopic signatures, indicative of kinetic fractionation (Craig et al., 1963), seems unlikely to contribute to the lithification and stabilization of these structures. By contrast, the correlation between some trace metal concentration and $\delta^{13}C$ in the thrombolites reflects the interplay between biotic and abiotic processes. The heavier carbon isotopic composition is indicative of a progressive depletion of available organic matter as it is used as an energy source by chemoheterotrophic microorganisms within the structure. Depletion of labile organic matter locally led to higher pore water alkalinities and $CO_3^{2-}$ activities which promoted the incorporation of $Mg^{2+}$ and other cations having similar enthalpies of hydration into secondary isotopically-heavier carbonate phases (e.g., Nissenbaum et al., 1972; Wright, 1999).

6. A modern analog to ancient gypsum-dominated microbialites

The microbialites from Laguna Pirata are comparable with massive, gypsumified stromatolitic structures from the mid-Miocene (Badeanian) of the eastern Ukraine and Poland (Peryt, 1996; Peryt et al., 2004; Babel, 2007), and also with less spectacular centimeter-scale examples of stromatolitic build-ups associated with gypsum that are known in the Messinian of western Cyprus, Sicily and Crete; Paleogene of Bresse, France, and Neogene of Egypt (Pierre and Rouchy, 1988; Rouchy and Monty, 2000, and references therein). Because gypsum is rarely found forming domal thrombolitic structures today (Rouchy and Monty, 2000), the thrombolites from Laguna Pirata offer a rare opportunity to examine the microbial role in the formation and preservation of gypsum-dominated microbialites.

These microbialites also allow us to extrapolate the biogeochemical processes that may have led to the development of microbialites in Late Archean to Proterozoic evaporitic environments, where comparable pseudomorphic fabrics occurred (El Tabakh et al., 1999; Pope and Grotzinger, 2003; Hardie, 2004; Sumner, 2004; Schröder et al., 2008), but for which the primary mineralogy remains largely unknown. For instance, the primary mineralogy of some meter-scale domes exhibiting pseudomorphic fabrics could have been either gypsum or aragonite, the latter being proposed based on petrographic and trace element data (Sumner and Grotzinger, 2000; Sumner, 2004; but see Hardie, 2003, 2004 for a discussion). In this regard, the relatively high concentrations of $Sr$ in pseudomorphic aragonite after gypsum in the thrombolites from Laguna Pirata (Table 3) suggest that using strontium concentrations to delineate primary mineralogy may only apply if the source of $Sr$ in the pseudomorphic calcite was seawater. If metastable aragonite forms after gypsum then the $Sr$ of calcite replacing the aragonite may completely mask that of the older sulfate precursor.
Thus, the use of strontium may not be clear-cut in resolving the issue of primary mineralogy of pseudomorphic fabrics in ancient microbialites.

7. Conclusions

1. In Laguna Pirata the production of EPS by phototrophic microbials and its subsequent degradation by an associated heterotrophic community promotes the accretion of gypsum-dominated thrombolites that grow along the shallow rim of the lagoon.

2. Within the thrombolites, spatially and temporally complex dissolution/precipitation reactions, mediated by the metabolic activity of anoxicogenic bacterial communities, lead to pseudomorphic replacement of gypsum by aragonite, accompanied by the occurrence of extracellular elemental sulfur accumulations and intragranular Mg-enriched microcrystalline cementation.

3. As a result of the ongoing microbially catalyzed early diagenetic reactions, metastable aragonite is prone to become successively replaced by more stable carbonate phases. These also lead to most extracellular S\textsuperscript{0} deposits within the fossil porous thrombolitic fabric to be cycled by sulfide dependent communities interacting with BSR.

4. Among the most influential parameters potentially affecting the progressive calcification of gypsum microbialites is the amount of organic compounds trapped during accretion because its degradation resulted in localized increased zones of alkalinity in which Mg-enriched calcites may subsequently precipitate.

5. The trace metal signatures in the secondary cements are correlative with degradation states of organic matter as inferred by mineralogical assemblages: aragonite + elemental sulfur vs. aragonite + Mg-calcite, and their elemental distribution.

6. By contrast, the abundance of gypsum and the degree of Mg-enrichment in the unlithified organic sediment formed in the shallowest parts of the lagoon suggests that conditions in this zone prevented the formation of complex photosynthetic microbial mats and thus, thrombolite development. As a consequence, the dissolved sulfide produced in the bottom sediment is likely to be oxidized to form sulfate, which in turn, reacts with dissolved Ca\textsuperscript{2+} to form gypsum granules (or gypsooids).

7. The timing and processes of microbially-induced replacement and lithification of modern gypsum-rich microbialites, and their resulting biogeochemical signatures, are crucial in trying to ascertain biogeochemical signatures in ancient microbialites, particularly considering that the ancient counterparts have been subjected to billions of years of post-depositional modifications.

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