



Biological and geological characterization of modern biofilms and microbial mats and comparison with similar lithified structures in Colombian Cretaceous formations

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ABSTRACT

Microorganisms may play an important role in the binding of sediments and the formation of sedimentary structures, by means of the formation of biofilms and microbial mats. In this work, filamentous cyanobacteria from three different environments were compared: a biofilm from a lacustrine environment with intervals of flooding/desiccation, a biofilm from a continental saline environment, and a microbial mat from a thermal spring. The optical identification of the cyanobacteria allowed to establish that in the biofilms there is a dominance of the Order Oscillatoriales, while in the microbial mat both cyanobacteria of the Orders Nostocales and Oscillatoriales dominated. Two rock samples isolated from the thermal spring which genesis was possibly influenced by the activity of cyanobacteria are described and classified. One of them is a travertine/microbial framestone with stromatolitic and thrombolytic texture. The second one is classified as a mudstone/microbial boundstone. Finally, a comparison between the sedimentary structures identified in those rocks with similar structures in the formations La Luna, Paja and Tetuán, deposited during regressive phases of the Colombian Cretaceous epyric sea, and microbial mat features previously described is performed. Based on morphological resemblance, fibrillar networks identified locally in those formations are interpreted as possible biolaminations originated from the activity of cyanobacteria.

Keywords: Cyanobacterium, extracellular polymeric substances (EPS), microbially-induced sedimentary structures (MISS), microbial mat features, biosignature, Geobiology.

Caracterización biológica y geológica de biopelículas y tapetes microbianos actuales y comparación con estructuras litificadas similares en formaciones cretácicas de Colombia

RESUMEN

Los microorganismos pueden jugar un papel importante en la cohesión de los sedimentos y la formación de estructuras sedimentarias, a través de la formación de biopelículas y tapetes microbianos. En el presente trabajo se compararon las cianobacterias filamentosas de tres ambientes diferentes: una biopelícula de un ambiente lacustre con intervalos de inundación/deseccación, una biopelícula de un ambiente continental salino, y un tapete microbiano aislado de una fuente termal. La identificación óptica de las cianobacterias permitió establecer que en las biopelículas predomina el Orden Oscillatoriales, mientras que en el tapete microbiano predominan a la vez cianobacterias de los Ordenes Nostocales y Oscillatoriales. Se describen y clasifican dos muestras de roca tomadas de la fuente termal cuya génesis posiblemente estuvo influenciada por la actividad de cianobacterias. Una de ellas es un travertino/ "framestone microbiana con textura estromatolítica y trombolítica. La segunda se clasificó como una lodolita/ "boundstone" microbiana. Finalmente, se realiza una comparación entre las estructuras sedimentarias identificadas en dichas rocas con estructuras similares en las formaciones La Luna, Tetuán y Paja, depositadas durante fases regresivas del mar epicontinental de Colombia durante el Cretácico, y las características de tapetes microbianos descritas previamente. Con base en las similitudes morfológicas, las redes fibrilares identificadas de manera localizada en dichas formaciones se interpretan como posibles biolaminas originadas por la actividad de cianobacterias.

Palabras clave: Cianobacteria, sustancias poliméricas extracelulares (EPS), estructuras sedimentarias inducidas por microorganismos (MISS), características de tapetes microbianos, biofirma, Geobiología.

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Introduction

The surface adhesion of microorganisms by means of extracellular polymeric substances (EPS) favors the formation of thin organic laminae known as biofilms (Marshall, 1992; Costerton *et al.*, 1995). These structures may grow to form thick stratified layers called microbial mats (Castenholz, 1994; Gerdes, 2010), fibrillar networks that often irreversibly bind filaments of cyanobacteria and sediments. They are characterized by an inner stratification with functional groups of microorganisms that coexist, generate symbiotic relationships and potentially modify the characteristics of sediments and sedimentary rocks (Walter, 1976 in Golubic *et al.*, 2000), particularly in extreme environments. Microbial mats have been regarded as advanced stages of a biofilm that build laminae on the top of layered surfaces and reflect gaps in sedimentation (Gerdes, 2010). The transition from a biofilm to a microbial mat involves several weeks of non-burial and the formation of fibrillar condensed networks (Schieber *et al.*, 2007), as well as the incorporation and stabilization of sediments (Stolz, 2000).

The interaction between EPS and sediments, either calcareous or siliciclastic, allows the preservation of characteristics that provide evidence of the presence of microorganisms. Those microbially-induced sedimentary structures (MISS) have been categorized into 17 different types and can be found not only in current environments but also in the lithological record (Noffke, 2009). In addition, Schieber *et al.* (2007) describes four main categories of microbial mat features in the rock record, from which the biolaminites are the most abundant. Both MISS and microbial mats are considered biosignatures, chemical or physical evidence of biological activity (Farmer and Des Marais, 1999; Summons *et al.*, 2011; Westall and Cavalazzi, 2011).

The study of MISS and microbial mat features is highly relevant to understand the paleobiological implications of the associations between microorganisms and sediments, the paleoecology of microorganisms and their contribution to the sedimentary record through the formation of structures and biominerals. Some other applications include: basin analysis and the detection of sea-level oscillations in sequence stratigraphy, oceanographic and paleoclimatic research, geochemical and paleoenvironmental reconstructions of the early Earth (Noffke, 2010), and the potential for the search of extraterrestrial life (Foster and Mobberley, 2010).

Studies on geobiology and astrobiology in Colombia are scarce and their development has not been extensively considered in the main geosciences research schools and centers that traditionally have given

more relevance to other areas such as geochemistry, petrology, geophysics, and oil and mineral deposits. This is one of the pioneering works in the geobiology and astrobiology lines of study at the Geosciences Department at the National University of Colombia. The aim of this study was to identify the filamentous cyanobacteria isolated from biofilms and microbial mats collected in three sampling sites, considered representative of present-day extreme environments, in order to assess their morphological variability and find correlations between the morphotypes and the environment. In addition, we analyzed the role of the cyanobacteria in the formation of the rocks in which they were growing, as an analog for the identification of potential microbial mat features on several Cretaceous formations in Colombia.

2. Data

The samples analyzed in this study were microbial mats and the rocks that they were growing on top of, collected from the thermal spring “Agua Caliente” at El Rosal (Cundinamarca), and biofilms from a channel with organic sludges adjacent to the salt mine at Nemocón (Cundinamarca) and a flooding land surface nearby the Colombian Agricultural Institute (ICA) inside the campus of the National University of Colombia, Bogotá (Figure 1). Table 1 indicates the sampling coordinates and Figure 2 shows the location of the sampling sites.

The rock samples collected with the microbial mat growing on top were macroscopically identified as a travertine (R-1) and a mudstone (R-2). The rocks, microbial mats and biofilms from each location are illustrated in Figure 3.

3. Methodology

1 cm² of the biofilm (less than 1 mm thickness), or 1 cm³ of the microbial mat (1cm thickness) collected at each location was separately dispersed on a glass slide with the aid of forceps. Nanopure water was added as necessary to dilute each sample until it was possible to distinguish cellular structures with transmitted light microscopy. The observation was made on a Nikon Eclipse E100 microscope at the Laboratory of Microbial Ecology of the National University of Colombia, Bogotá. Photographs at different scales were taken (Figures 4-6) and compared to the revised classification Castenholz (2002) and considering the taxonomic revisions of Komárek & Anagnostidis (2005), and Komárek *et al.* (2014).



Figure 1. Images of the sampling sites of biofilms and microbial mats analyzed in this study. (a). Thermal spring “Agua Caliente”, El Rosal (Cundinamarca). (b). Runoff channel with organic sludge adjacent to the Nemocón salt mine. (c). Flooding land Surface inside the campus of the National University of Colombia, Bogotá.

Table 1. Location and characteristics of the sampling site of biofilms and microbial mats analyzed in this study.

Sampling site	Coordinates (Bogotá origin)	Height (m)	Water temperature (°C) at the sampling time	Annual ranges of air temperature (°C)	Average air temperature (°C)
Thermal spring “Agua Caliente”, El Rosal (a)	4°55'44,09"N/ 74°15'39,16" W	2258	42	6.9-19.3	13.3
Salt mine, Nemocón (b)	5°3'55,10"N/ 73°52'41,08"W	2595	11	6.7-20.7	13.8
Flooding plain, UNAL (c)	4°38'6,72" N/ 74°5'11,43" W	2600	14	7.2-19.4	13.5

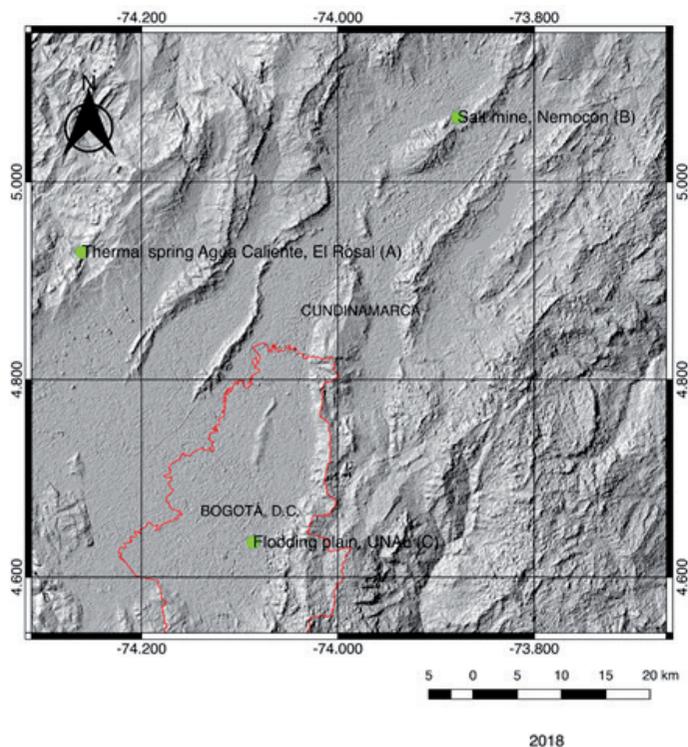


Figure 2. Satellite image illustrating the location of the sampling sites at the Department of Cundinamarca. (a). Thermal spring “Agua Caliente”, El Rosal. (b). Runoff channel with organic sludge near the salt mine of Nemocón. (c). Flooding plain, Universidad Nacional de Colombia. (QGis, 2018).

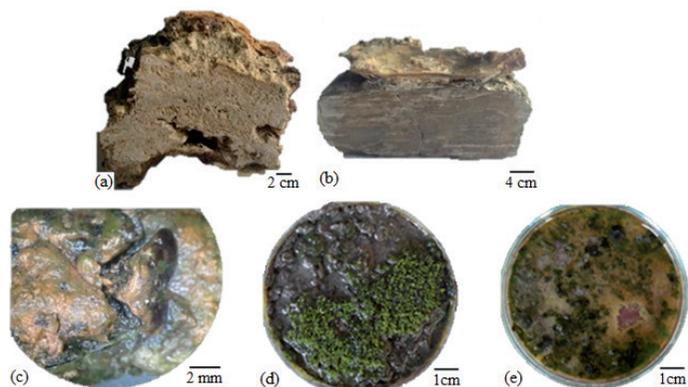


Figure 3. (a). Travertine from the thermal spring “Agua Caliente”, El Rosal. (b). Mudstone from the thermal spring “Agua Caliente”, El Rosal. (c). Microbial mat growing on top of the rock samples shown in a. and b. (d). Biofilm from a runoff channel with organic sludge adjacent to the Nemocón salt mine. (e). Biofilm from a flooding land surface nearby the Colombian Agricultural Institute (ICA) inside the campus of the National University of Colombia, Bogotá.

For assessing the presence of microbial mat features in the Colombian Cretaceous sedimentary record, we performed a petrographic analysis on rock samples from La Luna, Paja and Tetuán formations. These units were selected considering that they were formed in a shallow marine setting, the one where microbial mats have been mostly studied and described, and the previous observation of organic matter laminae in rocks from these formations (e.g. Sarmiento et al. 2015, Gaviria, 2016). Although the rocks analyzed in the present study are continental, we consider the comparison valid since the microbial mat features preserved in rocks are usually consistent over a wide range of environments (Schieber *et al.*, 2007). The samples from La Luna Formation (Turonian-Coniacian) were taken

from Quebrada La Sorda, Santander, where they are characterized by the dominance of foraminifera biomicrites with partial silicification, pseudospar and siliciclastic sediments of silt and sand sizes, mainly towards the top of the unit (Sarmiento *et al.* 2015). Tetuán Formation (Albian) samples were taken from Quebrada Motilona, Paicol, Huila, where it is composed of marls and biomicrites deposited during the second marine Cretaceous flooding (Gaona, 2015, Hay & Floegel, 2012, Keller, 2008 in Gaviria, 2016) and simultaneously with two different global anoxic events, which probably produced the high organic matter accumulations that characterize this unit (Villamil *et al.*, 1999 in Gaviria, 2016). Finally, samples from Paja Formation (Hauterivian-Late Aptian) were taken from the uppermost part of the intermediate level “Lodolitas abigarradas” near Villa de Leyva, Boyacá, characterized by fissile black mudstones interlayered with micrites, calcareous concretions and gypsum (Patarroyo, 2000).

To identify potential microbial mat features in samples R-1 and R-2 collected at the thermal spring “Agua Caliente”, as well as in the thin sections of rocks from the La Luna, Paja and Tetuán formations, the petrographical definition and characterization at Gerdes (2007), Schieber (1986), Schieber (2007) and Ulmer-Scholle *et al.* (2014) was used. For the characterization of sample R1 (travertine), neither the classifications of Folk (1959), Dunham (1962) or Embry & Klovan (1971), traditionally used for calcareous rocks, nor Mount’s (1985) classification for mixed rocks were considered. These classifications do not take into account fossils of microorganisms, so Burne & Moore (1987) classification for microbialites was used instead.

4. Results

4.1 Identification of cyanobacteria

The microscopical observation of cyanobacteria from the three sampling locations led to the morphological identification of organisms to order, and to genera in some cases. None unicellular cyanobacteria were recognized (Orders Chroococales and Pleurocapsales).

In the samples from the thermal spring “Agua Caliente”, five morphotypes of filamentous cyanobacteria were identified:

- Narrow trichomes (2.5 μm thickness), 5-8 μm length cells, abundant gas vesicles bound to the walls of the filaments. Order Oscillatoriales, unknown genus (Figure 4 (a), morphotype *).
- Narrow trichomes (< 2 thickness), 3.3-6.6 μm length cells. Order Oscillatoriales, Genus *Leptolyngbya* sp. (Figure 4 (a), morphotype **).
- Trichomes with helicoidal coiling in a close to partially unwinded helix, with a width of 1.4 to 2.9 μm . Crossed wills with nearly invisible cells. Order Spirulinales, Genus *Spirulina* sp. (Figure 4 (b)).
- Cyanobacteria with heterocysts, predominantly intercalary (inside the filaments). Vegetative cells spherical to ovoid. The trichomes have a slight coiling. Order Nostocales. Possible genera: *Nostoc* sp./ *Anabaena* sp. (Figure 4 (c)).
- Trichomes composed of vegetative cells with discoid shape. Presence of heterocysts and possible akinetes. Order Nostocales, genus *Nodularia* sp. (Figure 4 (d)).

At the biofilm from the runoff channel nearby the salt mine of Nemocón it was possible to recognize two morphotypes of filamentous cyanobacteria similar to those found at the thermal spring “Agua Caliente”:

- Order Oscillatoriales, unknown genus (Figure 5 (a), morphotype *); Order Oscillatoriales. Genus *Leptolyngbya* sp. (Figure 5 (a), morphotype **).
- Trichomes of 5-6 μm width, with discoid cells (wider than long). Order Oscillatoriales, genus *Oscillatoria* sp. (Figure 5 (b)).

Finally, at the flooding surface inside the National University of Colombia, Bogotá, one of the same morphotypes of filamentous cyanobacteria found at the channel nearby the salt mine of Nemocón was identified, assigned to the Order Oscillatoriales, Genus *Oscillatoria* sp. (Figure 6).

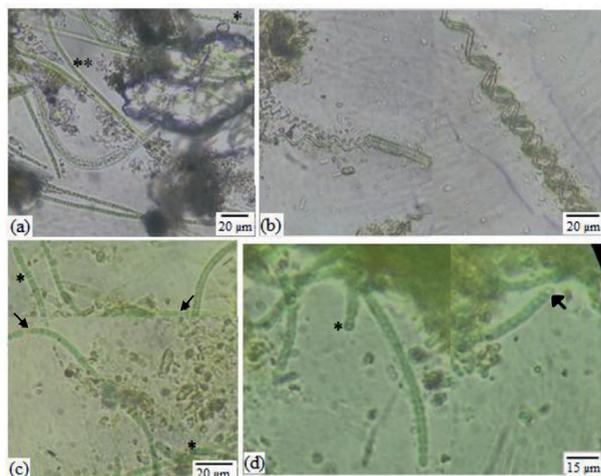


Figure 4. Light microscope photographs of the cyanobacteria found in the microbial mat collected at the thermal spring “Agua Caliente”, El Rosal (Cundinamarca), that allowed the morphological identification. (a). It is possible to differentiate two morphotypes of filamentous cyanobacteria: *, Order Oscillatoriales, unknown genus; **, Order Oscillatoriales, Genus *Leptolyngbya* sp. (b). Morphotype of filamentous cyanobacterium with narrow trichomes and closed to slightly uncoiled helicoidal coiling. Order Spirulinales, Genus *Spirulina*. (c). Filamentous cyanobacterium with recognizable heterocysts (light rounded cells, arrows) and possible akinetes (thick, strongly pigmented cells, asterisks). Order Nostocales, Genus *Nostoc* sp./*Anabaena* sp. (d). Filamentous cyanobacterium with discoid to ovoid cells. An apical heterocyst (arrow) and a possible akinete (asterisk) can be observed. Order Nostocales, Genus *Nodularia* sp. Each field of view was cut and fitted to show the more relevant characteristics.

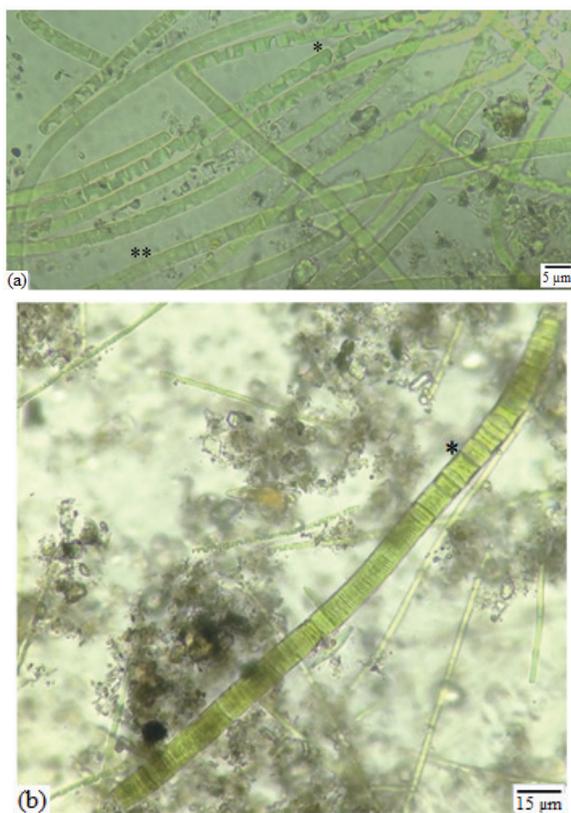


Figure 5. Light microscope photographs from the cyanobacteria found at the biofilm from the runoff channel with organic sludge near the salt mine of Nemocón (Cundinamarca). (a). Filamentous cyanobacteria; Two morphotypes were identified: *, Order Oscillatoriales, unknown genus; **, Order Oscillatoriales, Genus *Leptolyngbya* sp. (b). Order Oscillatoriales, Genus *Oscillatoria* sp. Abundant Oscillatoriales filaments from the morphotypes identified in a. can be observed in the background.

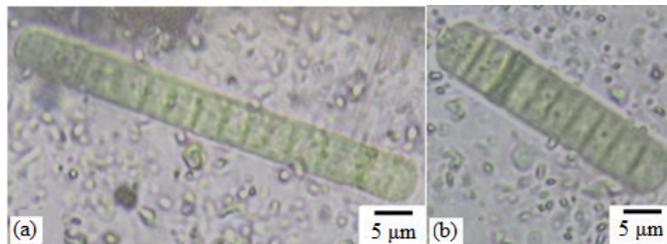


Figure 6. Light microscope photographs of cyanobacteria found in the biofilm retrieved from the flooding plain near ICA at National University of Colombia, Bogotá. (a) and (b). Order Oscillatoriales, Genus *Oscillatoria* sp.

4.2 Lithologic characterization of microbialites related to the microbial mat isolated from the thermal spring “Agua Caliente”, El Rosal (Cundinamarca)

The macroscopic observation of the samples R-1 and R-2 and the related microbial mat collected at the thermal spring “Agua Caliente” allowed the identification of processes of carbonate biomineralization, binding of quartz grains and a well-defined layering (Figures 7, 8 and 9, respectively). Sample R-1 was classified as a travertine according to the definition given by Pentecost (2005), and as a microbial framestone (Burne & Moore, 1987). It was possible to recognize brown lenses with a possible microbial origin, similar to the ones that form the microbial mat found on top of the rock. Layering is evident in some areas, while in some others the rock has a clotted texture (Figures 7 (a) and 8). Therefore, according to Kalkowski (1908) the rock has a stromatolitic texture in the layered areas, and it also has a dendritic texture in the zones with macroscopic patches (Aitken, 1967). Besides, a brownish tone can be observed in some layers, which could be ascribed to the presence of iron from pore fluids, that allowed the precipitation of hematite and sideritization of the carbonates nearby.

The observation of the sample R-2 allowed to establish the presence of terrigenous sediments and a very small fraction of calcareous material. Only at the uppermost part of the rock, it was possible to determine an association between the microorganisms from the mat and some crystals of carbonate and quartz. However, at the middle part of the sample layered structures with iron oxides were distinguished, similar to biolaminations (Figure 7 (b)). The observation of thin sections under the petrographic microscope allowed to determine that the sample is predominantly made of quartz grains (> 90%; 15% fine sand, 25% very fine sand, 60% mud). Besides, it contains iron oxides (5%), opaque minerals (1-3%), calcium carbonate (1-3%) and glauconite (<1%) (Figure 9 (a) and (b)). This sample was classified as a mudstone (Folk, 1959), a sandy mudrock (Mount, 1985), and a microbial boundstone (Burne & Moore, 1987).

The microbial mat that was found growing on the surface of samples R-1 and R-2 contains laminae with a thickness of less than 1 mm, and a green internal coloring, related to the photosynthetic pigments

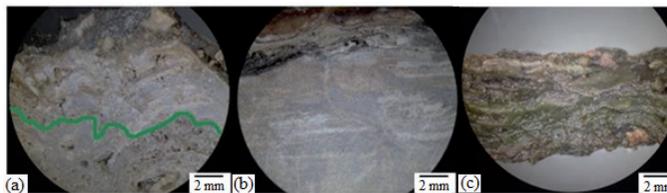


Figure 7. Mesoscale photographs of R-1 and R-2 samples, and the microbial mat growing on top of them, isolated from the thermal spring “Agua Caliente”, El Rosal (Cundinamarca). (a). R-1 sample. The stromatolitic (above the green contour) and thrombolitic (below the green contour) textures can be visualized inside the same rock body, as well as the microbial mat growing on the surface of the sample. (b). R-2 sample. It is possible to observe that this is a fine grain siliciclastic rock, with iron oxides lenses. The microbial mat growing at the surface of the sample can also be seen. (c). Transversal cut of the microbial mat growing on top of the samples R-1 and R-2. The orange tones suggest cyanobacterial pigment production and the presence of iron oxides, while the green layers suggest the presence of chlorophyll, also from cyanobacteria.

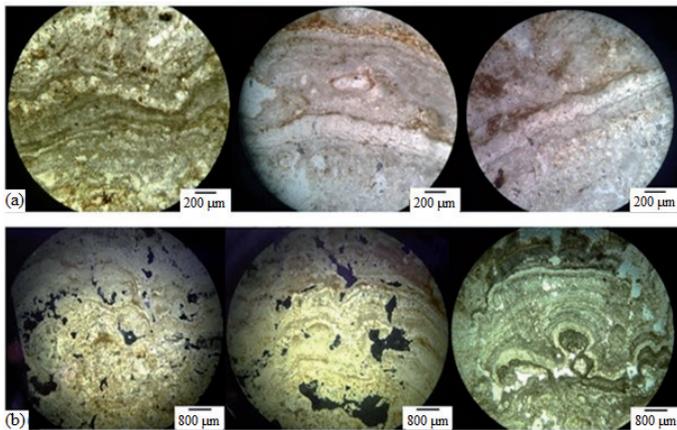


Figure 8. Microphotographs of thin sections of the R-1 sample, a travertine with a dominance of stromatolitic textures and some thrombolitic patches collected at the thermal spring “Agua Caliente”, El Rosal. (a). Inner section of the sample. Carbonate crystals were deposited between the laminae of organic matter, which is interpreted as *in situ* biomineralization, possibly aided by the presence of cyanobacteria. (b). Crossed section from the surface of the sample, over which the microbial mat was found growing. Brown laminae are thought to be lithified microbial mats, inside which carbonate crystals can be observed, as well as sideritization due to a potential iron enrichment.

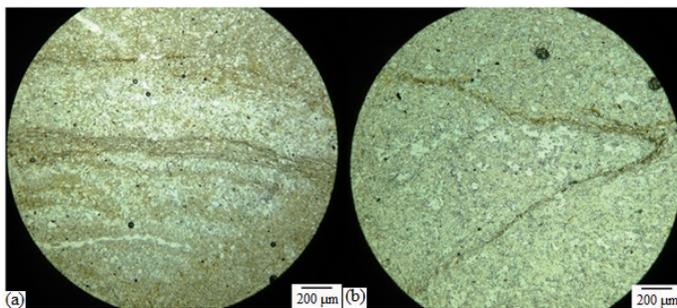


Figure 9. Microphotographs of thin sections of the R-2 sample, mudstone (Folk 1959) with iron oxides laminae and organic matter, or microbial boundstone. (a). and (b). Laminae and lenses rich in organic matter with iron oxides.

of cyanobacteria and with a brownish coloring at the borders (Figure 7 (e) and (f)). The microscopic observation of the mat allowed to establish the presence of iron oxides in the brown-reddish layers (Figure 10 (a)) and of calcium carbonates between the filaments formed by the cyanobacteria (Figure 10 (b)).

4.3 Identification of possible biolaminations and microbial mats in Cretaceous formations in Colombia

Potential lithified microbial mats were found in thin sections from the La Luna (Figure 11), Tetuán (Figure 12) and Paja (Figure 13) formations, that were interpreted based on the images from the Figures 8-10 and those presented in Schieber (1986), Schieber (2007), Gerdes (2007) and Ulmer-Scholle *et al.* (2014).

The most similar structure to a microbial mat from all the observed sections was the one from from the La Luna Formation. It consists of a network of light grey fibers, inside which it was possible to recognize carbonate crystals (Figure 11 (a) and (b)). Reddish brown continuous and discontinuous laminae, interpreted as biolaminations, were also identified. These laminae may contain microbial kerogen and carbonate aggregates inside the filament sheaths and networks (Figure 11 (c)-(g) and (h)). Light grey localized lenses with elongated surfaces, distinguishable from other structures similar to fibrillar networks, were observed in some sections (Figure 11 (i) and (j)).

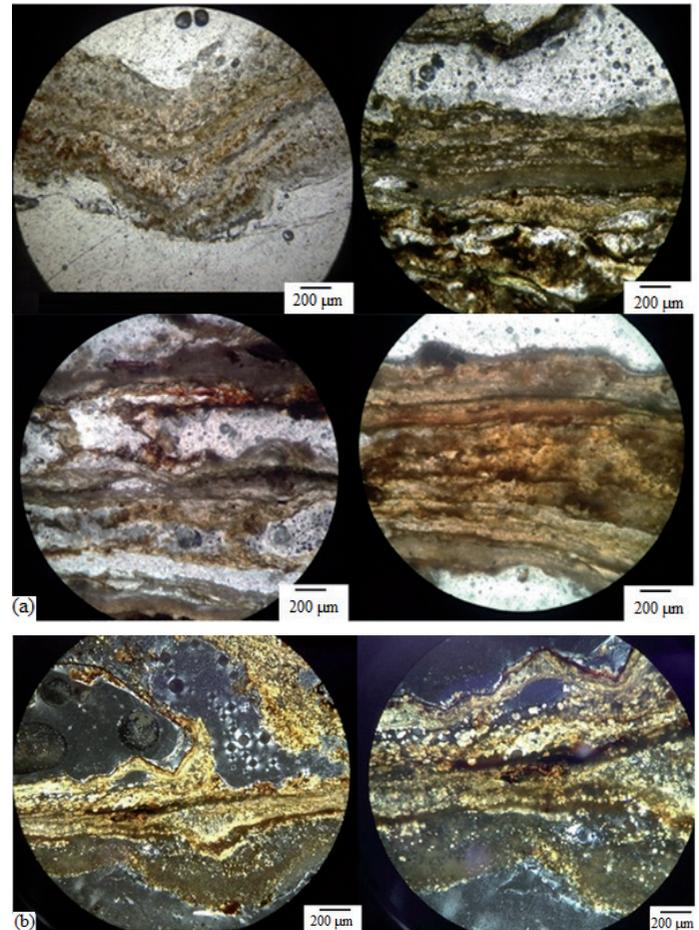


Figure 10. Microphotographs of thin sections of the microbial mat related to the samples R-1 and R-2 isolated from the thermal spring “Agua Caliente”, El Rosal (Cundinamarca). (a). Transversal cuts in which an internal lamination can be observed. (b). Precipitation of carbonate crystals between the laminae of microorganisms, that suggests the possible coexistence of processes of biomineralization and inorganic precipitation of the dissolved carbonate. XPL.

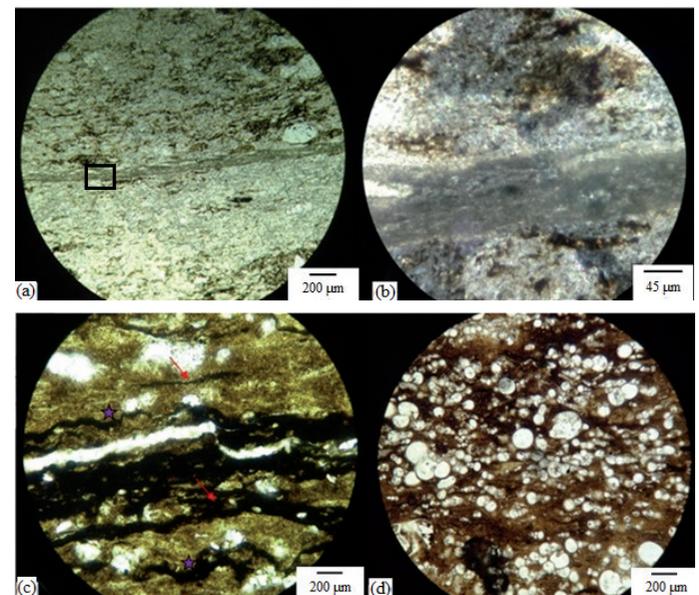


Figure 11. Microphotographs of thin sections of foraminifera biomicrites from the La Luna formation in which structures that resemble microbial mats can be observed. (Continue)

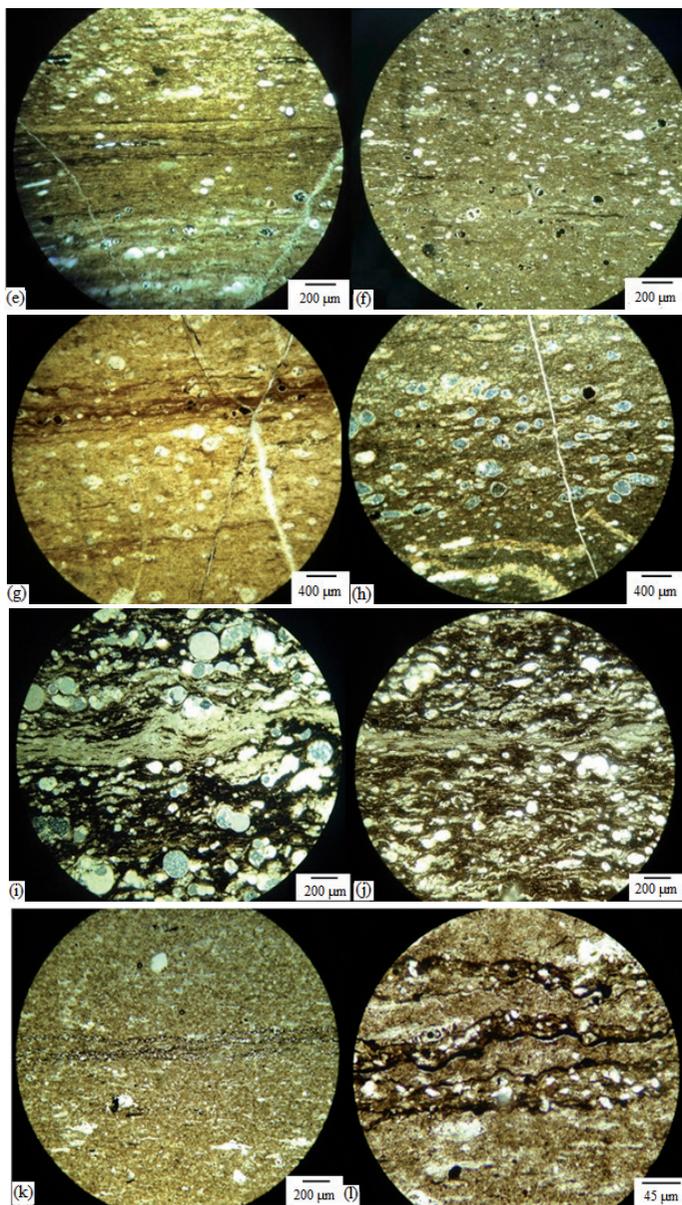


Figure 11. Microphotographs of thin sections of foraminifera biomicrorites from the La Luna formation in which structures that resemble microbial mats can be observed. (a). Potential microbial mat (center) in which a braided structure and a grey coloring distinguish it from the adjacent kerogen accumulations. (b). Zoom of the square region in (a), where carbonate crystals can be observed in the inner part. (c). Possible biolaminations with a microbial origin, pointed out with red arrows. The purple stars show similar structures, that may be interpreted as organic matter that follows stylolites. These can be differentiated because they are curved, with serrated, and are truncated against the minerals. (d). Accumulations of kerogen that acquire a texture of clear biolaminations braided towards the bottom, inside which interlaced fibers and foraminifera aggregates can be observed. (e), (f), (g) and (h). Laminae and parallel lenses of organic matter, similar to biolaminations and fragments of microbial mats. The continuity, parallelism, and zoning inside the sample are criteria that reinforce the hypothesis that these structures are lithified microbial mats. (i) and (j). Possible biolaminations surrounding a lighter central structure, that could evidence reworking inside the basin according to Schieber *et al.* (2010). (k) and (l). Laminar surfaces with accumulations of organic matter. The binding of grains, the presence of an inner network and the localized distribution suggest that these laminae have a biogenic origin, however, the curved and serrated shape of these surfaces does not allow to preclude a genesis related to stylolites.

On the other hand, the observation of possible biolaminations and biomineralizations associated to the presence of microorganisms in thin sections of the Tetuán Formation allowed the identification of laminar, discontinuous and linear dark-brown colored structures, that signal the presence of organic matter (Figure 12 (a) and (b)). Some of these form aggregates and very small and localized fibrillar networks (Figure 12 (e) and (f)). In one of the sections, the simultaneous presence of phosphatic lenses, mature kerogen, and possible biolaminations was observed, and apparent stylolites were identified (Figure 12 (c) and (d)).

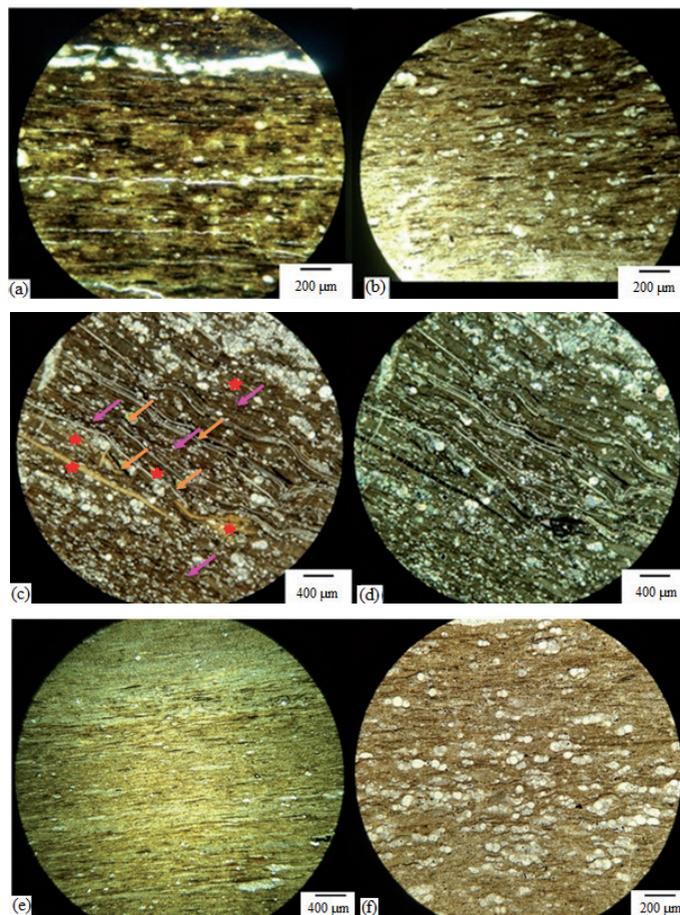


Figure 12. Microphotographs of thin sections of foraminifera biomicrorites and pseudosparites from the Tetuán formation. (a) and (b). Surfaces of organic matter accumulation relatively linear and continuous, that are interpreted as possible biolaminations. (c) and (d). Phosphatic laminae (red asterisks), laminae of accumulation of mature kerogen (orange arrows) interspersed with potential biolaminations (pink arrows). (e) and (f). Predominantly discontinuous lamination that might correspond to fragments of microbial mats. These laminae are less linear than those in (a) and (b) and sometimes are found surrounding foraminifera carapaces.

Observation of thin sections from the Paja Formation allowed to establish that these are rocks with flat parallel lamination and high fissionability, with a variable content of organic matter. Microphotographs show that its accumulation occurs in the form of sinuous fibrillar networks that produce dense to disperse laminae, concentrated in some regions of the samples (Figure 13 (a)) or disseminated, without any specific distribution (Figure 11 (b)). The laminae, that potentially have a microbial origin due to their high similarity to the structures reported in the literature, border the clay grains and the high interference tones in polarized light can be attributed to a high concentration of micas (Figure 13 (c)).

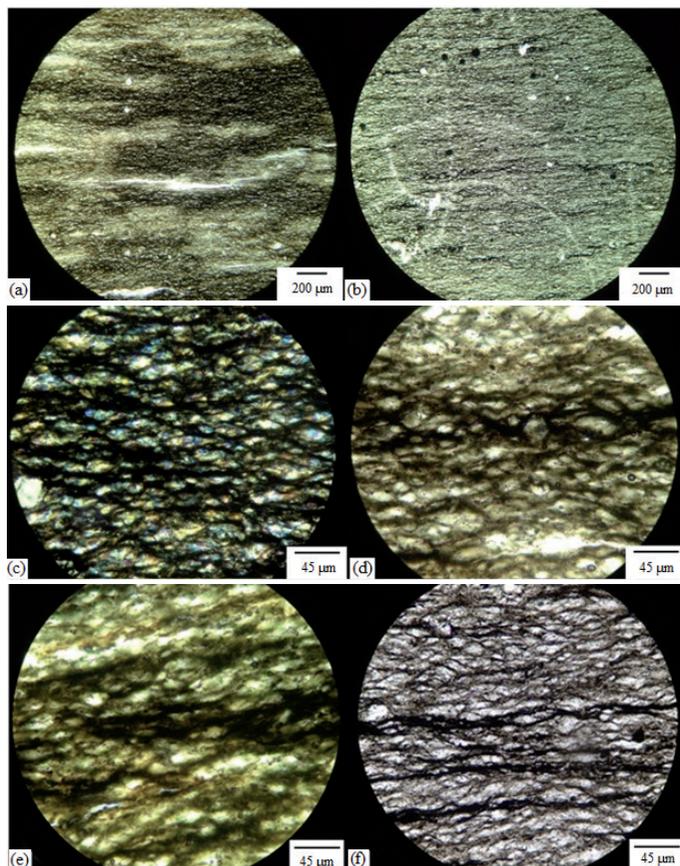


Figure 13. Microphotographs of thin sections of mudstones from the Paja formation. (a) and (b). Laminar and braided structures with organic matter, with a non-homogeneous distribution. (c), (d), (e) and (f). Detail of the structures observed in (a). In (c), the crystals do not have a defined shape and are entrapped inside the net formed by those structures, similar to the microbial mats described by Schieber (1986). We interpret the high interference tones as localized gypsum replacements. XPL.

5. Discussion

5.1. Lithologic characterization of microbialites associated with the microbial mat isolated from the thermal spring “Agua Caliente”, El Rosal (Cundinamarca)

Sample R-1 was classified as a travertine (Pentecost, 2005). In travertines, carbonate precipitation is mainly produced by the transference (evasion or invasion) of carbon dioxide from or to a source of groundwater that produces a calcium carbonate supersaturation, with nucleation and growth of crystals over an immersed surface. They can be stratified and the stratification is frequently irregular (Pentecost, 2005). It is produced because of compositional changes of the material dissolved in the water during their deposition -which is slow and often discontinuous- such as iron, organic matter, and other substances that precipitate together with the carbonate and may produce its characteristic band pattern (Ujueta, 1960).

Some travertine deposits have been identified in Colombia, mainly in the departments of Caldas and Boyacá, this last one being the one that registers the most travertines in the villages of Villa de Leyva (Ujueta, 1960), Firavitoba and Tibasosa. The geologic characterization of a travertine from Pesca was performed recently (Vargas, 2013). Because of their localization and distribution, travertine deposits in Colombia have been associated with the volcanic activity of domes and the ascent of hydrothermal fluids through fractures (Vargas, 2013). For the area of El Rosal-San Francisco-Subachoque, no travertines have been reported. The calcareous supply for the formation of these bodies possibly comes from the Seca Formation (Maastrichtian-Paleocene; De Porta, 1966), over which it is located.

In the microbial mat growing on the surfaces of the samples R-1 (travertine) and R-2 (mudstone), structures related to the microbial

growth together with the binding of sediments, such as biolaminations and biovarves, were identified (Schieber, 2004). The light laminae in biovarves have been related to the growth of coccoid cyanobacteria, which are the main EPS producers and may favor the precipitation of gypsum and carbonates (Gerdes, 2010).

Carbonates and iron oxides in microbial mats have been considered a product of possible simultaneous processes of inorganic precipitation and biomineralization, classified as structures produced by metabolic effects (Schieber, 2004). Photosynthesis may shift carbonate solubility enough to promote the precipitation of these minerals, which induces the formation of irregular ooids, disseminated grains, cementation in specific laminae, early diagenetic dolomite, etc. We also identified siderite, that was probably produced by a diagenetic substitution of calcium by iron in the carbonate structure. The layers with iron enrichment may be explained by the activity of sulfate-reducing bacteria. The binding of iron to polysaccharide sheaths promotes the fixation of carbon dioxide and limits photorespiration, and the iron hydroxides below the cyanobacteria layer protect them from an excess of sulfur and keep oxygen from reaching the anaerobic community of microorganisms (Schieber *et al.*, 2007).

The identification of microbial mats with similar textural characteristics in the samples R-1 (travertine) and R-2 (mudstone) provides an evidence of the ability of filamentous cyanobacteria to promote carbonate biomineralization and bind siliciclastic sediments. Therefore, the composition of the grains, the sedimentary textures, and the microbial mat features in any environment might be mainly influenced by its physicochemical properties and the input of sediment. We thus hypothesize that the species of cyanobacteria that colonize microbial mats could be very variable, since they could be interchangeable as long as their ecological role within the microbial community is fulfilled.

5.2 Cyanobacteria identification

Several groups of cyanobacteria have been found growing on travertine surfaces. This can be explained by the fact that many of these grow well in environments with high temperatures enriched in sulfur, variable light intensities, high carbon dioxide concentrations, and are resistant to desiccation (Castenholz, 2002). The cyanobacteria genera identified in the present study match those who have been recognized as the most predominant in fresh to brackish water environments with mild temperatures (Castenholz, 2002; Komárek and Anagnostidis, 2005; Komárek *et al.*, 2014). These cyanobacteria have also been recognized as some of the main contributors to the formation of MISS and modern microbial mats (e.g. Schieber *et al.*, 2014).

For the observation and morphological characterization of cyanobacteria, more advanced microscopy techniques had been used, such as confocal, phase-contrast, fluorescence and electronic microscopy (Castenholz, 2002). Getting a precise identification of cyanobacteria in different environments would be critical to know the diversity of these microorganisms, track the appearance of their characteristics and adaptations, and determine the conditions that drove the evolution of oxygenic photosynthesis, one of the most relevant events studied by geobiologists. The present is an exploratory, morphological study, and further work considering the use of molecular identification techniques is strongly encouraged to achieve a more precise discrimination and resolution of the cyanobacteria present in each location, given that the morphological identification may be subject to errors and does not allow to achieve the resolution needed to differentiate species, and in some cases, genera (Komárek *et al.*, 2014).

5.3 Identification of possible biolaminations and microbial mats in Cretaceous formations in Colombia

Most of the structures with a possible microbial origin identified in La Luna, Paja, and Tetuán formations are related to growth and binding of particles, such as biolaminations and biovarves, and with metabolic effects (biomineralizations) in the case of the La Luna and Tetuán formations. It is possible that the differences between the lithified and modern structures identified here and those that have been previously reported obey to two main factors: first, most reported MISS and microbial mat features have

been observed and classified on coastal and hypersaline environments (Schieber, 1986; Gerdes, 2007; Schieber, 2007; Ulmer-Scholle *et al.*, 2014), which might have contrasting conditions to the paleoenvironments in which the La Luna, Paja, and Tetuán formations were deposited. In addition, there are diagenetic phenomena that can alter the appearance of the structures observed in the rock record with respect to modern microbial mats and biofilms, from which is worth mentioning burial, compressive forces (that can produce faulting, fracturing and pressure dissolution), mineral replacement and weathering. These diagenetic processes can alter the integrity of lithified microbial mats, which makes it possible to find fragmented and transported fossil fragments inside of them. In the analyzed thin sections from La Luna Formation, light lenticular structures were found, that have been interpreted as intraclasts, produced by erosion and reworking from deep currents inside the basin (Schieber *et al.*, 2010). Conversely, in modern settings, deformed microbial mats with folds, bulges and roll-ups have been reported to form as a consequence of tidal currents (Cuadrado *et al.*, 2015), while sand layers interspersed within the microbial mats have been considered a record of highly energetic storm events (Cuadrado *et al.*, 2013). None of these processes can be discarded as a potential factor involved in the formation of the structures that we described.

It is also important to note that in some of the studied sections, the possible lithified microbial mats can be mistaken with surfaces in which dissolution of carbonates had occurred due to compressive forces (stylolites). Some criteria that can be used to differentiate them are the tendency of stylolites to be continuous, have curved and serrated edges and truncate carbonate crystals. However, the simultaneous presence of both structures cannot be discarded (Figure 11 (c), (k), and (l)). On the other hand, although the presence of phosphates and dissolution surfaces at the Tetuán Formation had been previously acknowledged (Gaviria, 2016), the present study is the first to question the nature of stylolites and some kerogen laminae in this unit and to propose a possible microbial origin for those structures. Finally, on the thin sections from Paja Formation highly continuous and abundant biolaminations were recognized (Figure 13 (d)-(f)), together with diagenetic gypsum at outcrop scale.

As previously stated, the La Luna, Paja and Tetuán formations are characterized by low-energy marine facies, with variations in depth and fossil content and with intermittency in the rates of sediment and continental matter supply. These characteristics have been previously regarded as ideal for microbial mat proliferation (Schieber, 1986). The finding of biolaminations with high similarity to those reported by Gerdes (2007), Schieber (1986), Schieber (2007) and Ulmer-Scholle *et al.* (2014), mainly in the La Luna and Paja formations is consistent with the depositional environment and constitutes the first morphological evidence of fossilization of microbial mats or their remnants in the rock record in Colombia.

It is very important to remark that observation and identification of MISS and microbial mat features should, whenever possible, be complemented with additional tests to provide confidence and robustness to their interpretation. The fact that morphology is an interpretative biogenicity criterium makes it susceptible to confusion with similar structures such as stylolites, intraclasts, and non-microbial kerogen. Some of the most used complementary analytical techniques are advanced microscopy techniques and geochemical analyses like chromatography and mass spectrometry to detect chemical biomarkers such as associated heavy elements and rare earth elements, as well as carbon, oxygen, sulfur and nitrogen isotopes in terrestrial samples studies (Westall, 2008).

6. Conclusions

The detailed observation of organic matter laminae in sedimentary geologic units, mainly in marine settings, has allowed the reinterpretation of some of these as potential microbial mat features. Most of these structures manifest themselves as continuous biolaminations, inside which a fibrillar mesh produced by the filaments of cyanobacteria can be differentiated, related to biomineralization and sediment binding. Diagenetic effects can alter the integrity of lithified microbial mats, which, together with the characteristics of the depositional environment, could account for the differences between the structures observed in the modern and the Cretaceous microbial mat features.

The observation of filamentous cyanobacteria isolated from the microbial mat from the thermal spring “Agua Caliente”, El Rosal (Cundinamarca) and from the biofilms collected from a runoff channel nearby the salt mine of Nemocón and a flooding plain at the National University of Colombia, Bogotá, allowed their identification until genus. Most of them belong to the order Oscillatoriales, that has a worldwide distribution in fresh, brackish and marine waters, with representatives that require or tolerate extremes of salinity and temperature.

Two rock samples from the thermal spring “Agua Caliente”, El Rosal (Cundinamarca), whose genesis was influenced by the growth of a microbial mat, were characterized. One of these, a travertine and microbial framestone, had stromatolitic and thrombolitic textures. In the second one, a mudrock and microbial boundstone, biolaminations and biomineralizations were identified. The comparison between the textures observed in a modern microbial mat and its associated rocks, and those reported in the literature, allowed the identification of potential microbial mat features in the La Luna, Paja and Tetuán formations. This finding is highly relevant given that it makes up the first report of biolaminations and biomineralizations produced by microorganisms in Cretaceous rocks in Colombia, and may be complemented with additional studies to perform paleoclimatic and sedimentological inferences about its depositional environment.

Despite the fact that the observation of microbial mat features is a strictly morphological criterium to assign a microbial origin to a sedimentary structure, it is a critical tool for the identification of biosignatures inside the frame of the search of life in extraterrestrial settings. However, we encourage performing additional tests in future studies to provide more reliability to the biogenicity interpretation.

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