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# Statistical trends in culture methods of endolithic bacteria extracted from Quartz, K-Feldspar (Jurassic) and Calcite (Quaternary) in semi-arid areas in Colombia

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## | A B S T R A C T |

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Microbial endolithic communities are ubiquitous in many deserts around the globe. They have been found in many different lithologies, including quartz, plagioclase and calcite. The use of culture methods in geomicrobiology is important because most bacteria are non-cultivable, which makes it very difficult to characterize and describe them. In this study, endolithic bacteria and yeast were cultured in three rock-forming minerals (quartz, K-feldspar and calcite) with standard culture methodologies. It was demonstrated that these endolithic microorganisms could grow in noble agar enriched with a mineral different from the one they were extracted. Microbes were also cultured in a nutritive broth under some physicochemical factors (sugar, salt, pH and temperature) to study their possible tolerance to different ecological conditions. These results were combined with Multiple Factorial Analysis to identify statistical trends between their morphologies (pigment, size and Gram stain), chemical elements and their tolerance to physicochemical factors. Our results show that it might exist a relationship between pigments in microbial colonies, their tolerance to pH9, salinity and temperature conditions; and that Gram-negative bacillus might have a high adaptive ability to different enriched minimum media. Characterizing microbial communities associated with lithic substrates in the laboratory could be helpful for future planning in the search for life on Mars.

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**KEYWORDS** | Endolithic microbial communities. Geomicrobiology. Microbial Ecology. Quartz. K-feldspar. Calcite.

## INTRODUCTION

Geomicrobiology is an emergent sub-discipline of geosciences that integrates techniques and concepts from microbial ecology, geology and biochemistry (Ehrlich *et al.*, 2015; Whitmeyer *et al.*, 2009). Its focal points are the interactions between microorganisms and

earth materials such as soil, rock-forming minerals and rocks, the atmosphere and the hydrosphere (Ehrlich *et al.*, 2015). One of the unanswered questions that emerge from geomicrobiology is whether the minerals can control microbial diversity or they only provide a microhabitat for some microorganisms (Rogers and Bennett, 2004).

The colonization of endolithic microorganisms is related to the bioreceptivity of rocks, which is the susceptibility of substrates to endolithic colonization (Guillitte, 1995). It is determined by the physical and chemical properties of the rock substrate, including mineral composition, permeability, presence of chemical compounds, the structure and distribution of pores, and other factors such as water retention capacity, pH, and exposure to climate and nutrient sources (Cockell *et al.*, 2009; Herrera *et al.*, 2009; Kelly *et al.*, 2011; Omelon *et al.*, 2007).

Although there is growing evidence that mineral composition may control biomass and bacterial community structures (Carson *et al.*, 2009; Certini *et al.*, 2004; Gleeson *et al.*, 2006; McNamara *et al.*, 2011; Rogers and Bennett, 2004), little is known about the role of elements within rock-forming minerals as a potential source of nutrients or energy for microorganisms. Phosphorous (P) and iron (Fe) are essential macronutrients for microbial metabolism (Madigan *et al.*, 2002). In some microhabitats (*e.g.* contaminated aquifers), silicate minerals that contained nutrients like P and Fe were preferentially colonized by microorganisms, in contrast to the silicates without them. The presence of P and Fe results in an increase in the biomass and biodegradation rate when both elements are present (Rogers and Bennett, 2004). Al, Si, and Ca have also driven the bacterial community structure within the system of crystals of quartz, K-feldspar, plagioclase, and muscovite in a Pegmatite Granite. These crystals were in proximity, yet distinct communities were detected on each mineral (with many ribotypes restricted to a single mineral type), indicating selective pressure by individual chemical elements on the bacterial population *in situ* (Gleeson *et al.*, 2006). Additionally, studies in microcosms when mica, basalt, and rock phosphate (phosphorite) were added to soil have shown that mineral addition to soil resulted in substantial changes in the microbial community structure, where authors conclude that when mineral substrates rich in K, Mg, Ca, or P are added to nutrient-poor soils, these support specific microbial communities (Carson *et al.*, 2009).

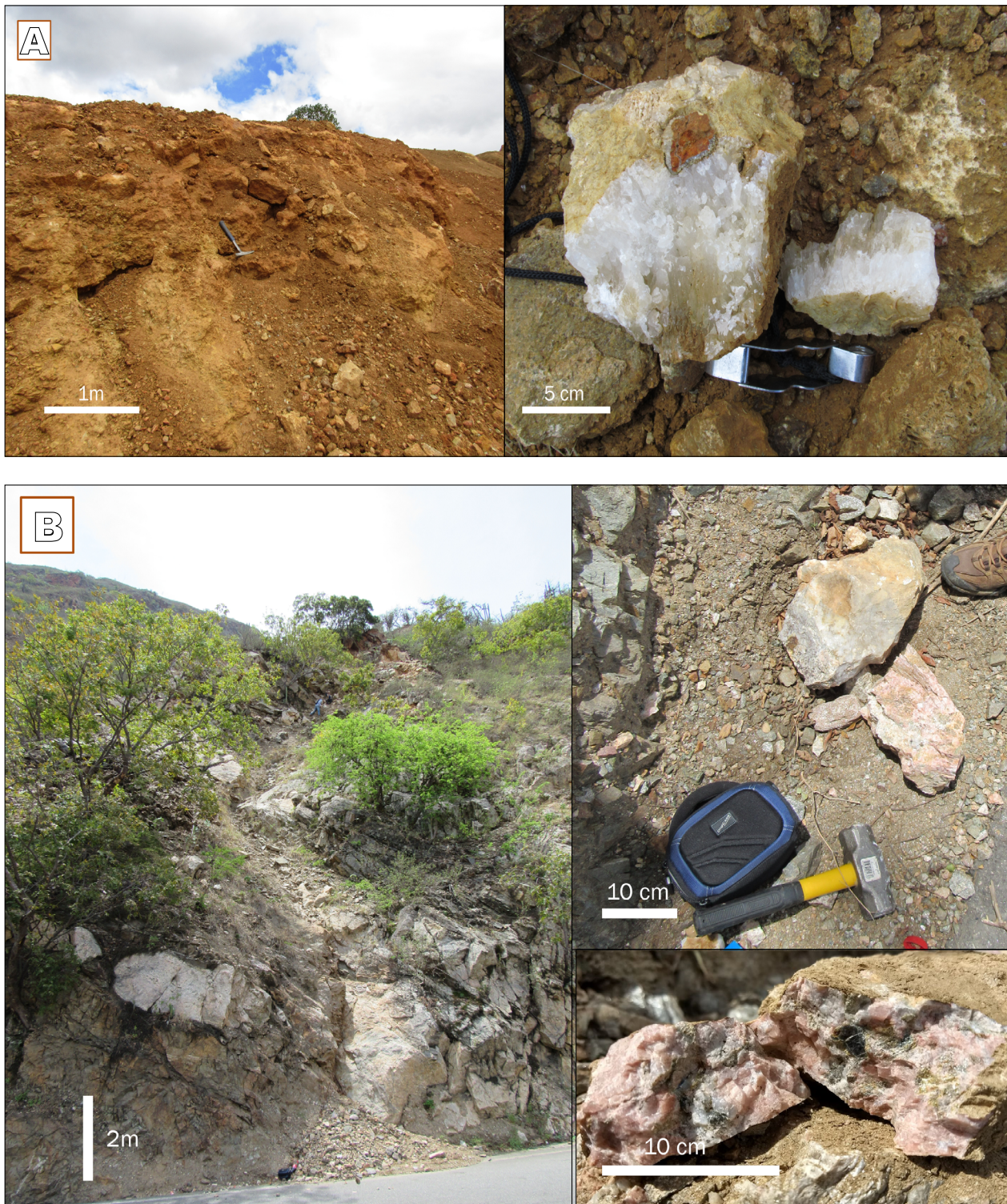
Endolithic microorganisms have been reported in many areas around the globe, some of them reported in warm hyper-arid and arid deserts such as Atacama, Mojave, Namib, and Sonora (Ascaso, 2002; Bell, 1993; Bungartz *et al.*, 2004; Dong *et al.*, 2007; Lacap *et al.*, 2011; Schlesinger *et al.*, 2003; Stomeo *et al.*, 2013; Vitek *et al.*, 2016), in cold deserts in the Arctic and Antarctic regions (Ascaso, 2002; Cockell and Stokes, 2004; Cowan *et al.*, 2010; Friedmann, 1980; Makhalanyane *et al.*, 2014; Omelon *et al.*, 2006; Smith *et al.*, 2000), and in deep subsoils and ocean trenches. In these extreme environments, microorganisms find protection against high and low temperatures, UV radiation and desiccation inside pores and fissures of

minerals and rocks (Bell, 1993; Wierzchos *et al.*, 2018). However, there are reports of endolithic microorganisms in inter-tropical zones (Gaylarde *et al.*, 2017), where humidity and solar radiation are significantly different from the above-mentioned biomes. Herein we report endolithic culturable bacteria and yeast-like fungi in an inter-tropical region in the semi-arid zones of Villa de Leyva, Boyacá, and Pescadero, Santander, in Colombia.

Within the rocks, microorganisms find a microhabitat that provides them with thermal buffering, physical stability, freeze-thaw events, and protection against incident UV radiation (Wierzchos *et al.*, 2012). Above all, as the rocks have networks of pores and fissures, water retention through them allows moisture availability (Wierzchos *et al.*, 2012). Dryness and aridity are unfavorable conditions for life. In desert zones, besides aridity, microorganisms also need to withstand lethal UV light, high and low temperatures and their rapid fluctuations, high rates of water evaporation, extended periods of desiccation, oligotrophic conditions and, often, high salinity levels (Cockell *et al.*, 2008; Wierzchos *et al.*, 2012). Even brief exposure to solar radiation can cause cell death within a few hours (Cockell *et al.*, 2008).

In Corzo-Acosta (2018), two field trips were performed to collect extensive samples of minerals that might host endolithic microorganisms in Villa de Leyva (Boyacá) and Pescadero (Aratoca, Santander), Colombia. Both areas were selected for being classified as semi-arid with low rainfall regimes and, in the case of Villa de Leyva, with substantial temperature changes (Fischer and Lüdders, 2002). Because both areas have a low rainfall regime, they develop poor vegetal cover and, therefore, depletion of soil (Fischer and Lüdders, 2002; Albesiano *et al.*, 2003; Instituto de Hidrología, Meteorología y Estudios Ambientales (IDEAM), 2017). These locations were selected because of the availability of big-size minerals and semi-arid conditions that may affect the biomass that can be formed inside the minerals (Meslier *et al.*, 2018). A travertine mine was selected for the calcite sample and a pegmatitic granite for Quartz and K-feldspar samples; this choice was made considering that all of them are present in big sizes (heavier than 3kg). Later small parts of these samples were used for X-ray diffraction analysis, elaboration of thin sections, and SEM. Endolithic culturable bacteria were isolated from three rock-forming minerals (calcite, K-feldspar, and quartz). A scratch was made from the inner surface with three replicates for each mineral to do a culture analysis (including Physicochemical factors in the growth and Gram Stain).

In this study, we use the data collected by Corzo-Acosta (2018) to uncover some possible relationships between the ability of microorganisms to grow in a mineral-enriched



**Figure 1.** A) Left, travertine mine in Villa de Leyva. Right, calcite samples. B) Left, outcrop 1.4km south of Pescadero. Right, pegmatitic samples for K-feldspar and quartz.

medium, the physicochemical factors and the morphology (macro and micro) of the cultured microorganisms. Culturing in nutritive agar and Gram Stain were used to

describe the morphology of the microorganisms; once extracted, they were cultured in Noble Agar enriched with the three minerals and in physicochemical factors. After that,

petrography and XRF analyses were carried out to describe the geochemical properties of minerals. A Multiple Factor Analysis (MFA) was implemented to identify simultaneous relations between the colonies with the abiotic factors and their growth in selective media.

Culturing methodology in microbial ecology plays an essential role as it provides crucial information regarding the physicochemical tolerances and metabolisms of microorganisms (Tang *et al.*, 2016). The study of terrestrial life distribution in endolithic environments serves as an approach to understanding the mineral-microbe association and the environmental limits of life on Earth (Bhattacharjee and Joshi, 2016). Furthermore, it is critical to assess the bioreceptivity of rock substrates or minerals on other planetary surfaces such as of Mars (Direito *et al.*, 2012), where quartz, feldspars and carbonates have been identified (Bandfield *et al.*, 2004; Carter and Poulet, 2013; Horgan *et al.*, 2020; Smith *et al.*, 2012).

## METHODS

### Sampling sites

The two study areas are located in Colombia, South America: i) Villa de Leyva (Boyacá) (5° 36' 24.12" N; 73° 31' 31.32" O) and ii) Pescadero (Aratoca, Santander) (6° 49' 0.62" N; 73° 0' 20.3" O). Both localities were selected due to the availability of big-size minerals of calcite in Villa de Leyva and quartz and K-feldspar in Corregimiento de Pescadero, Aratoca. Villa de Leyva is noticeable for its numerous Cretaceous sedimentary outcrops which are exposed in the Arcabuco Anticline. Over the Aptian mudstones of the Paja Formation (Fm.) lies unconformably a travertine deposit of Pleistocene age (Páramo-Fonseca *et al.*, 2019; Patarroyo *et al.*, 1997). This travertine deposit is associated with calcareous sources from Valanginian-Hauterivian, the Ritoque Fm., or Valanginian Rosablanca Fm. (Rojas and Sandy, 2019; Schemm-Gregory *et al.*, 2012). They tend to develop near springs, suggesting a close relationship between hydrothermal activity and travertine deposits. The travertine subject of this study is located 500m southeast of the main travertine quarry, it is small, and rests over the Valanginian marine siltstones of the Ritoque Fm. (Vargas *et al.*, 2014) Figure 1A.

Pescadero's location is in the Pescadero Granite, a pink fine grained granite, slightly porphyritic, and equigranular with K-feldspar and quartz fenocrystals. K-feldspar and quartz samples were collected in the Bucaramanga-Bogotá road, 1.4km south of the bridge across the Chicamocha River, in an outcrop with numerous Late Triassic–Early Jurassic pegmatite dikes intruding Esquistos de Silgara Fm.

(van der Lelij *et al.*, 2019; Navas, 1962; Zapata *et al.*, 2016) Figure 1B.

### Major Element Chemistry, Petrography and Electron Microscopy

Minerals, were powdered in an agate ball mill and then sifted with a grill of 100 $\mu$ m. The dust samples were dried at 105°C for 12 hours. Then mixed with a spectrometric wax (Merck) in a ratio of 10:1 and homogenized by agitation. Then three flat-surfaced discs of 37mm were built with a hydraulic press at 120kN per minute. Semi-quantitative Dispersive X-Ray Fluorescence (DXRF) analysis were carried out in a MagixPro PW-2440 Philips spectrometer, equipped with a rodio tube and a maximum power of 4KW. The software SemiQ 5 was used, making 11 scans to detect the present elements in the three samples, excluding H, Li, Be, N and O, using standard techniques (Ramsey *et al.*, 1995).

Two thin sections of each mineral were analyzed under a polarized microscope to ensure the samples were only made of one type of mineral and describe its optical properties. To make the thin sections, a mineral piece 5cm long, 2cm wide and 1cm thick, was polished until it was 100 $\mu$ m thick. The scratches resulting from the slimming process in the polisher were removed with 600 and 1000 carbide abrasive (Williams *et al.*, 1958). Subsequently, amber resin was added to paste the thinned mineral on a petrographic glass, and then polished again to 30 $\mu$ m thick.

High-resolution pictures were taken with a Field Electron and Ion Company (FEI) Quanta 200 Scanning Electron Microscopy (SEM) were split in half, and one of their surfaces was sputter-coated with Au/Pd to make them more conductive and obtain a better resolution through the scanning. Images were examined for evidence of pores, fissures and cracks where microorganisms may inhabit (Gleeson *et al.*, 2006).

### Bacterial Culturing and Gram Stain

To avoid contamination, samples of the three minerals were split and powdered from inner parts under sterile conditions in the laboratory according to the methodology described by Bhattacharjee and Joshi (2016). Samples of 10g of each mineral were obtained and stored in sterile containers to dilute plating on nutrient agar (Merck) and Gram stain methods. Each dilution on every microbiological procedure was carried out in triplicate.

A portion of the bacterial colony was removed from the Petri dish and fixed on a plate; then, the plate was flamed with the help of a lighter to set the microorganism and carried out on the Gram stain, as described in Dussault (1955).

**Table 1.** XRF major element composition (weight percent) of calcite, K-feldspar and quartz (mean of four replicates, normalized to 100%). Cells without data are 0%

Element or compound	Calcite %	K- Feldspar	Quartz
Al <sub>2</sub> O <sub>3</sub>	0,06	18,29	0,09
P <sub>2</sub> O <sub>5</sub>	0,02	0,04	0,03
MgO	0,03		
CaCO <sub>3</sub>	99,49		
Fe <sub>2</sub> O <sub>3</sub>	0,05	0,08	0,03
K <sub>2</sub> O		13,60	0,02
Na <sub>2</sub> O		2,63	0,03
CaO		2,41	0,47
Ba		0,20	
Cl		0,06	0,04
Rb		0,04	
Pb		0,03	
MnO		0,02	
Sr		0,02	
SO <sub>3</sub>		0,01	

### Physicochemical Factors in Growth

Isolated microorganisms extracted from each mineral were inoculated in nutrient broth (Merck) to test their growth response to varied pH (4, 7, 9), salt (NaCl) concentrations (1, 6.5, 15%), sugar (glucose) concentrations (10, 20, 30%) and different temperatures (4, 14, 37, 60, 100°C), according to standardized techniques (Breznak and Costilow, 2007). Each microbiological procedure was repeated in triplicate.

### Culturing in minimum media and culturing in cross-media

The structure of endolithic bacteria might be driven by the host mineral from which they were removed (Carson *et al.*, 2009; Gleeson *et al.*, 2006; Siebert *et al.*, 1996). All isolated colonies were cultured in a minimal medium made of powdered mineral dissolved in noble agar (Merck) with concentrations as follows: 10g (mineral)/1L (noble agar), 5g/L, 2.5g/L and 1.25g/L. All colonies were cultured in triplicate on each dissolution of minimal media based on the methodology developed by Bhattacharjee and Joshi (2016).

To study whether bacterial growth is restricted when the original composition changes, all isolated colonies were cultured in a minimal medium with a powdered mineral different from the mineral from which they were extracted (in the same concentrations as the Culture in Minimum Media and repeated in triplicate). Thus, bacteria originally extracted from quartz were cultured in K-feldspar and calcite medium, those removed from K-feldspar were cultured in quartz and calcite medium, and those extracted from calcite were cultured in quartz and K-feldspar medium.

### Statistical Methods

To test whether the growth ability of microorganisms in minimal enriched medium is related to their morphology (Gram stain, pigment and size) we applied a Multiple Factorial Analysis (MFA) to the collected data. Subsequently, we grouped the variables as follows: morphology, growth in minimal enriched medium (calcite, K-feldspar and quartz) and physicochemical factors. This step produced some elements with which, in the second step, we clustered the endolithic bacteria in seven groups to which we gave the main characteristics in terms of the variables that compound the groups.

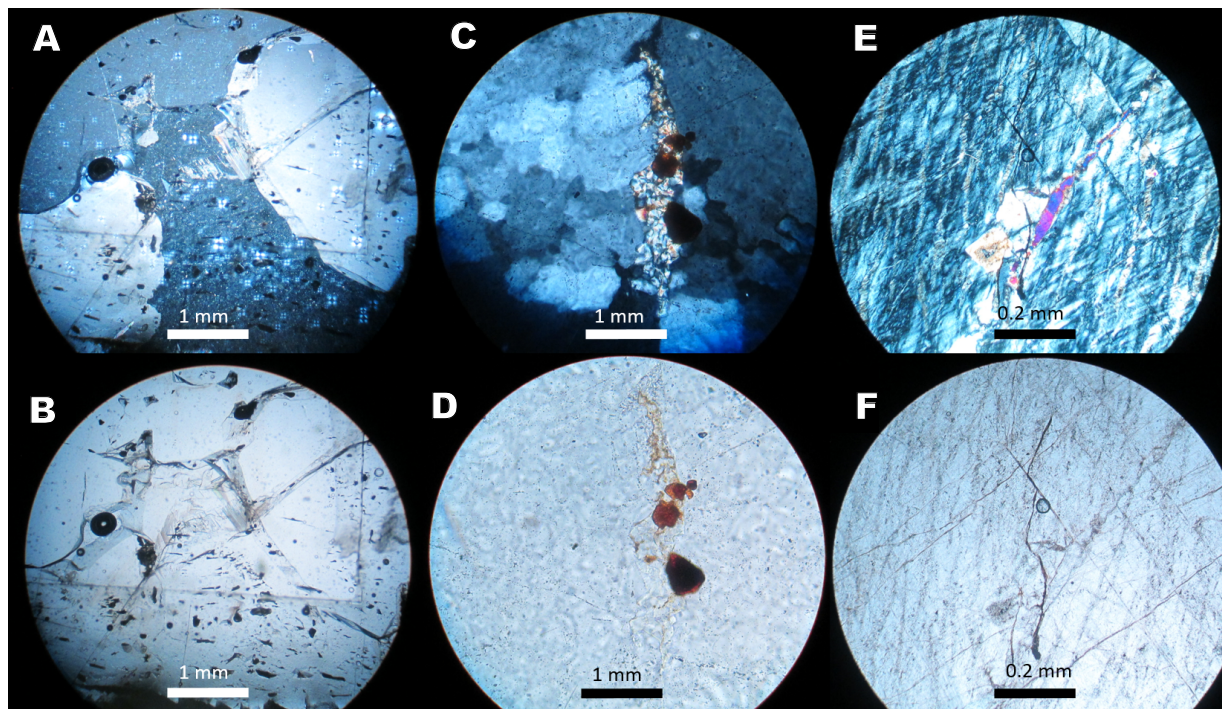
Both steps were repeated one time but using only the following groups of variables: morphology and growth in minimal enriched medium (separated by its chemical elements), to find possible relationships between rock components and the morphologies of the endolithic microorganisms.

Statistical analyses were carried out with R core team (R core Team *et al.*, 2017).

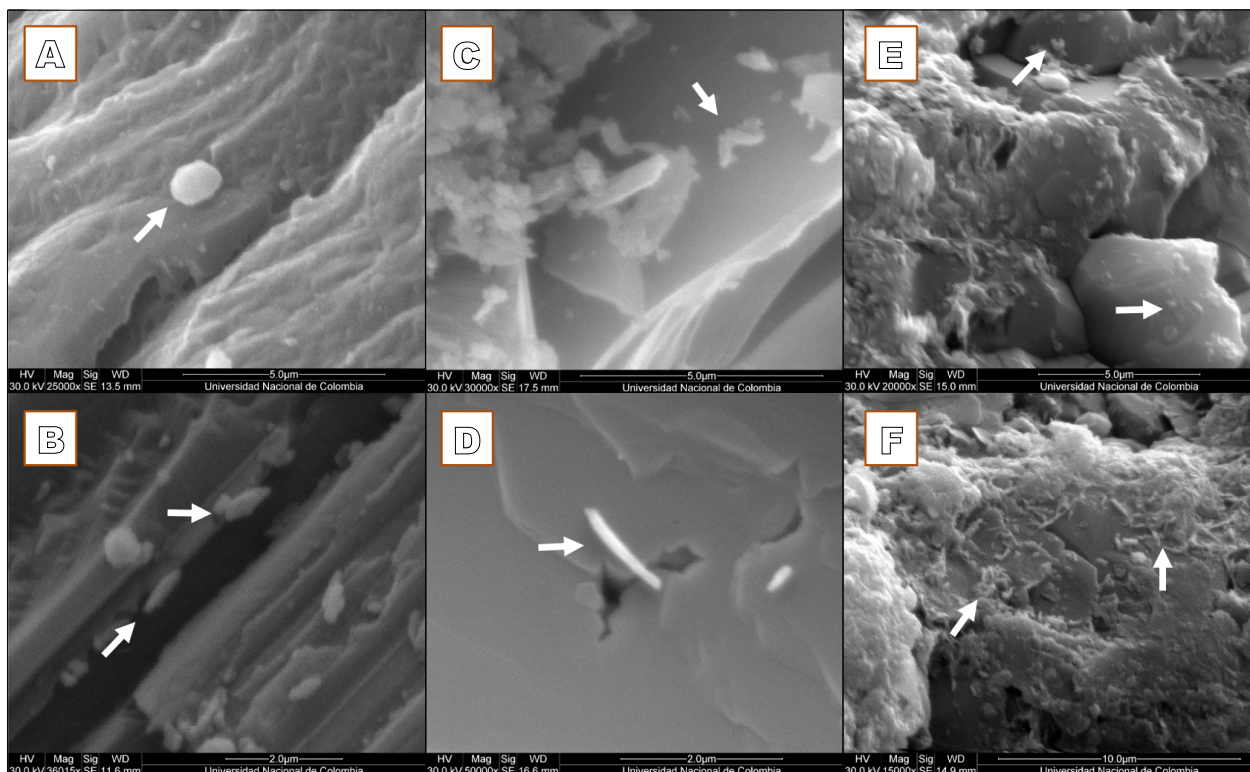
## RESULTS AND DISCUSSION

### Results of the XRF analysis and Petrography

Endolithic habitats have been traditionally considered places where microbial communities find refuge from environmental factors such as desiccation, radiation or lack of water (Bell, 1993; Friedmann, 1980). Furthermore, some elements within minerals and rocks are supposed to drive bacteria colonization (Boyd *et al.*, 2007; Carson *et al.*, 2009; Gleeson *et al.*, 2006; Rogers and Bennett, 2004). However, inside pure minerals, nutrient sources might be scarce because they offer fewer chemical elements than rocks in their crystal structure. XRF analyses of all minerals (calcite, K-feldspar and quartz) are shown in Table 1. Elemental compositions of quartz and feldspar are typical of those associated with granitic pegmatitic lithologies, and calcite is mainly composed of calcium carbonate. Note that data values below 0.05% are



**Figure 2.** A) Calcite in cross polarized light, zoom 4X. B) Calcite in plain polarized light. C) Quartz in cross polarized light, zoom 60X. D) Quartz in plain polarized light. E) K-feldspar in cross polarized light, zoom 4X. F) K-feldspar in plain polarized light.



**Figure 3.** SEM of inner mineral surfaces. A) Calcite showing a possible coccus, magnification 25000X. B) Calcite showing fissures and a possible bacillus, magnification 36015X. C) K-feldspar with a possible bacillus, magnification 30000X. D) K-feldspar surface with a pore and an inconclusive bacillus, magnification 50000X. E) Quartz with possible coccus, magnification 20000X. F) Quartz with grains and possible bacillus consortium, magnification 15000X.

**Table 2.** Colony and Gram Stain and Size and Pigment of each morphotype

Calcite			K-feldspar			Quartz		
Colony	Size	Pigment	Colony	Size	Pigment	Colony	Size	Pigment
C1B-	4	White	KF1B+	4	White	Q1B+	1	Colorless
C2B-	4	White	KF2B-	4	White	Q2B+	2	White
C3B-	1	White	KF3B-	1	White	Q3B+	1	White
C4B-	7	White	KF4B-	3	Yellow	Q4C+	1	White
C5C+	1.5	White	KF5B+	7	White	Q5B+	2	White
C6Y	2	Yellow	KF6B-	1.5	White	Q6B+	5	White
C7C+	1	Pink	KF7Y	2	Yellow	Q7Y	4	Pink
C8C+	2	Yellow	KF8B-	1	Pink	Q8B+	7	White
C9B-	4	Yellow	KF9C+	2	Yellow			
C10C+	3	White	KF10C+	4	Yellow			
C11C+	3	Yellow	KF11C-	3	White			
			KF12Y	3	Yellow			
			KF13B+	3	Yellow			

doubtful as the typical detection limit of the technique is about 0.1%.

Calcite crystals are anhedral with good exfoliation. The interference figure is negative uniaxial, and the macla is polysynthetic rhombohedral (Figure 2A, B)

The K-feldspar was identified as microcline because of its characteristic macla, which is typical of the environment where it was found and is associated with granitic pegmatites, and Sienites. It has peritization with exsolutions of plagioclase inside the feldspar. The plagioclase is slightly altered to sericite. Its interference figure is negative biaxial (Figure 2E, F).

The deformation of the edges between the quartz crystals showed that the quartz veins within the Granito de Pescadero have a low degree of metamorphism. The interference figure is positive uniaxial; a few crystals have a negative biaxial figure and correspond to feldspar. It has tiny crystals of sericite, muscovite, and goethite (Figure 2C, D).

### Scanning Electron Microscopy

Because all minerals seemed smooth, SEM helped to identify pores in the K-feldspar and quartz samples, and pores and fissures in the calcite sample. Empirically, it can be assumed that bacteria and yeast

might be cryptoendolithic in the case of K-feldspar and quartz, and cryptoendolithic and chasmoendolithic in the case of calcite. The low conductive nature of the three mineral samples made it difficult to have a high resolution image of the samples for the search for microorganisms. Although the pictures are inconclusive, bacterial consortia are present in the quartz pictures (Figure 3).

### Culturing of endolithic communities

A count of 33 colonies was obtained from the culture method. 11 colonies were isolated from the calcite sample, 13 from the K-feldspar sample, and 8 from the quartz sample. Among the 11 colonies of the calcite sample, we found 1 Gram-positive bacillus, 5 Gram-negative bacilli, 5 Gram-positive cocci, and 1 yeast-like fungus (Table 1). Included in the 13 colonies of the K-feldspar sample, we found 3 Gram-positive bacilli, 5 Gram-negative bacilli, 2 Gram-positive cocci, 1 Gram-negative coccus, the only one in the three mineral samples, and 2 yeast-like fungi. The eight colonies within the sample of quartz are Gram-positive: 6 Gram-positive bacilli, 1 Gram-positive coccus, and 1 yeast-like fungus. Table 2 shows the macroscopic pigments of the endolithic microbe colonies; there, we can see a clear tendency for quartz colonies to appear in white, and for most K-feldspar and calcite colonies to appear in white and yellow.

**Table 3.** Compiled results from the culture in selective media and physicochemical factors (sugar 30%, salt 15%, temperature 4°C and pH 4 were eliminated because non-single microbe grew under those conditions). Colonies that grew very well in the factors are marked with (+), colonies that grew with difficulty are marked with (+/-) and colonies that did not grow are marked with (-)

Colony	Grow in Calcite	Grow in KF	Grow in Quartz	Glucose 10%	Glucose 20%	Salt 1%	Salt 6.5%	pH 7 (37°C)	pH 9	T 14°C	T 60°C	T 100°C
C1B-	yes	yes	no	+	-	+	-	+	+	+	+	-
C2B-	yes	no	no	+	-	+	-	+	+	+/-	+/-	+/-
C3B-	yes	no	yes	+/-	-	+	-	+	+	+/-	+/-	+/-
C4B-	yes	yes	yes	+	-	+	-	+	+	+	+/-	+/-
C5C+	yes	yes	no	-	-	+	-	+	+/-	+/-	-	+/-
C6Y	yes	no	no	+/-	-	+/-	-	+	+/-	+	+	-
C7C+	yes	yes	no	+	+/-	+/-	-	+	-	+	-	-
C8C+	yes	no	yes	+/-	-	+/-	-	+	+	+	-	-
C9B-	yes	yes	no	+	-	+	+/-	+	+	+	+	-
C10C+	yes	no	yes	+	+/-	+	-	+	+	+/-	+	+/-
C11C+	yes	no	no	+	-	+/-	-	+	-	+/-	-	+/-
KF1B+	no	yes	yes	-	-	+/-	-	+	+/-	+/-	-	-
KF2B-	yes	yes	no	+/-	-	+/-	-	+/-	+/-	+/-	+/-	+/-
KF3B-	yes	yes	yes	+/-	-	-	-	+	+/-	+/-	+/-	+/-
KF4B-	no	yes	no	+/-	-	+/-	-	+/-	+/-	+/-	+/-	+/-
KF5B+	no	yes	no	+/-	-	+/-	-	+	+/-	+/-	+/-	+/-
KF6B-	no	yes	yes	+/-	-	+/-	+/-	+/-	+/-	+/-	+/-	+/-
KF7Y	yes	yes	no	+/-	-	+/-	-	+	+/-	+/-	-	-
KF8B-	no	yes	yes	+	+/-	+	+/-	+	+/-	+	+/-	+/-
KF9C+	no	yes	no	-	-	+/-	-	+	+/-	+/-	+/-	-
KF10C+	yes	yes	no	+/-	-	+	-	+/-	+/-	+/-	+/-	+/-
KF11C-	yes	yes	yes	+/-	-	+/-	-	+/-	+/-	+/-	+/-	-
KF12Y	no	yes	no	+/-	-	+	-	+	+/-	+	+/-	-
KF13B+	no	yes	yes	+	-	+	-	+	+/-	+	+/-	+/-
Q1B+	no	yes	yes	+/-	-	-	-	+	-	+	+/-	+/-
Q2B+	yes	yes	yes	+	-	+/-	-	+	+	+/-	+/-	+/-
Q3B+	yes	yes	yes	+/-	+	+	-	+	+	+/-	-	-
Q4C+	no	yes	yes	+	+	-	+/-	+	+	+	-	-
Q5B+	yes	no	yes	-	-	+/-	-	+	-	+	-	-
Q6B+	no	no	yes	+	+/-	+/-	-	+	+/-	+	-	-
Q7Y	yes	yes	yes	+	-	+/-	+/-	+	+/-	+/-	+/-	-
Q8B+	no	yes	yes	+/-	-	+/-	-	+/-	+/-	+	-	-

### Culture in selective media and Physicochemical analysis

Table 3 presents the physicochemical factors and the growth of each colony cultured in a minimal medium enriched with the mineral from where they were extracted. For an easier understanding of collected data, each territory has the initials of the mineral, a number and the Gram stain group. As all colonies grew in the medium enriched with the mineral from where they were extracted, endolithic

microorganisms may be controlled by the elements present in the three minerals.

The study realized by Carson *et al.* (2009) showed that structure of bacterial communities in soil is influenced by the mineral substrates in their microhabitat and that minerals in soil play a greater role in bacterial ecology than simply providing an inert matrix for bacterial growth. Moreover, the authors suggest that mineral heterogeneity in soil contribute to spatial variation in bacterial communities.



Previous works support this hypothesis. *Boyd et al. (2007)* showed that quartz, saprolite and hematite, extracted from an aquifer, selected different subdivisions of bacteria within the Proteobacteria. *Mauck and Roberts (2007)* and *Rogers and Bennet (2004)* have shown that different silicate minerals select diverse microbial communities with different structures colonizing their surfaces. And, *Gleeson et al. (2006)* found that in a weathered pegmatitic granite, the bacterial populations were associated with specific mineralogies (quartz, plagioclase, K-feldspar and muscovite). The abundance of the distinctive ribotypes of bacteria on the surface of the minerals is related to the presence of the chemical elements on the granite Ca, K, Na, P, Fe, Al and Si.

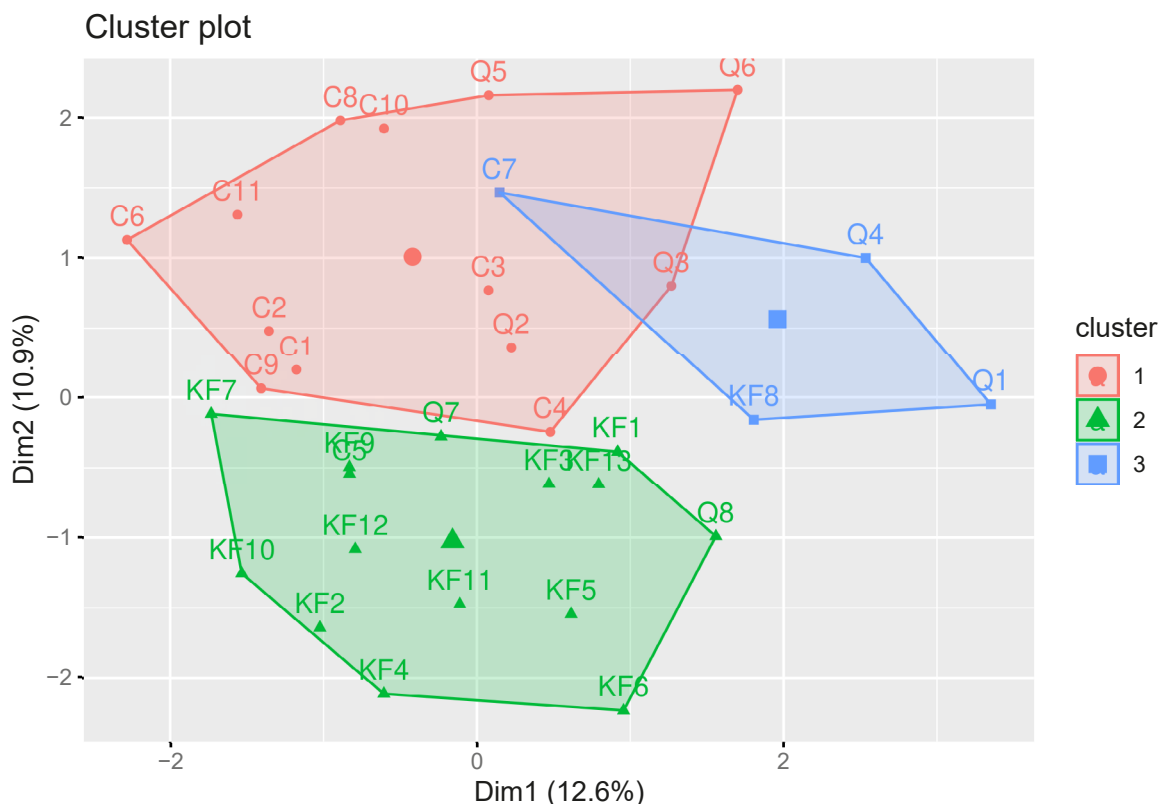
To sum up, it has been demonstrated that microbial lithobiontic communities can use P, K, Mg and Na (*Carson et al., 2009*); P and Fe (*Mauck and Roberts, 2007; Rogers and Bennett, 2004*) and Ca, K, Na, P, Fe, Al and Si (*Gleeson et al., 2006*) for their cellular functions..

The isolated colonies that grew up from calcite seem to be in equilibrium in coccus and bacillus: 5 Gram-negative bacillus, 1 Gram-positive bacillus, 6 Gram-positive coccus, and one yeast.

Quartz has mainly silica and small quantities of Al and Cl in its structure, which may restrict bacterial nutrition more than other minerals (in terms of mineral nutrients such as K, among others, *Gleeson et al., 2006*), possibly leading to less abundance found there: 6 Gram-positive bacillus, 1 Gram-positive coccus, and one yeast.

Microcline has high quantities of Si, Al and K and fewer quantities of Na, Ca, Ba and Fe; this implies a broader availability of nutrients for the microorganisms (*Gleeson, 2006; Rogers and Bennett, 2004*). This fact might explain the distribution in the morphologies found in their microbial communities, eight bacilli (3 Gram-negative and 5 Gram-positive), 2 Gram-positive cocci, 1 Gram-negative coccus, and two yeast-like fungi.

The finding of 4 yeast implies that diversity within endolithic habitats is variable. Yeast-like fungi are very well adapted to withstand unfavorable conditions. They can resist dryness and UV irradiation by expressing melanized thick walls as a stable character (*Selbmann et al., 2005*). This fact can be helpful in the case of yeast- found within translucent crystals in quartz and calcite analyzed in this study.



**Figure 4.** Hierarchical Cluster for the 32 morphotypes. Morphotype, pigment, and size vs. growth in minimum enriched medium and physicochemical factors.

The unique yeast-like fungus from calcite C6Y did not grow in the quartz medium not either in the K-feldspar medium. The two yeast-like fungi from the K-feldspar (FK7Y and FK12Y) did not succeed in the quartz medium, and only one of them (FK7Y) grew in the calcite medium. Finally, the yeast from quartz (Q7Y) was the only yeast that grew in K-feldspar and calcite media; thus, it was one of the strongest within the endolithic yeast-like fungi.

In Table 2, we can see that FK11C- is the only Gram-negative coccus among the 32 colonies. It was found within the K- feldspar (microcline), the mineral with the highest number of colonies (13). The K- feldspar is also the mineral with the highest availability of chemical elements (SiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, K<sub>2</sub>O, Na<sub>2</sub>O, CaO, Ba, Fe<sub>2</sub>O<sub>3</sub> and Cl) suggesting that the available nutrients control the diversity of endolithic microorganisms.

### Hierarchical clustering

We applied MFA and used the first five factors of this analysis to cluster the bacteria and discover possible influences of macroscopical (size, pigment) and microscopical (Gram stain) characteristics on the growing ability of the bacteria. The resulting groups are shown in Figure 4, represented on the two first components of the MFA.

Group 1 comprises 13 morphotypes (C1, C2, C3, C3, C6, C8, C9, C10, C11, Q2, Q3, Q5 and Q6) and it is distinguished because all its bacteria grew in high proportion under pH 7-37°C. Moreover, 8/13 (61.54%) grew in the K-feldspar enriched medium, and 9/13 (69.23%) grew in high proportion under pH9 nutritious broth. Furthermore, 12/13 (92.31%) grew in the calcite-enriched medium. These might imply that morphotypes that we can grow under pH7-37°C have a general advantage in adapting to K-feldspar enriched medium even though all of them were originally extracted from calcite or quartz. Calcite and quartz microbes that grow in a K-feldspar medium can also live successfully in alkaline conditions.

Group 2 comprises 15 morphotypes (C5, KF1, KF2, KF3, KF4, KF5, KF6, KF7, KF9, KF10, KF11, KF12, KF13, Q7 and Q8) and is distinguished because all its bacteria grew in the K-feldspar enriched medium. All of them grew in low proportion under pH9, and none grew in sugar 20%. Moreover, 12/15 (80.00%) grew in low proportion at T14°C, and 6/15 (40.00%) grew under pH7-37°C, which contrasts with the other 9/15 (60%) that grew in high proportion under pH7-37°C. Furthermore, 11/15 (73.33%) grew in low proportion under T60°C, and 10/15 (66.67%) grew in low proportion in sugar 10%. Therefore, microorganisms in Group 2 grew easily under pH 7 and 37°C. However, also most of them can adapt to temperatures as high as 60°C, which is interesting because it gives them



Figure 5. Hierarchical Cluster for the 32 morphotypes. Morphotype, pigment and size vs growth in minimum enriched medium.

the possibility to tolerate extreme changes in temperatures, and this is expected to happen in both semi-arid places, Pescadero and Villa de Leyva.

Group 3 is composed of 4 morphotypes (C7, KF8, Q1 and Q4) and is distinguished because all its bacteria have a size of 1mm and grew in high proportion under 14°C. Moreover, 2/4 (50%) didn't grow in salt 1% and have a pink pigment in the macroscopic colony. The size of 1 mm was one of the less standard sizes of all 32 morphotypes, and pink morphotypes have an anomalous behavior or pigment among all the colonies. Note that half of their morphotypes C7 and FK8) have a particular pink, and the other half (Q1 and Q4), which are not pink, didn't grow under salt 1%, which is a strange behavior as most of the 32 isolated morphotypes were able to succeed in this physicochemical factor (Table 3).

Considering that pigment and size in colonies and morphotype (Gram-positive/negative coccus or bacillus) might be driven by mineralogy, the following clustering only considers the morphology of the endolithic microorganisms and the chemical elements of the minerals that allow their growth. The resulting groups are shown in Figure 5 and represented in the two first components of the MFA.

Group 1 comprises six morphotypes (C6, C11, KF7, KF9, KF12 and KF14); 3/6 (50%) are yeast. It is distinguished because all its bacteria have a yellow pigment and didn't grow in the quartz enriched medium, which implies that there could be a relation between the yellow pigment and yeast endolithic microbes, as 3/4 of all yeast have this color, and the remaining one is pink. The yellow pigment could also be related to the impossibility of growing under a quartz-enriched medium. Note that none of the bacteria that were initially extracted from quartz has a yellow pigment in the colony form, so probably restriction in chemical elements (99.28% in quartz) could drive quartz morphotypes into white and colorless pigments (Table 2).

Group 2 comprises eight morphotypes (C1, C2, C5, C7, C9, KF2, KF10 and Q7) and is distinguished because all its bacteria grew in the calcite medium. 7/8 (87.5%) didn't grow in the quartz medium, and 6/8 (75%) have a size of 4 mm in their colony form, which could imply that the size of 4mm is related to an ability to grow under calcite-enriched medium and an inability to succeed in quartz supplemented medium.

Group 3 comprises seven morphotypes (C3, C8, C10, KF11, Q2, Q5 and Q6) and is distinguished because all its bacteria can grow in the quartz enriched medium, and 5/7 (71.43%) didn't grow in the K-feldspar enriched medium. Morphotypes of this group only have their ability to grow

under minimum enriched media in common but still share a Gram-positive morphology in their membranes.

Group 4 is composed of 11 morphotypes (C4, KF1, KF3, KF5, KF6, KF8, KF13, Q1, Q3, Q4 and Q8) and is distinguished because all its bacteria grew in the K-feldspar enriched medium and 9/11 (90.91%) grew also in the quartz enriched medium. Furthermore, 8/11 (71.3%) didn't grow in the calcite enriched medium, 6/11 (54.55%) are Gram-positive bacilli, 3/11 (27.27%) have a macroscopic size of 7 mm, and 5/11 (45.45%) have a length of 1mm, which is a rather diverse group. Although most of its member is bacillus except Q4, its members have different sizes: 1, 4 and 7mm, which might imply that Gram-positive bacillus is easily adaptable to minimum media enriched with at least two minerals and not that easy to adapt to calcite supplemented medium. Note that morphotypes cultured initially from calcite are Gram-negative bacillus or Gram-positive coccus (Table 2).

### Astrobiological Considerations

In this study, it was found endolithic bacteria and yeast in semi-arid zones with extreme climatic conditions. Daily irradiation in Villa de Leyva and Pescadero (4.5-5.0kWh/m<sup>2</sup> and 4.0-4.5kWh/m<sup>2</sup>, respectively) (Corzo-Acosta, 2018; IDEAM, 2017), might influence the morphotypes found in the calcite, K-feldspar and quartz. UV index radiation in these zones is 9-10, one of the highest in the Colombian territory, only overcome by the one in high elevation mountains such as Nevado del Ruiz, Nevado del Cocuy, and Paramo de Sumapaz.. Thus, microorganisms in these regions might have been induced to inhabit these microhabitats in calcite and quartz, which are translucent rocks that, like gypsum, quartzite, and/or obsidian, can easily protect them from harming UV radiations and allow photosynthesis at the same time (Cockell *et al.*, 2008; Herrera *et al.*, 2009; Hughes and Lawley 2003), and in the case of K-feldspar screening high doses of damaging solar radiation (Kapitulčinová *et al.*, 2015).

In some deserts, non-hygroscopic but microporous translucent gypsum crusts are found as potential substrates that can mitigate exposure to UV radiation and desiccation and allow microbial colonization (Cockell *et al.*, 2003; Oren, 1995). Calcite and quartz analyzed here possess pores and fissures and are translucent. Therefore, these rock-forming minerals could also mitigate UV radiation and desiccation exposure and allow microbial colonization in these Colombian semi-arid zones.

The importance of revealing knowledge of microbial communities that inhabit endolithic habitats is related to the field of Astrobiology (Wierzechos *et al.*, 2011, 2006). Inquiring what sort of microorganisms on Earth inhabit

specific minerals could help propose those lithologies as life detection targets on an extra-terrestrial surface such as Mars.

For instance, as the Atacama Desert has been proposed as one of the best Earth's analogs for Mars (McKay, 2003), there are many studies about the relations between lithobiontic microorganisms and the substrates present on its surface. For instance, Warren-Rhodes *et al.* (2006) point out the importance of understanding the limitations of microbial photosynthesis on quartz pebbles under dry conditions and its relevance to the question of life on the surface of Mars. Within those studies, other substrates, such as gypsum (Dong *et al.*, 2007), halite, granite, opaque cherts, and shocked volcanic rocks, are known to support cyanobacterial communities in terrestrial stony deserts and could serve as similar habitats on Mars (Cockell *et al.*, 2010). Given the discovery of quartz on Mars's Antoniadi Crater (Smith *et al.*, 2012), on the northern edge of the Syrtis Major shield volcano, which is co-located with plagioclase feldspar (probably associated with a felsic pluton that was later excavated by impacts (Bandfield *et al.*, 2004) and bands of carbonates detected in Jezero crater (Horgan *et al.*, 2020), finding of endolithic microbes within calcite, K-feldspar and quartz throughout this research may help to define which minerals may serve as microhabitat to possible Martian endolithic microbes. Moreover, there is a possibility of the presence of feldspar in the northern Hellas region, in the rims of large craters (including Holden), in a small valley in Xanthe Terra, and in the Nili Patera caldera of Syrtis Major (Carter and Poulet, 2013).

Seeing that some of the minerals mentioned later are related to impact craters, it is essential to point out that impact craters have a high potential for hosting life. According to Cockell *et al.* (2003), the lakes within the hydrologic depression associated with the crater bowl resulting from the impact may provide a new habitat for aquatic life. Once the crater begins to cool, melt rocks will provide a substrate for primary succession (primary succession on land occurs when the land is devoid of soil and the area is barren of life). On Mars, impact events have played an important role in the processing of the surface. Shocked rocks might provide a protected microhabitat (*e.g.* from UV radiation) for any putative indigenous life due to that porosity area and fractures are increased after the impact (Cockell *et al.*, 2003). Recently, liquid water was discovered in the Planum Australe region on Mars (Orosei, 2018). The data MARSIS (Mars Advanced Radar for Subsurface and Ionosphere Sounding) show that liquid water can be stable at relatively shallow depths (about 1.5km), providing a very important quality needed for life.

Considering that several robotic exploration missions during this decade are traveling (Mars 2020 Perseverance

Rover) or will travel to Mars (ExoMars 2022 and Tianwen-1) to investigate old or current biological traces on Mars surface and subsurface, it is of high relevance to study the endolithic microenvironments where life could host be protected from extreme physicochemical conditions (Böttger *et al.*, 2012; Fan *et al.*, 2021; Haltigin *et al.*, 2016). Studies like those presented here may help to give insights on what kind of adaptive strategies endolithic microorganisms might use to live in an inhospitable subsurface like Mars. This would be a practical approach to determining potential life detection targets and landing site strategies for Astrobiology purposes (Ehrenfreund *et al.*, 2011).

## CONCLUSIONS

In this study, some endolithic microorganisms extracted from calcite, K-feldspar and quartz have been cultured in nutritive agar and in a minimal medium enriched with the same mineral from which they were extracted and in minimal media enriched with different minerals. Most of the colonies (24 out of 32) grew in one or in the two media enriched with a different mineral from the one they were cultured originally. This suggests that organisms living inside minerals may successfully adapt to different lithologies.

The 32 colonies were cultured in nutritional broth at 4°C, 14°C, 37°C, 60° and 100°C. Any of them grew under 4°C, all grew at 14°C and 37°C, and some grew at 60°C and 100°C. This is the presumed behavior of endolithic microorganisms that live in semi-arid areas, where rock could protect them when the temperature decreases, but they must resist high midday temperatures in clear-cloudy skies. Considering this, calcite, K-feldspar and quartz have significant potential as life detection targets that, on the one hand, different microbial communities were found inside them and probably resisted high UV irradiation levels. On the other hand, those mineralogies (carbonates, feldspars, and quartz) are present on Mars. They can be selected as landing sites for future astrobiological research.

Noteworthy, none of the 33 morphotypes were able to grow under pH4 nutritive broth. Still, most of them can grow under pH9 nutritive broth. What is more, 9 of them grew in a high proportion under this alkaline condition, so possibly endolithic microorganisms in minerals could be alkalotolerant and therefore, this increases the interest in astrobiology exploration.

The clusters done as part of the methodology are very helpful in finding tendencies between pigment, size, morphology, and bacteria's ability to adapt to a wide range of temperatures, sugar and salt percentages or pH, and to nutritive

agar. Although these tendencies are not yet conclusive, we consider that the merit of this work lies in the use of multiple factorial analyses combined with basic culture techniques, which helps to study the behavior of live isolated colonies under different changes in physicochemical factors and thus to increase the understanding of the possible causalities between mineralogy and morphology developed for endolithic microorganisms. Finally, we suggest considering high elevation places in Colombia, such as Nevado del Ruiz Volcano, Páramo de Sumapaz, or Nevado del Cocuy, as future targets for studies like this, taking advantage of low temperatures and very high levels of ultraviolet radiation on their surfaces.

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## REFERENCES

- Albesiano, S., Rangel-Churio, J.O., Cadena, A., 2003. La vegetación del cañón del río Chicamocha (Santander, Colombia). *Caldasia*, 25(1), 73-99. DOI: 10.15446/caldasia
- Ascaso, C., 2002. Ecología microbiana de sustratos líticos. *Ciencia y medio ambiente*, 90-103. Last accessed: March 2021. Website: <http://hdl.handle.net/10261/111133>
- Bandfield, J.L., Hamilton, V.E., Christensen, P.R., McSween Jr., H.Y., 2004. Identification of quartzofeldspathic materials on Mars. *Journal of Geophysical Research: Planets*, 109(4), E10. DOI: <https://doi.org/10.1029/2004JE002290>
- Bell, R.A., 1993. Cryptoendolithic algae of hot semiarid lands and deserts. *Journal of Phycology*, 29(2), 133-139. DOI: <https://doi.org/10.1111/j.0022-3646.1993.00133.x>
- Bhattacharjee, K., Joshi, S.R., 2016. A selective medium for recovery and enumeration of endolithic bacteria. *Journal of microbiological methods*, 129, 44-54. DOI: <https://doi.org/10.1016/j.mimet.2016.07.026>
- Böttger, U., de Vera, J.P., Fritz, J., Weber, I., Hübers, H.W., Schulze-Makuch, D., 2012. Optimizing the detection of carotene in cyanobacteria in a martian regolith analogue with a Raman spectrometer for the ExoMars mission. *Planetary and Space Science*, 60(1), 356-362. DOI: <https://doi.org/10.1016/j.pss.2011.10.017>
- Boyd, E.S., Cummings, D.E., Geesey, G.G., 2007. Mineralogy influence's structure and diversity of bacterial communities associated with geological substrata in a pristine aquifer. *Microbial Ecology*, 54(1), 170- 182. DOI: <https://doi.org/10.1007/s00248-006-9187-9>
- Breznak, J.A., Costilow, R.N., 2007. Physicochemical factors in growth. In *¿? Methods for General and Molecular Microbiology*, 309-329.
- Bungartz, F., Garvie, L.A., Nash, T.H., 2004. Anatomy of the endolithic Sonoran Desert lichen *Verrucaria rubrocincta* Breuss: implications for biodeterioration and biomineralization. *The Lichenologist*, 36(1), 55-73. DOI: <https://doi.org/10.1017/S0024282904013854>
- Carson, J.K., Campbell, L., Rooney, D., Clipson, N., Gleeson, D.B., 2009. Minerals in soil select distinct bacterial communities in their microhabitats. *Federation of european microbiological societies( FEMS) Microbiology Ecology*, 67(3), 381-388. DOI: <https://doi.org/10.1111/j.1574-6941.2008.00645.x>
- Carter, J., Poulet, E., 2013. Ancient plutonic processes on Mars inferred from the detection of possible anorthositic terrains. *Nature Geoscience*, 6(12), 1008-1012. DOI: <https://doi.org/10.1038/ngeo1995>
- Certini, G., Campbell, C.D., Edwards, A.C., 2004. Rock fragments in soil support a different microbial community from the fine earth. *Federation of european microbiological societies( FEMS) Soil Biology and Biochemistry*, 36(7), 1119-1128. DOI: <https://doi.org/10.1016/j.soilbio.2004.02.022>
- Cockell, C., Osinski, G., Lee, P., 2003. The impact crater as a habitat: effects of impact processing of target materials. *Astrobiology*, 3181-191. DOI: <https://doi.org/10.1089/153110703321632507>
- Cockell, C.S., Stokes, M.D., 2004. Widespread colonization by polar hypoliths. *Nature*, 431(7007), 414-414. DOI: <https://doi.org/10.1038/431414a>
- Cockell, C.S., McKay, C.P., Warren-Rhodes, K., Horneck, G., 2008. Ultraviolet radiation-induced limitation to epilithic microbial growth in arid deserts—Dosimetric experiments in the hyperarid core of the Atacama Desert. *Journal of Photochemistry and Photobiology B: Biology*, 90(2), 79-87. DOI: <https://doi.org/10.1016/j.jphotobiol.2007.11.009>
- Cockell, C.S., Olsson, K., Knowles, E., Kelly, L., Herrera, A., Thorsteinsson, T., Marteinson, V., 2009. Bacteria in weathered basaltic glass, Iceland. *Geomicrobiology Journal*, 26(7), 491-507. DOI: <https://doi.org/10.1080/01490450903061101>
- Cockell, C.S., Osinski, G.R., Banerjee, N.R., Howard, K.T., Gilmour, I., Watson, J.S., 2010. The microbe–mineral environment and gypsum neogenesis in a weathered polar

- evaporite. *Geobiology*, 8(4), 293-308. DOI: <https://doi.org/10.1111/j.1472-4669.2010.00240.x>
- Corzo-Acosta J.A., 2018. Bacterias endolíticas cultivables en minerales (Cuarzo, Feldespato y Calcita) provenientes de muestreos geológicos en áreas de Villa de Leyva, Boyacá y Pescadero, Santander (Colombia). Master Thesis. National University of Colombia. Bogotá, Last accessed: 2 Dec 2022 Website: <https://repositorio.unal.edu.co/handle/unal/77435>
- Cowan, D.A., Khan, N., Pointing, S.B., Cary, S.C., 2010. Diverse hypolithic refuge communities in the McMurdo Dry Valleys. *Antarctic Science*, 22(6), 714-720. DOI: <https://doi.org/10.1017/S0954102010000507>
- Direito, S.O., Marees, A., Röling, W.F., 2012. Sensitive life detection strategies for low-biomass environments: optimizing extraction of nucleic acids adsorbing to terrestrial and Mars analogue minerals. Federation of European Microbiological Societies (FEMS). *Microbiology Ecology*, 81(1), 111-123. DOI: <https://doi.org/10.1111/j.1574-6941.2012.01325.x>
- Dong, H., Rech, J.A., Jiang, H., Sun, H., Buck, B.J., 2007. Endolithic cyanobacteria in soil gypsum: Occurrences in Atacama (Chile), Mojave (United States), and Al-Jafr Basin (Jordan) Deserts. *Journal of Geophysical Research: Biogeosciences*, 112, G2. DOI: <https://doi.org/10.1029/2006JG000385>
- Dussault, H.P., 1955. An improved technique for staining red halophilic bacteria. *Journal of Bacteriology*, 70(4), 484-485.
- Ehrenfreund, P., Röling, W.F.M., Thiel, C.S., Quinn, R., Sephton, M.A., Stoker, C., Kotler J.M., Direito, S.O.L., Martins, Z., Orzechowska, G.E., Kidd, R.D., van Sluis, C.A and Foing, B.H., 2011. Astrobiology and habitability studies in preparation for future Mars missions: trends from investigating minerals, organics and biota. *International Journal of Astrobiology*, 10(3), 239-253. DOI: <https://doi.org/10.1017/S1473550411000140>
- Ehrlich, H.L., Newman, D.K., Kappler, A., 2015. *Geomicrobiology* M. Dekker, 768pp.
- Fan, M., Lyu, P., Su, Y., Du, K., Zhang, Q., Zhang, Z., Dai, S., Hong, T., 2021. The Mars Orbiter Subsurface Investigation Radar (MOSIR) on China's Tianwen-1 Mission. *Space Science Reviews*, 217(1), 1-17.
- Fischer, G., Lüdders, P., 2002. Efecto de la altitud sobre el crecimiento y desarrollo vegetativo de la uchuva (*Physalis peruviana* L.). Bogotá, *Revista Comalfe*, 29(1), 1-10. Last accessed: December 2022 Website: [https://www.researchgate.net/profile/Gerhard\\_Fischer/publication/257363411\\_Efecto\\_de\\_la\\_altitud\\_sobre\\_el\\_crecimiento\\_y\\_desarrollo\\_vegetativo\\_de\\_la\\_uchuva\\_Physalis\\_peruviana\\_L/links/0c9605250b354583c4000000.pdf](https://www.researchgate.net/profile/Gerhard_Fischer/publication/257363411_Efecto_de_la_altitud_sobre_el_crecimiento_y_desarrollo_vegetativo_de_la_uchuva_Physalis_peruviana_L/links/0c9605250b354583c4000000.pdf)
- Friedmann, E.I., 1980. Endolithic microbial life in hot and cold deserts. *Limits of Life*, 10, 223-235. DOI: [https://doi.org/10.1007/978-94-009-9085-2\\_3](https://doi.org/10.1007/978-94-009-9085-2_3)
- Friedmann, E.I., Weed, R., 1987. Microbial trace-fossil formation, biogenous, and abiotic weathering in the Antarctic cold desert. *Science*, 236(4802), 703-705. DOI: 10.1126/science.11536571
- Gaylarde, C., Baptista-Neto, J.A., Ogawa, A., Kowalski, M., Celikkol-Aydin, S., Beech, I., 2017. Epilithic and endolithic microorganisms and deterioration on stone church facades subject to urban pollution in a sub-tropical climate. *Biofouling*, 33(2), 113-127. DOI: <https://doi.org/10.1080/08927014.2016.1269893>
- Gleeson, D.B., Kennedy, N.M., Clipson, N., Melville, K., Gadd, G.M., McDermott, F.P., 2006. Characterization of bacterial community structure on a weathered pegmatitic granite. *Microbial Ecology*, 51(4), 526-534. DOI: <https://doi.org/10.1007/s00248-006-9052-x>
- Guillitte, O., 1995. Bioreceptivity: a new concept for building ecology studies. *Science of the Total Environment*, 167(1-3), 215-220. DOI: [https://doi.org/10.1016/0048-9697\(95\)04582-L](https://doi.org/10.1016/0048-9697(95)04582-L)
- Haltigin, T., Lange, C., Mugnuolo, R., Smith, C., & iMARS Working Group (2016). (2018). iMARS Phase 2: a draft mission architecture and science management plan for the return of samples from Mars Phase 2 Report of the International Mars Architecture for the Return of Samples (iMARS) Working Group. *Astrobiology*, 18(S1). DOI: <https://doi.org/10.1089/ast.2018.29027.mars>
- Herrera, A., Cockell, C.S., Self, S., Blaxter, M., Reitner, J., Thorsteinsson, T., Arp, G., Dröse, W., Tindle, A.G., 2009. A cryptoendolithic community in volcanic glass. *Astrobiology*, 9(4), 369-381. DOI: <https://doi.org/10.1089/ast.2008.0278>
- Horgan, B.H., Anderson, R.B., Dromart, G., Amador, E.S., Rice, M.S., 2020. The mineral diversity of Jezero crater: Evidence for possible lacustrine carbonates on Mars. *Icarus*, 339, 113526. DOI: <https://doi.org/10.1016/j.icarus.2019.113526>
- Hughes, K.A., Lawley, B., 2003. A novel Antarctic microbial endolithic community within gypsum crusts. *Environmental Microbiology*, 5(7), 555-565. DOI: <https://doi.org/10.1046/j.1462-2920.2003.00439.x>
- Instituto de Hidrología, Meteorología y Estudios Ambientales (IDEAM) Atlas Interactivo-Radiación. Obtenido de Atlas Interactivo - Radiación. Last Accessed: 8 January 2021. Web: <http://atlas.ideam.gov.co/visorAtlasRadiacion.html>
- Kapitulčinová, D., Cockell, C.S., Patel, M., Ragnarsdóttir, K.V., 2015. The interlayer regions of sheet silicates as a favorable habitat for endolithic microorganisms. *Geomicrobiology Journal*, 32(6), 530-537. DOI: <https://doi.org/10.1089/ast.2019.2077>
- Kelly, L.C., Cockell, C.S., Herrera-Belaroussi, A., Piceno, Y., Andersen, G., DeSantis, T., Brodie, E., Thorsteinsson, T., Marteinsson, V., Poly, F., LeRoux, X. (2011). Bacterial diversity of terrestrial crystalline volcanic rocks, Iceland. *Microbial Ecology*, 62(1), 69-79. DOI: <https://doi.org/10.1007/s00248-011-9864-1>
- Lacap, D.C., Warren-Rhodes, K.A., McKay, C.P., Pointing, S.B., 2011. Cyanobacteria and chloroflexi-dominated hypolithic colonization of quartz at the hyper-arid core of the Atacama Desert, Chile. *Extremophiles*, 15(1), 31-38. DOI: <https://doi.org/10.1007/s00792-010-0334-3>
- van der Lelij, R., Spikings, R., Gerdes, A., Chiaradia, M., Vennemann, T., Mora, A., 2019. Multi-proxy isotopic tracing of magmatic sources and crustal recycling in the Palaeozoic

- to Early Jurassic active margin of North-Western Gondwana. *Gondwana Research*, 66, 227-245. DOI: <https://doi.org/10.1016/j.gr.2018.09.007>
- Makhalanyane, T.P., Pointing, S.B., Cowan, D.A., 2014. Lithobionts: cryptic and refuge niches. In: Cowan, D.A. (ed.). *Antarctic Terrestrial Microbiology*. Berlin, Heidelberg, Springer, 163-179. DOI: [https://doi.org/10.1007/978-3-642-45213-0\\_9](https://doi.org/10.1007/978-3-642-45213-0_9)
- Mauck, B.S., Roberts, J.A., 2007. Mineralogic control on abundance and diversity of surface-adherent microbial communities. *Geomicrobiology Journal*, 24(3-4), 167-177. DOI: <https://doi.org/10.1080/01490450701457162>
- McKay, C.P., Friedmann, E.I., Gómez-Silva, B., Cáceres-Villanueva, L., Andersen, D.T., Landheim, R., 2003. Temperature and moisture conditions for life in the extreme arid region of the Atacama Desert: four years of observations including the El Niño of 1997–1998. *Astrobiology*, 3(2), 393-406.
- McNamara, C.J., Perry, T.D., Bearce, K.A., Hernandez-Duque, G., Mitchell, R., 2006. Epilithic and endolithic bacterial communities in limestone from a Maya archaeological site. *Microbial Ecology*, 51(1), 51-64. DOI: <https://doi.org/10.1007/s00248-005-0200-5>
- Meslier, V., Casero, M.C., Dailey, M., Wierzbos, J., Ascaso, C., Artieda, O., McCullough, P.R., DiRuggiero, J., 2018. Fundamental drivers for endolithic microbial community assemblies in the hyperarid Atacama Desert. *Environmental microbiology*, 20(5), 1765-1781.
- Navas, J., 1962. Geología del Carbonífero al N. de Bucaramanga. *Boletín de Geología*, 11, 23-34.
- Omelson, C.R., Pollard, W.H., Ferris, F.G., 2006. Environmental controls on microbial colonization of high Arctic cryptoendolithic habitats. *Polar Biology*, 30(1), 19-29. DOI: <https://doi.org/10.1007/s00300-006-0155-0>
- Omelson, C.R., Pollard, W.H., Ferris, F.G., 2007. Inorganic species distribution and microbial diversity within high Arctic cryptoendolithic habitats. *Microbial Ecology*, 54(4), 740-752. DOI: <https://doi.org/10.1007/s00248-007-9235-0>
- Oren, A., Köhl, M., Karsten, U., 1995. An endoevaporitic microbial mat within a gypsum crust: zonation of phototrophs, photopigments, and light penetration. *Marine Ecology, Progress Series*, 128, 151-159. DOI: 10.3354/meps128151
- Orosei, R., Lauro, S.E., Pettinelli, E., Cicchetti, A., Coradini, M., Cosciotti, B., Paolo, F. Di., Flamini, E., Mattei, E., Pajola, M., Soldovieri, E., Cartacci, M., Cassenti, F., Frigeri, A., Giuppi, S., Martufi, R., Masdea, A., Mitri, G., Nenna, C., Noschese, R., Restano, M., Seu, R., 2018. Radar evidence of subglacial liquid water on Mars. *Science*, 361(6401), 490-493. DOI: 10.1126/science.aar7268
- Páramo-Fonseca, M.E., O'Gorman, J.P., Gasparini, Z., Padilla, S., Parra-Ruge, M.L., 2019. A new late Aptian elasmosaurid from the Paja Formation, Villa de Leiva, Colombia. *Cretaceous Research*, 99, 30-40. DOI: <https://doi.org/10.1016/j.cretres.2019.02.010>
- Patarroyo, P., Murillo, M.M., 1997. Nuevas Consideraciones en torno al Cabeceo del Anticlinal de Arcabuco, en cercanías de Villa de Leyva-Boyacá. *Geología Colombiana*, 22, 27-34. DOI: 10.15446/gc
- Patarroyo, P., 2008. La Formación Ritoque en la zona de Vélez (Santander-Colombia). *Geología Colombiana*, 33, 109-110. ISSN 0072-0992
- R Core Team, 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Ramsey, M.H., Potts, P.J., Webb, P.C., Watkins, P., Watson, J.S., Coles, B.J., 1995. An objective assessment of analytical method precision: comparison of ICP-AES and XRF for the analysis of silicate rocks. *Chemical Geology*, 124(1-2), 1-19. DOI: [https://doi.org/10.1016/0009-2541\(95\)00020-M](https://doi.org/10.1016/0009-2541(95)00020-M)
- Rogers, J.R., Bennett, P.C., 2004. Mineral stimulation of subsurface microorganisms: release of limiting nutrients from silicates. *Chemical Geology*, 203(1-2), 91-108. DOI: <https://doi.org/10.1016/j.chemgeo.2003.09.001>
- Rojas, A., Sandy, M.R., 2019. Early Cretaceous (Valanginian) brachiopods from the Rosablanca Formation, Colombia, South America: Biostratigraphic significance and paleogeographic implications. *Cretaceous Research*, 96, 184-195. DOI: <https://doi.org/10.1016/j.cretres.2018.12.011>
- Rondanelli, R., Molina, A., Falvey, M., 2015. The Atacama surface solar maximum. *Bulletin of the American Meteorological Society*, 96(3), 405-418. DOI: <https://doi.org/10.1175/BAMS-D-13-00175.1>
- Schemm-Gregory, M., Rojas-Briceño, A., Patarroyo, P., Jaramillo, C., 2012. First report of *Hadrosia Cooper*, 1983 in South America and its biostratigraphical and palaeobiogeographical implications. *Cretaceous Research*, 34, 257-267. DOI: <https://doi.org/10.1016/j.cretres.2011.11.005>
- Schlesinger, W.H., Phippen, J.S., Wallenstein, M.D., Hofmockel, K.S., Klepeis, D.M., Mahall, B.E., 2003. Community composition and photosynthesis by photoautotrophs under quartz pebbles, southern Mojave Desert. *Ecology*, 84(12), 3222-3231. DOI: <https://doi.org/10.1890/02-0549>
- Selbmann, L., De Hoog, G.S., Mazzaglia, A., Friedmann, E.I., Onofri, S., 2005. Fungi at the edge of life: cryptoendolithic black fungi from Antarctic desert. *Studies in Mycology*, 51(1), 1-32.
- Siebert, J., Hirsch, P., Hoffmann, B., Gliesche, C.G., Peissl, K., Jendrach, M., 1996. Cryptoendolithic microorganisms from Antarctic sandstone of Linnaeus Terrace (Asgard Range): diversity, properties and interactions. *Biodiversity & Conservation*, 5(11), 1337-1363. DOI: <https://doi.org/10.1007/BF00051982>
- Smith, M.C., Bowman, J.P., Scott, E.I., Line, M.A., 2000. Sublithic bacteria associated with Antarctic quartz stones. *Antarctic Science*, 12(2), 177-184. DOI: <https://doi.org/10.1017/S0954102000000237>
- Smith, M.R., Bandfield, J.L., 2012. Geology of quartz and hydrated silica-bearing deposits near Antoniadi Crater, Mars. *Journal of Geophysical Research: Planets*, 117(E6), 177-184. DOI: <https://doi.org/10.1029/2011JE004038>
- Stomeo, F., Valverde, A., Pointing, S.B., McKay, C.P., Warren-Rhodes, K.A., Tuffin, M.I., Seely, M., Cowan, D.A., 2013.

- Hypolithic and soil microbial community assembly along an aridity gradient in the Namib Desert. *Extremophiles*, 17(2), 329-337. DOI: [https://doi.org/10.1007/s00792-](https://doi.org/10.1007/s00792-007-00351-x)
- Tang, Y., Cheng, J.Z., Lian, B., 2016. Characterization of endolithic culturable microbial communities in carbonate rocks from a typical karst canyon in Guizhou (China). *Polish Journal of Microbiology*, 65(4), 413-423.
- Vargas, M., Patarroyo, P.C., 2014. Caracterización Geológica Del Travertino Localizado Al Noroccidente Del Municipio De Pesca, Boyacá. Universidad Nacional de Colombia. Unpublished Bachelor Thesis. National University of Colombia. Bogotá. Last accessed: 2 December 2022. Website: [https://www.researchgate.net/profile/Marianela-Vargas-3/publication/304252053\\_CHARACTERIZACION\\_GEOLOGICA\\_DEL\\_TRAVERTINO\\_LOCALIZADO\\_AL\\_NOROCCIDENTE\\_DEL\\_MUNICIPIO\\_DE\\_PESCA\\_BOYACA/links/576aaca808aefcf135bd3827/CARACTERIZACION-GEOLOGICA-DEL-TRAVERTINO-LOCALIZADO-AL-NOROCCIDENTE-DEL-MUNICIPIO-DE-PESCA-BOYACA.pdf](https://www.researchgate.net/profile/Marianela-Vargas-3/publication/304252053_CHARACTERIZACION_GEOLOGICA_DEL_TRAVERTINO_LOCALIZADO_AL_NOROCCIDENTE_DEL_MUNICIPIO_DE_PESCA_BOYACA/links/576aaca808aefcf135bd3827/CARACTERIZACION-GEOLOGICA-DEL-TRAVERTINO-LOCALIZADO-AL-NOROCCIDENTE-DEL-MUNICIPIO-DE-PESCA-BOYACA.pdf)
- Vítek, P., Ascaso, C., Artieda, O., Wierzchos, J., 2016. Raman imaging in geomicrobiology: endolithic phototrophic microorganisms in gypsum from the extreme sun irradiation area in the Atacama Desert. *Analytical and Bioanalytical Chemistry*, 408(15), 4083-4092. DOI: <https://doi.org/10.1007/s00216-016-9497-9>
- Warren-Rhodes, K.A., Rhodes, K.L., Pointing, S.B., Ewing, S.A., Lacap, D.C., Gomez-Silva, B., Amudson, R., Friedmann, E.I., McKay, C.P., 2006. Hypolithic cyanobacteria, dry limit of photosynthesis, and microbial ecology in the hyperarid Atacama Desert. *Microbial Ecology*, 52(3), 389-398. DOI: <https://doi.org/10.1007/s00248-006-9055-7>
- Warren-Rhodes, K.A., Kevin, L.R., Boyle, L.N., Pointing, S.B., Chen, Y., Liu, S., Zhu, P., McKay, C.P., 2007. Cyanobacterial ecology across environmental gradients and spatial scales in China's hot and cold deserts. *Federation of european microbiological societies (FEMS) Microbiology Ecology*, 61(3), 470-482. DOI: <https://doi.org/10.1111/j.1574-6941.2007.00351.x>
- Whitmeyer, S.J., Mogk, D.W., Pyle, E.J., 2009. An introduction to historical perspectives on and modern approaches to field geology education. *Geological Society of America Special Papers*, 461, vii-ix.
- Wierzchos, J., Ascaso, C., McKay, C.P., 2006. Endolithic cyanobacteria in halite rocks from the hyperarid core of the Atacama Desert. *Astrobiology*, 6(3), 415-422. DOI: <https://doi.org/10.1089/ast.2006.6.415>
- Wierzchos, J., Cámara, B., de Los Rios, A., Davila, A.F., Sánchez Almazo, I.M., Artieda, O., Wierzchos, K., Gómez-Silva, B., McKay C., Ascaso, C., 2011. Microbial colonization of Calcium sulfate crusts in the hyperarid core of the Atacama Desert: implications for the search for life on Mars. *Geobiology*, 9(1), 44-60. DOI: <https://doi.org/10.1111/j.1472-4669.2010.00254.x>
- Wierzchos, J., Ríos, A.D.L., Ascaso, C., 2012. Microorganisms in desert rocks: the edge of life on Earth. *International Microbiology* 15, 173-183. DOI: <http://dx.doi.org/10.2436/20.1501.01.170>
- Wierzchos, J., Casero, M.C., Artieda, O., Ascaso, C., 2018. Endolithic microbial habitats as refuges for life in polyextreme environment of the Atacama Desert. *Current Opinion in Microbiology*, 43, 124-131. DOI: <https://doi.org/10.1016/j.mib.2018.01.003>
- Williams, H., Turner, E., Gilbert, Ch., 1958. An introduction to the study of rocks in thin sections. *WH. Freeman and Company*, San Francisco, 406pp.
- Zapata, G., Correa, A., Rodríguez, G., Arango, M., 2016. Catálogo de unidades litoestratigráficas de Colombia. Granito de Pescadero, (Cordillera Oriental Departamento Santander). *Servicio Geológico Colombiano*, 55pp.

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