REMOTE LASER-INDUCED BREAKDOWN SPECTROSCOPY OF BACTERIAL GROWTHS IN CARBONATE ROCKS IN A MARTIAN-LIKE ATMOSPHERE

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ABSTRACT

Understanding the past habitable environments of Mars increases the requirement to recognize and examine modern analogues and to evaluate the mechanisms that may preserve biosignatures in them. The phenomenon that originates and preserves possible microbial biosignatures in mineral phases is of particular interest in astrobiology. On Earth, the precipitation of carbonate matrices can be mediated by bacteria. Besides microbialites and other sedimentary structures, carbonate formations can be observed in certain karstic caves. The present work is focused on the remote LIBS characterization of cyanobacteria, exploring the possibilities for identification and discrimination on carbonate substrates. For this purpose, the extremophile cyanobacterium *Chroococcidiopsis* sp. (collected from the Nerja Cave, Malaga, Spain) was analyzed under laboratory-simulated Martian conditions in terms of chemical composition and gas pressure. LIBS results related to acquired molecular emission features allowed bacterial differentiation from the colonized mineral substrate. In addition, the limits of detection were estimated with a laboratory-grown culture of the cyanobacterium *M. aureginosa*. Our results reveal LIBS's capability to detect biological traces under simulated Mars conditions. Additionally, the time-resolved analysis of the biological samples demonstrates the selection of optimal temporal conditions as a critical parameter for the preferential acquisition of molecular species in organics material.

KEYWORDS: LIBS, Cyanobacteria; Simulated Mars conditions; Biosignatures.

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1. INTRODUCTION

The concept of habitability on Mars can be approached from different working perspectives, from the in-situ analysis by means of the restricted instrumentation implemented on the rovers, the use of simulators that mimic Martian atmospheric conditions, to the study of Martian analogues on Earth (e.g., Macey *et al*., 2023).

A central aspect of planetary missions conducted on Mars and of particular interest in astrobiology is the search for possible traces left by microorganisms, assuming they have adapted and persisted over time, mainly in mineral phases of sedimentary rocks (e.g., Carrier *et al*., 2020; Westall et al., 2015). The hydrated saline deposits of carbonates and sulfates recently discovered on the Red Planet can potentially trap and preserve biological records formed by halophilic extremophiles on Earth (e.g., Barbieri and Stivaletta, 2011, Bosak *et al*., 2021). In this sense, the presence of these minerals could provide unique opportunities to study possible signatures of prebiotic chemical processes on Mars (e.g., Zhu and Dittrich, 2016; McMahon *et al*., 2018) and could constitute primary evidence for fossil Martian life. From an astrobiological perspective, a question that demands an answer is whether traces of life have ever existed on Mars and if so, how the possible microbial biosignatures can be preserved under extreme conditions (e.g. Warren-Rhodes *et al*., 2019). Necessarily, the limits of habitability in the planet have to be evaluated from such extremophile organisms, since they are the most likely to persist over time, and in specific environments with enough shelter conditions. Recently, a report from Astrogeology Science Center has listed "The Mars Global Cave Candidate Catalog" (https://www.usgs.gov/news/caves-mars) with more than a thousand cave-entrance candidates on Mars, including karst terrains, emphasizing the importance of caves in the future of Mars exploration since they can provide shelter from the entire range of harsh surface conditions, maintaining stable microclimates inside. On Earth, many of the earliest chemical, textural and morphological evidence of life are found in carbonates and carbonate-hosted phases. Thus, carbonate bedrock in karstic formations constitutes a specific environment frequently colonized by microorganisms. Among the most frequent phototrophic microorganisms colonizing rocks and sediments in caves are cyanobacteria. The bio-receptivity of these substrates allows the formation of biofilms as a complex aggregation of these cyanobacteria colonies growing on solid substrates and forming an extracellular coating of polymeric substances, which enables them to adapt to and survive in extreme environment conditions (e.g., Warscheid and Braams, 2000; Di Pippo *et al*., 2009). For specific substrates, some species present the capacity to penetrate a few millimeters below the surface, thus colonizing the rocks in an endolithic way (e.g., Walker and Pace, 2007).

In the last few years, laser-induced breakdown spectroscopy (LIBS) has emerged as a powerful tool for space exploration (e.g., Cremers, 2014; Fortes and Laserna 2010; Sallé et al., 2005; Fortes et al., 2013 Gasda et al., 2015). First ChemCam (the remote sensing instrument installed on NASA Curiosity rover) and then SuperCam (installed on the Mars 2020 Perseverance rover) include both LIBS systems among the analytical techniques onboard. In this latter case, the LIBS device uses a focused laser beam to interrogate rocks and soils remotely at distances up to 10 m from the rover (e.g., Wiens *et al*., 2021; Maurice *et al*, 2021). In the last decades, several reports concerning the identification of microbiological samples by LIBS have been published (e.g., Samuels *et al*., 2003; Baudelet *et al*., 2006; Saari *et al*., 2015; Singh, VK *et al*., 2018). The use of atomic and molecular signals as markers of the macro and micronutrients has been the most demanded strategy in this particular application (e.g., Morel *et al*., 2003; Zivkovic *et al*., 2017). In addition, the use of chemometric tools has improved LIBS potential and addressed the discrimination and classification of bacterial strains (e.g., Blanchette *et al*., 2022; Rehse, 2019). Other studies have evaluated changes in the emission spectra caused by stress during cell growth, exposure to pollutants or UV radiation (e.g., Mohaidat *et al*., 2011, 2012). It should be noted that much more specific aspects of LIBS analysis of these characteristics have also been investigated, such as the contribution of the background gas or the influence of the substrate (e.g., Multari *et al*., 2012; Gottfried, 2011). Previous studies on the LIBS emissions patterns of organic compounds (e.g., Dequaire *et al*., 2017; Cabalín *et al*., 2020; Delgado *et al*., 2020, 2021) provide the starting point for the new experiments discussed here.

The present paper aims to study different cyanobacteria strains by LIBS in a Martian-like atmosphere, exploring the possibilities for their identification and discrimination on carbonate substrates. For this purpose, *Chroococcidiopsis* sp. and *Microcystis aureginosa* cyanobacteria were inspected under simulated Martian conditions in the laboratory. Results allowed the bacteria identification and their differentiation from the colonized substrate. In addition, *Chroococcidiopsis* sp. was employed to optimize LIBS analysis parameters for an accurate emission features interpretation. A time-resolved analysis of LIBS emissions demonstrated the relevance of a careful evaluation of plasma persistence to accurately observe molecular emissions derived from the biological samples. In order to evaluate LIBS's detection power for this particular application, the limit of detection (LODs) of *Microcystis aureginosa* was also estimated.

2. EXPERIMENTAL SET-UP

2.1. Instrumentation

The remote LIBS instrument used for the analysis of bacterial growths has been described in a recent paper (e.g., Delgado *et al*., 2021), so only a brief description is given here. The fundamental wavelength of a Q-switched Nd:YAG laser (Quantel Brilliant B; 1064 nm; 20 Hz; 5 ns pulse width) was directed via a reflecting mirror and a dichroic mirror (with high reflectivity for 1064 nm at 45º and transparent to the emission of the plasma), the latter being placed just before the focusing lens. The laser beam was focused onto the sample surface by a BK7 planoconvex lens, 800 mm focal length. The output energy was set at 15-30 mJ pulse^{-1} and the crater diameter was measured in 350 µm. In order to simulate Mars conditions, the sample was placed inside a stainless-steel vacuum chamber (≈ 10 L volume) at a pressure of 7 mbar. Plasma emission was observed though a 4.5" in diameter fused silica window and was coaxially collected by a modified Maksutov-Cassegrain telescope (F1900 mm, f 1/15) located at 200 cm from the sample. A collector lens, placed in front of the telescope, focused the plasma light into the entrance slit (10 µm) of a 303 mm focal-length Czerny-Turner spectrograph (Andor, model Shamrock SR-303i-A) with an optical fiber (600 µm diameter; N.A: 0.22). The detector, coupled with an intensified CCD camera (Andor, i-star CCD, model DH740-25F-03) and equipped with an 1800 lines/mm holography grating (blaze at 300 nm), allows a spectral resolution of ~0.07 nm, covering a total spectral range of 46 nm.

The number of laser shots delivered for LIBS measurements was established depending on the nature of the studies and samples in each case. Sampling position was refreshed continuously using a rotating stage (at angular speed of 9.5×10^{-2} rad s^{-1}).

2.2. Samples

In this work, different species of cyanobacteria, *M. aeruginosa* (cultured sample) and *Chroococcidiopsis* sp. (natural sample) were selected for LIBS analysis.

M. aureginosa strain was provided by the Department of Botany and Plant Physiology of the University of Malaga. It is characterized by small cells, generally about 4-5 µm in size, lacking individual sheaths. BG-11 (C3061, Sigma-Aldrich) was selected as a culture medium, diluted at 50% in deionized tissue culture grade water, and the pH was adjusted to 7.2 with NaOH. Then, the culture was incubated in a climate-controlled chamber at 20ºC for 46 days, under a continuous photon flux density of 60 µmol·m^{-2}·s^{-1} over the waveband 400–700 nm provided by cool-white fluorescent lamps. A volume of 40 mL of each sample was centrifuged (5000g's of force, 10 min), and then the supernatant was removed. Once the cells were extracted, the cyanobacteria population was estimated to be $1.9\cdot10^{6}$ cells mL^{-1}. This value was estimated by measuring the absorbance of the cell culture at 750 nm. For LIBS analysis, aliquots of 2 µL were deposited on a magnesite foil and then dehydrated in an oven at 30ºC for 1 hour. This step was repeated 3 times to ensure the thickness and concentration of the biofilm (e.g., Melero-Jiménez *et al*., 2020).

The adaptive potential of this cyanobacteria under different rates of environmental change -mainly regarding its salinity resistance- has been deeply studied (e.g., Melero-Jiménez *et al*., 2020)**.** The interest in *Chroococcidiopsis* sp. lies in its exceptional resilience to desiccation, UV-irradiation, salt toxicity and high temperature, with minimum nutrient requirements. Moreover, they can become desiccated and recover under favorable conditions, thus resisting fluctuations in humidity while surviving in the most extreme habitats for long periods (e.g., Merino *et al*., 2019, Cockell *et al*., 2005, Caiola *et al.*, 1993).

Under suitable light conditions, photosynthetic microorganisms are capable of growing on the walls and speleothems of a cave. In the Cave of Nerja (Malaga), *Chroococcidiopsis* sp develops preferentially on humid and porous substrates or into the cavities and cracks, taking refuge in times of extreme dryness. Their thick mucilaginous sheath expands or contracts depending on water availability, joining substrate particles contributing to its breakdown (e.g., Del Rosal *et al*., 2014). Its presence disrupts the natural formation of speleothems and could damage their surface, where phototrophic biofilms are mixed with the substrate.

The colonization of the substrate by the cyanobacteria, which presents spherical geometry, is shown in Figure 1. SEM micrographs were acquired following the analysis protocol for biological samples. Sample processing involved fixation in glutaraldehyde (0.2%) and paraformaldehyde (2%) in 0.1 M cacodylate buffer. To improve the conductivity of the samples, they were postfixed in a solution of osmium tetroxide (1%). Dehydration was performed by ethanol gradient and drying by critical point. Finally, the samples were coated with carbon and observed by the microscope. The observation of the samples was carried out using a Scanning Electron Microscope with back-scattered electrons (BSE) and energy dispersive X-rays (EDX) (Quanta 200, FEI þ EDAX). X-ray spectra were acquired at an accelerating voltage of 20 kV, with a beam current of 10 Torr and an acquisition time of 30 s. This microorganism is chasmoendolytic, i.e., it appears in aggregates and also tends to occupy cavities and fractures in the substrate (e.g., Del Rosal *et al*., 2016; Del Rosal *et al*., 2020). EDX analysis revealed the presence of C and O as major components, whereas Ca and Mg, corresponding to the substrate composition, were detected in minor concentrations.

For laboratory analysis, attending to criteria of sampling representativeness, morphology, and surface alteration degree, biofilm samples were selected and gently scratched with a sterile scalpel from the surfaces at different locations in the dolomitic system of the Cave of Nerja, and then transported to the laboratory for characterization by LIBS analysis. Previously, a microscopic inspection analysis was carried out to verify the conservation state of *Chroococcidiopsis* sp. Its morphotype was identified using an Axioplan optical microscope (Carl Zeiss, Oberkochen, Germany) and images were captured using an AxioCam MRc5 digital camera.

Figure 2A reveals the sampling location inside the Nerja Cave. In this area, the bedrock was exposed to natural light, and the biofilm was directly scratched from the walls, thus presenting the rests of the mineral substrate of dolomitic marble as presented in Figure 2B. In Figure 2C, the well-preserved conservation state of microorganisms is corroborated from optical microscope image.

3. RESULTS

3.1. Characterization of cyanobacteria by LIBS

3.1.1 Emitting species of cyanobacteria (M. aeruginosa and Chroococcidiopsis **sp***)*

The chemical characterization of *M. aeruginosa* and *Chroococcidiopsis* sp was performed by LIBS. Selected spectral ranges of both cyanobacteria are shown in Figure S1. Elements identified in both strains are summarized in Table 1. As indicated in the table, for *M. aeruginosa* some emission lines (Ca, Cu, Fe, K, Mg, and Na) can be also associated to the culture medium (BG11). On the other hand, in the *Chroococcidiopsis* sp. biofilm from Nerja Cave, the presence of Ca, Mg, Fe and Sr could be attributed to the dolomitic marble of the substrate. Additionally, molecular emissions of CN and C_2 were detected during the LIBS analysis, in addition to the listed atomic and ionic emissions. The main formation routes of these molecular species have been extensively described in previous works (e.g., Cabalín *et al*., 2020).

On top of that, LIBS spectra of carbonate-based substrate and *Chroococcidiopsis* sp. in Martian atmosphere (7 mb) are depicted in Figure S2 for comparative purposes. The emission lines of C(I) 247.8 nm, H(I) 656.6 nm, CN 388.3 nm ($\Delta\nu$=0) and C_2 473.7 nm ($\Delta\nu$=1) are labelled in the spectra. As demonstrated, this biofilm presents a remarkable increase of emission intensity of C(I) when related to the substrate signal, mainly associated to the native carbon of the microorganism structure. A prominent CN emission band is observed for cyanobacteria emission spectrum compared to mineral substrate, result of the direct release of the dimer or ulterior recombination with atomic N in the plasma plume. A weak emission corresponding to the C_2 Swan system was also detected in the Mars atmosphere, an observation compatible with the presence of organic carbon recombined in the plasma or directly released from the rupture of aromatic molecules present in the cells. The presence of H emission was correlated to the organic origin of the biofilm (e.g., Rapin *et al*., 2016), as confirmed in Figure S3, which shows the compared LIBS spectral window of H(I) at 486.1 nm emission line for the *Chroococcidiopsis* sp. bacteria and the mineral substrate.

3.1.2 Effect of timing conditions on the detection of cyanobacteria

Considering the highly dynamic and complex nature of plasma, a correct optimization of the detection conditions could allow to discriminate the origin of molecular emissions. In this context, a time-resolved LIBS experiment was performed to elucidate whether the signal emission derives from the organic material or from the substrate. Figure 3 presents the LIBS signal obtained at delay times ranging from 0 ns to 5000 ns with 200 ns intervals. The kinetics of C (I), CN, C_2, and H was monitored during their lifetime so that the early stages coming from molecular fragmentation could be differentiated from the late stages after recombination processes in the laser-induced plasma. As seen in left panel of Figure 3, no molecular emissions are observed from the inorganic substrate. In this case, molecular emission is mainly due to the late dimerization of carbon, whereas a small amount of CN is observed due to the reaction of inorganic carbon with the limited amount of nitrogen present in the atmosphere (3%). Atomic signals from hydrogen and oxygen (not shown) decay faster than that of carbon, which persists beyond 5 μs. The different behavior presented by the atomic species (C, H and O) may be due to their electron energy level, according to the NIST database (e.g., Cousin *et al*., 2011). For instance, for carbon at 247.9 nm the energy of the lower level involved in this transition is considerably low (21,648 cm^{-1}), and therefore easier to return to the ground state.

In *Chroococcidiopsis* sp (right panel), the maximum intensity for the molecular species was achieved at early stages. However, normalized intensities demonstrate the presence of late strong contributions from CN and C_2 which result from both C and N of bacterial origin. Thus, maximum CN emission is reached at 0.6 μs, although a residual contribution still remains after 5 μs. The bottom panels of Figure 3 represent the main emissive contribution at each time in the inorganic substrate and the biofilm. In the graph, the signal intensity of each emission line was normalized to the sum of the net intensities of each of the species present in the spectrum, C, CN, C_2 and H. As seen in the substrate, carbon emission is dominant during the entire plasma lifetime; meanwhile the presence of H and CN was only noticeable in the first temporal stages. In contrast, the contribution of molecular species to the entire laser-induced plasma is more than evident in the case of biofilm.

3.1.3 M. aeruginosa deposited on magnesite

To demonstrate the capability of LIBS for the detection of cyanobacteria in carbon-containing mineral phases, an experiment was designed to establish whether the C_2 and CN signals came from the substrate (magnesite), the culture medium (Bg11), or the bacteria. Figure 4 depicts the comparative LIBS spectra corresponding to the $\Delta\nu=1$ transition of C_2 Swan System and the $\Delta\nu=0$ transition of CN Violet System for i) magnesite, ii) magnesite + Bg11 and iii) magnesite + Bg11 + *M. aeruginosa*. LIBS analysis was performed at 7 mb in CO_2 atmosphere. As shown, although the deposit of Bg11 presents a detectable C_2 signal, the complete sequence of the Swan System is only discernible in the *M. aeruginosa* spectrum. No molecular emission was observed in magnesite. These results suggest that the molecular signals derive from the release of nitrogenous functional groups and hydrocarbon fragments from the biofilm structure (Delgado *et al.*, 2022).

3.1.4 Quantification of M. aeruginosa

In order to simulate the presence of cyanobacteria deposits or residues in rocks, 3 small craters were drilled by delivering 200 laser shots in specific positions on a magnesite slab. Crater produced were about 350 μm in diameter and 1 mm in depth. Then, 2 μL aliquots of *M. aeruginosa* (see details of the protocol in the experimental section) were deposited inside the craters and analyzed by LIBS. After one laser pulse, the surface of magnesite foil was not altered, but the deposited biofilm was removed. Net intensities in a raster along these deposits are depicted in Figure 5. As seen, the CN emission considerably increased in the positions where the M. aeruginosa was deposited (indicated with arrows). The atomic emission line Mg (I) 382.9 nm was also included in the graph for comparative purposes. In contrast, the Mg signal intensity is more significant in the pure magnesite locations, although this element is marginally found throughout all sampling points, as it is one of the macronutrients present in higher proportion in cyanobacteria medium.

4. DISCUSSION

4.1 Implications for organic biosignatures detection

LIBS spectra from cyanobacteria revealed the presence of the crucial elements for the life such as carbon, oxygen, nitrogen and hydrogen, which are precursors of the organic building blocks of cells. In addition, some macronutrients –concentration in dry matter >0.1%– (K, Ca, Mg, Si), micronutrients –concentration in dry mater <0.1%– (Fe, Na) (e.g., Epstein, 1972) and metalloids such as Al, Ba and Sr, were also identified. In particular, the presence of barium and strontium in cyanobacteria is of crucial interest since it suggests its involvement in biomineralization processes (e.g., Couradeau *et al*., 2012). It is remarkable the presence of molecular emissions related to organic materials, i.e., CN and C_2. However, the dolomitic marbles of the Nerja Cave's wall are composed almost exclusively of dolomite, although there are also samples containing calcite (in the form of isolated grains or thin veins). In this sense, a prime challenge was to ascertain whether other possible sources of CN and C_2 are contributing to the observed signals. For instance, CN may be formed by a reaction of organic carbon and residual atmospheric nitrogen (~3%), whereas C_2 could derive from carbonate decomposition or the recombination of dissociated atmospheric carbon. Research using isotopologues as model compounds demonstrated that those contributions are possible, although they produce only marginal signals (e.g., Delgado *et al*., 2021).

The laser-induced plasma under Martian conditions presents a complex temporal evolution that influences the acquired LIBS spectrum. Regarding this effect, optimization of temporal conditions could serve to discern whether the signal emission derives from the organic material or from the substrate. The selection of optimal temporal conditions is crucial for the preferential acquisition of molecular species in organics, thus removing signals coming from the inorganic substrate (e.g., Delgado, *et al*., 2014, 2016). According to Figure 3, 600 ns delay time and 3 μs integration time were chosen as timing values for further studies of biological samples. With the purpose of verifying the observations discussed above, the spectral response obtained from the mineral substrate and the cyanobacteria were compared. Net intensity ratios for the main atomic (C, H) and molecular (CN, C_2) emission signals in the time window covering 600 to 3000 ns were calculated. Considering that the intensity of the carbon line at 247.9 nm is practically constant for both mineral substrate and cyanobacteria in CO_2-rich atmosphere and consequently not very sensitive to the organic carbon concentration of the sample (e.g., Anderson *et al*., 2017), this element could be used as normalization parameter of the LIBS signal studied (e.g., Rapin *et al*., 2016). The results demonstrated that the most significant changes in the ratios obtained are for the CN/C and C_2/C. In both correlations, these values are practically negligible (0.13 and 0.02, respectively) in the substrate in comparison with those obtained in the biological sample (5.71 and 0.516). Therefore, the balance of these emitting species could be an indicator of interest for the discrimination of organic matter present in mineral samples.

4.2. Allusions to quantitative detection of cyanobacteria (LOD of M. Aeruginosa)

Once demonstrated that LIBS is able to detect biological material on carbonate-based substrates, the question of quantifying these particular cyanobacteria that give rise to CN and C_2 signals raised. It is known that the extreme conditions on Mars reduce the probability of detecting cyanobacteria on the surface of the red planet. A priori, it seems that one realistic possibility might be in cavities and cracks in the bedrock, especially in carbonate units. To assess the detection power of LIBS for this particular application, the limit of detection (LOD) for the cultured bacteria (*M. Aeruginosa)* was calculated. The LOD was estimated for the $\Delta\nu=0$ transition of CN Violet System at 388.3 nm (extracted from Fig. 5), using the following expression (e.g., Klostermeier *et al*., 2005):

$$LOD = \frac{K \ x \ C \ x \ RSD_{Bck}}{\frac{S_{net}}{S_{Bck}}} \ x100 \qquad (1)$$

where K takes the value of 3 for a confidence interval of 99.5%, the RSD_{Bck} is the percent relative standard deviation of the background, S_{net} is the maximum net intensity of CN molecular emission at 388.3 nm, S_{Bck} is the background signal measured in one side of the spectral line, and C (expressed in mg) is the mass of cyanobacteria exposed to the laser radiation. In our case, C was estimated according to the proportion of the biofilm affected by the incident radiation (which depends on the spot size, 350 µm in diameter) and considering that the number of cyanobacteria deposited in each inclusion was ~80×10^6 cells (estimated after the centrifugation of 40 mL of bacterial suspension, following the protocol described in the experimental section) (e.g., Blanchette *et al*., 2022). Table 2 summarizes the most relevant information for the three inclusions of cyanobacteria. According to these results, the number of cells necessary to detect LIBS signal varied between 2.1×10^6 and 4.5×10^6. These values are in good agreement with those reported by

Paulick *et al*., 2019, who studied E. coli bacteria deposited on filters and estimated a limit of detection, using strong atomic lines of P, Mg and Ca, to be of approximately 0.1 $x10^6$ cells (expressed as CFU, colony forming unit), per laser shot. Taking into account the characteristics of the studied molecular emissions, which exhibit significantly lower intensities, our results seem to be in reasonable agreement. It should be noted that the tracking of molecular species allows an analysis that is less dependent on the nature of the mineral substrate besides less sensitive to the presence of common elements among the sample and the support material.

Further, by considering that in a fresh culture, the equivalence of 10^6 cells is approximately 1 mg in mass (e.g., Melero-Jiménez *et al*., 2020) it is possible to calculate the limit of detection in mass units as indicated in the table. Finally, estimated mass (in mg) of dry carbon content in M. aeruginosa and considering that the carbon represents 42% of the dry extract and water, 80% of bacterial cells, LODs ranging from 17.8 µg to 31.5 µg were calculated. These results evidence the capability of LIBS for the detection of trace biological material in simulated Mars conditions.

5. CONCLUSIONS

Investigation of terrestrial analogs may provide fundamental insights on searching for biosignatures on Mars and for the development of planetary exploration strategies. In this work the ability of LIBS for the remote identification of bacterial communities has been investigated. Acquisition of LIBS spectra from different cyanobacteria with extremophile features in their colonization habitat in Martian atmospheric conditions together with identification of the main organic emission features was possible in all tested cases. Carbonate matrices (despite having a contribution of inorganic carbon) did not cause molecular emission, a result that helps in the use of LIBS for detection of organics. In this regard, the time-resolved study demonstrates that the selection of optimal temporal conditions is crucial for the preferential acquisition of molecular species in organics, thus removing signals coming from the inorganic substrate.

Results presented here are quite promising in an astrobiology context since they faced the challenging task of identifying cyanobacteria by LIBS in a real scenario such as a karstic formation, where carbonate mineralization by microorganisms is feasible. Thus, results under Martian conditions suggest that emitting species characteristic of organic matter could be used as a potential indicator of the presence of a preserved biological matter.

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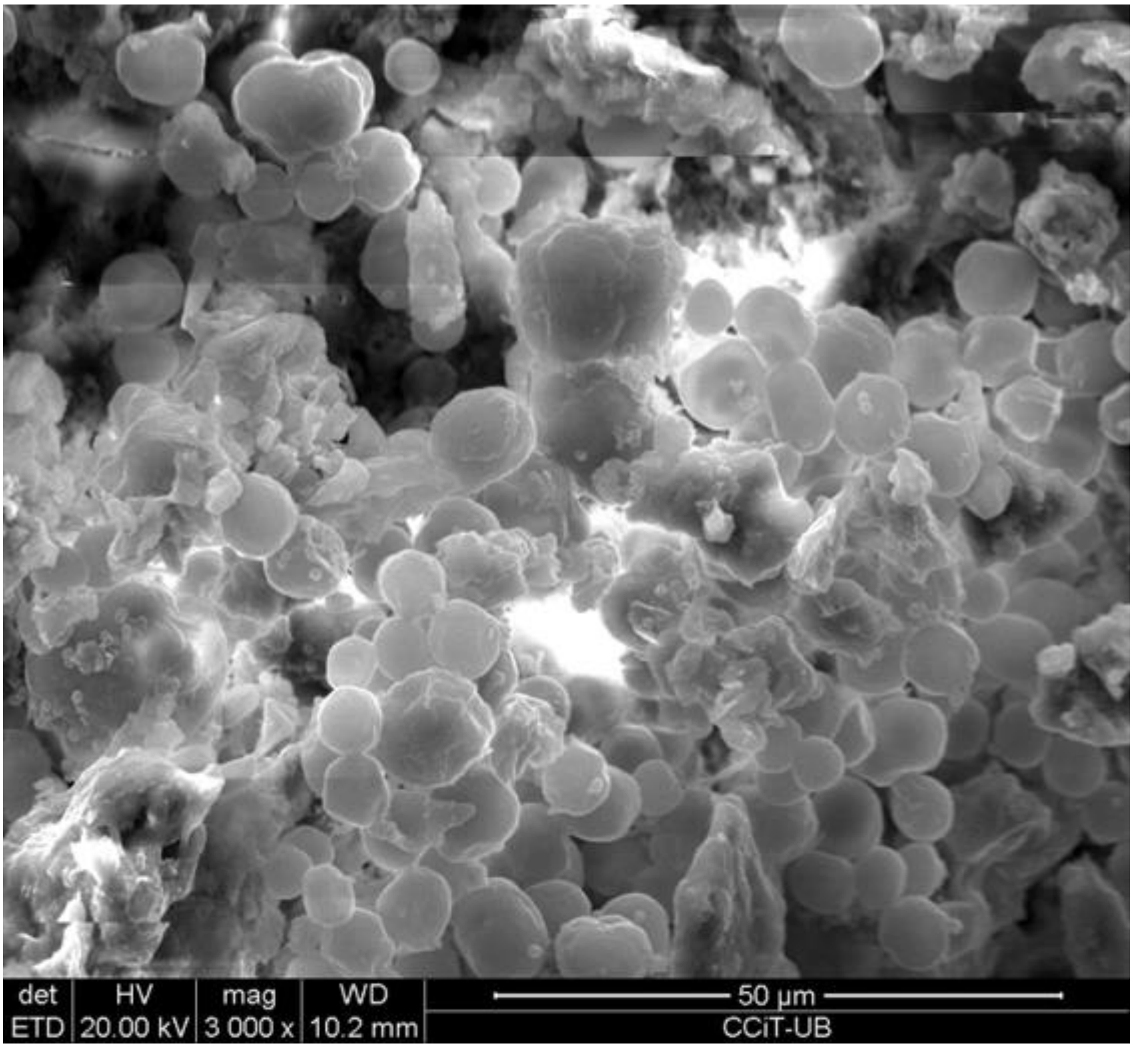

Figure 1. SEM microphotographs showing the colonization of the mineral substrate by *Chroococcidiopsis* sp. The protocol carried out to obtain this image is described in the experimental section.

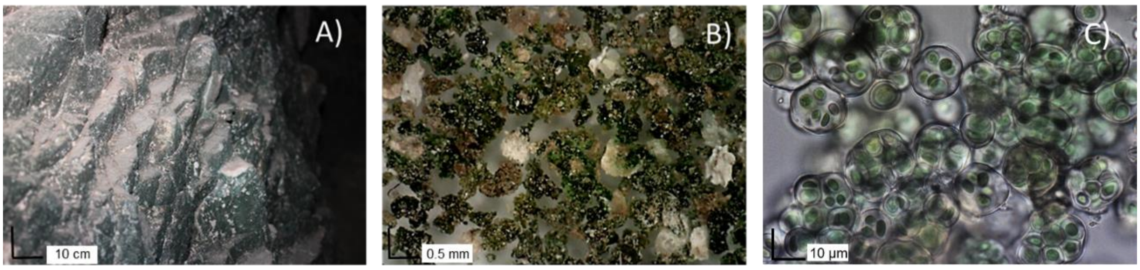

Figure 2. A) *Chroococcidiopsis* sp. biofilm from Nerja Cave. Detail of the vertical dolomite wall from which the cyanobacteria sample was collected; B) imagen acquired by a Dino lite digital miscroscope with an 80X magnification of collected biofilm sample with presence of substrate grains/fragments; C) imagen obtained with the optical microscope described in the experimental section of *Chroococcidiopsis* sp strain.

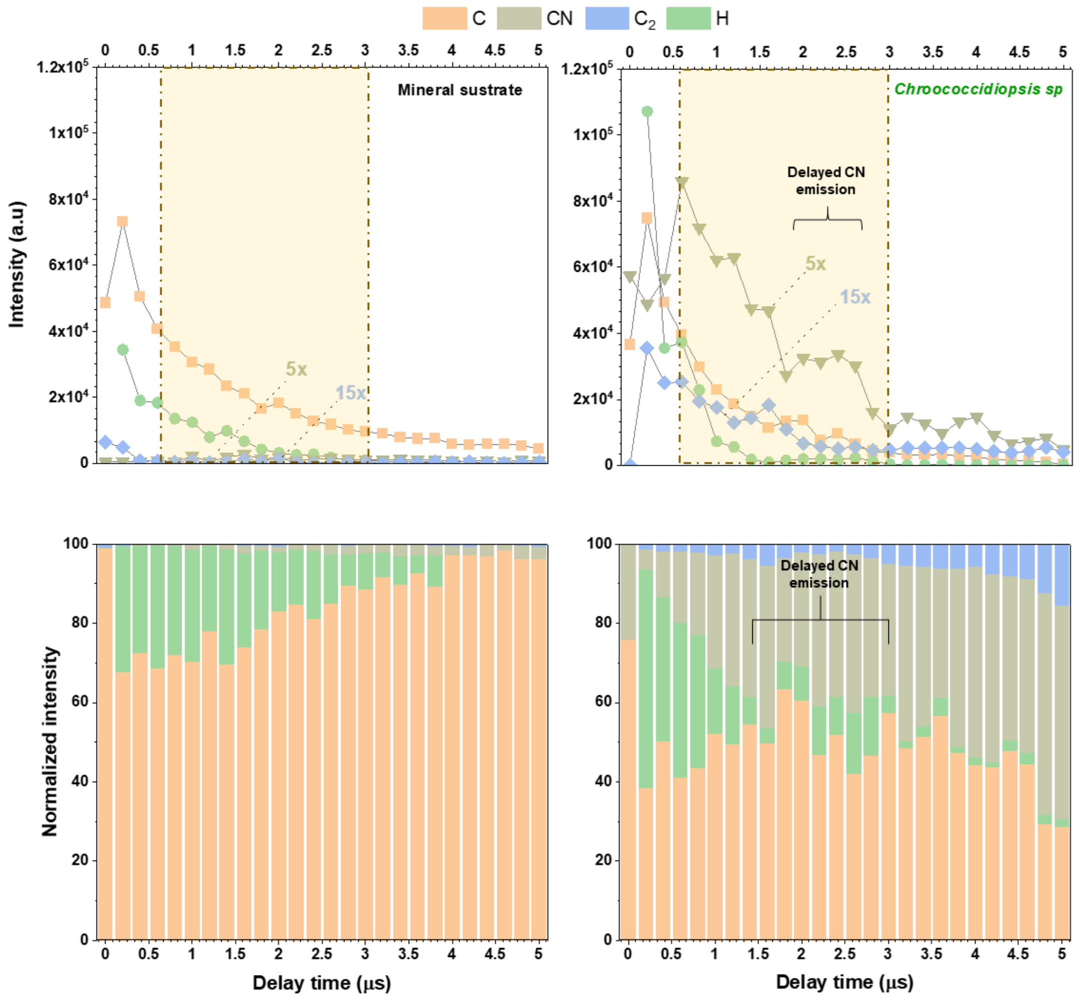

Figure 3. Evolution of net intensities from each individual emission species during plasma lifetime for both mineral substrate (left) and cyanobacteria *Chroococcidiopsis* sp. (right) under simulated Martian conditions (7mb pressure, Mars gas mixture). LIBS signal was obtained with

an integration time of 200 ns and a laser pulse energy of 30 mJ and accumulation of ten laser shots. For a better visualization, CN and C_2 signals were multiplied by a factor of 5 and 15, respectively. The graphs below depict normalized intensity of the sum of C, CN, C_2 and H from the spectrum up to a delay time of 5 μs.

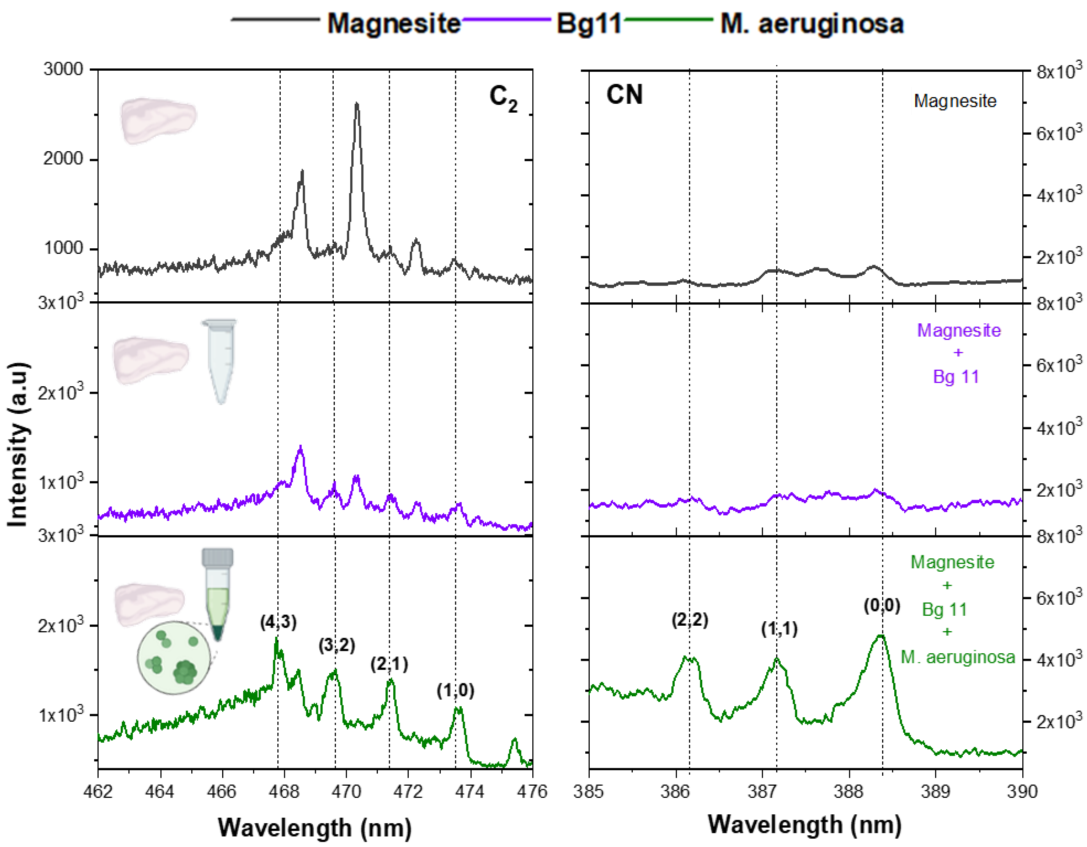

Figure 4. Comparative LIBS spectra of C_2 and CN molecular emission systems in deposits of Bg11 and *M. aeruginosa* on magnesite. Chemical analyses were performed under a CO_2 atmosphere using one laser shot. Integration and delay time were fixed at 5 μs and 750 ns respectively. The most intense electronic transitions of such molecular sequences were labelled in the spectra.

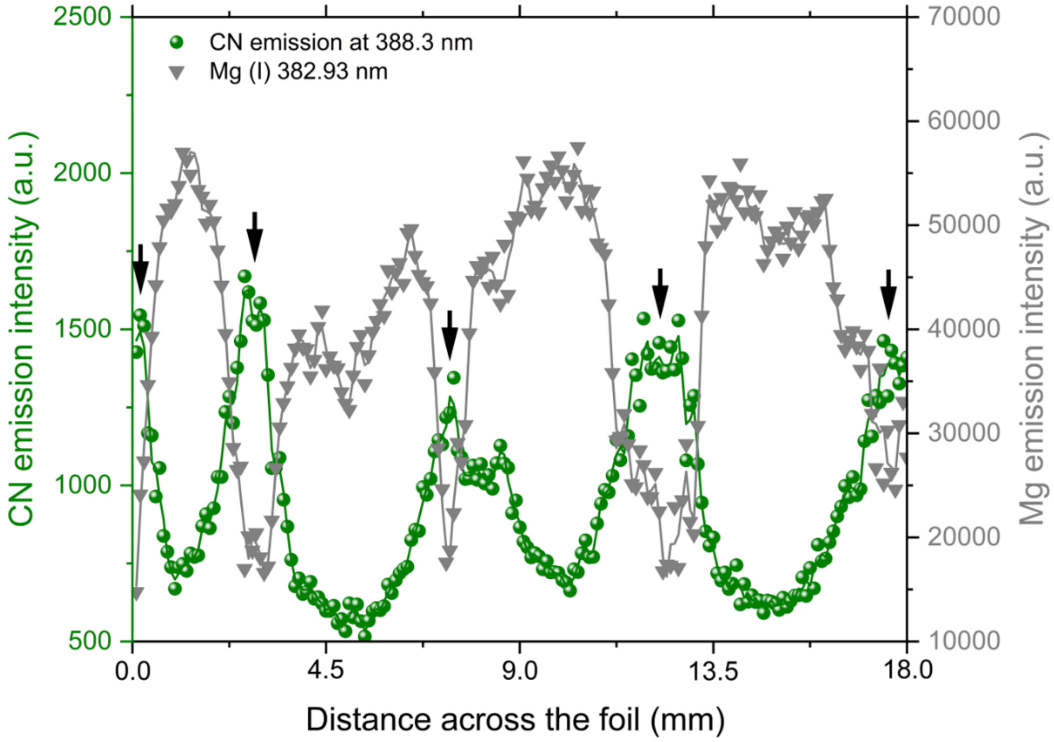

Figure 5. Evolution of CN (Δν=0) and Mg I emission intensities acquired along the sampling path. Experiment was carried out in simulated Martian conditions (7mb pressure, Mars gas mixture) using one laser shot per position. Integration and delay time were fixed at 5 μs and 750 ns respectively. Positions with bacteria deposits are indicated with arrows.

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