LASER-INDUCED BREAKDOWN SPECTROSCOPY OF BACTERIAL GROWTHS IN CARBONATE ROCKS UNDER SIMULATED MARTIAN CONDITIONS. T. Delgado, L.M. Cabalín, F.J. Fortes, L. García-Gómez and J.J. Laserna, UMALASERLAB, Departamento de Química Analítica, Facultad de Ciencias, Universidad de Málaga, Campus de Teatinos s/n, 29071 Málaga, España, e-mail address: laserna@uma.es

Overview: If there was life in the past on the Red Planet, specific biosignatures might still be present only if preservation conditions have been favorable for them [1]. The recognition of the habitable past on Mars increases the exigency to identify and characterize modern analogs of these surroundings and to evaluate the mechanisms that can conserve biosignatures in them. Of particular interest are the processes that originate and safeguard possible microbial-type biosignatures in mineral phases [2]. The research on microbial survival in simulated extraterrestrial environments is of great relevance for future missions to Mars. Thus, satellite images have confirmed the existence of calcium and magnesium carbonates on the Jezero's crater rim.

In Earth, carbonated matrices are capable of precipitating assisted by bacteria. Apart from stromatolites and other sedimentary formations, this phenomenon can be observed in certain karstic caves [3]. In this sense, the present study aimed to characterize cyanobacteria by LIBS, exploring the possibilities for its identification and discrimination on carbonate substrates. For this purpose, *Chroococcidiopsis* bacterial growths were in-situ analyzed in the Cave of Nerja (Malaga, Spain) and then inspected under simulated Martian conditions in laboratory. This specific bacterial strain represents a useful model to study the biological responses to extreme desiccation conditions on Earth and expand our knowledge about terrestrial organisms' survival potential in extraterrestrial environments [4].

Experimental: For the present study, *Chroococcidiopsis* cyanobacteria supported on dolomite matrices were analyzed by a man-portable LIBS instrument designed and constructed in the Laser Laboratory at the University of Malaga (UMALASERLAB). The overall system consists of a hand-held probe, a main unit, and the laser power supply. The spectrometer and the computer components are enclosed in the main unit, which consists of a specially adapted backpack.

The hand-held probe encloses a compact laser head and the focusing and collection optics. The plasma was generated using a Q-switched Nd:YAG laser, operating at its fundamental wavelength, and the laser pulse energy was 50 mJ. Then, the plasma emission was directly collected by a fiber-optic cable and guided to the entrance slit of a compact spectrometer located at the main unit of the instrument. A LabView script was designed to allow spectra acquisition and composition depth profile visualization of any particular spectral line in real time.

Samples: Growths of *Chroococcidiopsis* cyanobacteria were *in situ* analyzed in the interior of the Cave of Nerja (Malaga, Spain). The interest in this oxyphotobacteria lies in its ability to survive in extreme atmospheric conditions [5].

For laboratory analysis, attending to criteria of sampling representativeness, morphology, and surface alteration degree, samples were selected, scratched from the wall, and then transported to the laboratory for its subsequent LIBS analysis from different locations in the Cave of Nerja.

Results and discussion: Cyanobacteria are microorganisms mainly composed of C, H, O, and other macronutrients such as N, K, Ca, Mg, P, S, and Si. Figure 1A illustrates one of the sampling points of Chroococcidiopsis cyanobacteria inside the Nerja Cave. This area receives natural light from a hole connecting the exterior with the top of one of the cave galleries. The attached inset shows a detail of the rock vertical wall where the cyanobacteria colonies were found.

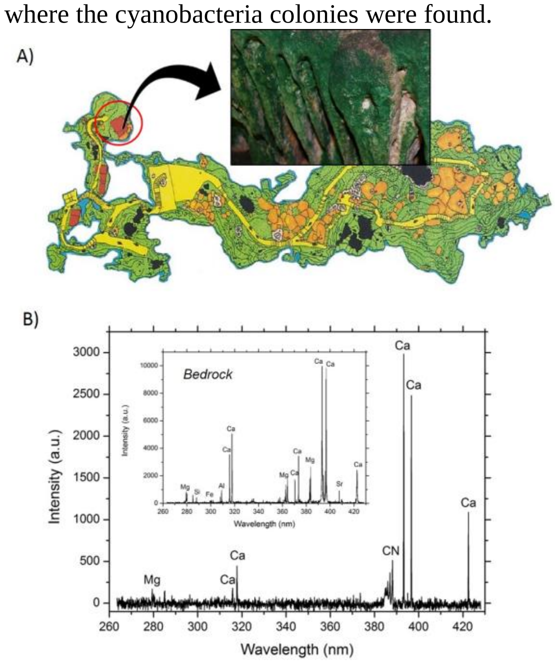

Figure 1. A) Map illustrating one of the sampling points inside Cave of Nerja. B) LIBS spectra of Chroococcidiopsis cyanobacteria during field analysis.

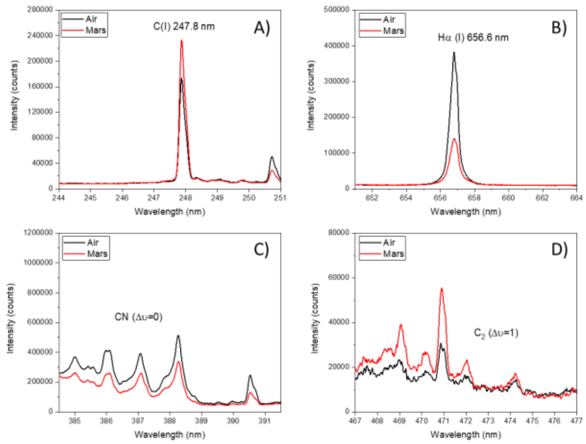

Figure 2. LIBS spectra of the Chroococcidiopsis cyanobacteria collected inside Nerja Cave acquired at reduced pressure (7mb) in both air and Martian atmospheres showing the emission lines A) C (I) 247.8 nm; B) H_α (I) 656.6 nm, and the emission bands C) CN (Δυ=0); D) C_2 (Δυ=1).

Figure 1B shows LIBS spectra of Chroococcidiopsis cyanobacteria during field analysis, in the 265-430 nm spectral range. The CN violet system centered at 388.3 nm is also well identifiable in the spectra which could be related with the presence of organic material in the inspected sample. On the other hand, the bedrock is characterized by emission lines of Ca, and Mg and other minor elements including Al, Fe, Si and Sr.

Once detected the presence of molecular emission in our spectra, a set of samples from the Cave of Nerja was transported to the UMALASERLAB for an exhaustive LIBS characterization. In laboratory, samples were analyzed inside a vacuum chamber under Martian conditions. Figure 2 shows comparative LIBS spectra of this cyanobacteria acquired in air and Martian atmospheres, for several spectral ranges corresponding to most representative emission features. As shown Figure 2A, the emission intensity of C (I) line at 247 nm was higher under Martian conditions. This increased intensity can be directly attributed to the contribution of the carbon from breakdown of atmospheric CO_2. The observation of H (I) at 656.3 nm (Fig. 2B) in absence of hydrogen confirms the detection of this species coming from cyanobacteria. Likewise, especially relevant is the presence of the CN emission in the Martian atmosphere, closely related to the recombination of organic carbon with atmospheric nitrogen (Fig. 2C). On the other hand, a weak emission corresponding to the C_2 Swan system was detected (Fig. 2D), an observation compatible with the presence of organic matter.

A prime challenge to this study 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 reaction of organic carbon and atmospheric nitrogen, whereas C_2 could derive from decomposition of carbonates. A recent study on molecular signatures in LIBS spectra using isotopologues as model compounds [6] has demonstrated that these contributions are possible, although they produce only marginal signals. Close to the limit of detection of the organic compound these signals may be detrimental [6].

In the case of cyanobacteries, the presence of films or other multicellular communities may represent concentrations high enough for the detection of molecular signatures in the associated LIBS spectra as demonstrated here.

Conclusions: Carbonate mineralization by cyanobacteria is of major significance in geology. In this sense, the results presented here are quite promising and faced the challenging task of identifying cyanobacteria by LIBS from a real scenario such as a karstic formation. Thus, results under Martian conditions suggest that some found emitting species, characteristic of organic matter, could be used as a potential indicator of the presence of a biological residue.

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