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Raman microspectroscopy as a tool for identifying biosignatures in speleothem microbialites

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Introduction

Raman spectroscopy [RS] is a non-destructive and high-resolution technique to detect molecules and mineral phases. This technique is effective in differentiating organic and inorganic compounds at a (sub-) micrometer scale [1]. Combined with other techniques (*e.g.,* Petrography, Scanning Electron Microscopy [SEM]) RS can be useful in identifying biosignatures in rocks.

The expected biosignatures in sedimentary rocks include fatty acids, amino acids, saccharides, and carbonaceous matter [2].

We used RS to investigate biosignatures in speleothems (calcite crusts) that are possible markers of microbial communities found in a cave system in Northeast Brazil. These crusts occur in the Catão cave, a 100-meter-long cave situated in São Desidério, west of the State of Bahia.

RS is a non-usual technique for this type of sedimentary deposits and shows efficient results in identification of organic compounds. This study aims to comprehend microbe-rock interactions in extreme environments (low-light incidence). Additionally, these speleothems might be examples of Holocenic microbialites that evolved in caves, elucidating biologically induced or mediated mineral precipitation in low temperature systems.

Methods

The samples of calcite crust from Gruta do Catão were partitioned in 8 petrographic thin sections, analyzed using a petrographic microscope with reflected and transmitted light and magnification up to 1000x with a coupled camera.

Samples were categorized into microfabrics, and filaments were identified in specific micritic layers. Thin section I7 was chosen due to the abundance of filaments in micritic layers. Filaments closer to the thin section's surface were selected as targets for RS.

Five targets were settled in a Raman Horiba microscope. Experiments were performed using laser length of 785 nm (IPEN). Equipment parameters were variable depending on the lens magnification and product obtained. Objectives of 10, 50 and 100x were used, with acquisition time varying between one and four seconds, and up to twenty accumulations. Incident energy on samples was 25 and 50 mW, and beam diameter ~1 µm.

Raman spectra processing was performed via baseline subtraction and smoothing in Origin. In addition, Raman spectra analysis included hierarchical clustering via the Hyperspec R-code. This clustering revealed data groups of similar spectral features, where peaks were identified using a non-linear iterative curve fitting code used in Matlab ® (peakfit of ISignal: https://terpconnect.umd.edu/~toh/spectrum/). This non-linear fit used a set of Lorentzian plus Gaussian peaks and yielded an error of ~3% regarding spectrum representative of cluster D.

Results and Discussion

Columnar microfabrics observed in thin sections contain fine laminations defined by intercalations of micritic and microsparitic layers. **Figure 1a** shows a caption of thin section I7 with arrows indicating micritic laminae. The targets indicated by squares show abundant filaments, which occur in micritic layers, with length varying from 10 to 50 µm. **Figure 1b** shows a filament encountered in target 3 (red in Figure 1a), where Raman spots were shot.

The spectra indicate calcite as the only mineral phase encountered in the specified targets (peaks: 155, 287, 712, 1087, 1437 cm^{-1} [3]. Although calcite peaks present the most intense signals, the interval between 1200 and 1700 cm^{-1}, where organic bounds vibrate, register numerous peaks.

Hierarchical clustering revealed three data groups of similar spectral features, presented in **Figure 2**. Spectra 01, 03 and 06 were grouped in Cluster A; spectra 02, 04 and 05 in Cluster B; spectrum 07 in Group C (outlier), and spectra 08, 09 and 10 in Cluster D.

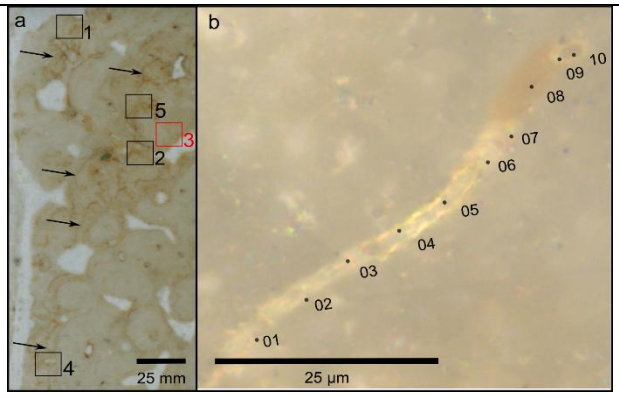

Figure 1. (a) caption of thin section I7, arrows indicate micritic laminae, squares indicate targets, red target indicate location for (b). (b) Filament showing Raman spots.

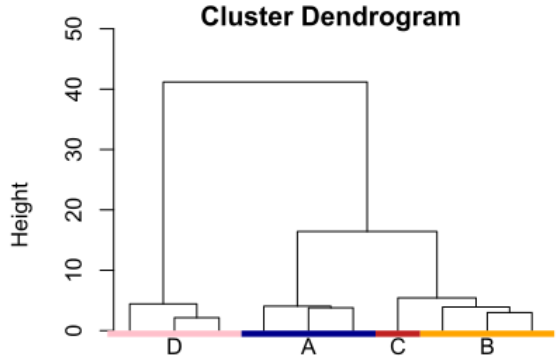

Figure 2. Cluster A: spectra 01, 03, 06; Cluster B: spectra 02, 04, 05; Cluster C: spectrum: 07; Cluster D: spectra 08, 09, and 10.

Deconvolution of Cluster D's medium curve revealed ten peaks in the range between 1200 and 1600 cm -1 (**Figure 3**, peaks: 1240, 1292, 1335, 1368, 1437, 1492, 1546, 1573 cm·¹).

We suggest that those peaks represent several organic bonds as found in [4], including: C-C-N bend, C-N stretch, N-H bend in amide III (1240 and 1292 cm·¹); (Metal) N-heterocycle ring vibration and/or calcite (1437 cm·¹); C-H antisymmetric deformation (peak 1492 ± 53 FWHM cm·¹ which includes 1483 cm·¹ peak from Wiemann et al., 2020); Pyridine-like ring stretch (1573 cm·¹). Peaks 1335, 1368, 1546 cm·¹, were not yet identified.

Based on these preliminary results, we suggest that the identified peaks correspond to peptides and/or proteins preserved in the filaments, which were not entirely altered by diagenesis. Similar Raman spectra were obtained in the porous carbonate micritic matrix, also indicating the presence of proteins.

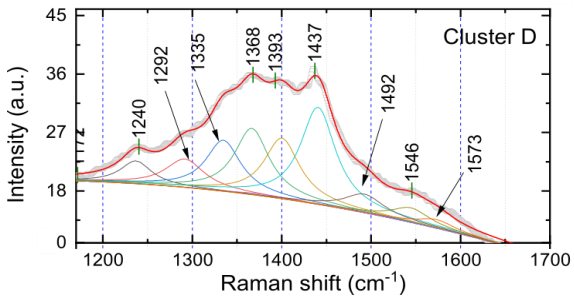

Figure 3. Medium baseline corrected Raman curve for Cluster D in gray; curve fit in red; defined peaks indicated by Raman shift number, with approximate error of ~3%.

Conclusions and future directions

The possible presence of peptides and/or proteins in the filaments and the micritic matrix suggests their biological origin. The matrix probably provided an organic substrate in which filaments evolved during deposition in subaqueous conditions.

The transport of the filaments and organic matter to the depositional site is unlikely due to their small size, high fragility and abundance, and the absence of associated detritic material. Diagenesis in surface-temperature and young age of the samples [5] could be the providers of good taphonomic conditions and material preservation.

As an on-going study, the characterization of alternative clusters and targets will provide additional data regarding filament and matrix composition. Therefore, Raman outputs advocate for application of different tools such as small-angle x-ray scattering to determine co-location of organics into porous matrices. Also, we will focus on quantification of OM via maldi-tof and SEM imaging.

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