Microbial diversity and geochemical characterization of Fe-rich caves in Diamantina (MG) as environmental models for habitability on Mars

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Abstract

Iron-rich caves are little-explored environments that can be used as analogs to Martian environments, such as the lava tubes protected from radiation that could harbor chemolithoautotrophic life. Understanding how life can thrive in these environments could provide us with information about the present and ancient habitability of Mars. Through metabarcoding, and mineral and geochemical characterization, we observed that silica-rich environments presented large populations of microorganisms from the Alphaproteobacteria and Gammaproteobacteria classes (phylum Proteobacteria). However, a tendency to increase the presence of the phylum Actinomycetota was observed upon silica amorphization (formation of opal-AN) and the presence of nitrate. A large presence of the phylum Acidobacteriota was detected in locations with low pH, and a tendency for a greater presence of the phylum Actinomycetota and Planctomycetota appears in a higher presence of iron and manganese, respectively. Metagenomic analyzes of selected samples will still be carried out focusing on the search for genes related to sulfur, nitrogen, and carbon metabolisms. The results highlight the significance of iron-rich caves with distinct environmental characteristics as an important ecosystem in an astrobiological context.

Résumé

Les grottes riches en fer sont des environnements peu explorés qui peuvent servir d'analogues aux environnements martiens, tels que les tubes de lave identifiés sur la planète, des sites protégés des radiations qui pourraient abriter une vie chimiolithoautotrophe. Comprendre comment la vie est capable de se développer dans ces environnements peut nous fournir des informations sur l'habitabilité actuelle et ancienne de Mars. Grâce aux techniques génomiques et à la caractérisation minérale et géochimique, il a été possible d'observer des tendances dans les taxons qui colonisent des lieux présentant des caractéristiques environnementales similaires. Les environnements riches en silice présentent de grandes populations d'organismes de l'embranchement des protéobactéries. Cependant, une tendance à l'augmentation de la présence du phylum Actinomycetota a été observée lors de l'amorphisation de la silice (formation d'opale-AN) et de la présence de nitrate. Une forte présence d'organismes du phylum Acidobacteriota a été détectée dans les endroits à faible pH, et une tendance à une plus grande présence des phylum Actinomycetota et Planctomycetota apparaît dans les endroits où la présence de fer et de manganèse, respectivement, est plus élevée. Des analyses métagénomiques d'échantillons sélectionnés seront encore effectuées à la recherche de gènes liés à une meilleure compréhension des métabolismes dominants. Les résultats soulignent l'importance de lieux tels que les grottes riches en fer en tant qu'environnement important dans un contexte astrobiologique.

1. Introduction

Caves are among the most unique and poorly studied environments on Earth. Due to the harsh conditions observed in many caves, including nutrient depletion and low light levels, these ecosystems are considered extreme environments. Therefore, it is worth paying special attention

to the microbial communities existing in these unique systems with such a variety of geological contexts and mineralogical compositions.

Studies have explored microbial communities in different subterranean ecosystems, but little is known about the diversity and survival strategies. Cave mineralogy is closely related to the organisms that thrive in this environment, and recent studies have uncovered potential relationships between microorganisms and their ecological processes, such as chemolithoautotrophic metabolisms (BENDIA et al. 2022).

This provides support for discussing its implications for the search for life forms existing beyond our planet in iron-rich environments, especially on Mars (SAURO et al. 2020). Martian lava tubes are volcanic caves on Mars that are believed to have formed as a result of fast-moving basaltic lava flows associated with volcanism. Studies of bacterial communities in centuries-old caves and lava tubes on the island of Hawaii have shown that they are more diverse than scientists expected, and may help us understand how life may have existed on Mars and ancient Earth (LÉVEILLÉ & DATTA 2010).

Environments similar to Martian lava tubes are becoming of interest to research to assess the habitability of the red planet. Thus, places such as iron-rich caves, such as those found in the region between Serro and Morro do Pilar, in the state of Minas Gerais, become little-explored analogues for understanding the ways in which life, as we know it, could develop the survival strategies necessary to inhabit places on the Martian surface. Lava tubes are especially interesting because they are protected from ultraviolet radiation and cosmic rays but, like the iron-rich caves on Earth, lack nutrients and energy sources such as sunlight (SAURO et al. 2020).

The assessment of bacterial and archaeal diversity, taxonomic composition, metabolic prediction of these caves using high-throughput 16S rRNA gene sequencing will help us in the search for the diversity of microbial groups involved in different biogeochemical cycles, including reductive pathways and oxidative processes related to carbon, sulfur, nitrogen and iron. The interpretation of these data, together with a mineralogical mapping of the environment, could reveal the microbial diversity in cave ecosystems, contributing to elucidating potential metabolisms in analogous extraterrestrial oligotrophic systems, such as subterranean environments on Mars (BENDIA et al. 2022). In this context, identifying specific microbial groups linked to this cave environment helps to reveal information about local habitability in an astrobiological context.

2. Materials and methods

Three caves were visited in the region between Serro and Morro do Pilar, in the state of Minas Gerais, in July 2023, totaling 12 samples collected. The first cave (Cave_01), with low iron content, had 3 samples collected, while the second (Cave_02) and the third cave (Cave_03), rich in iron, had 4 and 5 samples collected, respectively.

For 16S gene analyzes, genomic material from collected samples was extracted using the DNeasy PowerSoil Pro Kit (QIAGEN©, Hilden, Germany). After DNA extraction, the integrity of the genomic material was verified by 1% (v/v) agarose gel electrophoresis and the concentration was assessed using the Qubit dsDNA HS kit (Thermo Fisher Scientific, Waltham, MA, USA). DNA quality was verified with NanoDrop ND1000 (Thermo Scientific, USA). Large-scale sequencing of the 16S rRNA gene was performed with the genomic material on the Illumina Miseq platform (2 x 300 bp), with 100,000 reads of coverage. The V4-V5 regions of Bacteria and Archaea were amplified using the universal primers 515f/926r (Caporaso et al., 2011; Parada et al., 2015). The reads obtained from sequencing were processed with the QIIME2 software version 2019.10 (Bolyen et al., 2019). The taxonomic classification was assigned to the amplicon sequence variants (ASVs) using Silva database v. 138.

The local mineralogy was assessed via X-ray diffraction (XRD), while accessory mineral identification was performed by micro-Raman. The XRD analyzes was performed in Bruker equipment, D8 Advance Da Vinci model with LYNXEYE detector and TWIN-TWIN optics, using a Cu-Kα1 source and 2θ ranging from 5° to 65°. The micro-Raman measurements were carried out using a Renishaw InVia Reflex equipment coupled to a Leica DM2500M microscope, using a 785 nm (diode laser, Renishaw)-500 mW- with 5% of its power. Each individual spectrum was collected using 30 accumulations of 1 s each, with the removal of cosmic rays. The software Wire 4.4, Fityk 1.3.1, and OriginPro 8 were used to collect, analyze, and plot the data, respectively. Standards of adenine, cytosine, guanine, thymine, and uracil (Sigma Aldrich) were also measured and used as a comparison to help the identification of the mineral deposits.

The geochemistry was obtained by synchrotron techniques such as micro-X-ray fluorescence (micro-XRF) and XANES (for iron and manganese speciation). The micro-X-ray fluorescence (micro-XRF) and X-ray absorption near edge structure (XANES) analyzes were performed at Carnaúba beamline (Sírius/CNPEM). The XRF spectra were acquired in white beam mode, micro focusing (beam size of 1 x 1 μm^2), and using a silicon drift detector (SDD). Each spectrum was acquired with approximately 180 s of accumulation in flyscan mode and the data was processed using the PyMCA software. X-ray absorption near edge structure (XANES) spectra were collected in transmission mode at the Carnaúba beamline, around the Fe K-edge (7112 eV) and Mn k-edge (6539 eV) at room temperature and ambient pressure. The XANES absorption curves obtained were later normalized using the Athena XAS data processing software. Samples with enough humidity/water were tested for its pH using pH strips (KASVI).
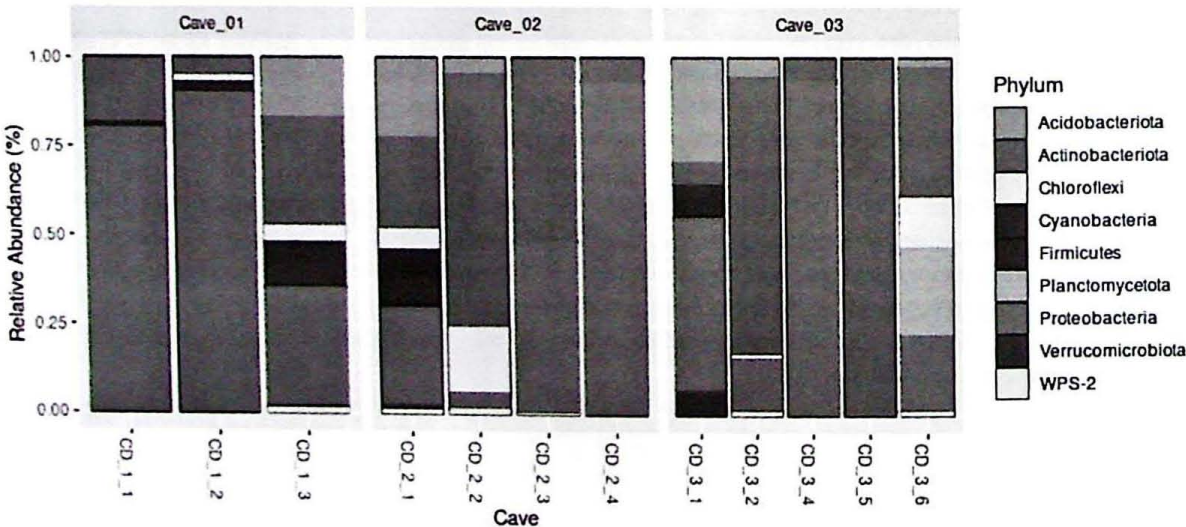

Figure 1: *Relative abundance of the most representative phyla of Bacteria and Archaea (abundance greater than 0.1%) in samples from Cave_01, Cave_02, and Cave_03 through 16S gene sequencing.*

3. Results

The 16S gene sequencing revealed a great diversity of microorganisms in all collected samples. After sequencing the 16S gene, it was possible to recover a total of 1211573 DNA sequences from the 12 analyzed samples, representing an average of 44873.07 ± 12129.61 sequences per sample. The sequences obtained were classified into 6,205 ASVs, representing an average of 1445.815 ± 682.7496 ASVs per sample. The total of 6,135 classified ASVs belonged to the Bacteria domain and 70 ASVs belonged to the Archaea domain. The dominant phylum of the Bacteria domain was Proteobacteria (46.71%), followed by Actinobacteriota (36.60%), Acidobacteriota (7.16%), Chloroflexi (3.97%), Planctomycetota (2.04%), Cyanobacteria (1.68%), Verrucomicrobiota (0.80%), WPS-2 (0.57%) and Firmicutes (0.23%). The Archaea communities were mainly represented by the phyla Crenarchaeota (0.61%), Euryarchaeota (0.0005%), Halobacterota (0.0338%), and Thermoplasmatota (0.0076%). At class level, Alphaproteobacteria (45.86%) was the most abundant group in the sampled caves, followed by Actinobacteria (33.84%), Acidobacteriae (7.25%), Ktedonobacteria (3.30%), Thermoleophilia (2.88%), Planctomycetes (2.04%), Cyanobacteriia (1.69%), Gammaproteobacteria (0.97%), Verrucomicrobiae (0.80%), WPS-2 (0.58%), JG30-KF-CM66 (0.32%), Bacilli (0.23%) and Nitrososphaeria (0.19%) (Fig.1).

The presence of quartz was observed in all samples, but it was predominant in the iron-poor cave (Cave_01), along with feldspar and illite. All iron-rich caves (Cave_02 and Cave_03) presented the minerals goethite and hematite, along with other iron oxides and oxyhydroxides, such as lepidocrocite, that were more sporadic. Among the iron-rich caves visited, the most developed one (Cave_03) had the presence of coralloids, rich in mixtures of iron minerals, in addition to the presence of small structures of hyaline opal (opal-AN), on which the presence of nitrate (NO^{3-}) was identified (sample CD_3_5). Acidic samples, like sample CD_1_3 (pH between 3 and 4) showed a higher concentration of Acidobacteriota.

While in the less developed cave (Cave_02), the iron was mostly in its most oxidized form (Fe^{3+}), the most developed cave (Cave_03) presented some points with reduced iron ion (Fe^{2+}), which increases the possibilities of metabolisms linked to iron oxidation-reduction cycles. The most significant concentrations of manganese, another element associated with biogeochemical cycles and chemolithoautotrophic metabolisms, were also observed in this cave. At this location, mixtures of manganese in the oxidative states Mn^{2+}, Mn^{3+} and $Mn4^+$ were observed (sample CD_3_6).

4. Discussion

The analyses of the taxonomic composition of the bacterial and archaeal communities indicated the presence of a great microbial diversity, distributed in more than 40 phyla. Despite the distinction between the composition of the caves (quartzite and iron), the microbial communities did not present significant differences in species richness and diversity among the three caves analyzed. However, it is possible to observe that the samples richest in quartz present a greater abundance of organisms from the phylum Proteobacteria (notoriously having numerous species capable of carrying out N2 fixation and metabolism of C1 compounds), visible in Cave_01 and in points rich in quartz, such as coralloids in Cave_02 (sample CD_2_4) and Cave_03 (sample CD_3_4). The sample with the presence of opal-AN and nitrate (sample CD_3_5) (Fig.2) shows a greater abundance of organisms from the phylum Actinobacterota, greater than all other samples. These predominances are in agreement with what was reported by SAURO et al. (2018). According to them, the increase of the amorphization of the silica (parallel to a possible increase of external organic matter) was consonant with higher abundance of Actinomycetota. Samples from iron-rich caves (Cave_02 and Cave_03) also showed a higher abundance of Actinomycetota. Although most of them are heterotrophic, feeding on organic carbon, some are also known to fix nitrogen and to have chemolithoautotrophic activities exhibiting nitrate-dependent iron oxidation.

On the other hand, there does not seem to be a relationship between the microbiota and variations in the presence of Fe^{2+} and Fe^{3+}. Samples with lower pH and higher concentration of iron (such as CD_1_3, with pH = 3 to 4) showed also a higher concentration of Acidobacteriota than the other samples, a correlation described, for example, by KIELAK et al. (2016). The sample with the highest presence of manganese (CD_3_6) stands out for the presence of the phylum Planctomycetota, in a higher quantity than the other samples. Studies have demonstrated the direct link (LOMAKINA et al. 2023) and presence of genes linked to manganese oxidation (SUAREZ et al. 2023) and the group of Planctomycetota.

Metagenomic analyzes of selected samples from these iron-rich caves will still be carried out in the search for genes related to metabolisms that provide insights on the habitability of these environments.
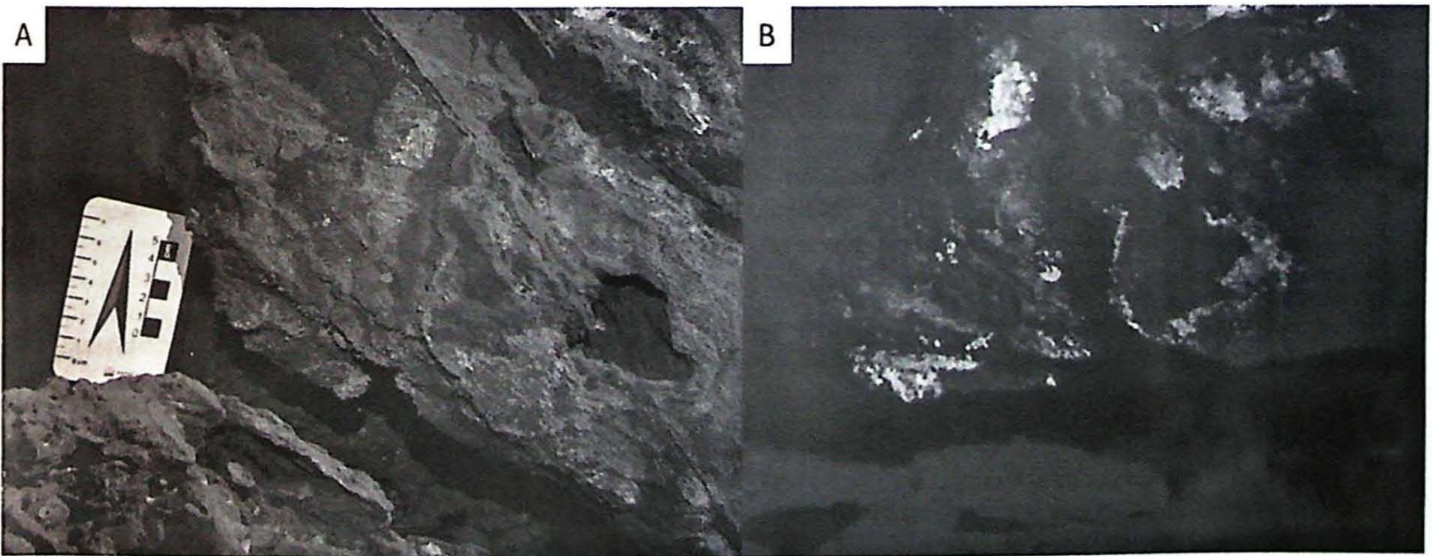

Figure 2: Coralloids rich in opal-AN and nitrate over an iron-rich wall of Cave_03 (sample CD_3.5). A. Visible light view of the region containing opalized coralloids. B. A slightly tilted view of the same area under ultraviolet light shows the fluorescence of the opalized coralloids (brightness and contrast slightly enhanced for better observation of the fluorescence).

5. Conclusion

In this work, it was possible to demonstrate a great diversity of species in the studied cave environments. Although preliminary, these results expand our knowledge about the microbial communities that colonize iron-rich caves compared to environments richer in silica.

The results demonstrate a clear relationship between the progress of silica amorphization and the composition of the microbial population, related to the geochemistry of the microenvironments. The process of progressive silica amorphization seems to lead to a change in the community from the predominance of Proteobacteria to Actinomycetota. In addition, local changes in pH and local aspects of silica solubility may result in bacterial metabolic processes related to chemolithoautotrophic activities, for example, inorganic nitrogen transformation and iron oxidation. The presence of iron and manganese also seems to exert pressure for a change in the microbial community, leading to higher concentrations of Actinomycetota and Planctomycetota, respectively.

As a follow-up, we intend to conduct a more detailed metagenomic study of some selected selections, opening doors to a wide range of metabolisms and survival strategies possible in this environment. Together, these results will be able to demonstrate that, in an analogous way, Martian environments, such as lava tubes, could support an ecosystem similar to those found in the researched region, expanding the habitability of these places for life as we know it. This highlights the importance of places such as iron-rich caves as an environment to be studied in detail to expand our knowledge in an astrobiological context.

Acknowledgments

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