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Halobacterium salinarum as a model for the study of biosignatures aimed at the investigation of planetary systems

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Abstract. The exploration of exoplanets is limited by the large distances, preventing in situ investigation, but allowing remote explorations in search of biosignatures associated with life. Despite the promising scenario, little is known about the infrared absorption bands of biological molecules associated with extremophiles. Thus, this research aimed to characterize the DNA molecule of *Halobacterium salinarum* NRC-1 through the infrared spectroscopy technique. The results show that absorption bands centered at 1240cm $^{-1}$, 1228 cm^{-1}, 1050 cm^{-1}, and 968 cm^{-1} are relevant in the search for signals compatible with life.

Resumo. A exploração de exoplanetas é limitada pelas grandes distâncias, impedindo a investigação in situ, mas permitindo explorações remotas em busca de bioassinaturas ligadas à vida. Apesar do cenário promissor, pouco se conhece sobre as bandas de absorção no infravermelho de moléculas biológicas ligadas aos extremófilos. Assim, essa pesquisa teve como objetivo caracterizar a molécula de DNA da *Halobacterium salinarum* NRC-1 por meio da técnica de espectroscopia no infravermelho. Os resultados mostram que bandas de absorção centradas em 1240 cm^{-1}, 1228 cm^{-1}, 1050 cm^{-1} e 968 cm^{-1} são relevantes na busca por sinais compatíveis com vida.

Keywords. Astrobiology – Infrared: general – Techniques: spectroscopy

1. Introduction

Astrobiology is understood as a multidisciplinary field of science aimed at investigating the origin, evolution, and possible futures of life both on Earth and beyond. Currently, according to NASA's Exoplanet Exploration Program (ExEP), there are already more than 6,000 confirmed exoplanets, a number that strengthens the hypothesis of the existence of worlds located in orbits compatible with habitable or extremophile zones around their host stars (Bernardes, 2012).

The possibility of finding extraterrestrial life on Mars or other celestial bodies has long intrigued humanity. Despite Mars presenting hostile conditions, such as low temperatures, scarce liquid water, and a rarefied atmosphere, the planet may still harbor extremophile life forms (Bernardes, 2012). The presence of these microorganisms, associated with molecules such as DNA, RNA, chlorophyll, and carotenoids, constitutes relevant biomarkers for the indication of life. Thus, bodies exhibiting particle ejection mechanisms similar to those on Earth may have their atmospheres contaminated by biological materials (Bernardes, 2018).

The exploration of exoplanets remains limited due to vast distances and technological constraints that prevent in situ investigations. Nevertheless, upcoming space telescopes are expected to enable atmospheric analyses and the identification of life-related biosignatures. In recent years, species of Halobacterium salinarum, a halophilic microorganism, have been identified in evaporites of the Sergipe Basin, Brazil, and the detection of similar deposits on Mars reinforces the possibility that microorganisms with comparable adaptations may exist in Martian sediments and be detected by future missions (Hornemann et al., 2017; Bernardes, 2018; Olga, 2021). Thus, the creation of a database of DNA absorption bands of archaea aims to support future space missions in the search for biosignatures, focusing on extremophile halophilic archaea that inhabit hypersaline environments with NaCl concentrations of 4–5 mol, far exceeding that of seawater (Leuco et al., 2015).

2. Methodology

The archaeon was cultivated in a nutrient medium composed of NaCl (250 g/L), $MgSO_4 \cdot 7H_2O$ (20 g/L), KCl (2 g/L), sodium citrate (3 g/L), peptone (10 g/L), and distilled water. The process was carried out in the laboratory in an incubator at 37 °C, under conditions of high luminosity and agitation between 125 and 250 rpm. The procedure was completed when the exponential growth phase was reached ($O.D._{600}$ nm = 0.5). The DNA isolation and purification processes performed in this work followed the *DNeasy Blood and Tissue Kit (Qiagen)* protocol, a method based on the selective adsorption of DNA through silica membranes. After the DNA extraction procedure, samples containing 100 ng/μL were analyzed using a PerkinElmer Spotlight 400 Series spectrophotometer, with variable resolution from 16 to 0.50 cm^{-1}, covering the range from 4000 cm^{-1} to 400 cm^{-1} with a resolution of 4 cm^{-1}, in absorbance mode, with 32 scans under controlled temperature between 18 and 20 °C. The spectra were processed using *OriginPro software (version 2023, OriginLab Corporation)*, with the objective of applying common treatments for absorption band identification, such as normalization, baseline correction, smoothing, second-derivative tests, and Gaussian deconvolution (Bernardes, 2018; Savitzky and Golay, 1964).

3. Results

The stages of the research, from DNA extraction and purification to the absorption spectrum of the DNA of the archaeon *Halobacterium salinarum*, are presented in Figure 1. For analysis purposes, the spectrum was divided into four ranges: from 1791 to 1480 cm^{-1}, from 1480 to 1161 cm^{-1}, from 1161 to 920 cm^{-1}, and from 920 to 460 cm^{-1}. The region between 4000 and 1797 cm^{-1} was not considered due to the presence of an extremely intense band associated with water absorption. In the range from 1791 to 1480 cm^{-1}, characteristic amide absorptions are observed, mainly corresponding to the N–H and C–H stretching modes of functional groups such as carboxyl, carbonyl, and ketone present

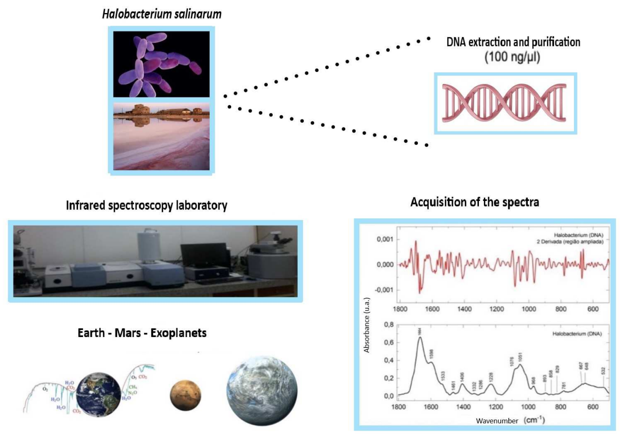

Figure 1. Stages of the investigation, from the extraction and purification of *Halobacterium salinarum* DNA, through the acquisition and processing of the spectra, to data analysis and the identification of the main bands associated with the biological material of the archaeon.

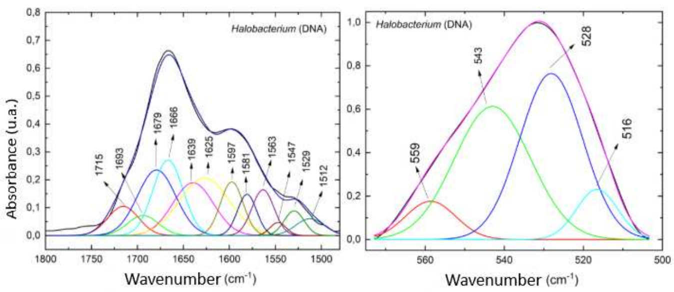

Figure 2. Absorption spectra of the amide I band and sugar/sugar–phosphate vibrations, respectively, of the archaeon *Halobacterium salinarum*

in proteins and peptides. In this region, the bands related to amide I and amide II typically occur around 1664 and 1598 cm^{-1}, respectively (Hornemann et al., 2017; Ricachenevsky, 2020).

The region referred to as "mixed," between 1480 and 1161 cm^{-1} (Hornemann et al., 2017), presents bands associated with proteins, lipids, and phosphate compounds. The absorptions recorded between 1161 and 920 cm^{-1} correspond mainly to vibrations of the sugar–phosphate backbone of the DNA chain. Finally, the bands present between 920 and 460 cm^{-1} are related to conformations of the sugar unit. The spectra and their respective deconvolutions were examined separately based on the following spectral ranges: (i) amide bands, (ii) the "mixed" region, (iii) vibrations of the sugar–phosphate backbone, and (iv) vibrations associated with the sugar and the sugar–phosphate framework. In Figure 2, the deconvolution of some bands that concentrate relevant information is observed, such as those related to the structural conformation of DNA (sugar/sugar–phosphate vibrations) and the amide I band.

Table 1 compiles some of the most representative absorption bands of the DNA of the extremophile archaeon *Halobacterium salinarum*, forming a database intended for the identification of biosignatures. The table lists the central wavenumber of each band and its respective assignment, such as ribose stretching, with a peak near 1050 cm^{-1} (C–O).

Table 1. Some absorption bands of the DNA of the archaeon *Halobacterium salinarum*.

Band (cm^{-1})	Fragments	Designation
1664	C=O	Primary amide stretching
1533	N-H	Secondary amide bending, Stretching C-N
1461	CH_3	Asymmetric lipid bending
1218	PO_2^-	Antisymmetric stretching of DNA
1228	PO_2^-	Asymmetric DNA stretching
1116	C–O	Ribose stretching
1050	C–O	Ribose stretching
968		Skeletal vibrations of DNA ribose–phosphate
648	N–H	Secondary amide

The data demonstrate that infrared spectroscopic characterization is a viable approach for studying microorganisms, highlighting the importance of repositories containing representative absorption bands associated with biological processes as potential evidence of life in other environments. In this context, the detection of bands near 1228, 1050, and 968 cm^{-1} would constitute consistent evidence of halophilic extremophiles on Mars or in exoplanetary atmospheres. Moreover, the simultaneous identification of multiple bands may reduce false positives, as characteristic absorption patterns differ from signals produced by atmospheric gases or other materials present on planetary surfaces and atmospheres.

4. Conclusion

This study identified biological markers associated with the DNA of the extremophile microorganism Halobacterium salinarum using infrared spectroscopy in the 4000–400 cm^{-1} range. The results indicate that specific absorption bands are relevant for detecting signals compatible with the presence of life, since DNA or its fragments are considered irrefutable evidence of biological activity. In this context, absorptions related to DNA skeletal motions and ribose/DNA stretching modes may indicate habitability conditions in the investigated environment, both in in situ missions, such as those on Mars, and in remote observations of exoplanetary atmospheres.

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