Preservation of carotenoids in salts and Mars regolith in various conditions

Dominik Horký (1), Thomas Leya (2), Jean-Pierre de Vera (3) and Mickaël Baqué (1)

(1) German Aerospace Center (DLR), Institute of Planetary Research, Planetary Laboratories Department, Berlin, Germany, (2) Fraunhofer Institute for Cell Therapy and Immunology, Branch Bioanalytics and Bioprocesses IZI-BB, Extremophile Research & Biobank CCCryo, Potsdam, Germany (3) German Aerospace Center (DLR), Space Operations and Astronaut Training, Microgravity User Support Center (MUSC), Köln, Germany

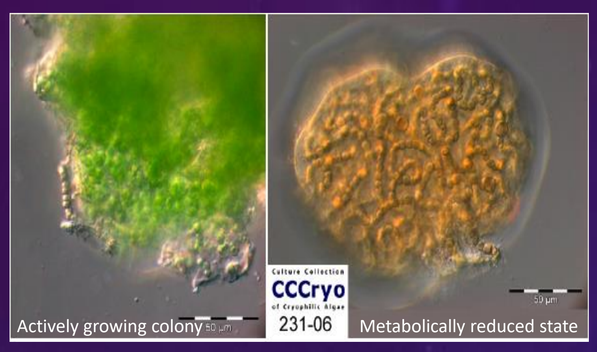
Introduction

The search for **life on Mars** requires new tools and techniques. Among them, **Raman spectroscopy** is a powerful and non-destructive method for detecting biosignatures during missions to Mars such as NASA's **Perseverance** and ESA/ROSCOSMOS's **Rosalind Franklin** rovers. It is therefore important to study the detection possibilities of **model biosignatures** and their preservation in various conditions over time in order to guide future missions and interpret future data. Cyanobacterial photoprotective pigments (namely **carotenoids**) have been extensively used as suited targets for such measurements and to serve as biosignature models thanks to their **stability and easy identification** by Raman spectroscopy.

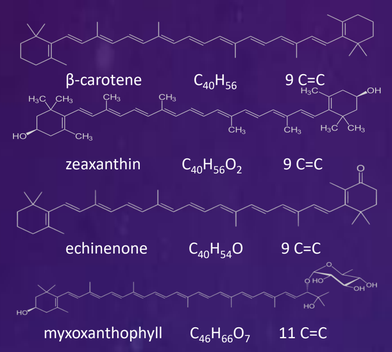
Carotenoid decomposition can be caused by **oxidation**[1] (prevented by higher humidity) and **irradiation** (prevented by lower humidity[2]). Carotenoids seem to be decomposing at different rates in different sets of conditions and on different matrices.

During the preparation phase of **BioSigN** (BioSignatures and habitable Niches) we explore the possibility that different matrices enhance or diminish preservation of detectable carotenoid signal under different storage conditions. Both pure molecular **β-carotene** and cyanobacterium ***Nostoc sp.*** (strain CCCryo 231-06) were used.

Nostoc sp. (CCCryo 231-06) isolated from Antarctica



Carotenoids in *Nostoc* sp.



Previous experiments

BIOMEX[3] (2020)

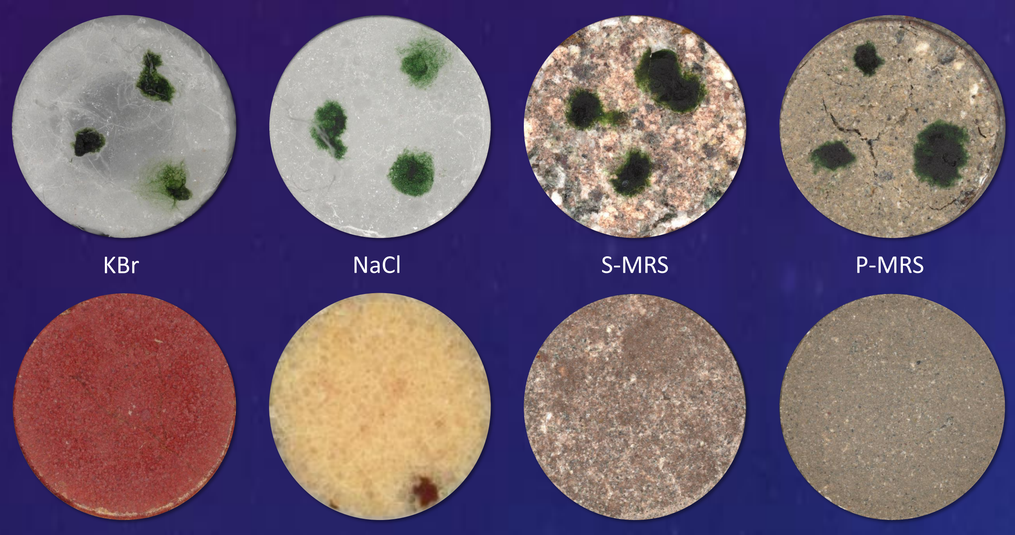
β-carotene (pure molecule)
Space conditions
KBr, NaCl, S-MRS, P-MRS

Controls stored in:
22 °C, <0.01% RH (dry)
4 °C, ~36% RH (fridge)

Starlife[2] (2015)

Nostoc sp.
γ radiation
S-MRS, P-MRS, free culture

22 °C, <0.01% RH (dry)
-18 °C, ~38% RH (freezer)



Sample preparation

Pellets were pressed with 4.5 Mpa

- **KBr** (non-reactive to Raman laser)
- **NaCl** (analog to brines and salty regions on Mars)
- **S-MRS** (Sulfatic Mars Regolith Simulant, present Mars)
- **P-MRS** (Phyllosilicatic Mars Regolith Simulant, ancient Mars)

On each pellet, cultured *Nostoc sp.* (strain 231-06, Fraunhofer IBMT, Potsdam) was streaked three times and dried for 24 hours.

The samples were kept in three different environments:

- **22 °C, <0.01% RH (dry)**
- **4 °C, ~36% RH (fridge)**
- **-18 °C, ~38% RH (freezer)**

Controls from BIOMEX and Starlife were measured as well.

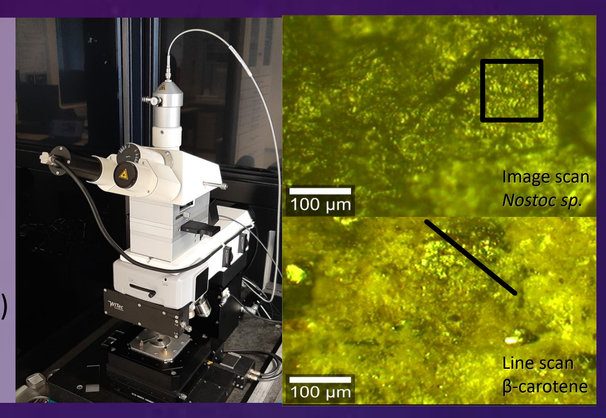
Goals

Carotenoid decomposition can be accelerated or decelerated by various factors, such as **humidity**[4], **temperature** or **oxygen** presence[1]. The goal of this work is to untangle the factors affecting the loss of carotenoid signal. This is important for two different reasons:

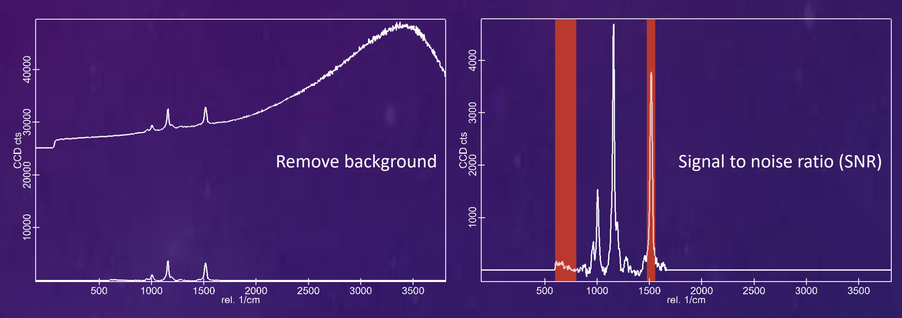
1) **Better controls** for future missions, such as **BioSigN**, and separating the effects of storage from the effects of the experiment
2) **Building a database** for biosignatures detectability in **Mars conditions** and on Mars

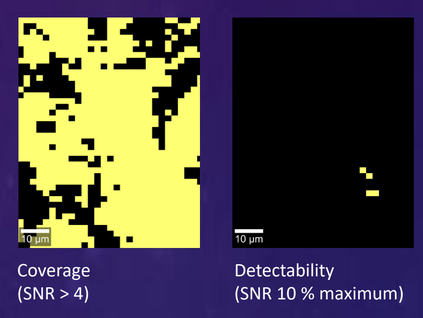
Experimental setup

Microscope:	confocal Witec alpha300		
Spectrometer:	4-5 cm^{-1} spectral resolution		
	600 l/mm grating		
Objective:	10x		
Excitation:	**532 nm**		
Laser power:	**1 mW**		
Scan type:	Image scan	70x70 µm	30x30 pts. (*Nos.*)
	Line scan	200 µm	10 pts. (β-car.)
Integration:	1x 1s (*Nos.*), 5x 1s (β-car.)		



Analysis





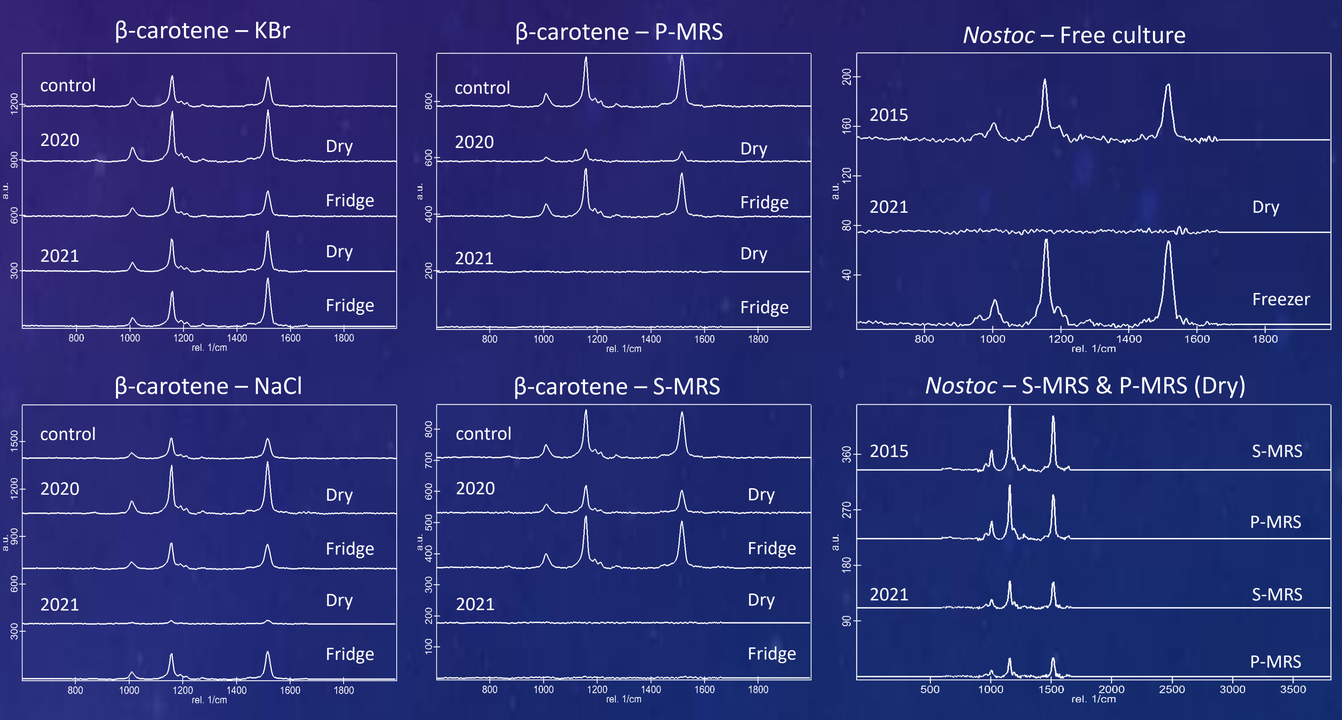
Results

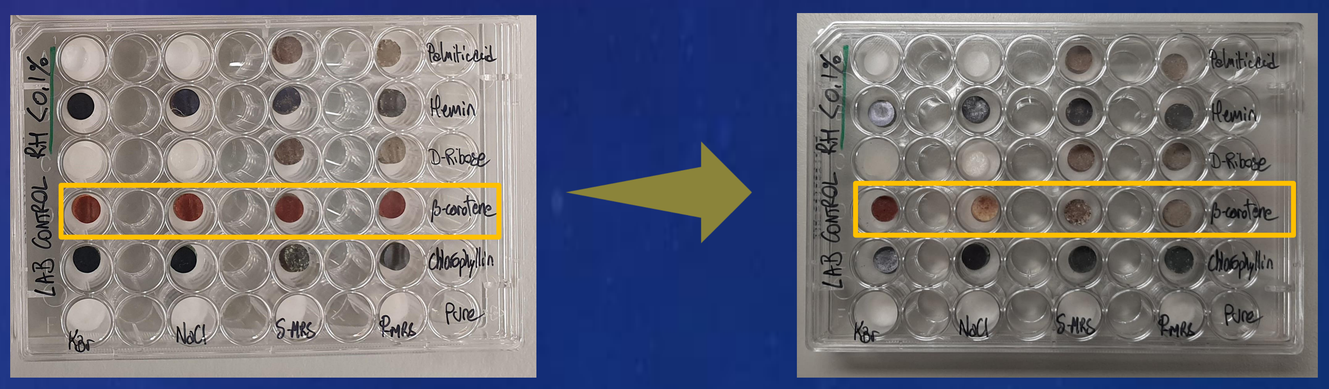
β-carotene (1 year)

- **KBr**
  - no significant change (both dry and fridge)
- **NaCl**
  - **Dry** - significant **decrease**
  - **Fridge -** no significant change
- **S-MRS** and **P-MRS**
  - complete **loss** (both dry and fridge)

Nostoc sp. (6 years)

- **Free** culture
  - **Freezer** – **increase**
  - **Dry** – complete **loss**
- **S-MRS** and **P-MRS**
  - **Dry** – significant **decrease**





Discussion and outlook

- **long-term** experiments (1 and 6 years)
  - **better preserved in cold and humid** rather than dry and warm conditions
  - **better preserved in salts** rather than Mars simulants
  - **better preserved in the cells** of the *Nostoc* cultures rather than as a free molecule
- **short-term** experiment (monthly)
  - **preliminary results**
    - **initial increase** in signal strength **followed by decrease**
- Future
  - **Raman measurements will continue monthly**
  - **Fluorescence microscopy** to observe **photosynthetic pigments** on *Nostoc sp.*

Additionally, salt nodules (NaCl) from Atacama desert will be studied to determine the possibility of carotenoid preservation and detection in them and similar formations on Mars.

References

(1) Neto, RO Teixeira, et al. (1981) "Oxygen Uptake and β-Carotene Decoloration in a Dehydrated Food Model." *Journal of Food Science* 46.3: 665-669. (2) Moeller, R., Raguse, M., Leuko, S., Berger, T., Elisabeth Hellweg, C., Fujimori, A., Okayasu, R., Horneck, G. & the STARLIFE research group (2017) STARLIFE – an international campaign to study the role of galactic cosmic radiation in astrobiological model system. *Astrobiology* vol17,2, pp.101-109. (3) de Vera, J.-P., Alawi, M., Backhaus, T., Baqué, M., Billi, D., Böttger, U., Berger, T., Bohmeier, M., Cockell, C., Demets, R., et al. (2019). Limits of Life and the Habitability of Mars: The ESA Space Experiment BIOMEX on the ISS. Astrobiology 19, 145–157, (4) Chou, Hung-en, and Breene, William M. (1972) "Oxidative decoloration of β-carotene in low-moisture model systems." *Journal of Food Science* 37.1: 66-68.