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RESEARCH ARTICLE

Biosignature preservation in FaRLiP cyanobacteria after prolonged desiccation and its relevance to space missions searching for life

Giorgia Di Stefano[1], Mickael Baqué[2], Jean-Pierre Paul de Vera[3], Micol Bellucci[4], Manuele Ettore Michel Gangi[4] and Daniela Billi[1]

[1]Department of Biology, University of Rome Tor Vergata, Rome, Italy
[2]Department of Planetary Laboratories, Astrobiological Laboratories, German Aerospace Center (DLR), Institute of Planetary Research, Berlin, Germany
[3]German Aerospace Center (DLR), Space Operations and Astronaut Training, MUSC, Cologne, Germany
[4]Italian Space Agency, Rome, Italy
Corresponding author: Daniela Billi; Email: billi@uniroma2.it

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Abstract

Two desert cyanobacterial strains, *Chroococcidiopsis* sp. CCMEE 010 and CCMEE 130, capable far-red light photoacclimation (FaRLiP), were investigated for the stability of biosignatures after six years of desiccation. Biosignature detectability was demonstrated by confocal laser scanning microscopy and Raman spectroscopy thus highlighting that these two FaRLiP cyanobacteria are a novel reservoir of an array of pigments, encompassing canonical chlorophyll *a*, far-red shifted chlorophylls, phycobilins and carotenoids. The recorded signals were comparable to those of dried cells of *Chroococcidiopsis* sp. CCMEE 029, CCMEE 057 and CCMEE 064, not capable of FaRLiP acclimation and previously reported for biosignature stability and survivability after exposure to space and Mars-like conditions during the BIOMEX (BIOlogy and Mars EXperiment) and BOSS (Biofilm Organisms Surfing Space) low Earth orbit missions. Since infrared-light driven photosynthesis has implications for the habitability of Mars as well as exoplanets, the stability of far-red shifted chlorophylls in dried *Chroococcidiopsis* is a prerequisite for future experimentations under simulated planetary conditions in the laboratory or directly into space. It is anticipated that post-flight investigations of FaRLiP cyanobacteria as part of the BioSigN (Bio-Signatures and habitable Niches) space mission will contribute to gather novel insights into biosignature degradation/stability and thus prepare future planetary exploration missions to Mars. In addition, the scored viability of strains CCMEE 010 and CCMEE 130 after prolonged desiccation is relevant to investigate life endurance under deep space conditions, as planned by the BioMoon mission that aims to expose dried and rehydrated extremophiles on the Moon surface after exposure to deep space.

Contents

Introduction

There is strong evidence that many potentially habitable worlds exit in our galaxy: in the Solar System environments that might have hosted life in the past or even today have been identified. Those include the surface of early Mars, the sub-surface of present-day Mars, the oceans of the icy moons Europa and Enceladus, or even the clouds of Venus, along with thousands of exoplanets orbiting in the habitable zone of their star (Styczinski *et al*., 2024; Cockell *et al*., 2024). Hence, understanding how biosignatures change over time and how they are modified by space conditions is critical for space exploration missions searching for life (Dartnell *et al*., 2012; Dartnell and Patel, 2014).

Since everything we know about biology derives from Earth, microorganisms living in extreme environments, the so-called extremophiles, are the best-case scenario to identify protective biomolecules that can serve as biomarkers and to investigate biosignature stability/degradation under planetary simulations in the laboratory or in space (Martins *et al*., 2017; Jorge-Villar and Edwards, 2013; Wilhelm *et al*., 2018). Microbial pigments are promising biosignatures because they can be easily detected by Raman spectroscopy (Jehlička *et al*., 2022), carotenoids, chlorophylls, phycocyanins and scytonemin have been all detected in extreme habitats and included in a biosignature library of Raman spectra (Varnali and Edwards, 2014). Extremophiles are not only a valuable reservoir of biosignatures but also model systems to evaluate the habitability of other planets (Merino *et al.,* 2019). For example, increasing dryness in deserts causes a shift from edaphic to lithic communities, so that it has been proposed that during the loss of surface habitability of Mars, if life ever occurred it may have retreated to sub-surface niches (Davila and Schulze-Makuch, 2016).

Cyanobacteria of the genus *Chroococcidiopsis* colonize lithic niches in extremely dry deserts, and since their discovery they have been pointed as a model organism to search for life on Mars (Friedmann and Ocampo, 1976). Although there is no general agreement if photosynthesis ever occurred on Mars (Cockell and Raven, 2004; Westall *et al*., 2015), the capability of certain cyanobacteria of using far-red light to drive photosynthesis offer a new scenario for the habitability of Mars (Antonaru *et al*., 2023; Billi *et al*., 2022). On Earth these cyanobacteria inhabit niches depleted in visible light (VL) and enriched in far red light (FRL) and have developed an adaption known as far-red light photoacclimation (FaRLiP) consisting in the remodeling of the photosynthetic apparatus and production of far-red shifted chlorophylls (Gan and Bryant, 2015). In particular, the colonization of FaRLiP cyanobacteria of rocks and caves (Behrendt *et al*., 2015; Antonaru *et al*., 2023) has implications for the habitability of Mars since a putative photosynthetic life form might have retreated to sub-surface niches and caves.

Moreover, far-red photosynthesis has implications for the habitability of exoplanets. Exoplanets orbiting M stars have a light spectrum peaking in the far-red and infrared that might support oxygenic photosynthesis (Lehmer *et al*., 2021). The feasibility of oxygenic photosynthesis under M-dwarf light has been pointed out by documenting the capability of cyanobacteria and more complex photosynthetic organisms to grow and produce oxygen under laboratory simulations of M-dwarf light (Battistuzzi *et al*., 2023, 2023a).

The current knowledge on how biosignatures respond to space and Mars-like conditions has been largely achieved thanks to space missions that used the ESA-EXPOSE facility installed outside the International Space Station (ISS) allowing the exposure of extremophiles and their molecules to space and Mars-like conditions (Cottin *et al*., 2017). The BIOMEX (BIOlogy and Mars EXperiment) space experiment showed that after exposure for 469 days to Mars-like simulations, out of seven biomolecules, only three (chlorophyllin, quercetin and melanin) were still detectable on UV-exposed

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Table 1. *List of* Chroococcidiopsis *sp. CCMEE strains used in this study*

CCMEE strain	Sampling site	Rock substrate/ colonization	FaRLiP capability	Air-dried storage (years)
010	Negev Desert, Israel	Granite/chasmoendolithic	yes	6
029	Negev Desert, Israel	Limestone/ chasmoendolithic	no	10
057	Sinai Desert, Egypt	Granite/chasmoendolithic	no	6
064	Sinai Desert, Egypt	Stone pavement/hypolithic	no	6
130	Canyonlands, Utah	Sandstone/cryptoendolithic	yes	6

samples although with a reduced Raman signal, while slightly reduced Raman signals occurred in biomolecules mixed with regoliths to mimic sub-surface environments (Baqué *et al*., 2022).

The desert cyanobacterium *Chroococcidiopsis* sp. CCMEE 029 was exposed to space and Mars-like simulations along with other extremophiles during the BIOMEX experiment (de Vera *et al*., 2019). Post-flight analyses of dried cells mixed with Martian mineral analogs revealed detectable pigments and genomic DNA thanks to the UV shielding provided by the regoliths (Billi *et al*., 2019a). Three desert strains of *Chroococcidiopsis*, namely CCMEE 029, CCMEE 057 and CCMEE 064, were exposed to space and Mars-like simulations as dried biofilms during the BOSS (Biofilm Organisms Surfing Space) space mission (Cottin and Rettberg, 2019). Post-flight analyses revealed unbleached photosynthetic pigments in the bottom layers of the biofilms that were shielded against UV radiation by top layer-cells (Billi *et al*., 2019b). However, none of these *Chroococcidiopsis* strains were capable of FarLiP adaption (Billi *et al*., 2022; Antonaru *et al*., 2023).

Novel insights into biosignature detectability of extremophiles under simulations of Mars- and icy moon-like conditions will be delivered by the BioSigN (Bio-Signatures and habitable Niches) space mission that will use the foreseen Exobio facility to be installed outside the ISS (2027-2028), thus preparing future planetary exploration missions to Mars, Enceladus and Europa (de Vera and Baqué, 2024).

The overarching goal of the present work was to investigate the suitability for the of BioSigN space mission of two desert strains of *Chroococcidiopsis*, namely CCMEE 010 and CCMEE 130, capable of FaRLiP acclimation and both possessing far-red shifted chlorophylls (Antonaru *et al*., 2023; Billi *et al*., 2022). Since BioSigN will expose dried microorganisms, the assessment of desiccation tolerance and stability of sub-cellular dried components is mandatory. Therefore, these two strains were investigated for biosignature detectability and survival after 6 years of storage in the air-dried state. The detectability of photosynthetic pigments and genomic DNA was assessed at the single-cell level by using confocal laser scanning microscopy (CLSM), while carotenoids were detected with Raman spectroscopy. Then, biomarker detectability was compared with that of dried cells of *Chroococcidiopsis* strains that were exposed to space and Mars-like conditions during the BIOMEX and BOSS space experiments (Billi et al., 2019a, b). Biosignature detectability was assessed in strains CCMEE 057 and CCMEE 064 after 6 years of air-drying, and in CCMEE 029 after 10 years. Finally, the occurrence of survivors in all five strains was evaluated after 72 h-rehydration by using an indirect method based on Calcein-AM and by assessing their capability to enter cell division after transfer into a fresh growth medium.

Materials and methods

Cyanobacterial strains and sample preparation

The five *Chroococcidiopsis* strains used in this study are part of the Culture Collection of Microorganisms from Extreme Environments (CCMEE) established by E. Imre Friedmann and Roseli Ocampo-Friedmann (Table 1) and were cultured in BG11 medium in 50-mL vented flasks placed in an

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incubator at 25°C, without shaking. Cultures under visible light were exposed to a photon flux density of 20 µmol m^{-2} s^{-1} provided by white tubular led lights (OSRAM LEDs). Cultures under far-red light were exposed to a photon flux density of 5 µmol m^{-2} s^{-1} provided by far-red tubular led lights (OSRAM LEDs). Dried samples were prepared by filtering cell aliquots on Millipore filters and air-dried overnight in a sterile hood and stored in sealed plastic bags in the dark under room conditions.

Confocal laser scanning microscopy

Cells were recovered from small fragments (about 2 mm^2) by using 500 µL BG-11 medium and after centrifugation resuspended in 20 µL Phosphate Buffered Saline (PBS) buffer containing 1.5% agarose and immobilized onto a microscopy slide and observed with a confocal laser scanning microscope (CLSM, Olympus Fluoview 1000) by using a 60X objective. Photosynthetic pigments were imaged with a 635 nm laser and collecting the fluorescence emission from 650 to 680 nm for phycobilisomes and chlorophylls. Genomic *DNA was visualized at the CLSM with a 405*-nm excitation laser after *staining with Hoechst as* follows: cells were harvested by gentle centrifugation and resuspended in 1 mL PBS with *Hoechst* 33342 (Thermo Fisher Scientific Inc.) at *final concentration of* 5 µg/ml and incubated in the dark at room temperature for 15 minutes. Then the cells were washed once with PBS buffer and resuspended in 20 µL PBS buffer containing 1.5% agarose for slide preparation. CLSM lambda scans were obtained by using a 488-nm excitation laser and collecting the emission from 550 to 800 nm. Curve plotting was performed using the GraphPad Prism program (GraphPad Software, San Diego, CA).

For cell viability, small fragments (about 2 mm^2) of dried samples were inoculated in BG-11 medium under optimal growth conditions for 72 h. Calcein staining was performed as follows: cells were harvested by gentle centrifugation and resuspended in 500 µL of fresh BG-11 containing 10 µL Calcein-AM (Thermo Fisher Scientific Inc.; 1 mg/mL dimethylsulfoxide). The suspension was incubated in the dark at room temperature for 90 minutes *(*Mullineaux *et al*., 2008*)*. Then cells were washed three times with PBS buffer, resuspended in 20 µL PBS buffer containing 1.5% agarose for slide preparation and observed at the CLSM with a 488-nm excitation laser.

Raman Spectroscopy: Set-up and spectra parameters

Raman measurements were performed with a confocal WITec alpha300 Raman microscope operating at room temperature, under ambient atmospheric conditions. The Raman laser excitation wavelength was 532 nm and the spectral resolution of the spectrometer 4–5 cm^{-1}. A Nikon 10× objective, with a 0.25 numerical aperture, was used to focus the laser on a 1.5 µm spot. The surface laser power was set at 1 mW. A spectral calibration was performed with a pure silicon test sample. Spectra were acquired directly on fragments of about 2 mm^2 of the Millipore filters for the dried samples and on 10 µL air-dried drops on a microscopy glass slide for the liquid samples. Acquisition time was kept between 0.5 and 1 s to avoid signal saturation from photosynthetic pigments' fluorescence with 1 accumulation. Single spectra, line scans and image scans with up to 30 µm × 30 µm and up to 400 image points (only for selected samples) were obtained thus collecting a minimum of 50 measurements per sample. The spectra were visualized directly with the instrument's software (Control5) and processing was further implemented with Python library RamanSpy (Georgiev *et al*., 2024) for spectra pre-processing (cosmic ray removal, cropping and background subtraction) and plotting of the average spectra from the n>50 measurements.

Results

Detection of photosynthetic pigments and genomic DNA

The detectability of biosignatures was investigated in all the samples by CLSM (Figure 1). The visualization of the FaRLiP strains *Chroococcidiopsis* sp. CCMEE 010 and CCMEE 130 with a 635-nm laser revealed an intense pigment autofluorescence indicating high content of phycobiliproteins and

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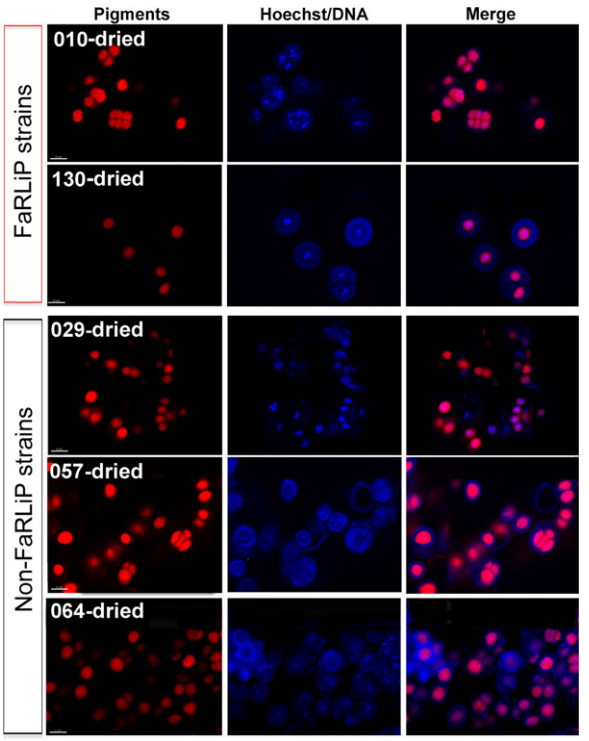

Figure 1. *CLSM imaging of photosynthetic pigments (635-nm excitation laser) and* Hoechst*-stained nucleoids (405-nm excitation laser) in* Chroococcidiopsis*. Strains CCMEE 010 and CCMEE 130 were grown under far-red light and desiccated for 6 years. Strains CCMEE 029, CCMEE 057 and CCMEE 064 were grown under visible light and desiccated for 6 years (057 and 064) or 10 years (029). Bar = 5 μm.*

chlorophylls in most of the cells of both strains, although cells with a reduced autofluorescence were also visualized. By applying a 405-nm laser, *Hoechst*-stained nucleoids could be identified in each cell regardless of the intensity of pigment autofluorescence. A blue-fluorescent envelope occurred around dried cells of strain CCMEE 130 but not in strain CCMEE 130.

Similarly, dried samples of strains CCMEE 029, CCMEE 057 and CCMEE 064 showed cells with either an intense or reduced pigment autofluorescence, each one with *Hoechst*-stained nucleoids. Images with the 405-nm laser revealed the presence of a blue-fluorescent envelope around *Hoechst*-stained dried cells of CCMEE 057 and CCMEE 064, that was absent in hydrated cells of all both strains (not shown).

Spectral features of photosynthetic pigments

The stability of the far-red shifted chlorophylls in *Chroococcidiopsis* sp. CCMEE 010 and CCMEE 130 grown for two weeks under far-red light and then air-dried and stored for 6 years, was evaluated by CLSM-λscan analysis by using excitation with a 488-nm laser (Figure 2). In strain CCMEE 010 the emission spectrum was similar in shape and intensity in both dried cells and hydrated control. A peak at 650–660 nm due to phycobiliproteins, mainly allophycocyanin, and one peak in the 675–695 nm range due to chlorophyll *a*. An additional peak in the 720–750 nm range corresponding to far-red shifted chlorophylls was also detected in both dried and hydrated cells of strains CCMEE 010 and CCMEE 130 (Figure 2A). A similar spectrum was obtained for dried and hydrated cells of strain CCMEE 130 (Figure 2B).

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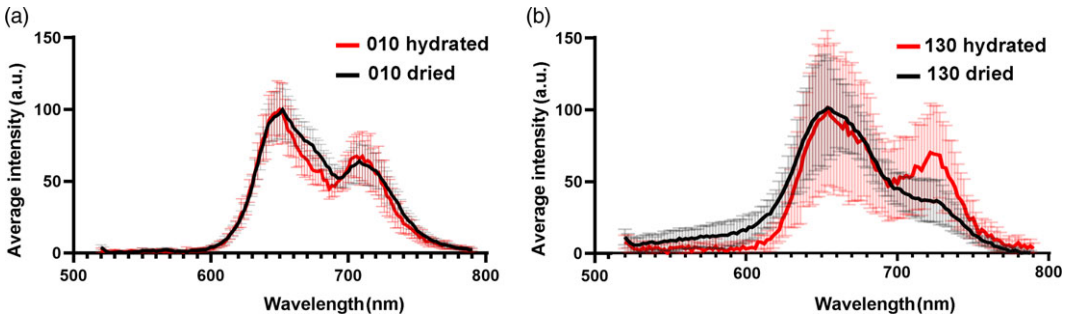

Figure 2. *CLSM-lambda-scan of photosynthetic pigments in dried FaRLiP* Chroococcidiopsis*. Strain CCMEE 010 (A) and CCMEE 130 (B). Cells were grown under far-red light and desiccated for 6 years; hydrated cells were grown in liquid cultures under far-red light and used as control. Graphs represent normalized fluorescence intensity versus emission wavelength.*

Raman signal of carotenoids

The effect of prolonged desiccation on the detectability of carotenoids in *Chroococcidiopsis* sp. CCMEE 010 and CCMEE 130 was determined by Raman analyses using a 535-nm laser for excitation by comparing cells grown under far-red light and then air-dried for 6 years with hydrated used as control (Figure 3). Each sample showed a typical Raman signal mainly due to carotenoids with three distinct peaks at 1009, 1150 and 1515 cm^{-1}, corresponding to in-plane rocking modes of CH_3, groups attached to the polyene chain coupled with C-C bonds, and in-phase C-C stretching ($\nu 2$) and C=C (ν_1) vibrations of the polyene chain in carotenoids, respectively. The spectra were normalized for clarity. No evident differences occurred in the intensity of the carotenoid main peaks among dried and hydrated cells of strains CCMEE 029, CCMEE 057 and CCMEE 064.

Detection of surface pigments

The presence of fluorescent pigments observed in the envelope of dried cells of strain CCMEE 130 after *Hoechst* staining at the CLSM (Figure 1) was further evaluated in the absence of any staining. Cells grown under far-red light showed a blue autofluorescence of the envelope when excited with a 405-nm laser and a red autofluorescence of photosynthetic pigments when excited with a 635-nm laser (Figure 4A). The CLSM λscan with a 405-nm laser of three regions of interest selected in the cell envelope yielded a spectrum with a peak of faint intensity at 430–435 nm possibly due to scytonemin (Klicki *et al*., 2018), while the fourth region of interest selected in the cytoplasm showed an intense peak in 675–695 nm range due to photosynthetic pigments (Figure 4C).

As shown in Figure 4C, no Raman signal for to scytonemin or scytonin, was detected, that generally have similar spectra with bands near 1600, 1550, 1400, 1300 and 1180 cm^{-1} (Edwards *et al*., 2023).

Survival after prolonged desiccation

The Calcein staining was used to investigate the viability of *Chroococcidiopsis* sp. CCMEE 010 and CCMEE 130 grown for two weeks under far-red light and then air-dried for 6 years (Figure 5). Before the staining dried cells were rehydrated under optimal growth conditions because the assay is based on a nonfluorescent dye that is converted by esterases into green-fluorescent Calcein. The imaging with the CLSM using a 488-nm excitation laser revealed a strong green, fluorescent signal throughout the cytoplasm of the hydrated controls of both strains CCMEE 010 and CCMEE 130 indication the viability of the cells. When dried samples were rehydrated for 2 hs no esterase activity was detected (not shown). After 72-h hydration both strains CCMEE 010 and CCMEE 130 showed a dot-like green, fluorescent

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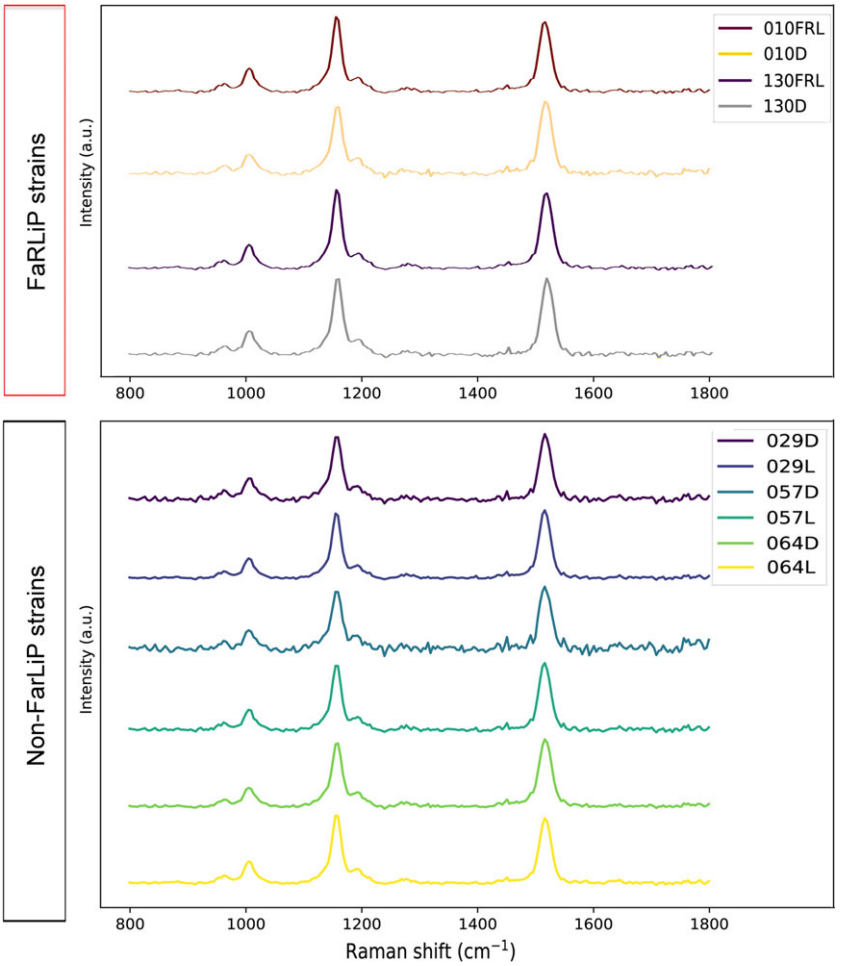

Figure 3. *Raman spectra from* Chroococcidiopsis. *Strains CCMEE 010 and CCMEE 130 were grown under far-red light and desiccated for 6 years (D); hydrated controls were grown in liquid cultures under far-red light (L). Strains CCMEE 029, CCMEE 057 and CCMEE 064 were grown under visible light and desiccated for 6 years (057 and 064) or 10 years (029) (D); hydrated controls were grown in liquid cultures under visible light (L).*

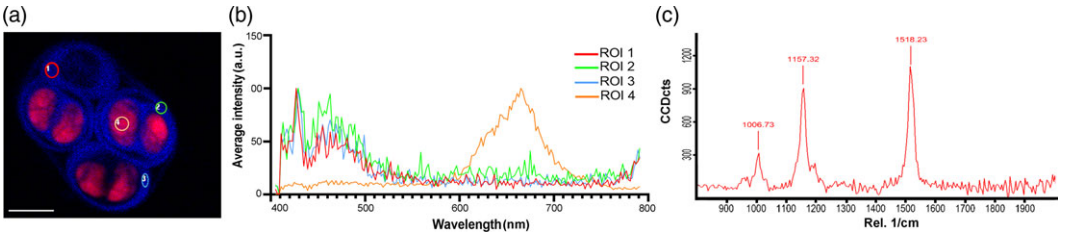

Figure 4. *CSLM and Raman analysis of* Chroococcidiopsis *sp. CCMEE 130 grown under far-red light. Merge image of optical sections obtained with a 405-nm and 635-nm laser (B); spectral profiles of four regions of interest (ROI) excited with a 405-mn laser (B). Raman spectrum obtained with a 532-nm laser. Bar = 10 μm.*

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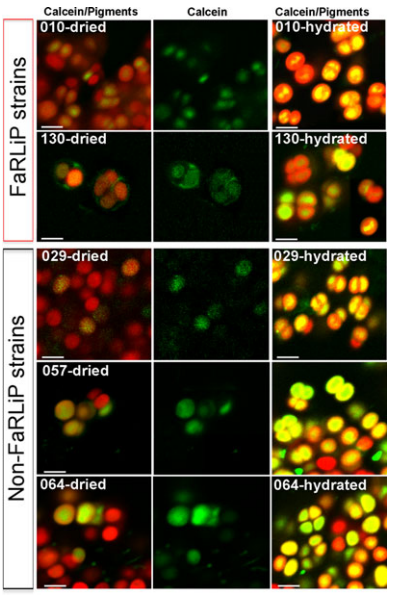

Figure 5. *Viability of* Chroococcidiopsis *examined by Calcein staining. Merge images of photosynthetic pigments (635-nm excitation laser) and* Calcein*-stained cells (488-nm excitation laser). Dried cells and hydrated controls of strains CCMEE 010 and CCMEE 029 were grown under far-red light; dried and hydrated controls of strains CCMEE 029, CCMEE 057 and CCMEE 064 were grown under visible light. Bar = 5 μm.*

signal in about 20% of the cellular population, regardless the presence of the photosynthetic pigment autofluorescence. Similarly, after 72h-rehydration, esterase activity was detected in strains CCMEE 029, CCMEE 057 and CCMEE 064.

Discussion

The biosignature detectability in *Chroococcidiopsis* sp. CCMEE 010 and CCMEE 130, two desert strains of capable of FaRLiP acclimation (Antonaru *et al*., 2023; Billi *et al*., 2022), after 6 years of desiccation was demonstrated. The combined use of CLSM and Raman spectroscopy highlighted the permanence of canonical chlorophyll *a*, far-red shifted chlorophylls, phycobilins and carotenoids as well as of *Hoechst*-stained genomic DNA, all considered unambiguous traces of life (Malaterre *et al*., 2023). No evident variation in biosignature detectability occurred between dried cells of these two strains and strains CCMEE 029, CCMEE 064 and CCMEE 057 that were previously exposed to space and to Mars-like conditions (Billi *et al*., 2019a, b). Such feature of *Chroococcidiopsis* sp. CCMEE 010 and CCMEE 130 provides a prerequisite necessary for the implementation into the BioSigN space mission that will investigate survival and biomarker detectability in dried extremophiles exposed to Mars- and open space conditions by using the foreseen ESA's Exobio facility outside the ISS (de Vera and Baqué, 2024).

CLSM imaging of dried CCMEE 010 and CCMEE 130 revealed the permanence of phycobiliproteins and chlorophylls due to their intrinsic fluorescence, that was comparable to that of dried CCMEE 029, CCMEE 064 and CCMEE 057. Moreover, the peak typical of far-red shifted chlorophylls was identified with CLSM-λscan in dried CCMEE 010 and CCMEE 130 acclimated to far-red light before desiccation. Therefore, these two FaRLiP strains are a unique reservoir of pigments to be investigated under planetary simulations to be performed in the laboratory or in space. The detectability of pigment autofluorescence is relevant in a scenario in which fluorescence microscopy

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and flow cytometry have been proposed as a potential technology for *in situ* life detection on icy moons and polar ice caps of Mars (Nadeau *et al*., 2008; Wallace *et al*., 2024). The fact that *Hoechst*- stained DNA was detected in dried *Chroococcidiopsis* cells after years of air-drying is relevant since the feasibility of using fluorescent dye labeling as a tool for life detection has been proposed in combination with the detection of intrinsically fluorescent molecules for searching sign of life on Mars (Nadeau *et al*., 2008). Moreover, Nanopore sequencing is currently under validation in diverse environments to support the search for nucleic-acid based life beyond Earth (Carr *et al*., 2020; Sutton *et al*., 2019).

Raman spectra of dried strains CCMEE 010 and CCMEE 130 showed only a slightly reduced intensity of the carotenoid peaks compared to hydrated controls, thus suggesting the capability of these two desert cyanobacteria to efficiently stabilize sub-cellular components as reported for strain CCMEE 029 (Baqué *et al*., 2020). The Raman detectability of carotenoids is relevant since miniaturized Raman instrumentation has the potential to be used in planetary exploration rovers (Edwards *et al*., 2021). Currently on Mars NASA Perseverance rover is using two miniaturized Raman spectrometers (Maurice *et al*., 2021; Razzell Hollis et al., 2022) while the ESA Rosalind Franklin rover to be launched in 2028 is equipped with a Raman Laser Spectrometer (Rull *et al*., 2017; Rull and Martínez-Frías, 2006). A Raman instrumentation has been suggested for the NASA Europa Lander Mission, a conceptual study to search for life on Europa by using *in situ* techniques (Hand *et al*., 2022).

The stability of sub-cellular components in dried cells of the two *Chroococcidiopsis* FaRLiP strains makes them a novel reservoir of biosignatures to be investigated and contribute to future planetary exploration missions to Mars as well as to biosignature detection on exoplanets. In fact, pigments like canonical chlorophylls, far-red shifted chlorophylls and carotenoids might target life beyond the photosynthetic one, just because on Earth, microbial pigmentation has been developed for different purposes beyond light capture (Barreto *et al*., 2023). Therefore, FaRLiP cyanobacteria are relevant for searching biosignatures of photosynthetic life powered by infra-red light in sub-surface environments, but also of non-photosynthetic, pigmentated life in sub-surface environments supported by chemical energy (Cockell *et al*., 2016). Moreover, since the absorption and reflection of light harvesting pigments can serve as surface biosignatures for exoplanets (Schwieterman *et al*., 2018), FaRLiP cyanobacteria are suitable model system for laboratory simulations to investigate the boundary conditions of the habitability of exoplanets around M stars and detectability of exotic photosynthetic life. Because in literature theoretical investigations and some indices are even considering a potential of photosynthesis in deep sea and hydrothermal areas (Beatty *et al*., 2005; Yurkov *et al*., 1999), the potential of photosynthesis in the deep sea using far IR cannot be neglected. Therefore, a small likelihood to postulate the presence of photosynthesizing organisms in the icy ocean worlds in our solar system could be possible (Fisher *et al*., 2024).

CLSM imaging of strain CCMEE 130 suggested the presence of scytonemin-like compounds that were secreted in the cell envelope and that yielded an emission at about 430–435 nm when exited with a 405-nm laser (Klicki *et al*., 2018). Such a capability is relevant since scytonemin is a UV-absorbing pigment that possesses also antioxidant properties (Sen and Mallick, 2022). However, the presence of scytonemin, or scytonin, in the envelope of CCMEE 130 grown under far-red light was not confirmed by Raman spectroscopic probing. Nevertheless, the production of UV-screening compounds in FaRLiP cyanobacteria under far-red light is largely unknown and reported so far only for *Chlorogloeopsis fritschii* sp. PCC 6912 (Llewellyn *et al*., 2020). So, it could be speculated that, if produced the amount of scytonemin in CCMEE 130 was not enough to be detected. Indeed, the synthesis of scytonemin has been reported for two desert *Chroococcidiopsis* strains in response to other stress rather than UV radiation, for instance under osmotic stress in the absence of UV radiation (Dillon *et al*., 2002; Casero *et al*., 2021), but also in response of periodic desiccation under UV radiation (Fleming and Castenholz, 2007).

Finally, the fact the number of survivors scored among *Chroococcidiopsis* sp. CCMEE 010 and CCMEE 130 desiccated for 6 years was comparable to that of CCMEE 029 after for 4 years of desiccation (Billi, 2009), further supports their suitability for implementation in the BioSigN space mission that foresees one-year exposure of dried extremophiles to Mars- and icy-moon simulation outside the ISS.

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Moreover, based on the comparable stability of their sub-cellular components with that of strains CCMEE 029, CCMEE 064 and CCMEE 057 already tested in space, it is anticipated that post-flight analysis might contribute to gather novel insights into survival potential and biosignature detectability. The biosignature stability and survivability scored in the present work after prolonged desiccation are an important prerequisite to future investigation on how extremophiles respond to deep space, as proposed by the BioMoon space mission that aims to expose to the lunar environment dried cells as well as cells that will be rehydrated on the Moon after exposure to deep space (Cockell *et al.*, 2024).

Author contributions. Conceptualization: D.B.; Methodology, G.d.S.; M.Ba.; Formal Analysis: G.d.S. and M.Ba.; Writing – Original Draft Preparation: G.d.S.; D.B.; Writing – Review & Editing: D.B., M.Ba, M.Be.; M.E.M.G. and J.P.d.V.; Supervision: D.B.; Funding Acquisition: D.B. All authors have read and agreed to the published version of the manuscript.

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