

# Preservation of Biomarkers from Cyanobacteria Mixed with Mars Like Regolith Under Simulated Martian Atmosphere and UV Flux

Mickael Baqué<sup>1</sup> · Cyprien Verseux<sup>1</sup> · Ute Böttger<sup>2</sup> ·  
Elke Rabbow<sup>3</sup> · Jean-Pierre Paul de Vera<sup>2</sup> ·  
Daniela Billi<sup>1,4</sup>

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**Abstract** The space mission EXPOSE-R2 launched on the 24th of July 2014 to the International Space Station is carrying the BIOMEX (BIOlogy and Mars EXperiment) experiment aimed at investigating the endurance of extremophiles and stability of biomolecules under space and Mars-like conditions. In order to prepare the analyses of the returned samples, ground-based simulations were carried out in Planetary and Space Simulation facilities. During the ground-based simulations, *Chroococcidiopsis* cells mixed with two Martian mineral analogues (phyllosilicatic and sulfatic Mars regolith simulants) were exposed to a Martian simulated atmosphere combined or not with UV irradiation corresponding to the dose received during a 1-year-exposure in low Earth orbit (or half a Martian year on Mars). Cell survival and preservation of potential biomarkers such as photosynthetic and photoprotective pigments or DNA were assessed by colony forming ability assays, confocal laser scanning microscopy, Raman spectroscopy and PCR-based assays. DNA and photoprotective pigments (carotenoids) were detectable after simulations of the space mission (570 MJ/m<sup>2</sup> of UV 200–400 nm irradiation and Martian simulated atmosphere), even though signals were attenuated by the treatment. The fluorescence signal from photosynthetic pigments was differently preserved after UV irradiation, depending on the thickness of the samples. UV irradiation caused a high

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✉ Daniela Billi  
billi@uniroma2.it

<sup>1</sup> Department of Biology, University of Rome Tor Vergata, Rome, Italy

<sup>2</sup> German Aerospace Center (DLR), Institute of Planetary Research, Berlin, Germany

<sup>3</sup> Radiation Biology Department, German Aerospace Center (DLR), Institute of Aerospace Medicine, Köln, Germany

<sup>4</sup> Dipartimento di Biologia, Università di Roma “Tor Vergata”, Rome, Italy

background fluorescence of the Martian mineral analogues, as revealed by Raman spectroscopy. Further investigation will be needed to ensure unambiguous identification and operations of future Mars missions. However, a 3-month exposure to a Martian simulated atmosphere showed no significant damaging effect on the tested cyanobacterial biosignatures, pointing out the relevance of the latter for future investigations after the EXPOSE-R2 mission. Data gathered during the ground-based simulations will contribute to interpret results from space experiments and guide our search for life on Mars.

**Keywords** Astrobiology · Cyanobacteria · Biosignatures · Martian regoliths · EXPOSE-R2

## Introduction

Cyanobacteria are among the oldest life forms on Earth, with earliest evidence in the fossil record between 3.5 and 2.8 billion years ago (Schopf 2002; Schwartzman et al. 2008; Bosak et al. 2013), at a time when environmental conditions (noteworthy water availability and UV irradiation) are thought to be similar on the Earth and on Mars (McKay 1997; Wynn-Williams and Edwards 2000). If life exists on present-day Mars, it is likely underground and photosynthesis-independent, given the harsh conditions at the surface where solar light is available (see, e.g., Westall et al. 2013; Cockell 2014). However, if environmental conditions on Mars were life-permissive long enough for life to originate there (McKay 2010; Westall et al. 2013; Cockell 2014) and to evolve metabolic systems as complex as oxygenic photosynthesis, and/or if life was exchanged between the two planets (see, e.g., Nicholson 2009), remains from life forms analogous to desiccation- and radiation-resistant cyanobacteria or other photosynthetic microorganisms might still exist and be detectable on Mars.

Since the Viking landers in 1976, no hardware landed on Mars was dedicated to the search for life there. However, two rovers planned to be sent to Mars in the near future will be so: the ExoMars (Barnes et al. 2006; Vago et al. 2006) and Mars 2020 (Fries et al. 2010) rovers will carry instruments to detect organic molecules and potential biomarkers on Mars in 2018–2020. Miniaturized Raman spectrometers such as the Raman Laser Spectrometer (Barnes et al. 2006; Vago et al. 2006; Hutchinson et al. 2014) will be on-board the ExoMars rover, while an UV Raman spectrometer will be on-board Mars2020 (Tarcea et al. 2007; Fries et al. 2010).

Studies on terrestrial life in extreme environments guide our instrument choices and target biosignatures for future missions to Mars (Horneck 2000; Rothschild and Mancinelli 2001). Extensive field and laboratory investigations have focused on carotenoids and photosynthetic pigments in Mars terrestrial analogues, for instance via the development of Raman and fluorescence emission techniques (Edwards et al. 2005a; Vítek et al. 2009a; Smith et al. 2012; Jorge-Villar and Edwards 2013; Groemer et al. 2014). In preparation for future missions to Mars, a focus was given to their optimization in the presence of Martian analogue minerals (Vítek et al. 2009a; Böttger et al. 2012; Vandenabeele et al. 2012; Jehlička et al. 2014) along with the assessment of potential biosignature degradation under Mars-like conditions (Dartnell et al. 2011, 2012; Dartnell and Patel 2013; Stromberg et al. 2014).

In addition, technologies based on laser excitation of autofluorescent biomolecules have been proposed to survey potential target regions on Mars before further analysis (Griffiths et al. 2008; Storrie-Lombardi and Sattler 2009; Dartnell and Patel 2013; Smith et al. 2014b). In this context, cyanobacteria are relevant models as they contain

photoprotective and antioxidant molecules (e.g., carotenoids), ultraviolet screening compounds (e.g., scytonemin), photosynthetic pigments (e.g., chlorophyll *a* and phycobiliproteins) and their degradation products (e.g., porphyrins), which have been classified as high priority targets for biomolecule detection (Parnell et al. 2007). Among them  $\beta$ -carotene is of particular interest due to its stability and easy identification by Raman spectroscopy (due to a strong Raman resonance effect), even when embedded in a mineral matrix (e.g., Wynn-Williams and Edwards 2000; Edwards et al. 2005b; Vitek et al. 2010; Böttger et al. 2012; Vandenabeele et al. 2012). Moreover, even fossilized carotenoids (such as carotane) remain easily identifiable by Raman spectroscopy (Marshall and Marshall 2010); carotenoids and fossil carotenoids (such as carotane) were identified with GC/MS and Raman spectroscopy in 1.44 million-year-old halite brine inclusions (Winters et al. 2013) and in 1.64-billion-year-old samples, respectively (Lee and Brocks 2011).

Likewise, DNA is also considered a high priority biosignature target due to its ubiquity in organisms on Earth (Parnell et al. 2007; Lyon et al. 2010; Martins 2011). Instruments for its detection on Mars, based on biochip technologies (Parro et al. 2008; Martins 2011; Parro et al. 2011; Sims et al. 2012) or PCR methods (Isenbarger et al. 2008; Carr et al. 2013), are under development.

To ensure unambiguous identification of potential biomarkers, degradation of biosignatures by environmental stressors and potential interference of surrounding minerals should be taken into account. In this context, the BIOlogy and Mars EXperiment (BIOMEX) aims at investigating not only the endurance of extremophiles mixed with Lunar and Martian mineral analogues, but also the stability/degradation of their macromolecules under space and Mars-like conditions (de Vera et al. 2012). BIOMEX is one of the four astrobiology experiments of the European Space Agency (ESA) EXPOSE-R2 mission, started on the 24th of July 2014 with its launch to the International Space Station (ISS) and its installation on the 18th of August 2014 on the outside of the Russian Zvezda module. In the EXPOSE-R2 facility, the astrobiological samples will be exposed to space and simulated Martian conditions for 12–18 months (de Vera et al. 2012).

Among the selected extremophiles and macromolecules for the BIOMEX experiment is a desert cyanobacterial strain, *Chroococidiopsis* sp. CCMEE 029. The endurance of this cyanobacterium under extreme conditions including deserts on Earth (Friedmann 1980; Grilli Caiola and Billi 2007; Billi et al. 2013), prolonged laboratory air-dry storage (at least up to 4 years; Billi 2009), high doses of ionizing radiation (up to 15 kGy; Billi et al. 2000) and UVC irradiation (up to 13 kJ/m<sup>2</sup>; Baqué et al. 2013b) makes it a relevant model when assessing biosignature stability over prolonged periods under Martian-simulated conditions.

In the present paper, we investigated the role on the preservation of cyanobacterial biosignatures of Phyllosilicatic Mars Regolith Simulant (P-MRS) and Sulfatic Mars Regolith Simulant (S-MRS). P-MRS and S-MRS reflect minerals formed during the Noachian (Early Mars) and Hesperian/Amazonian (Late Mars) epochs, respectively (Bibring et al. 2006; Mustard et al. 2008; Böttger et al. 2012; de Vera et al. 2012; Ehlmann and Edwards 2014). Our main motivation is to contribute to the search for traces of life forms naturally present on Mars, but these biosignatures might also be useful for the detection of human cyanobacterial contamination of Mars caused by human activity. Such contamination might come from spacecraft contaminated on the ground or, in a more distant future, leak from a biological life support system (see Verseux et al. 2015). As a secondary objective, we also

assessed the survival of *Chroococcidiopsis* spp. under space- and Mars-like stress factors. Such data can be elements in the debate around the lithopanspermia theory (for a review, see Nicholson 2009), but might also be useful in the assessment of the risk of Mars's contamination by terrestrial cyanobacteria. The experiments reported here were performed in the framework of ground-based Scientific Verification Tests (SVTs), using facilities of the Planetary and Space Simulation (PSI) at the DLR-Institute of Aerospace Medicine in Köln (Germany) and for analysis the Astrobiology Raman Lab at the DLR-Institute of Planetary Research in Berlin (Germany) and the Department of Biology of the University of Rome Tor Vergata (Italy), in preparation of future analyses of samples from the BIOMEX experiment. Dried cells of *Chroococcidiopsis* sp. CCMEE 029 mixed with P-MRS or S-MRS were exposed to a Mars-like atmosphere in combination or not with  $570 \text{ M J/m}^2$  of polychromatic UV radiation (200–400 nm). This UV dose simulated the one expected in a 1-year exposure mission in low Earth orbit, or 383 sols on the Martian surface (Cockell et al. 2000; Rabbow et al. 2012; Dachev et al. 2014; Rabbow et al. 2014). After exposure, the detectability of biosignatures from photosynthetic pigments (chlorophyll *a* and phycobiliproteins), the photoprotective pigment  $\beta$ -carotene and DNA, was evaluated by confocal laser scanning microscopy, Raman spectroscopy and PCR-based assay, respectively. In addition, cyanobacterial survival was assessed by testing colony forming ability.

## Material and Methods

### Culture Conditions, Martian Mineral Analogues and Sample Exposure

*Chroococcidiopsis* sp. CCMEE 029 was isolated by Roseli-Ocampo Friedmann from cryptoendolithic growth in sandstone in the Negev Desert (Israel). The strain is part of the Culture Collection of Microorganisms from Extreme Environments (CCMEE) established by E. Imre Friedmann and now maintained at the Department of Biology of the University of Rome Tor Vergata. Cyanobacteria were grown under routine conditions at 25 °C in BG-11 medium under a photon flux density of  $40 \mu\text{mol/m}^2/\text{s}$  provided by fluorescent cool-white bulbs with a 16/8 h light/dark cycle.

Multilayered planktonic samples were obtained by plating pellets from 2-month-old liquid cultures ( $\sim 2 \times 10^9$  cells) on top of BG-11 agarized medium and mixed (or not) with  $\sim 0.2$  g of a Mars regolith analogue mixture, either Phyllosilicatic Mars Regolith Simulant (P-MRS) or Sulfatic Mars Regolith Simulant (S-MRS). The composition of the two Mars mineral analogues (Table 1) was prepared by the Naturkunde-Museum (Berlin) in the framework of the Helmholtz-Alliance “Planetary Evolution and Life”. Size distribution of the two mineral mixtures is reported in Fig. 1. Samples were allowed to dry before cutting disks to the size of the exposure carrier cells ( $\sim 115 \text{ mm}^2$ ) under sterile conditions. Following the accommodation plan scheduled for the EXPOSE platform, SVTs allowed only one replicate per sample (de Vera et al. 2012).

Samples were exposed inside the PSI chamber to a simulated Martian atmosphere (95.55 %  $\text{CO}_2$ , 2.70 %  $\text{N}_2$ , 1.60 % Ar, 0.15 %  $\text{O}_2$ ,  $\sim 370$  ppm  $\text{H}_2\text{O}$ , Praxair Deutschland GmbH) to a final pressure of 780 Pa for 89 days, in combination or not with polychromatic UV (200–400 nm) radiation produced by the solar simulator SOL2000. The dose of  $570 \text{ MJ/m}^2$  was reached by running the SOL2000 for 125 hours at  $1271 \text{ W/m}^2$ . Neutral density filters attenuating 99.9 % of the radiation spectrum (0.1 % ND filter) were used for samples exposed

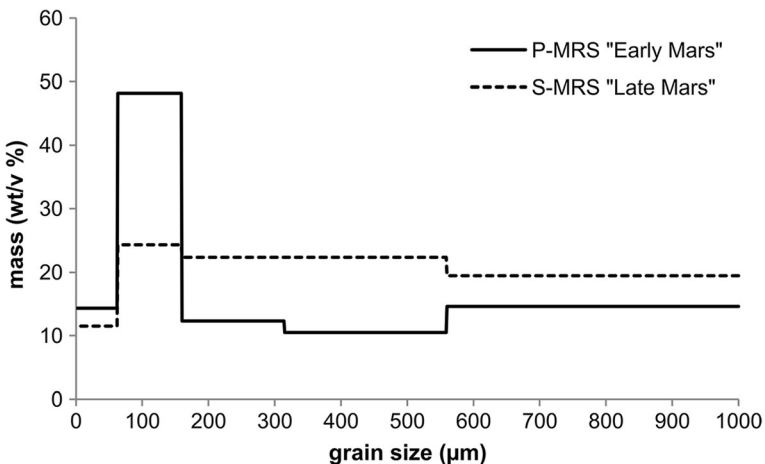
**Table 1** Mineralogical composition of S-MRS and P-MRS in weight/volume percent (modified from Böttger et al. 2012)

Component	P-MRS (wt/v %)	S-MRS (wt/v %)
Pyroxene, Plagioclase, Amphibole, Ilmenite (Gabbro)	3	32
Olivine(Mg,Fe) <sub>2</sub> SiO <sub>4</sub>	2	15
Quartz SiO <sub>2</sub>	10	3
Hematite Fe <sub>2</sub> O <sub>3</sub>	5	13
Montmorillonite [(Na,Ca) <sub>0.33</sub> (Al,Mg) <sub>2</sub> Si <sub>4</sub> O <sub>10</sub> (OH) <sub>2</sub> x H <sub>2</sub> O]	45	–
Chamosite [(Fe <sup>2+</sup> ,Mg,Fe <sup>3+</sup> ) <sub>5</sub> Al(Si <sub>3</sub> Al)O <sub>10</sub> (OH,O) <sub>8</sub> ]	20	–
Kaolinite Al <sub>2</sub> Si <sub>2</sub> O <sub>5</sub> (OH) <sub>4</sub>	5	–
Siderite Fe(CO <sub>3</sub> )	5	–
Hydromagnesite Mg(CO <sub>3</sub> )	5	–
Goethite FeO(OH)	–	7
Gypsum Ca(SO <sub>4</sub> )x2H <sub>2</sub> O	–	30

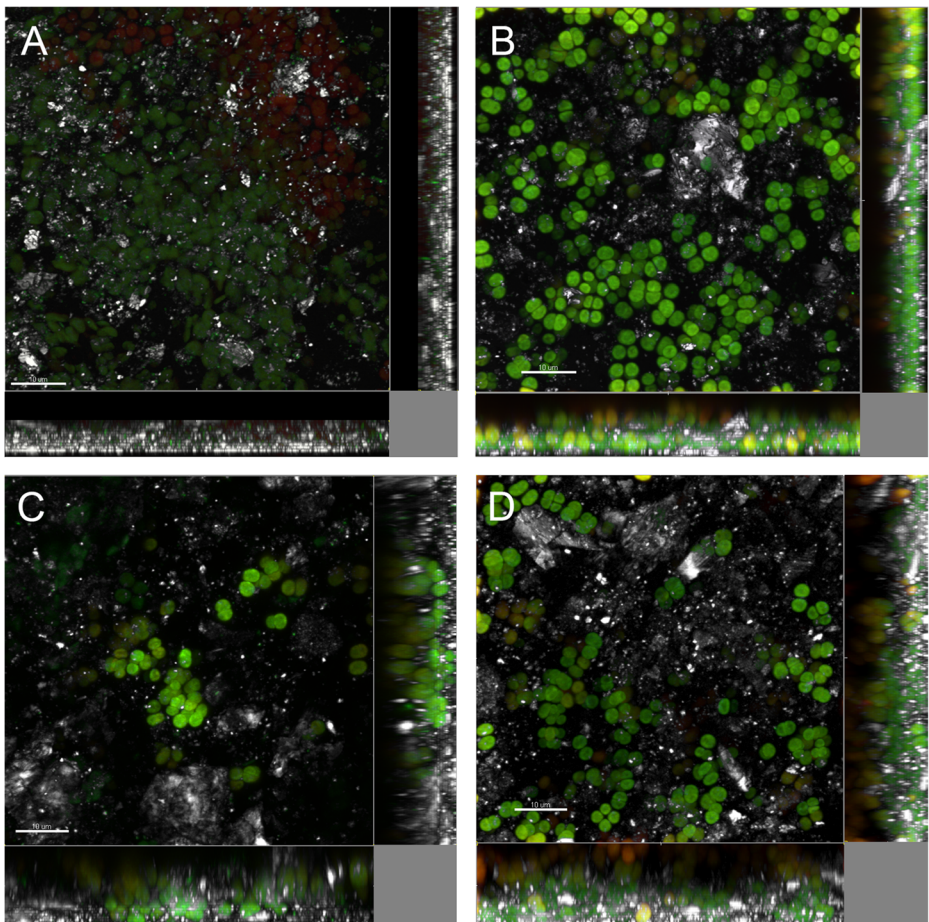
without minerals. Irradiation was performed at intervals during working hours to allow constant monitoring of the temperature during irradiation to avoid the risk of sample heating. Temperature varied from  $-25$  to  $30$  °C throughout the experiment but remained below  $10$  °C during irradiation times. Laboratory controls were kept at DLR in the dark, at room temperature.

### Confocal Laser Scanning Microscopy

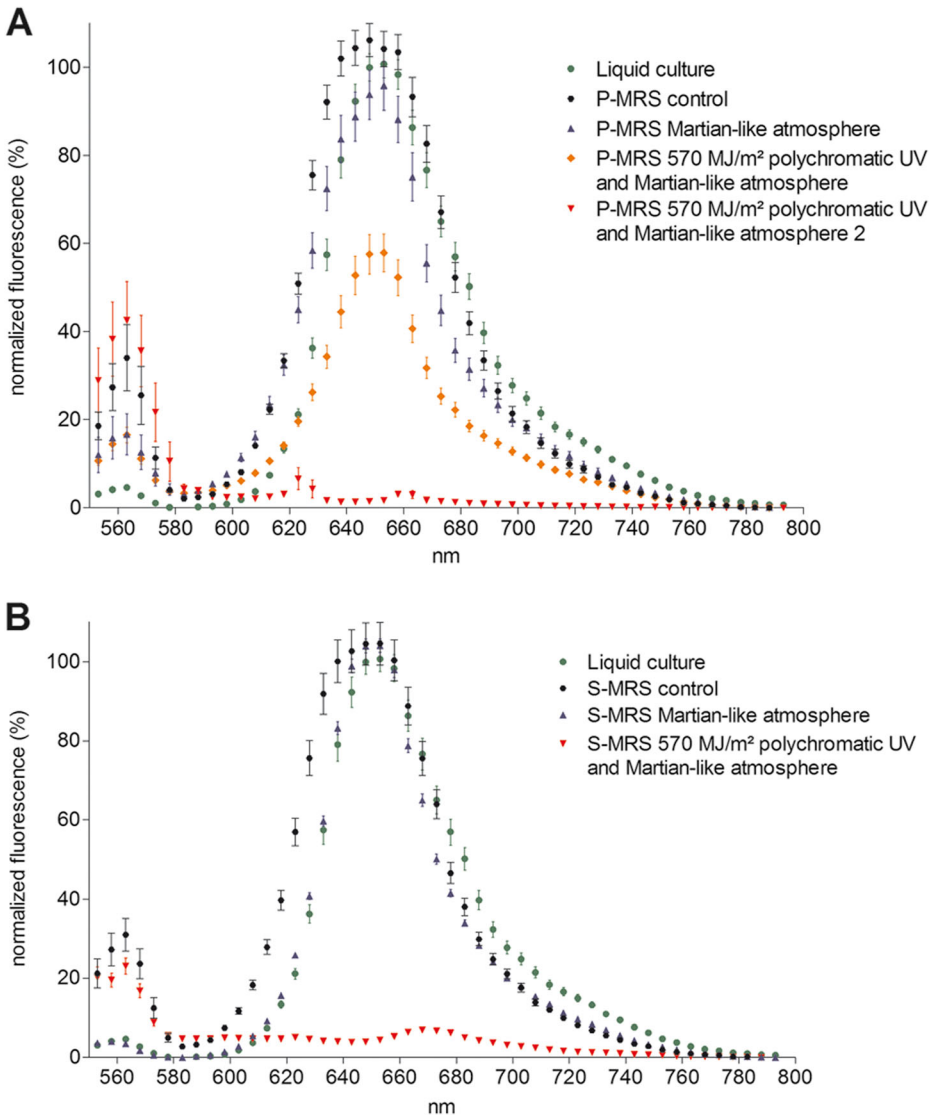
Microscopy analyses were performed on fragments of approximately  $2\text{ mm}^2$ , using a confocal laser scanning microscope (CLSM; Olympus Fluoview 1000 Confocal Laser Scanning System) coupled with spectral analysis (CLSM-Ascan). Photosynthetic pigment chlorophyll *a* and phycobiliproteins autofluorescence was investigated by successively exciting the samples with 543-nm and 635-nm lasers, and collecting the

**Fig. 1** Size distribution of the mineral mixtures determined by sieving

emitted fluorescence in the 555–609 and 655–755 nm channels, respectively. Mineral reflectance was recorded after excitation at 488 nm by collecting the reflective signal between 490 and 510 nm. Three-dimensional images were captured every 0.5  $\mu\text{m}$  and processed with Imaris v. 6.1.0 software (Bitplane AG Zürich, Switzerland) to obtain maximum intensity projections. The spectral analysis of regions of interest (ROI) was performed using the 543-nm laser at 54 % of the maximum power (0.54 mW) and collecting the emission from 543 to 800 nm. Mean fluorescence intensity (MFI) was measured on  $n \geq 15$  cells from three different areas. Spectral analyses on minerals were performed using the 488 and 543-nm lasers at 1 mW and collecting the emission of ROIs from 488 to 800 nm and from 543 to 800 nm, respectively. Curve plotting and normalization were performed using the GraphPad Prism program (GraphPad Software, San Diego, CA).



**Fig. 2** CLSM images with lateral projections showing mineral reflectance (white color) and autofluorescence of phycobiliproteins and chlorophyll *a* (555–609 and 655–755 nm channels) of dried *Chroococcidiopsis* cells mixed with S-MRS (a, b) or P-MRS (c, d) after exposure to simulated Martian atmosphere (b, d) or in combination with 570 MJ/m<sup>2</sup> of polychromatic UV (a, c). Scale bar is 10  $\mu\text{m}$



**Fig. 3** CLSM-λscan with 543 nm laser at 0.54 mW of photosynthetic pigments in *Chroococcidiopsis* cells from liquid culture, or mixed with P-MRS (a) or S-MRS (b) mineral analogues, unexposed (control) or exposed to all simulated Martian conditions (570 MJ/m<sup>2</sup> polychromatic UV and simulated Martian atmosphere) or simulated Martian atmosphere alone. Data points represent normalized fluorescence intensity at 653 nm ± standard error for  $n \geq 15$  cells as a function of emission wavelength

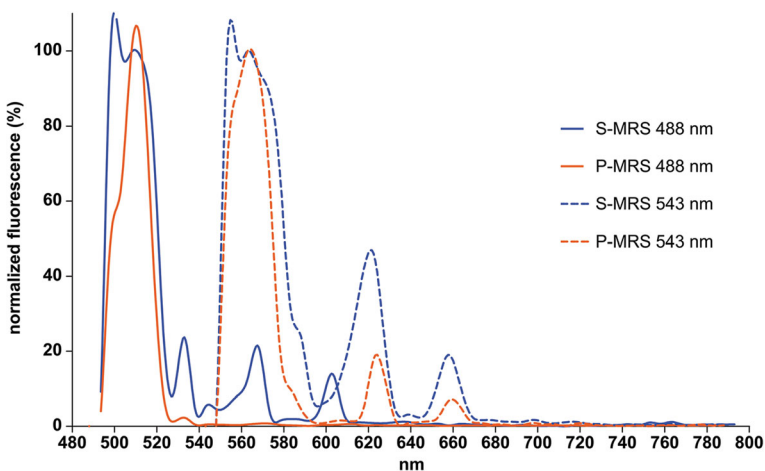
### Raman Confocal Microscopy

Raman measurements were performed in the Astrobiology-Raman-Lab at the DLR-Institute of Planetary Research (Berlin) with a confocal Raman microscope (Witec alpha300 R system, <http://www.witec.de/en/products/raman/alpha300r/>) at room temperature under ambient atmospheric conditions. The Raman laser excitation

**Table 2** Fluorescence emission at 563 nm and 653 nm of photosynthetic pigments in *Chroococidiopsis* cells from liquid culture and dried sample (controls) and of dried cells mixed with P-MRS or S-MRS exposed to Mars simulations. Data are expressed as percent of the emission intensity at 653 nm of liquid culture ( $\pm$  standard error for  $n \geq 15$  cells)

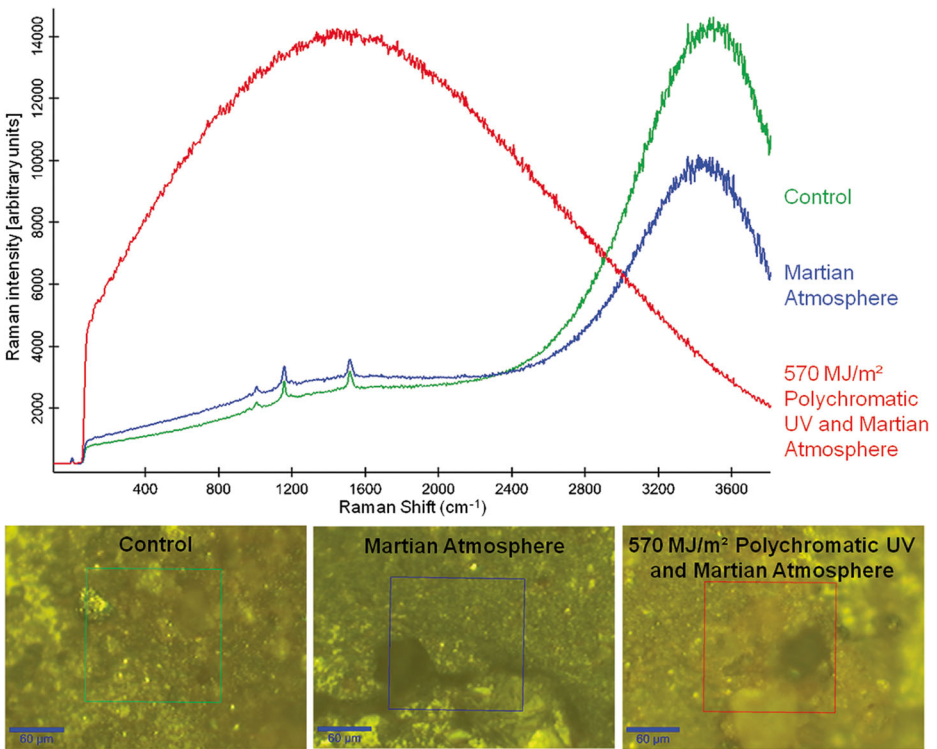
	Liquid culture		Dried sample Control	Dried sample 570 MJ/m <sup>2</sup> +Martian atmosphere	Martian atmosphere
563 nm	4.6 $\pm$ 0.4	P-MRS	34.0 $\pm$ 7.5	16.5 $\pm$ 1.8 42.5 $\pm$ 8.8	16.7 $\pm$ 4.6
		S-MRS	31.0 $\pm$ 4.1	23.0 $\pm$ 2.0	3.5 $\pm$ 0.5
653 nm	100.7 $\pm$ 3.2	P-MRS	104.1 $\pm$ 4.1	57.9 $\pm$ 4.3 1.6 $\pm$ 0.1	95.8 $\pm$ 5.6
		S-MRS	104.6 $\pm$ 5.3	4.4 $\pm$ 0.1	101.0 $\pm$ 1.7

wavelength was 532 nm, which is the value proposed for the RLS instrument on ExoMars, and the surface laser power 1 mW, a compromise between measurement time in the lab and the RLS irradiance values (Hutchinson et al. 2014). The spectral resolution of the spectrometer was 4–5 cm<sup>-1</sup>. A Nikon 10X objective was used to focus the laser on a 1.5  $\mu$ m spot. Based on the optimizations made by Böttger et al. (2012) for the detection of  $\beta$ -carotene in the same mineral matrices, acquisition time was kept at 1 s to avoid signal saturation from chlorophyll fluorescence with 1 accumulation. Single spectra, line scans and image scans with up to 150 $\times$ 150  $\mu$ m and up to 2500 image points were obtained. The coverage of the  $\beta$ -carotene signal on image scans was calculated in two steps. First, signal recognition was achieved using the width filter function of the WITec Project software, centered on the three main peaks of the  $\beta$ -carotene spectrum (990<1009<1030, 1140<1159<1170 and 1490<1518<1550 cm<sup>-1</sup>), to generate image masks so that white areas denote  $\beta$ -carotene



**Fig. 4** CLSM- $\lambda$ scan of P-MRS (orange) or S-MRS (blue) mineral analogues spotted on a glass slide with a 488-nm (plain lines) and 543-nm (dashed lines) laser at 1 mW



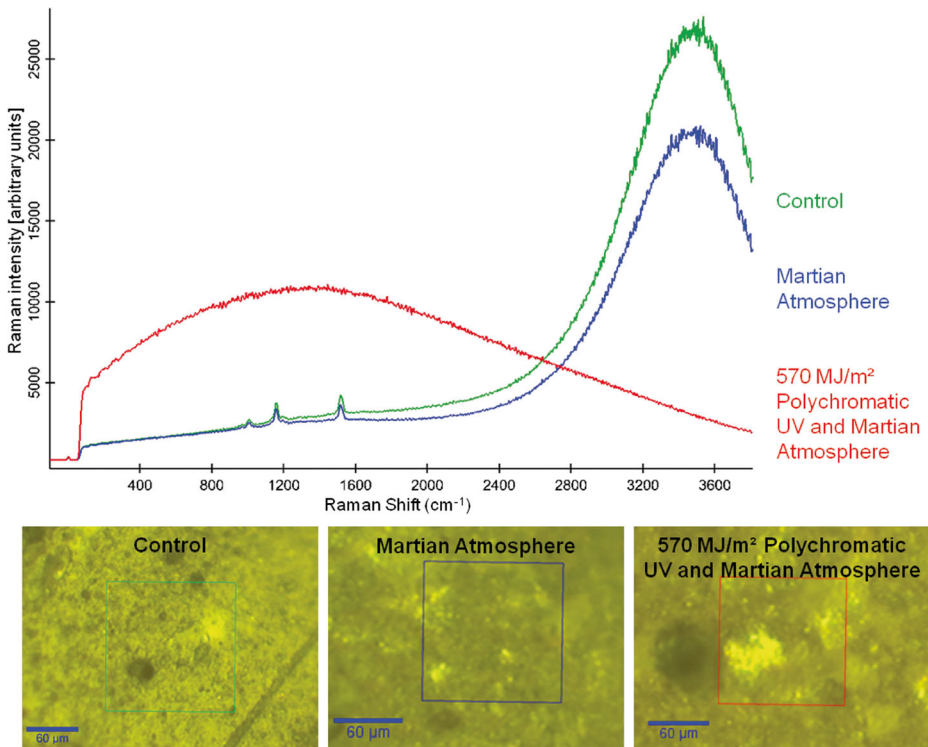


**Fig. 5** Raman spectra of  $\beta$ -carotene in *Chroococcidiopsis* mixed with S-MRS and exposed to Martian UV flux and atmosphere. *Top*: average uncorrected Raman spectra recorded on image scans of unexposed control and after exposure to simulated Martian atmosphere and after exposure to 570 MJ/m<sup>2</sup> full UV and simulated Martian atmosphere. The same scale was applied to all spectra. *Bottom*: microscopy images with the areas used for the image scans highlighted by *color squares* (scale bar is 60  $\mu$ m)

signal and black areas its absence. Then, the resulting images were processed with the threshold function of Adobe Photoshop to calculate the coverage percentage of white versus black areas.

**Random Amplification of Polymorphic DNA (RAPD) Assay**

Small fragments (about 2 mm<sup>2</sup>) of control and exposed dried samples were resuspended in 50  $\mu$ L of sterile MilliQ water, washed twice by centrifuging 10 min at 10,000 rpm and resuspended in 20  $\mu$ L sterile MilliQ water. They were then subjected to three cycles of freeze-thawing (-80 °C for 10 min and 60 °C for 1 min) and boiled for 10 min. After centrifugation, 5  $\mu$ L of lysed cell suspensions were used for PCR amplification with the HIP1-CA primer (5'-GCGATCGCCA-3'). PCR conditions were as follows: 94 °C for 3 min, 30 cycles at 94 °C for 30 s, 37 °C for 30 s and 72 °C for 1 min, and 72 °C for 7 min. Genomic DNA from 1-month-old cultures of *Chroococcidiopsis* was used as control. PCR products were loaded on a 1.5 % agarose gel and electrophoresis was run at 90 V in TAE buffer. RAPD patterns were revealed under UV lamp with ethidium bromide staining.



**Fig. 6** Raman spectra of  $\beta$ -carotene in *Chroococidiopsis* mixed with P-MRS and exposed to Martian UV flux and atmosphere. *Top*: average uncorrected Raman spectra recorded on image scans of unexposed control and after exposure to simulated Martian atmosphere and after exposure to 570 MJ/m<sup>2</sup> full UV and simulated Martian atmosphere. The same scale was applied to all spectra. *Bottom*: microscopy images with the areas used for the image scans highlighted by *color squares* (scale bar is 60  $\mu$ m)

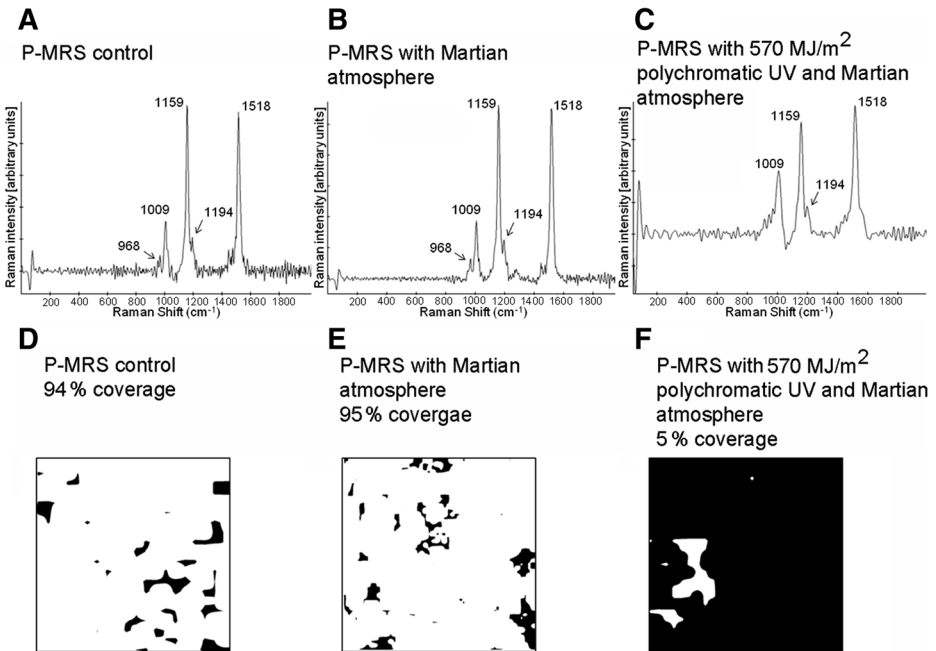
## Survival Assessment

Irradiated samples were diluted in BG11 and plated on agarized BG11 medium. Plates were incubated under routine conditions and formed colonies were scored. *Chroococidiopsis* usually forms cell aggregates of 2 to 10 cells; each aggregate was here counted as one colony forming unit (CFU). Due to the low amount of samples exposed when no survivors were scored on the first attempt, the number of cells per plate was increased from 10<sup>6</sup> to 10<sup>8</sup>.

## Results

### Remnants of Photosynthetic Pigments as Revealed by CLSM Imaging and Spectral Analysis

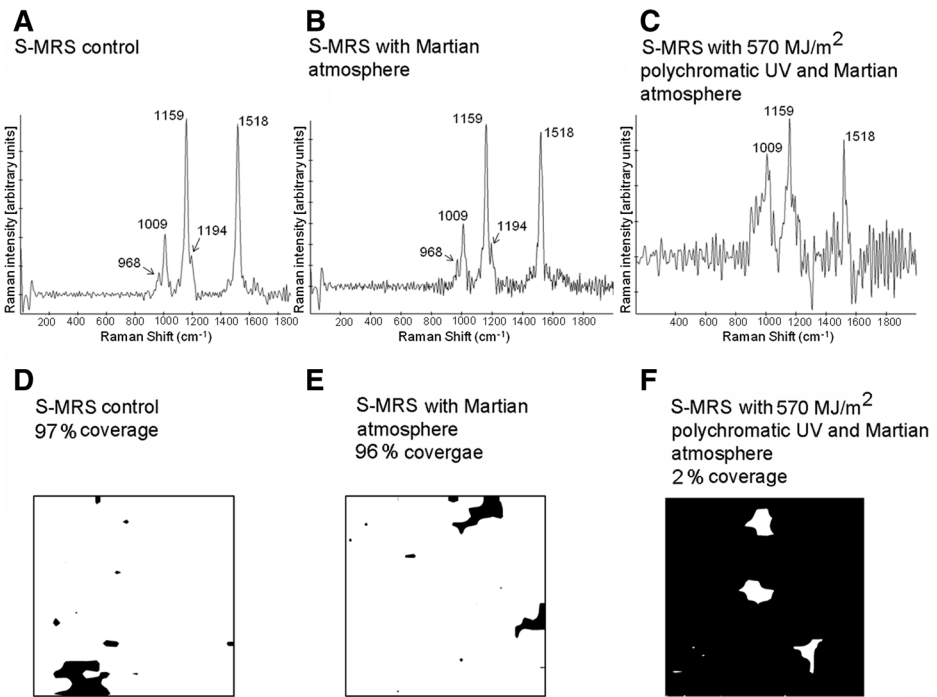
CLSM imaging of dried samples of *Chroococidiopsis* sp. CCMEE 029 mixed with minerals revealed that cells were non-homogeneously associated with S-MRS (Fig. 2a and b) or P-MRS (Fig. 2c and d). The experimental set-up yielded dried samples of about 10  $\mu$ m, corresponding



**Fig. 7** Raman spectra of  $\beta$ -carotene in *Chroococcidiopsis* mixed with S-MRS and exposed to Martian UV flux and atmosphere. *Top row*: background corrected spectra for  $\beta$ -carotene peaks in unexposed cells (a), after exposure to simulated Martian atmosphere (b) and after exposure to 570 MJ/m<sup>2</sup> polychromatic UV and simulated Martian atmosphere (c). *Bottom row*: reconstructed  $\beta$ -carotene signal coverage of the image scans (100  $\times$  100  $\mu$ m) of images shown in Fig. 5 in unexposed cells (d), after exposure to simulated Martian atmosphere (e) and after exposure to 570 MJ/m<sup>2</sup> polychromatic UV and simulated Martian atmosphere (f). Coverage was calculated using the Width Filter function of the WITec Project software in combination with the threshold filter function of Adobe Photoshop (varied according to real spectra positions). *White areas* denote  $\beta$ -carotene signal presence, *black areas* its absence

to 2–3 cell layers when mixed with S-MRS (Fig. 2a and b), but were significantly thicker when mixed with P-MRS with a total thickness of about 15  $\mu$ m corresponding to 4–5 cell layers (Fig. 2c and d). This is probably due to the different grain size distribution in the two prepared analogues, P-MRS presenting thinner grains than S-MRS (Fig. 1). After exposure to simulated Martian atmosphere, CLSM analysis revealed that no morphological alteration occurred in dried cells mixed with S-MRS (Fig. 2b) or P-MRS (Fig. 2d) compared to controls (not shown). On the other hand, exposure to simulated Martian atmosphere in combination with 570 MJ/m<sup>2</sup> of polychromatic UV irradiation resulted in bleached photosynthetic pigments and altered cell morphology in cells mixed with S-MRS (Fig. 2a) or with P-MRS (Fig. 2c, upper left corner). However, cells with an unaltered morphology and unbleached photosynthetic pigments were scored in samples mixed with P-MRS (Fig. 2c, middle).

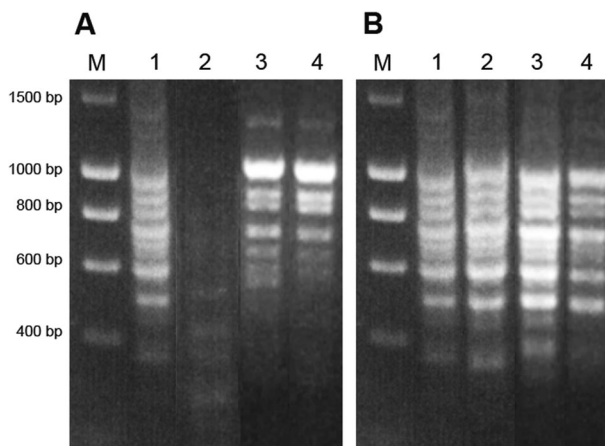
CLSM- $\lambda$ scan with a 543-nm laser was used to reveal the effect of simulated Martian atmosphere and polychromatic UV radiation on the emission spectrum of the photosynthetic pigments of dried cells of *Chroococcidiopsis* sp. CCMEE 029 mixed with P-MRS (Fig. 3a) or S-MRS (Fig. 3b). Cells from liquid culture showed an emission spectrum with a peak at 650–660 nm due to the overlapping emission of phycobiliproteins (phycocyanin and allophycocyanin) and chlorophyll *a* (Roldán et al. 2004). The emission spectrum of dried cells without minerals was identical to that of liquid controls (not shown), whereas the emission spectrum of unexposed dried cells mixed with P-MRS or S-MRS presented an



**Fig. 8** Raman spectra of  $\beta$ -carotene in *Chroococcidiopsis* mixed with P-MRS and exposed to Martian UV flux and atmosphere. *Top row:* background corrected spectra for  $\beta$ -carotene peaks in unexposed cells (a), after exposure to simulated Martian atmosphere (b) and after exposure to 570 MJ/m<sup>2</sup> polychromatic UV and simulated Martian atmosphere (c). *Bottom row:* reconstructed  $\beta$ -carotene signal coverage of the image scans (100  $\times$  100  $\mu$ m) of images shown in Fig. 6 in unexposed cells (d), after exposure to simulated Martian atmosphere (e) and after exposure to 570 MJ/m<sup>2</sup> polychromatic UV and simulated Martian atmosphere (f). *White and black areas* as in Fig. 7

altered shape, shifted towards lower wavelengths starting from 580 nm instead of 600 nm (Fig. 3a and b). However, the maximum emission intensity at 653 nm was not significantly different to that of controls, with  $104.1 \pm 4.1$  % for P-MRS and  $104.6 \pm 5.3$  % for S-MRS at 653 nm (Table 2). In addition, the emission spectrum of dried cells mixed with P-MRS or S-MRS showed a peak at 563 nm that was significantly higher than that of control,  $34.0 \pm 7.5$  % for P-MRS and  $31.0 \pm 4.1$  % for S-MRS compared to  $4.6 \pm 0.4$  % for cells in liquid culture (Table 2). These differences might be due to minerals interfering with fluorescence excitation and emission; indeed, the maximum projection images showed a high reflectance of the minerals (Fig. 2b and d). Furthermore, when mineral particles spotted on a glass slide without cells were excited with the 543-nm laser, an emission peak at 543 nm, extending to  $\sim$ 580 nm, occurred and can be attributed to reflectance (Fig. 4). Comparable results were obtained with the same cyanobacterial strain mixed with anorthosite (Baqué et al. 2014), which is a dominant component in Lunar regolith (Ashwal 1993).

The maximum emission intensity of the emission spectrum of the photosynthetic pigments of dried cells mixed with P-MRS or S-MRS was not affected by the exposure to simulated Martian atmosphere (representing  $95.8 \pm 5.6$  and  $104.0 \pm 1.7$  % of the maximum emission intensity of the spectrum of cells from liquid culture, respectively). However, the lower wavelength shift observed in unexposed dried cells mixed with minerals was less evident in S-MRS than in P-MRS samples. This was probably due to a reduced mineral interference in



**Fig. 9** PCR-based evaluation of DNA persistence in dried *Chroococcidiopsis* under Martian UV flux and atmosphere. **a** PCR fingerprint from liquid culture (lane 1), impaired PCR products from dried cells without minerals exposed to 570 MJ/m<sup>2</sup> with 0.1 % ND filter and simulated Martian atmosphere (lane 2) and altered PCR fingerprints of dried cells exposed to 570 MJ/m<sup>2</sup> (without ND) and simulated Martian atmosphere and mixed with S-MRS (lane 3) or P-MRS (lane 4). **b** PCR fingerprint from liquid culture (lane 1), PCR fingerprint from dried cells without minerals exposed to simulated Martian atmosphere (lane 2) or in the presence of S-MRS (lane 3) or P-MRS (lane 4). Lanes M: DNA marker

the regions selected for the analysis (not shown), as supported by the lower emission at 563 nm of S-MRS ( $3.5 \pm 0.5$  %) than P-MRS ( $16.7 \pm 4.6$  %).

Exposure to simulated Martian atmosphere combined with 570 MJ/m<sup>2</sup> of polychromatic UV resulted in an overall bleaching of the photosynthetic pigments in cells mixed with minerals, as pointed out by a reduction in the emission peak at 653 nm (compared to the liquid control) for S-MRS and P-MRS down to, respectively,  $1.6 \pm 0.1$  and  $4.4 \pm 0.1$  % (Fig. 3). However, the non-homogeneous cell-mineral association and the thin layering of the dried samples (Fig. 2) impaired an easy discrimination between top and bottom layers, as previously reported for thick-dried biofilms (Baque et al. 2013a). CLSM-λscan analysis showed cells in P-MRS sample with an emission peak at 653 nm reduced to either  $57.9 \pm 4.3$  or  $1.6 \pm 0.1$  %, thus reflecting the presence of differently shielded cells as shown in Fig. 2c. Results are summarized in Table 2.

Moreover, some fluorescent minerals were spotted on both Martian analogues with two small emission peaks at 620 and 660 nm, as shown on the fully exposed sample with the P-MRS analogue (Fig. 3a). Mineral fluorescence from both preparations (without cells) was therefore further investigated with both the 488 and 543 nm lasers. The emission spectra of both mineral analogues produced fluorescent peaks, most notably around 620 and 660 after 543 nm excitation. In addition, high reflectance peaks, extending from 490 to 520 nm after 488 nm excitation and from 550 to 580 nm after 543 nm excitation, were observed (Fig. 4).

### Preservation of β-Carotene as Revealed by Raman Spectroscopy

The typical Raman shifts of β-carotene at about 1009, 1159 and 1518 cm<sup>-1</sup> were detected in spectra of *Chroococcidiopsis* sp. CCMEE 029 mixed with S-MRS (Figs. 5 and 7a) or P-MRS (Figs. 6 and 8a). The 1518 and 1159 cm<sup>-1</sup> bands are strong and characteristic for the C=C and C–C stretching vibrations while the smaller feature at 1009 cm<sup>-1</sup> corresponds to the rocking

modes of the CH<sub>3</sub> groups (Vitek et al. 2010). Chlorophyll *a* fluorescence is also present as a broad band centered at 3500 cm<sup>-1</sup> due to the 532 nm excitation laser used.

Average spectra generated from image scans described the global behavior of the samples before and after exposure to the Martian simulations (Figs. 5 and 6). The β-carotene features were dominant in both unexposed mineral matrices (Figs. 5 and 6, control, green spectra) and occurred after exposure to simulated Martian atmosphere (Figs. 5 and 6, blue spectra). However, the combination of simulated Martian atmosphere with 570 MJ/m<sup>2</sup> of polychromatic UV caused the disappearance of β-carotene features from the average spectra for cells mixed with S-MRS (Fig. 5, red spectrum) or P-MRS (Fig. 6, red spectrum). In the same way, the strong chlorophyll *a* fluorescence was present in unexposed samples (controls), as expected from a typical cyanobacterial spectrum (Figs. 5 and 6, green spectra), but its intensity was slightly reduced after exposure to simulated Martian atmosphere only (Figs. 5 and 6, blue spectra) and completely disappeared after exposure to all simulated Martian conditions (570 MJ/m<sup>2</sup> polychromatic UV and simulated Martian atmosphere) for both mineral matrices (Figs. 5 and 6, red spectra). Characteristic minerals of both analogues could be identified in spite of using a non-optimal low acquisition time (1 s), as described by Böttger et al. (2012), but β-carotene and chlorophyll completely dominated the overall spectra (not shown). Another surprising feature noted for both mineral matrices (but stronger for the S-MRS analogue) was the high increase in fluorescence background with a maximum around 1400 cm<sup>-1</sup> after exposure to polychromatic UV (Figs. 5 and 6, red spectra) which is not connected to the above mentioned chlorophyll *a* fluorescence but equivalent to the 575 nm emission noted on Fig. 4.

Nevertheless, the identification of the three characteristic bands of β-carotene remained possible even on the highly fluorescent spectra of UV-exposed samples (presented as background corrected spectra), with either of both S-MRS (Fig. 7c) and P-MRS (Fig. 8c) analogues. However, the high fluorescent background impaired the optimization of the signal-to-noise ratio by increasing acquisition time or accumulations.

Figures 7 and 8 present the background corrected spectra and the images derived from Raman scans of cells mixed with S-MRS and P-MRS analogues, respectively, for the three conditions tested: control (A and D), exposure to simulated Martian atmosphere (B and E) and all simulated Martian conditions (C and F). The coverage of β-carotene's three main peaks was calculated by extracting the areas of appearance from the Raman images generated by the 2500 scans and treated with the width filter function of the WITec Project software. Due to the unequal distribution of the fluorescent background in UV exposed samples, classical filters such as peak area, center of mass or intensity filters failed to represent an accurate coverage of the β-carotene signal contrary to the width filter. The resulting images, processed with the threshold function of Adobe Photoshop, allowed for β-carotene coverage percentage calculation (Figs. 7 and 8, D E F). The presence of the β-carotene bands (denoted by the white areas) was verified for each position and the typical resulting spectra are shown in Fig. 7d, e and f for S-MRS and Fig. 8d, e and f for P-MRS. This process allowed us to confirm the omnipresent coverage of the β-carotene bands for unexposed samples, present in ~97 % of the image scan for S-MRS (Fig. 7d) and ~94 % for P-MRS (Fig. 8d). Consistently with previous results, exposure to the simulated Martian atmosphere did not impair β-carotene coverage, with values virtually identical to the unexposed samples for either S-MRS (~96 % coverage, Fig. 7e) or P-MRS (~95 % coverage, Fig. 8e). Exposure to 570 MJ/m<sup>2</sup> of polychromatic UV irradiation

in combination with simulated Martian atmosphere, however, dramatically decreased  $\beta$ -carotene coverage in our samples where the values dropped down to  $\sim 2$  % for S-MRS (Fig. 7f) and  $\sim 5$  % for P-MRS (Fig. 8f). The three main peaks of  $\beta$ -carotene are visible with a slightly better signal-to-noise ratio on the spectrum of the P-MRS sample (Fig. 8c) than on the spectrum of the S-MRS sample (Fig. 7c), probably due to the lower fluorescent background noted on the P-MRS average spectrum.

### DNA Detection by PCR-based Assay and Survivability

The persistence of DNA in dried cells of *Chroococcidiopsis* sp. CCMEE 029 exposed to Martian-simulated conditions was assessed by testing its suitability as template in a PCR-based assay (Fig. 9). Positive amplification of DNA from dried cells mixed with the two Martian analogues was verified for unexposed controls (not shown), even though the composition of the mineral matrix (especially clay containing minerals such as montmorillonite in P-MRS; see Table 1) is known to be problematic (Alvarez et al. 1998; Direito et al. 2012).

When dried cells without minerals were exposed to  $570 \text{ kJ/m}^2$  of polychromatic UV radiation ( $570 \text{ MJ/m}^2$  with 0.1 % ND filter) combined with a simulated Martian atmosphere, no PCR amplicons were yielded (lane 2). When cells mixed to S-MRS and P-MRS were exposed to  $570 \text{ MJ/m}^2$  of polychromatic UV radiation (without ND filter), PCR fingerprints were obtained although with altered profiles (lanes 3–4) compared to PCR fingerprints obtained from the liquid control (lane 1). However, dried cells of *Chroococcidiopsis* sp. CCMEE 029, mixed or not with P-MRS and S-MRS, lost their colony-forming ability when exposed to polychromatic UV irradiation (with or without ND filter) in combination with a simulated Martian atmosphere.

On the other hand, PCR profiles virtually identical to the control (lane 1) were yielded when dried cells were exposed to simulated Martian atmosphere (for 89 days) in the absence (Fig. 9b, lane 2) or presence of S-MRS (lane 3) or P-MRS (lane 4) and was correlated by the scoring of survivors, regardless of the presence of a mineral matrix.

### Discussion

The BIOMEX experiment is aimed at exposing extremophiles and biomolecules to space and Martian-like conditions in low Earth orbit, the latter being simulated in one of the trays of the ESA EXPOSE-R2 facility containing a simulated Martian atmosphere. After ground-based simulations, the detectability of biomarkers (photosynthetic pigments,  $\beta$ -carotene and DNA) was investigated in dried cells of *Chroococcidiopsis* sp. CCMEE 029 mixed or not with P-MRS or S-MRS after exposure to  $570 \text{ MJ/m}^2$  of polychromatic UV irradiation (combined with a simulated Martian atmosphere), as expected in 1 year in LEO (Rabbow et al. 2012, 2014) or 383 sols on the Martian surface (Cockell et al. 2000; Schuerger et al. 2003).

Exposure to  $570 \text{ MJ/m}^2$  of polychromatic UV irradiation led to a strong reduction (1–4 % of control values) of the emission spectrum of photosynthetic pigments in *Chroococcidiopsis* sp. CCMEE 029 mixed with P-MRS or S-MRS. This pointed out that both the 4–5 cell layers obtained when mixing with P-MRS, and the 2–3 cell layers obtained when mixing with S-MRS, did not provide efficient shielding against UV radiation. Consistently, when dried monolayers of *Chroococcidiopsis* sp. CCMEE 029 were exposed to  $72 \text{ KJ/m}^2$  of a Martian-like UV flux, the pigment autofluorescence dropped to less than 5 % of controls (Cockell et al.

2005). Nevertheless, among cells mixed with P-MRS, pigment autofluorescence was only reduced to 60 % of control values. This might be a consequence of the thinner grain-size of P-MRS that impaired the preparation of homogeneous samples, thus leading to thicker and protected areas in the samples. Hence, although photosynthetic pigments are expected to bleach when exposed on the Martian surface for a period as long as 383 sols, they might retain their fluorescence if sufficiently shielded with minerals.

In the present work, the reflectance of S-MRS and P-MRS minerals, when excited with a 543-nm or a 488-nm laser, impaired the identification of fluorescent breakdown products of the photosynthetic pigments. Indeed, photolytic destruction of photosynthetic pigments was reported in *Synechocystis* sp. PCC 6803 exposed to UV and ionizing radiation, yielding fluorescent breakdown products with an emission peak at ~450 nm (Dartnell et al. 2011; Dartnell and Patel 2013). An emission peak at 563 nm, probably due to chlorophyll photolysis, occurred in unshielded *Chroococcidiopsis* sp. CCME029 exposed to 800 MJ/m<sup>2</sup> of polychromatic UV irradiation. It was however absent from cells mixed with anorthosite, a lunar mineral analogue, as shown by the lambda scan spectra obtained on mineral-free areas to avoid mineral reflectance (Baqué et al. 2014, MB personal communication). P-MRS and S-MRS have autofluorescence peaks at 620 and 660 nm and, although it was not possible to obtain cell pigment spectra in mineral-free areas, the typical emission peaks of phycocyanin, allophycocyanin and chlorophyll *a* in UV-exposed *Chroococcidiopsis* were detected.

Fluorescent signals were also reported for Martian relevant minerals when excited in the UV (266 and 355 nm) and visible (532 nm) regions. In particular, calcium perchlorate has a strong fluorescent peak at around 600 nm after 532 nm excitation (Smith et al. 2014b). Hence, a more thorough analysis is needed to insure that Martian soil does not interfere with the identification of selected autofluorescent molecules. This might be relevant when using the foreseen instrumentation on-board the ESA/Roscosmos mission, for example the UV/Vis camera filters or the VIS-NIR spectrometer Ma\_Miss (Mars Multispectral Imager for Subsurface Studies) (De Angelis et al. 2014).

In the present study, the persistence of the  $\beta$ -carotene signature in dried *Chroococcidiopsis* was assessed by using a confocal Raman microscope with an excitation wavelength of 532 nm, as foreseen for the RLS instrument on-board the ExoMars rover. In unexposed cells,  $\beta$ -carotene spectrum and chlorophyll *a* fluorescence were the dominant signals and remained virtually identical to controls after exposure to simulated Martian atmosphere. On the contrary, after 570 MJ/m<sup>2</sup> of polychromatic UV irradiation, in cells mixed with S-MRS or P-MRS the  $\beta$ -carotene signal dropped to 2 and 5 % of control, respectively. The lowest  $\beta$ -carotene amount detected was about 4 pg/ $\mu$ g of sample (4 mg/kg), based on an estimation of the carotenoid content in *Chroococcidiopsis* (0.1 pg/cell) and considering a homogeneous repartition of the cells on the investigated areas ( $2.10^9$  cells on 115 mm<sup>2</sup>).

In relevant Martian minerals the detection limits of pure  $\beta$ -carotene were reported to go down to 0.1 mg/kg in an evaporitic matrix with a 514.5 nm laser (Vítek et al. 2009a) and 1 mg/kg in halite with a 785 nm laser (Vítek et al. 2009a; Vandenabeele et al. 2012). Hence, after the EXPOSE-R2 space mission in *Chroococcidiopsis* shielded with Martian regolith, a lower  $\beta$ -carotene amount might be detected. In fact, during the EXPOSE-E and EXPOSE-R missions, the exposure to about 570 MJ/m<sup>2</sup> of polychromatic UV 200–400 nm, resulted in the failure of the Raman analyses to detect  $\beta$ -carotene in epilithic cyanobacterial biofilms (Cockell et al. 2011) and in *Chroococcidiopsis* sp. CCME029 layered on rocks, as opposite to UV-shielded samples (Bryce et al. 2014).



UV irradiation is one of the main biological damaging factors encountered in space or on the Martian surface. However, as Mars lacks an intrinsic magnetic field (possibly lost, at least 3.5 Gya ago), ionizing radiation of solar energetic protons (SEP), and galactic and solar cosmic rays (GCRs and SCR) reach today's Martian surface and subsurface with high energies. Curiosity reported a GCR dose rate of 76 mGy/year on the Martian surface and of 8.7 mGy/year at 2 m under the surface (Hassler et al. 2013; Farley et al. 2014). This suggested that although over geological time scales dormant microorganisms might be inactivated even at depths between 1 and 2 m below the Martian surface, transient habitable conditions might allow them to repair the accumulated damages (Dartnell et al. 2007; Hassler et al. 2013). This data also gives clues on biosignature preservation over geological timescales, suggesting that organic matter could be preserved in recently exposed rocks or soils (Ming et al. 2014), such as mudstone at Gale crater's surface (in situ radiometric measurements revealed its exposure time of  $78 \pm 30$  million years (Farley et al. 2014)). In particular, gamma-rays were shown to diminish  $\beta$ -carotene Raman signal after 15 kGy and to destroy it after 150 kGy (Dartnell et al. 2012). Therefore, Raman identification of  $\beta$ -carotene would be impaired after  $\sim 2$  million years on the Martian surface and after  $\sim 17$  million years at 2 m under the surface, corresponding to the foreseen ExoMars drill depth.

On the other hand, an ionizing radiation dose of 675.4  $\mu$ Gy/day (resulting from GCR, energetic protons of the inner radiation belt and electrons from the outer radiation belt (Dachev et al. 2014)) was recorded during the EXPOSE-R mission, hence the EXPOSE-R2 mission will simulate an exposure of  $\sim 30$  years (in a 12-month mission) to  $\sim 40$  years (in an 18-month mission) at a 2 m depth on Mars.

The observed reduction of the  $\beta$ -carotene signal in thin layers of *Chroococcidiopsis* mixed with the Martian regolith simulants after exposure to a UV dose simulating half a Martian year on the Martian surface suggested that remains of carotenoid-containing life forms close to the surface would quickly lose their Raman signature. On the contrary, under thicker UV-shielding materials, Raman signature destruction is driven by the ionizing radiation environment and could persist for several million years.

In our study, UV irradiation caused an augmentation in the mineral fluorescence background, which was more pronounced in S-MRS but did not impair the  $\beta$ -carotene identification in *Chroococcidiopsis* exposed to a dose corresponding to that received in 383 sols. Nevertheless, it might have detrimental implications for the identification of biomarkers and mineral context on the highly UV irradiated Martian surface. Blue-green excitation is known to induce a strong fluorescent background in Raman spectra (Edwards et al. 2013), but a 532 nm excitation, as foreseen for ExoMars's RLS, enhances the Raman signal by producing a strong resonance effect on  $\beta$ -carotene molecules due to their conjugated C=C double bonds (e.g., Gill et al. 1970; Marshall et al. 2007; Vitek et al. 2009b).

In order to evaluate the contribution of Martian regolith to the preservation of DNA in dried *Chroococcidiopsis* cells exposed to polychromatic UV irradiation in combination with a simulated Martian atmosphere, DNA detection was attempted by a PCR-based assay. A dose of 570 kJ/m<sup>2</sup> of polychromatic UV irradiation (570 MJ/m<sup>2</sup> attenuated with a 0.1 % ND filter) impaired the DNA detectability, whereas amplicons were yielded in thin layer of cells (from 2–3 cell layers to 4–5 cell layers) mixed with P-MRS or S-MRS and exposed to 570 MJ/m<sup>2</sup> of polychromatic UV irradiation, simulating half a Martian year. Consistently, the amplificability of DNA in *Chroococcidiopsis* sp. CCMEE 029 mixed with anorthosite and exposed to 800 MJ/m<sup>2</sup> of polychromatic UV irradiation was previously reported (Baqué et al. 2014). On Earth, DNA was recovered from samples

between 400,000 years and 1.5 million years old (Pääbo et al. 2004; Sankaranarayanan et al. 2014); on Mars, DNA might remain detectable at least for several million years in the presence of UV shielding regolith, and amid the extreme dryness and low temperatures (Willerslev et al. 2004).

Even though the protection provided by P-MRS or S-MRS allowed biosignature detection in *Chroococcidiopsis* exposed to Martian simulated conditions, no survivors were scored after polychromatic UV radiation ( $570 \text{ MJ/m}^2$ ); survivors were scored only after a 3-month exposure to simulated Martian atmosphere regardless of the mineral matrix. This lack of survival is in agreement with a scenario in which several features might affect *Chroococcidiopsis*'s survival, including the thickness of cell envelopes, the presence of UV shielding minerals, or the abundance of extracellular polymeric substances (EPS) produced by biofilms as opposite to planktonic cultures (Baqué et al. 2014). In the present work, planktonic cultures were dried while mixed with minerals, the thickness not exceeding  $15 \mu\text{m}$  (corresponding to 4–5 cell layers). This thickness did not guarantee the survival even of bottom layers cells when exposed to  $570 \text{ MJ/m}^2$  of polychromatic UV. It was previously reported that in *Chroococcidiopsis* biofilms exposed to UVC radiation, damage occurred in the first 3–4 cell layers and that dried biofilms better survived  $570 \text{ kJ/m}^2$  of polychromatic UV radiation compared to dried planktonic multilayers (Baqué et al. 2013a).

For the EXPOSE-R2 space mission, neutral density filters were applied to reduce the received dose to 0.1 % of the total flux, thus leading to an expected dose of  $570 \text{ kJ/m}^2$  of UV 200–400 nm for a 1-year mission. The reduced dose could simulate light conditions in some natural hypolithic communities (Warren-Rhodes et al. 2013; Smith et al. 2014a) or evaporite communities below 2 mm of gypsum (Stromberg et al. 2014). The mineral matrix composition and thickness is moreover relevant when considering target Martian habitats (Martinez-Frias et al. 2006) because different rocks provide different UV shielding for hypolithic communities (Smith et al. 2014a). A basalt dust (basalt is one of the main components of Martian dust) with a thickness of  $\sim 300 \mu\text{m}$  reduces a polychromatic UV flux to 0.1 % of its incident value (Caro et al. 2006). However, their role in effectively protecting potential biological remains and their consequent impact on biosignatures identification has been poorly studied and are among the investigated subjects of the BIOMEX experiment.

In conclusion, insights into the preservation potential of cyanobacterial biosignatures in a Martian environment were obtained from *Chroococcidiopsis* exposed to Martian simulations carried out in the frame of the BIOMEX space mission. Polychromatic UV irradiation simulating 1 year in LEO or 383 sols on the Martian surface ( $570 \text{ MJ/m}^2$ ) differently impaired the detectability of the investigated biosignatures. The  $\beta$ -carotene Raman signal was always detectable regardless of the layer thickness of the cell mixed with regolith simulants (up to  $15 \mu\text{m}$ ). Photosynthetic pigments were strongly bleached in both 2–3 cell layers and in 4–5 cell layers, whereas autofluorescence could be detected in a few cells occurring in thicker layer yielded when mixing the cells with the thinner-sized grains of P-MRS. DNA was a suitable template for PCR amplification regardless of whether cells were mixed with P-MRS or S-MRS, whereas it was not amplifiable in the absence of minerals. When the analysis of the samples exposed to simulated Martian conditions outside the ISS inside the EXPOSE-R2 facility will be carried out, new insights will be gathered on the influence of all the space parameters on *Chroococcidiopsis*'s survival and preservation of its cellular components.

In the present work, the radiation received within 383 sols on the Martian surface was estimated using the average UV flux of  $\sim 50 \text{ W/m}^2$  predicted by previous models (Cockell et al. 2000; Schuerger et al. 2003). However, according to the latest Curiosity measurements, this is probably an overestimation since the maximum UV irradiance (200–380 nm) recorded at midday at Gale’s crater was  $\sim 20 \text{ W/m}^2$  (Gómez-Elvira et al. 2014). Hence the results inferred from the Martian simulation during the EXPOSE-R2 space mission could suggest longer preservation of potential biosignatures on Mars. In addition, since the Martian analogues showed a strong fluorescence when investigated by Raman analysis, further investigation is needed to ensure unambiguous biosignature detection in future Mars missions.

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