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ASTROBIOLOGY

Detection of Macromolecules in Desert Cyanobacteria Mixed with a Lunar Mineral Analogue After Space Simulations

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Abstract In the context of future exposure missions in Low Earth Orbit and possibly on the Moon, two desert strains of the cyanobacterium *Chroococcidiopsis*, strains CCMEE 029 and 057, mixed or not with a lunar mineral analogue, were exposed to fractionated fluencies of UVC and polychromatic UV (200–400 nm) and to space vacuum. These experiments were carried out within the framework of the BIOMEX (BIOlogy and Mars EXperiment) project, which aims at broadening our knowledge of mineral-microorganism interaction and the stability/degradation of their macromolecules when exposed to space and simulated Martian conditions. The presence of mineral analogues provided a protective effect, preserving survivability and integrity of DNA and photosynthetic pigments, as revealed by testing colony-forming abilities, performing PCR-based assays and using confocal laser scanning microscopy. In particular, DNA and pigments were still detectable after 500 kJ/m^2 of polychromatic UV and space vacuum (10^{-4} Pa), corresponding to conditions expected during one-year exposure in Low Earth Orbit on board the EXPOSE-R2 platform in the presence of 0.1 % Neutral Density (ND) filter. After exposure to high UV fluencies (800 MJ/m^2) in the presence of minerals, however, altered fluorescence emission spectrum of the photosynthetic pigments were detected, whereas DNA was still amplified by PCR. The present paper considers the implications of such findings for the detection of biosignatures in extraterrestrial conditions and for putative future lunar missions.

Keywords Astrobiology · Extreme environments · Expose-R2 · Biosignatures · Lunar regolith

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Introduction

On December, 14th 2013, the Moon landing of the Chinese Space Agency Chang'e 3 lander, carrying the Yutu rover, put an end to a 40 year gap with no soft landing on our closest celestial neighbor. Yet the Moon, and particularly the lunar surface, has always offered outstanding opportunities for increasing our knowledge of our solar system and our ability to explore it (Crawford et al. 2012). It also has potential as an astrobiology platform for testing instrumentation and carrying life resistance studies within the search for remnant or extinct life on Mars (Carpenter et al. 2012; de Vera et al. 2012).

Indeed, Mars still remains the prime target for searching extraterrestrial life. Simulating the parameters of its surface is still highly challenging: these include low atmospheric pressure and temperatures (resulting in liquid water instability), lower gravity than Earth's, and a lack of both a magnetic field and an ozone layer allowing harmful - in a biological sense - UV radiation, cosmic rays and solar energetic particles to reach the surface (Cockell et al. 2000; Patel et al. 2004). Mars analogues on Earth regarding extreme temperatures and prolonged absence of liquid water have been identified in hot and cold deserts such as the Dry Valleys in Antarctica and the Atacama desert in Chile, where life takes refuge within or under rocks (Friedmann 1980; Bahl et al. 2011). However, no terrestrial analogue exists with an atmospheric pressure, gravity and radiation comparable to that of Mars. Simulations in ground-based chambers and in Low Earth Orbit (LEO) are consequently used to plan future life detection missions, to test instruments and to understand how biosignatures are affected by extraterrestrial conditions (Demets et al. 2005; Rabbow et al. 2009, 2012).

In this context, the Moon could prove invaluable: being outside the influence of the Earth's magnetosphere, its surface is subject to high solar and galactic irradiation similar to that on Mars and could therefore represent a test platform for instrumentation and life resistance studies (Carpenter et al. 2012; de Vera et al. 2012). More generally, the Moon is considered a valuable site to address a wide range of life science and astrobiology questions dealing with i) the understanding of the habitability of the Earth through time, ii) the appreciation of the possibility of life elsewhere in the universe, and iii) research that advances the human exploration and settlement of space (Crawford et al. 2012).

In addition theories of the potential existence of preserved organic molecules in Lunar Ice relevant to the origin of life (Schulze-Makuch 2013) are in the focus of future analysis and need detection technologies with reference to life. In parallel the search for proves or falsification of successful interplanetary transfer of life or biogenic material from the Early Earth to the Moon (Armstrong et al. 2002) through asteroid impacts (Lithopanspermia theory within the Earth-Moon system) could also be a challenging work to be realized on the surface of the Moon where technology of life detection is needed.

Technologies currently used for detecting traces of life on Mars, such as the one onboard NASA's Mars Science Laboratory (Mahaffy et al. 2012), are based on gas chromatography mass spectrometers. Other approaches include a miniaturized Raman spectrometer, planned as part of the Pasteur payload in the ESA-Roscosmos ExoMars mission (Barnes et al. 2006; Vago et al. 2006), antibody microarray devices such as the Signs of Life Detector instrument (Parro et al. 2008, 2011) proposed for the IceBreaker mission (McKay et al. 2013), the Life Marker Chip (Sims et al. 2012) planned for the ExoMars mission, Polymerase Chain Reaction (PCR)-based methods for targeting ribosomal RNA genes and DNA sequencing (Isenbarger et al. 2008; Carr et al. 2013). Complementary technologies based on laser induction of biomolecule autofluorescence were proposed to survey potential target regions before further analysis (Dartnell and Patel 2013). All the above implies that degradation by environmental stress and potential interference from surrounding minerals should be taken into account. Due to

their chlorophyll and phycobiliproteins, photoautotrophs such as cyanobacteria are choice organisms for such studies.

The BIOlogy and Mars EXperiment (BIOMEX) project aims at investigating the resistance of selected extremophiles (mixed or not with lunar and Martian mineral analogues), and the stability/degradation of their macromolecules, when exposed to space and Mars-like conditions simulated in ground-based facilities and in LEO (de Vera et al. 2012). This experiment is part of the EXPOSE-R2 space mission that reached the International Space Station (ISS) on July 24th, 2014 on board the space cargo Progress 56, whereas on August 18th, the EXPOSE-R2 facility was installed outside the ISS on the Russian Svezda module for a 12–18 months exposure to space and Mars simulated conditions in LEO. One of the objectives of BIOMEX is to yield useful data for optimizing exploration missions and avoiding possible pitfalls during the detection of life markers, in the contest of future Mars exploration missions (Carpenter et al. 2012; de Vera et al. 2012).

BIOMEX involves biological systems isolated from terrestrial Martian analogues and/or shown to be resistant to selected factors encountered on Mars and in space. Among these factors is extreme desiccation. Besides affecting biological systems that would be exposed to Mars's surface, this is relevant in the context of radiation studies, evidence having shown that desiccation and radiation resistance are correlated. Radiation resistance is considered a consequence of adaptation to dehydration (Slade and Radman 2011); this could explain certain organisms' resistance to radiation doses much higher than what they receive on Earth. For example, desiccation-tolerant cyanobacteria from the genus *Chroococcidiopsis* isolated from extremely arid environments can withstand up to 15 kGy of ionizing radiation (Billi et al. 2000) and 13 kJ/m^2 of UVC radiation (Baqué et al. 2013b). In addition, *Chroococcidiopsis* radioresistance is enhanced when in a dried, ametabolic state: dried monolayers tolerated 30 kJ/m^2 of a simulated Mars UV flux (Cockell et al. 2005).

In the present paper, we report the effects of ground-based simulations conducted in the context of BIOMEX's preparative phase on two desert strains of *Chroococcidiopsis*, namely CCMEE 029 from the Negev Desert (Israel) and CCMEE 057 from the Sinai desert (Egypt). Dried cells, mixed or not with a lunar regolith analogue, were exposed to monochromatic UVC radiation, polychromatic UV radiation and a combination of simulated space vacuum and UV radiation. Exposed cells were then tested for detectability of DNA and autofluorescence of photosynthetic pigments by methods based on, respectively, PCR and confocal laser scanning microscopy. Finally, survivability was assessed by testing the colony-forming ability.

Material and Methods

Culture Conditions, Lunar Mineral Analogue and Sample Preparation

Chroococcidiopsis sp. CCMEE 029 (N6904) and CCMEE 057 (S6e) were isolated by Roseli-Ocampo Friedmann from, respectively, cryptoendolithic growth in sandstone in the Negev Desert (Israel) and chasmoendolithic growth in granite in the Sinai Desert (Egypt). Both strains are currently kept at the Department of Biology, University of Rome "Tor Vergata", as part of the Culture Collection of Microorganisms from Extreme Environments (CCMEE) established by E. Imre Friedmann. Cyanobacteria were grown under routine conditions at 25 °C in BG-11 medium under a photon flux density of 40 μmol/m^{2}s^1 provided by fluorescent cool-white bulbs with a 16-h/8-h light/dark cycle.

Multilayered planktonic samples were obtained by plating pellets obtained from 2-month-old liquid cultures, on the top of BG-11 agarized medium and mixed (or not) with anorthosite

from the Ukrainian shield (Korosten Pluton, Zhytomyr region) (Mytrokhyn et al. 2003), here referred to as "lunar mineral analogue". Samples were allowed to dry before cutting disks to the size of the exposure carrier cells (~110 mm^2) under sterile conditions.

Test Facilities and Exposure Conditions

Ground-based simulations, carried out in the framework of Experiment Verification Tests (EVTs) and Scientific Verification Tests (SVTs), were performed using the Planetary and Space Simulation facilities (PSI) of the Institute of Aerospace Medicine (German Aerospace Center, DLR, Köln, Germany). EVT tests were performed in triplicate and laboratory controls were kept at DLR in the dark, at room temperature (RT). For SVTs, the real accommodation plan was followed and only one replicate per sample was consequently possible. Tests facilities and exposure conditions were as reported in Table 1. The applied fluency corresponds to one year of exposure outside the ISS, as estimated from previous EXPOSE data and simulations (Rabbow et al. 2012), and the use of ND filter as planned for the real mission.

Random Amplification of Polymorphic DNA (RAPD) Assay

Control and exposed cells were resuspended in sterile MilliQ water (50 μL), washed twice by centrifuging 10 min at 10,000 rpm and resuspended in 20 μL 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 μL of lysed cell suspensions were used for genomic PCR amplification with the HIP1-CA primer (5′-GCGATCGCCA-3′). PCR conditions were as follows: 1 cycle at 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 1 cycle at 72 °C for 7 min. Genomic DNA from one-month-old cultures of

Table 1 Parameters obtained using the Planetary and Space Simulation facilities at DLR (Köln, Germany) for ground-based simulations

EXPOSE-R2 simulation experiments	
Experiment verification tests part 1 (EVT1)	
UVC (254 nm) irradiation with Hg low pressure lamp at 80 mW/cm^2	0 J/m^2 10 J/m^2 (1 s) 100 J/m^2 (12 s) 1,000 J/m^2 (2 min 5 s) 10,000 J/m^2 (20 min 50 s)
Experiment verification tests part 2 (EVT2)	
Polychromatic UV irradiation (200–400 nm) with SOL2000 at 1,370 W/m^2.	0 kJ/m^2 1.5×10^3 kJ/m^2 (18 min) 1.5×10^4 kJ/m^2 (3 h) 1.5×10^5 kJ/m^2 (30 h) 5.0×10^5 kJ/m^2 (99 h) 8.0×10^5 kJ/m^2 (148 h)
Scientific verification tests (SVTs)	
Vacuum (2×10^{-4} Pa) for 89 days + polychromatic UV irradiation (200–400 nm) with SOL2000 at 1,370 W/m^2, attenuated with 0.1 % neutral density filter.	5×10^2 kJ/m^2 99 h SOL2000
Control	1 atm air, dark, room temperature

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 then revealed under UV lamp after ethidium bromide staining.

Confocal Laser Scanning Microscopy

Microscopy analyses were performed using a confocal laser scanning microscope coupled with spectral analysis (CLSM-λscan). Small fragments (about 2 mm^2) were put onto slides and examined using a CLSM (Olympus Fluoview 1000 Confocal Laser Scanning System). Autofluorescence of photosynthetic pigments (chlorophyll *a* and phycobiliproteins) and minerals was investigated by successively exciting the samples with 488-nm, 543-nm and 635-nm lasers, and collecting the emitted fluorescence in three channels: 503–524 nm, 555–609 nm and 655–755 nm. Three-dimensional images were captured every 0.5 μm 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 553 to 800 nm, and mean fluorescence intensity (MFI) was measured. Curve plotting was performed using the GraphPad Prism program (GraphPad Software, San Diego, CA).

Survival Assessment

Chroococcidiopsis 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 10^6 to 10^8 CFU.

Results

PCR-based Detection of DNA in Dried Cells Exposed to UVC and Polychromatic UV Radiation

After UVC radiation, genomic DNA from dried *Chroococcidiopsis* sp. CCMEE 029 and CCMEE 057 exposed without lunar mineral analogue was used as PCR template in RAPD assays. Reduced band profiles were produced at fluencies above 100 J/m^2 (Fig. 1a, c). In particular, no amplicons were obtained from strain CCMEE 029's DNA after 10 kJ/m^2 of UVC irradiation (Fig. 1a, lane 6), suggesting abundant DNA damage. By contrast, genomic DNA from cells mixed with the lunar mineral analogue yielded PCR band profiles after exposure to each UVC fluency (Fig. 1b, d), showing a shielding effect provided by the lunar mineral analogue.

The exposure to polychromatic UV radiation of dried *Chroococcidiopsis* sp. CCMEE 029 without mineral resulted in the lack of PCR amplicons after RAPD assays after each dose, these ranging from 1.5×10^3 kJ/m^2 to 8×10^5 kJ/m^2 (Fig. 1e). By contrast, amplicons were consistently obtained when cells were mixed with the lunar mineral analogue (Fig. 1f); RAPD patterns were altered compared to those of unexposed cells, although increasing polychromatic UV doses were not paralleled by increasing alteration of RAPD patterns. Similar results, except for a higher overall resistance of cells without mineral, were obtained from strain CCMEE 057 (not shown).

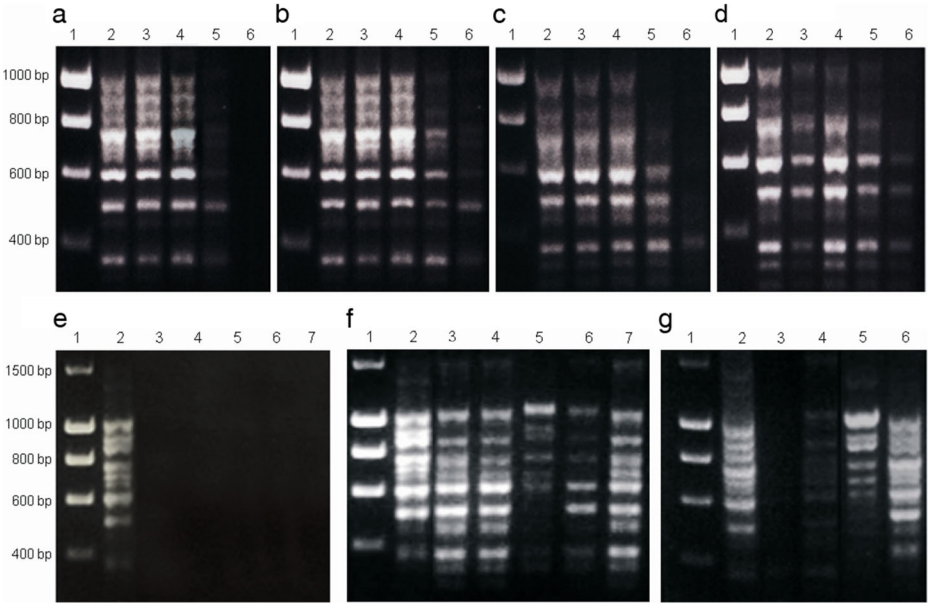

Fig. 1 RAPD of *Chroococcidiopsis* sp. CCMEE 029 (**a**, **b**) and CCMEE 057 (**c**, **d**) exposed to UVC without (**a**, **c**) and with (**b**, **d**) lunar mineral analogue; lane 1: DNA ladder, lane 2: dried control, lanes 3–6: UVC (10, 100, 1,000 and 10,000 J/m^2). RAPD of CCMEE 029 exposed to polychromatic UV without (**e**) and with lunar mineral analogue (**f**); lane 1: DNA ladder, lane 2: dried control, lanes 3–7: polychromatic UV (1.5×10^3, 1.5×10^4, 1.5×10^5, 5×10^5, and 8×10^5 kJ/m^2). Effect of space simulation on PCR amplification of CCMEE 029 (**g**): dried cells (lane 2), cells without mineral exposed to polychromatic UV (5×10^2 kJ/m^2) and vacuum (lane 3) and vacuum (lane 4); cells mixed with lunar mineral analogue and exposed to polychromatic UV and vacuum (lane 5) and vacuum (lane 6)

PCR-based Detection of DNA in Dried Cells Exposed to Space Simulation

RAPD assays on genomic DNA from dried *Chroococcidiopsis* sp. CCMEE 029 exposed to 5×10^2 kJ/m^2 (5×10^5 kJ/m^2 attenuated with 0.1 % ND filter) polychromatic UV radiation combined with space vacuum (2×10^{-4} Pa for 89 days) yielded almost undetectable PCR amplicons when cells were exposed without mineral (Fig. 1g, lane 3); PCR amplicons were clearly visible, however, when cells were exposed in the presence of lunar mineral analogue (Fig. 1g, lane 5). RAPD profiles were less affected by exposure to vacuum only, especially for cells mixed with lunar mineral analogue that lead to unaltered PCR profiles (Fig. 1g, lane 6).

Emission Spectra of Photosynthetic Pigments in Dried Cells Exposed to Polychromatic UV Radiation

CLSM 3-D images of dried samples of *Chroococcidiopsis* sp. CCMEE 029 mixed with lunar mineral analogue showed that cells occurred as thin layers where top cells provided UV shielding to bottom ones. In addition, cells were differently associated with the minerals, thus receiving varying degrees of protection (Fig. 2c).

CLSM-λscan analyses of unexposed dried cells excited with a 543-nm laser produced emission spectra of photosynthetic pigments with a peak at 650–660 nm (Fig. 2b) due to the overlapping emission of phycobiliproteins (phycocyanin and allophycocyanin) and chlorophyll *a* (Roldán et al. 2004). This spectrum proved to be similar in shape and height (98.6±

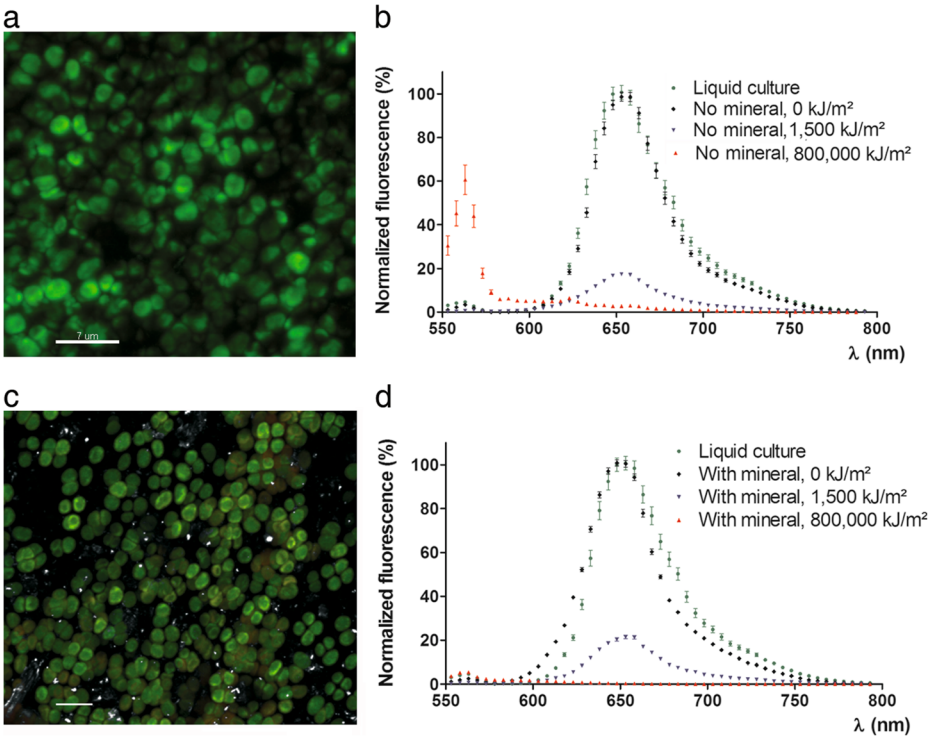

Fig. 2 CLSM imaging of dried *Chroococcidiopsis* sp. CCMEE 029 in the absence (**a**) or presence (**c**) of lunar mineral analogue showing bleached and unbleached pigments after exposure to 1.5×10^3 kJ/m^2 respectively; phycobiliproteins and chlorophyll *a* (555–609 and 655–755 nm channels), lunar mineral analogue (503–524 nm channel, grey color); *bar* = 7 μm. CLSM-λscan of photosynthetic pigments of *Chroococcidiopsis* sp. CCMEE 029 cells from liquid culture, unexposed dried cells (0 kJ/m^2) and dried cells exposed to polychromatic UV (1.5×10^3 and 8×10^5 kJ/m^2), in the absence (**b**) or presence (**d**) of lunar mineral analogue. Graphs represent normalized fluorescence intensity versus emission wavelength. Data points show normalized fluorescence intensity ± standard error for $n\geq15$ cells

2.0 % at λ_{em}=653 nm) to that of cells from liquid culture. After 1.5×10^3 kJ/m^2 of polychromatic UV irradiation without mineral, the 650–660 nm peak height dropped to 17.4±0.4 % (Fig. 2b) and cells exhibited bleached photosynthetic pigments (Fig. 2a). After the highest fluency, 8×10^5 kJ/m^2, the 650–660 nm peak proved to be almost undetectable (2.8±0.5 %) and an emission peak occurred at about 560 nm, with a height about 15 times that of liquid or dried controls (Fig. 2b).

The emission spectrum of dried cells mixed with lunar mineral analogue differed from those described above. First, the spectrum of the unexposed sample started at 580 nm (instead of 600 nm as in cells without mineral), whereas the maximum emission remained identical to control (100.4±1.6 % at λ_{em}=653 nm) (Fig. 2d). After 1.5×10^3 kJ/m^2 of UV radiation, cells mixed with lunar mineral analogue showed an emission spectrum higher than those without (21.5±0.8 % vs 17.4±0.4 %). After the highest dose (8×10^5 kJ/m^2) the emission peak at 560 nm was not detected in the presence of lunar mineral analogue: emission at this wavelength was not significantly different than that of control (5.4±0.3 % compared to 4.6 ±0.4 % at λ_{em}=563 nm, Fig. 2d), and photosynthetic pigment bleaching was comparable to that of cells without minerals with an overall flat spectrum (0.3±0.1 % at λ_{em}=653 nm). Results are summarized in Table 2.

Table 2 Fluorescence emission at 563 nm and 653 nm of photosynthetic pigments in dried *Chroococcidiopsis* exposed to space simulations with or without lunar mineral analogue; data are expressed as percent of control values±standard error for n≥15 cells

		Liquid culture	0 kJ/m^2	1.500 kJ/m^2	800.000 kJ/m^2	500 kJ/m^2 + vacuum	vacuum
563 nm	No mineral	4.6±0.4	0.6±0.1	3.4±0.6	60.6±6.8	N.D.	N.D.
	With mineral		2.5±0.2	0.7±0.1	5.4±0.3	2.8±0.5	2.5±0.2
653 nm	No mineral	100.7±3.2	98.6±2.0	17.4±0.4	2.8±0.3	N.D.	N.D.
	With mineral		100.4±1.6	21.5±0.8	0.3±0.1	29.2±2.7	93.6±3.2[1]

N.D. not determined

[1] maximum at 648 nm : 96.0±3.3 %

Emission Spectra of Photosynthetic Pigments in Dried Cells Exposed to Space Simulation

Dried cells of *Chroococcidiopsis* sp. CCMEE 029 mixed with lunar mineral analogue and exposed to 5×10^2 kJ/m^2 of polychromatic UV (5×10^5 kJ/m^2, attenuated with 0.1 % ND filter) combined with vacuum (2×10^{-4} Pa for 89 days) underwent photosynthetic pigment bleaching, as shown by the CLSM-λscan analysis with a 543-nm laser. The emission spectra of photosynthetic pigments had a peak at 653 nm reduced to 29.2±2.7 % of that of unexposed cells (Fig. 3a). Cells exposed to space vacuum only were unbleached, with an emission spectrum of photosynthetic pigments comparable to control (Fig. 3a). However, as noted for unexposed cells mixed with the lunar mineral analogue, the peak shape was distorted with, in this case, a maximum of emission at 648 nm (instead of 653 nm) and a peak height of 96.0± 3.3 % (Table 2). The 686 nm- centered shoulder was reduced in cells mixed with the mineral analogue. Figure 3b shows CLSM images corresponding to the maximum emission peaks of the photosynthetic pigments in cells exposed to 5×10^2 kJ/m^2 and 2×10^{-4} Pa and only to vacuum.

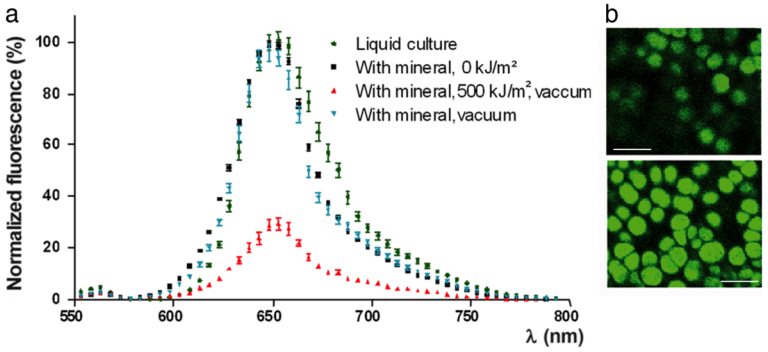

Fig. 3 CLSM-λscan of photosynthetic pigments in *Chroococcidiopsis* sp. CCMEE 029 from liquid culture, and in dried cells mixed with lunar mineral analogue unexposed (0 kJ/m^2), or exposed to space simulation (5×10^2 kJ/m^2 and 2×10^{-4} Pa) and vacuum. Data points represent normalized fluorescence intensity at 653 nm versus emission wavelength. Data points show normalized fluorescence intensity ± standard error for $n\geq15$ cells (**a**). CLSM images corresponding to the maximum emission peaks of the photosynthetic pigments excited with the 543-nm laser in cells exposed to (5×10^2 kJ/m^2 and 2×10^{-4} Pa) (**b**, top) and vacuum (**b**, bottom); *bar* = 7 μm

Survivability of Dried Cyanobacteria Mixed with Minerals Under Space Simulations

After exposure to any fluency of UVC radiation, dried cells of *Chroococcidiopsis* sp. CCMEE 029 and CCMEE 057 retained their colony-forming ability, upon rehydration. At the lowest polychromatic UV doses (up to 1.5×10^3 kJ/m^2) only cells mixed with the lunar mineral analogue formed colonies. No colonies were formed at the higher polychromatic UV fluencies.

When *Chroococcidiopsis* sp. CCMEE 029 was exposed to 5×10^2 kJ/m^2 (5×10^5 kJ/m^2, 0.1 % ND filter) in combination with space vacuum, colony-forming ability was lost. However, cells exposed to space vacuum only formed colonies upon rehydration.

Discussion

As a preparative phase of the BIOMEX experiment of the EXPOSE-R2 space mission on the ISS and of future lunar missions, dried cells of the desert cyanobacteria *Chroococcidiopsis* sp. CCMEE 029 and CCMEE 057 were mixed with a lunar regolith analogue and exposed to simulations of different space conditions. The effects on DNA degradation, bleaching of the photosynthetic pigments and cell survival were assessed, respectively, by testing the genomic DNA as PCR template by RAPD, performing confocal scanning laser microscopy analyses and assessing the colony-forming ability. When assessing the preservation potential of putative biosignatures in extraterrestrial environments the geochemical environment is of particular importance, since the lunar highland regolith is mainly composed of anorthosite, in this paper we used anorthosite from the Ukrainian shield (Mytrokhyn et al. 2003; Kozyrovska et al. 2006) to simulate real exposure of putative biosignatures to a lunar environment. The presence of this lunar regolith analogue had an overall protective effect against polychromatic UV radiation, resulting in the yielding of PCR amplicons (up to 8×10^5 kJ/m^2), in the pigment preservation, although with qualitatively altered emission spectra and in a positive colony-forming ability (up to 1.5×10^3 kJ/m^2).

UVC radiation exposure of dried cells from both strains in the absence of minerals led to a dose-dependent genomic DNA degradation, detectable by RAPD assay at 100 J/m^2, and lack of visible products from strain CCMEE 029 exposed to 10 kJ/m^2. Even at this dose, cells exhibited positive colony-forming ability. The fact that cells formed colonies even when the genomic DNA was highly damaged is in line with the capability of this cyanobacterium to repair extensive DNA damage (Billi et al. 2000, 2011). The presence of the lunar mineral analogue guaranteed DNA protection: detectability was enhanced, with amplicons generated from both strains exposed to 10 kJ/m^2 of UVC radiation.

Chroococcidiopsis sp. CCMEE 029 was shown to be highly resistant to polychromatic UV radiation (Cockell et al. 2005). In the present study, the lowest tested fluency (1.5×10^3 kJ/m^2) corresponded approximately to the dose reaching Mars' surface at the equator in 1 day (Cockell et al. 2000), while the highest (8×10^5 kJ/m^2) corresponded to that expected during an 18-month EXPOSE-R2 mission. Based on data from previous missions (Rabbow et al. 2012), this dose was estimated to be 8×10^5 kJ/m^2, resulting from an average flux of about 16 W/m^2 for 18 months. It should be noted that even though the same total fluency was reached here, it was done by applying a higher flux (1,370 W/m^2) for a shorter time (148 h). Although the dose-to-exposure-time ratio may be critical for metabolically active organisms, desiccated cells will accumulate damage and activate their repair mechanisms only upon rehydration, as previously reported (Baqué et al. 2013a). Hence, the use of short exposure times under high flux allowed the simulation of longer time periods, as needed for biosignature degradation assessment.

Exposure of dried *Chroococcidiopsis* to 1.5×10^3 kJ/m^2 of polychromatic UV radiation without minerals caused a loss of colony-forming ability, suggesting that accumulated DNA damage exceeded repair capability. Consistent with this, RAPD assays yielded no amplicons. When mixed with lunar mineral analogue, however, cells retained viability although altered RAPD patterns highlighted DNA damage. At fluencies higher than 1.5×10^3 kJ/m^2 of polychromatic UV radiation, in spite of the relatively preserved genomic DNA integrity, no survivors were scored. It could be speculated that the primary cause of the lethal effects was reactive oxygen species (ROS) produced by UV radiation and the induced damage to proteins (Daly 2009; Slade and Radman 2011). For an Arctic permafrost community exposed to simulated martian UV flux, a reduction in biomolecules (proteins and DNA) and bacterial survivors at depths >1.5 mm was reported and ascribed to reactive oxygen species formed from traces of atmospheric oxygen and water vapor in the simulation chamber and from water film on soil grains (Hansen et al. 2009). In our experimental setup, cells were mixed together with the lunar mineral analogue hence forming 3–4 cell layers in combination with minerals for a total depth of about 15 μm (as shown by CLSM imaging). The main damaging factor of EVTs and SVTs was UV irradiation (either at a single wavelength, 254 nm, or as the full UV spectrum from 200 to 400 nm) due to its direct (absorption by DNA and proteins) or indirect (production of reactive oxygen species) effects on living organisms; whereas vacuum is well known to induce dehydration. The achieved results further support the effective shielding of anorthosites as a lunar mineral analogue but also the resilience of *Chroococcidiopsis* cells to extraterrestrial simulated conditions.

In the present work, increasing polychromatic UV doses were not paralleled by increasing alteration of RAPD patterns, likely as a consequence of the heterogeneous repartition of cells around the lunar mineral analogue, leading to unequal shielding. A quantitative evaluation of the accumulated DNA damage by using real-time quantitative polymerase chain reaction (qPCR) as previously reported for *Chroococcidiopsis* (Baqué et al. 2013a, b) was not performed due to the low purity of the yielded DNA. Indeed, DNA extraction from samples mixed with minerals is known to be problematic due to the adsorption of DNA to different types of clays (Direito et al. 2012).

Polychromatic UV radiation caused a strong decrease in the intensity of the emission spectrum of phycobiliproteins and chlorophyll *a* of the cyanobacterial photosystem. In the absence of lunar mineral analogue, the lowest dose applied (1.5×10^3 kJ/m^2) reduced the fluorescence emission peak to 17 % of its initial value. A decrease to a level of 35 % was reported (Dartnell and Patel 2013) for *Synechocystis* sp. PCC 6803 after 64 h Mars equivalent UV dose (around 10^4 kJ/m^2, assuming a flux of 50 W/m^2; Cockell et al. 2005). At the highest fluency used in our study (8×10^5 kJ/m^2), the fluorescence emission of the photosynthetic pigments around 650 nm was lost and a peak appeared at 560 nm, suggesting an accumulation of photolytic products. Consistently, accumulation of fluorescent chlorophyll catabolites (FCCs) proposed to derive from chlorophyll and resulting in an increased fluorescent emission at 450 nm, was reported in *Synechocystis* sp. PCC 6803 exposed to either ionizing radiation or one-hour Mars equivalent UV dose (Dartnell et al. 2011; Dartnell and Patel 2013). Since photosynthetic pigments and their breakdown products (e.g., porphyrins) are considered as high priority targets in putative biosignatures to guide future search for life missions (Parnell et al. 2007), additional studies are needed to confirm the nature of the species causing the altered photosynthetic pigment emission spectrum observed in the present work.

The protective effect conferred by the lunar mineral analogue was confirmed by the fluorescence results at the lowest polychromatic UV fluency (1.5×10^3 kJ/m^2): irradiation in the presence of the mineral reduced the photosystem peak emission to 21 % instead of to 17 %. Similar conclusions can be drawn from exposure to space simulations (combining UV

irradiation and vacuum exposure) and to vacuum alone. The presence of lunar mineral analogue led to an increased DNA preservation, as shown by RAPD patterns. Photosynthetic pigments fluorescence was not altered by vacuum alone and dropped to about 30 % after space simulation (Tab. 2).

This result supports the fact that mixing cells with anorthosites provide them an effective shielding, but also the resilience of *Chroococcidiopsis* sp. CCMEE 029 and CCMEE 057 to extraterrestrial simulated conditions. Even though our results provide insights into the influence of a lunar environment on biosignature modification, not all conditions found on the Moon can be reproduced in ground-based simulations; further insights are expected from future experiments in LEO and ultimately on the Moon. Our neighbor could also be used as a test-bed for biology-based technologies that will support human space exploration, including biological modules of life support systems based on in situ resource utilization (ISRU). Lunar ISRU has indeed been proposed as a potential on-site source of valuable products including food, biofuels, oxygen and various chemicals (Olsson-Francis and Cockell 2010; Montague et al. 2012). The presence of lunar mineral analogue on agarized BG-11 medium did not impair *Chroococcidiopsis*'s growth (data not shown); furthermore CCMEE 029 proved able to grow in distilled water containing only anorthosite and nitrogen (Olsson-Francis and Cockell 2010). Hence we anticipate that this cyanobacterium might contribute to the development of biological ISRU processes on the Moon.

In conclusion, this work deepened our knowledge on *Chroococcidiopsis*'s potential to survive under extraterrestrial constraints when mixed with a lunar mineral analogue, by demonstrating its survival under a UV dose simulating 4 h of full irradiation (1.5×10^3 kJ/m^2) in LEO (or 8 h of a Mars UV flux). Whereas, when subjected only to space vacuum (2×10^{-4} Pa for 89 days) *Chroococcidiopsis* not only survived but showed no degradation to their cellular constituents (DNA and pigments). At the highest polychromatic UV fluencies (800 MJ/m^2) corresponding to 18 months in LEO, the presence of lunar mineral analogue led to different detectability of DNA and pigments, preserving DNA amplification but leading to altered photosynthetic pigment emission spectra. DNA and pigments were still detectable after space vacuum (10^{-4} Pa) and 500 kJ/m^2 of polychromatic UV, corresponding to one-year exposure in LEO in the presence of 0.1 % ND filter as planned for the EXPOSE-R2 space mission. The qualitative effect of minerals on fluorescence spectra underlines the need for taking into account geological data when developing biosignature databases; more generally, for further investigation into mineral-microorganism interaction. With the accomplishment of the BIOMEX experiments in space, these investigations will not only help preparing our search for life but will also have implications for future cyanobacteria-based space applications, including bioleaching and life support systems.

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References

Armstrong J, Wells L, Gonzalez G (2002) Rummaging through earth's attic for remains of ancient life. Icarus 160:183–196. doi:10.1006/icar.2002.6957

Bahl J, Lau MCY, Smith GJD, Vijaykrishna D, Cary SC, Lacap DC, Lee CK, Papke RT, Warren-Rhodes KA, Wong FKY, McKay CP, Pointing SB (2011) Ancient origins determine global biogeography of hot and cold desert cyanobacteria. Nat Commun 2:163. doi:10.1038/ncomms1167

Baqué M, de Vera J-P, Rettberg P, Billi D (2013a) The BOSS and BIOMEX space experiments on the EXPOSE-R2 mission: endurance of the desert cyanobacterium *Chroococcidiopsis* under simulated space vacuum, Martian atmosphere, UVC radiation and temperature extremes. Acta Astronaut 91:180–186. doi:10.1016/j.actaastro.2013.05.015

Baqué M, Viaggiu E, Scalzi G, Billi D (2013b) Endurance of the endolithic desert cyanobacterium *Chroococcidiopsis* under UVC radiation. Extremophiles 17:161–169. doi:10.1007/s00792-012-0505-5

Barnes D, Battistelli E, Bertrand R, Butera F, Chatila R, Del Biancio A, Draper C, Ellery A, Gelmi R, Ingrand F, Koeck C, Lacroix S, Lamon P, Lee C, Magnani P, Patel N, Pompei C, Re E, Richter L, Rowe M, Siegwart R, Slade R, Smith MF, Terrien G, Wall R, Ward R, Waugh L, Woods M (2006) The ExoMars rover and Pasteur payload phase a study: an approach to experimental astrobiology. Int J Astrobiol 5:221–241. doi:10.1017/S1473550406003090

Billi D, Friedmann EI, Hofer KG, Grilli Caiola M, Ocampo-Friedmann R (2000) Ionizing-radiation resistance in the desiccation-tolerant cyanobacterium *Chroococcidiopsis*. J Appl Environ Microbiol 66:1489–1492. doi:10.1128/aem.66.4.1489-1492.2000

Billi D, Viaggiu E, Cockell CS, Rabbow E, Horneck G, Onofri S (2011) Damage escape and repair in dried *Chroococcidiopsis* spp. from hot and cold deserts exposed to simulated space and Martian conditions. Astrobiology 1:65–73. doi:10.1089/ast.2009.0430

Carpenter JD, Fisackerly R, De Rosa D, Houdou B (2012) Scientific preparations for lunar exploration with the European Lunar lander. Planet Space Sci 74:208–223. doi:10.1016/j.pss.2012.07.024

Carr CE, Rowedder H, Vafadari C, Lui CS, Cascio E, Zuber MT, Ruvkun G (2013) Radiation resistance of biological reagents for in situ life detection. Astrobiology 13:68–78. doi:10.1089/ast.2012.0869

Cockell CS, Catling DC, Davis WL, Snook K, Kepner RL, Lee P, McKay CP (2000) The ultraviolet environment of mars: biological implications past, present, and future. Icarus 146:343–359. doi:10.1006/icar.2000.6393

Cockell CS, Schuerger AC, Billi D, Friedmann EI, Panitz C (2005) Effects of a simulated Martian UV flux on the cyanobacterium, *Chroococcidiopsis* sp. 029. Astrobiology 5:127–140. doi:10.1089/ast.2005.5.127

Crawford IA, Anand M, Cockell CS, Falcke H, Green DA, Jaumann R, Wieczorek MA (2012) Back to the moon: the scientific rationale for resuming lunar surface exploration. Planet Space Sci 74:3–14. doi:10.1016/j.pss.2012.06.002

Daly MJ (2009) A new perspective on radiation resistance based on *Deinococcus radiodurans*. Nat Rev Microbiol 7:237–245. doi:10.1038/nrmicro2073

Dartnell LR, Patel MR (2013) Degradation of microbial fluorescence biosignatures by solar ultraviolet radiation on Mars. Int J Astrobiol 13:112–123. doi:10.1017/S1473550413000335

Dartnell LR, Storrie-Lombardi MC, Mullineaux CW, Ruban AV, Wright G, Griffiths AD, Muller J-P, Ward JM (2011) Degradation of cyanobacterial biosignatures by ionizing radiation. Astrobiology 11:997–1016. doi:10.1089/ast.2011.0663

De Vera J-P, Boettger U, Noetzel RDLT, Sánchez FJ, Grunow D, Schmitz N, Lange C, Hübers H-W, Billi D, Baqué M, Rettberg P, Rabbow E, Reitz G, Berger T, Möller R, Bohmeier M, Horneck G, Westall F, Jänchen J, Fritz J, Meyer C, Onofri S, Selbmann L, Zucconi L, Kozyrovska N, Leya T, Foing B, Demets R, Cockell CS, Bryce C, Wagner D, Serrano P, Edwards HGM, Joshi J, Huwe B, Ehrenfreund P, Elsaesser A, Ott S, Meessen J, Feyh N, Szewzyk U, Jaumann R, Spohn T (2012) Supporting mars exploration: BIOMEX in low earth orbit and further astrobiological studies on the moon using Raman and PanCam technology. Planet Space Sci 74:103–110. doi:10.1016/j.pss.2012.06.010

Demets R, Schulte W, Baglioni P (2005) The past, present and future of Biopan. Adv Space Res 36:311–316. doi:10.1016/j.asr.2005.07.005

Direito SOL, Marees A, Röling WFM (2012) Sensitive life detection strategies for low-biomass environments: optimizing extraction of nucleic acids adsorbing to terrestrial and Mars analogue minerals. FEMS Microbiol Ecol 81:111–123. doi:10.1111/j.1574-6941.2012.01325.x

Friedmann EI (1980) Endolithic microbial life in hot and cold deserts. Orig Life 10:223–235. doi:10.1007/BF00928400

Hansen AA, Jensen LL, Kristoffersen T, Mikkelsen K, Merrison J, Finster KW, Lomstein BA (2009) Effects of long-term simulated Martian conditions on a freeze-dried and homogenized bacterial permafrost community. Astrobiology 9:229–240. doi:10.1089/ast.2008.0244

Isenbarger TA, Carr CE, Johnson SS, Finney M, Church GM, Gilbert W, Zuber MT, Ruvkun G (2008) The most conserved genome segments for life detection on earth and other planets. Orig Life Evol Biosph 38:517–533. doi:10.1007/s11084-008-9148-z

Kozyrovska NO, Lutvynenko TL, Korniichuk OS, Kovalchuk MV, Voznyuk TM, Kononuchenko O, Zaetz I, Rogutskyy I, Mytrokhyn OV, Mashkovska SP, Foing BH, Kordyum VA (2006) Growing pioneer plants for a lunar base. Adv Space Res 37:93–99. doi:10.1016/j.asr.2005.03.005

Mahaffy PR, Webster CR, Cabane M, Conrad PG, Coll P, Atreya SK, Arvey R, Barciniak M, Benna M, Bleacher L, Brinckerhoff WB, Eigenbrode JL, Carignan D, Cascia M, Chalmers RA, Dworkin JP, Errigo T, Everson P,

Franz H, Farley R, Feng S, Frazier G, Freissinet C, Glavin DP, Harpold DN, Hawk D, Holmes V, Johnson S, Jones A, Jordan P, Kellogg J, Lewis J, Lyness E, Malespin CA, Martin DK, Maurer J, McAdam AC, McLennan D, Nolan TJ, Noriega M, Pavlov AA, Prats B, Raaen E, Sheinman O, Sheppard D, Smith J, Stern JC, Tan F, Trainer M, Ming DW, Morris RV, Jones J, Gundersen C, Steele A, Wray J, Botta O, Leshin LA, Owen T, Battel S, Jakosky BM, Manning H, Squyres S, Navarro-González R, McKay CP, Raulin F, Sternberg R, Buch A, Sorensen P, Kline-Schoder R, Coscia D, Szopa C, Teinturier S, Baffes C, Feldman J, Flesch G, Forouhar S, Garcia R, Keymeulen D, Woodward S, Block BP, Arnett K, Miller R, Edmonson C, Gorevan S, Mumm E (2012) The sample analysis at Mars investigation and instrument suite. Space Sci Rev 170:401–478. doi:10.1007/s11214-012-9879-z

McKay CP, Stoker CR, Glass BJ, Davé AI, Davila AF, Heldmann JL, Marinova MM, Fairen AG, Quinn RC, Zacny KA, Paulsen G, Smith PH, Parro V, Andersen DT, Hecht MH, Lacelle D, Pollard WH (2013) The Icebreaker life mission to mars: a search for biomolecular evidence for life. Astrobiology 13:334–353. doi:10.1089/ast.2012.0878

Montague M, McArthur GH, Cockell CS, Held J, Marshall W, Sherman LA, Wang N, Nicholson WL, Tarjan DR, Cumbers J (2012) The role of synthetic biology for *in situ* resource utilization (ISRU). Astrobiology 12:1135–1142. doi:10.1089/ast.2012.0829

Mytrokhyn OV, Bogdanova SV, Shumlyanskyy LV (2003) Anorthosite rocks of fedorivskyy suite (Korosten Pluton, Ukrainian Shield). Current problems in geology. Kyiv National University, Kyiv, pp 53–57

Olsson-Francis K, Cockell CS (2010) Use of cyanobacteria for in-situ resource use in space applications. Planet Space Sci 58:1279–1285. doi:10.1016/j.pss.2010.05.005

Parnell J, Cullen D, Sims MR, Bowden S, Cockell CS, Court R, Ehrenfreund P, Gaubert F, Grant W, Parro V, Rohmer M, Sephton M, Stan-Lotter H, Steele A, Toporski J, Vago J (2007) Searching for life on mars: selection of molecular targets for ESA's Aurora ExoMars mission. Astrobiology 7:578–604. doi:10.1089/ast.2006.0110

Parro V, Rivas LA, Gómez-Elvira J (2008) Protein microarrays-based strategies for life detection in astrobiology. Space Sci Rev 135:293–311. doi:10.1007/s11214-007-9276-1

Parro V, de Diego-Castilla G, Rodríguez-Manfredi JA, Rivas LA, Blanco-López Y, Sebastián E, Romeral J, Compostizo C, Herrero PL, García-Marín A, Moreno-Paz M, García-Villadangos M, Cruz-Gil P, Peinado V, Martín-Soler J, Pérez-Mercader J, Gómez-Elvira J (2011) SOLID3: a multiplex antibody microarray-based optical sensor instrument for *in situ* life detection in planetary exploration. Astrobiology 11:15–28. doi:10.1089/ast.2010.0501

Patel MR, Bérces A, Kerékgyárto T, Rontó G, Lammer H, Zarnecki JC (2004) Annual solar UV exposure and biological effective dose rates on the Martian surface. Adv Space Res 33:1247–1252. doi:10.1016/j.asr.2003.08.036

Rabbow E, Horneck G, Rettberg P, Schott J-U, Panitz C, L'Afflitto A, von Heise-Rotenburg R, Willnecker R, Baglioni P, Hatton J, Dettmann J, Demets R, Reitz G (2009) EXPOSE, an astrobiological exposure facility on the international space station - from proposal to flight. Orig Life Evol Biosph 39:581–598

Rabbow E, Rettberg P, Barczyk S, Bohmeier M, Parpart A, Panitz C, Horneck G, von Heise-Rotenburg R, Hoppenbrouwers T, Willnecker R, Baglioni P, Demets R, Dettmann J, Reitz G (2012) EXPOSE-E: an ESA astrobiology mission 1.5 years in space. Astrobiology 12:374–386. doi:10.1089/ast.2011.0760

Roldán M, Thomas F, Castel S, Quesada A, Hernández-Mariné M (2004) Noninvasive pigment identification in single cells from living phototrophic biofilms by confocal imaging spectrofluorometry. J Appl Environ Microbiol 70:3745–3750. doi:10.1128/AEM.70.6.3745-3750.2004

Schulze-Makuch D (2013) Organic molecules in lunar ice: A window to the early evolution of life on Earth. In: de Vera JP, Seckbach J (eds) Cellular origins, life in extreme habitats and astrobiology vol 28, Habitability of other planets and satellites Springer Netherlands, pp 333–345. doi:10.1007/978-94-007-6546-7-7

Sims MR, Cullen DC, Rix CS, Buckley A, Derveni M, Evans D, Miguel García-Con L, Rhodes A, Rato CC, Stefinovic M, Sephton MA, Court RW, Bulloch C, Kitchingman I, Ali Z, Pullan D, Holt J, Blake O, Sykes J, Samara-Ratna P, Canali M, Borst G, Leeuwis H, Prak A, Norfini A, Geraci E, Tavanti M, Brucato J, Holm N (2012) Development status of the life marker chip instrument for ExoMars. Planet Space Sci 72:129–137. doi:10.1016/j.pss.2012.04.007

Slade D, Radman M (2011) Oxidative stress resistance in *Deinococcus radiodurans*. Microbiol Mol Biol Rev 75:133–191. doi:10.1128/MMBR.00015-10

Vago J, Gardini B, Kminek G, Baglioni P, Gianfiglio G, Santovincenzo A, Bayón S, van Winnendael M (2006) ExoMars - searching for life on the red planet. ESA Bull 126:16–23