

Research Paper

Effects of a Simulated Martian UV Flux on the Cyanobacterium, *Chroococcidiopsis* sp. 029

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ABSTRACT

Dried monolayers of *Chroococcidiopsis* sp. 029, a desiccation-tolerant, endolithic cyanobacterium, were exposed to a simulated martian-surface UV and visible light flux, which may also approximate to the worst-case scenario for the Archean Earth. After 5 min, there was a 99% loss of cell viability, and there were no survivors after 30 min. However, this survival was approximately 10 times higher than that previously reported for *Bacillus subtilis*. We show that under 1 mm of rock, *Chroococcidiopsis* sp. could survive (and potentially grow) under the high martian UV flux if water and nutrient requirements for growth were met. In isolated cells, phycobilisomes and esterases remained intact hours after viability was lost. Esterase activity was reduced by 99% after a 1-h exposure, while 99% loss of autofluorescence required a 4-h exposure. However, cell morphology was not changed, and DNA was still detectable by 4',6-diamidino-2-phenylindole staining after an 8-h exposure (equivalent to approximately 1 day on Mars at the equator). Under 1 mm of simulant martian soil or gneiss, the effect of UV radiation could not be detected on esterase activity or autofluorescence after 4 h. These results show that under the intense martian UV flux the morphological signatures of life can persist even after viability, enzymatic activity, and pigmentation have been destroyed. Finally, the global dispersal of viable, isolated cells of even this desiccation-tolerant, ionizing-radiation-resistant microorganism on Mars is unlikely as they are killed quickly by unattenuated UV radiation when in a desiccated state. These findings have implications for the survival of diverse microbial contaminants dispersed during the course of human exploratory class missions on the surface of Mars. **Key Words:** Mars—Simulated UV flux—*Chroococcidiopsis*—Cyanobacterium. *Astrobiology* 5, 127–140.

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INTRODUCTION

THE DESICCATION-TOLERANT, ionizing-radiation-resistant cyanobacterium, *Chroococcidiopsis* [Order *Pleurocapsales* (Rippka *et al.*, 1979); Order *Chroococcales* (Komárek and Anagnostidis, 1998)], occurs around the globe, for example, in the hot Negev desert in Israel and the cold Ross Desert of the Antarctic (Friedmann and Ocampo-Friedmann, 1985; Büdel and Wessels, 1991). It is unicellular or forms few-celled packets, and is non-motile. In its natural environment *Chroococcidiopsis* often inhabits microscopic fissures (chasmoliths) and structural cavities (cryptoliths) of rocks, or it forms biofilms at the stone-soil interface under pebbles of desert pavements (hypoliths; for terminology see references in Golubic *et al.*, 1981). In environments such as the extreme arid regions of the Negev desert, where conditions are too extreme for eukaryotes and many other prokaryotes, it may be the only phototrophic organism to persist. Because water in hot deserts is available for just a few days a year, *Chroococcidiopsis* has evolved to cope with prolonged desiccation stress (Grilli Caiola *et al.*, 1993). Viable cells were recovered from long-term (over 5 years) laboratory-dried cells of *Chroococcidiopsis* from Negev rocks, characterized by a thick cell envelope along with other changes in cell morphology and ultrastructure (Grilli Caiola *et al.*, 1993).

Compared with other cyanobacteria, it is slow growing and does not fix nitrogen under aerobic conditions (Rippka *et al.*, 1979). Desert forms of *Chroococcidiopsis* can tolerate prolonged nutrient limitation and starvation, and exhibit cellular morphological changes that lead to the synthesis of thick cell envelopes under stressed nutrient conditions (Billi and Grilli Caiola, 1996a,b). Desiccation-tolerant forms of *Chroococcidiopsis* retain viability after exposure to 15 kGy of ionizing radiation (Billi *et al.*, 2000).

Photoautotrophy, such as that exhibited by the cyanobacteria, is a successful means of gathering energy and carbon, and it is hypothesized that photoautotrophy was an early innovation on the Earth (Buick, 1992). The lack of an ozone shield prior to the oxygenation of Earth's atmosphere would have allowed wavelengths of UV radiation as low as 200 nm to have reached the surface (Kasting, 1987). A similar situation exists on the planet Mars today (Kuhn and Atreya, 1979). It is estimated that the absence of an ozone shield on

Mars increases the rate of damage to DNA caused by UV radiation up to three orders of magnitude higher than it is on Earth (Cockell *et al.*, 2000; Córdoba-Jabonero *et al.*, 2003; Patel *et al.*, 2003). The higher flux of UV radiation is highly damaging to the spore-forming bacterium *Bacillus subtilis* (Mancinelli and Klovstad, 2000; Schuerger *et al.*, 2003). Schuerger *et al.* (2003), using a robust Mars UV simulation, showed that the viability of spores of *B. subtilis* was reduced by more than 99% after 15 s; after 15 min no survivors were recovered.

Three questions are posed by the putative high UV flux on early Earth and the known high UV flux on Mars: (1) How would direct exposure to a UV flux from 200 nm and higher wavelengths affect the viability and preservation potential of phototrophs? (2) What is the importance of shielding in a micro-habitat? (3) How would the present-day martian UV flux affect extremophilic organisms inadvertently transported to Mars on spacecraft or accidentally released from human habitats on the martian surface? Although phototrophs are not expected to be carried on robot spacecraft, an understanding of how their component biomolecules respond to UV irradiation provides a model that describes the mechanisms by which microorganisms in the martian environment would be destroyed.

In this study, we examined the effects of a simulated martian UV flux on the survival of desiccated *Chroococcidiopsis* sp. 029 cells and its component biomolecules. We investigated the effects of micro-habitats on the responses to these environmental conditions.

EXPERIMENTAL APPROACH

Organism and culture conditions

Chroococcidiopsis strain 029 (isolated from cryptolith growth in Nubian sandstone in the Negev desert) was obtained from the University of Rome "Tor Vergata." The organism was grown in BG (Blue-Green)-11 medium (Rippka *et al.*, 1979) for 1 month, and cells in a 0.2-ml volume at a cell density of 0.5 Absorbance Units at 750 nm (1 cm light path) were plated onto 2% BG-11 agar plates. All cultures and plates were grown under an 18:6 h light/dark period under a light intensity of 50 $\mu\text{mol}/\text{m}^2/\text{s}$. After 3 months, cells were aseptically harvested from the plates and

homogeneously mixed into 4 ml of sterile H₂O to a final absorbance value of 0.5 at 750 nm (1 cm light path). For exposure to the UV light source, 30 μ l of cell suspension was pipetted onto borosilicate glass discs (Glass Precision Engineering, Bedfordshire, UK) of dimensions 6 mm diameter \times 1.75 mm thickness to generate monolayers of cells (Fig. 1). The number of discs prepared corresponded to the study to be undertaken. The discs were enclosed in a Petri dish with a single vented lid and left to dry at 22°C.

Mars simulations and calculation of UV flux

Experiments to study the destruction of biomolecules in micro-habitats (experiments 2 and 3) were conducted within a Mars Simulation Chamber (MSC) previously described by Schuerger *et al.* (2003) (Fig. 2). The MSC system is located at the Kennedy Space Center, Florida. The MSC is a stainless steel low-pressure cylin-

drical chamber with internal dimensions measuring 1.5 m long \times 0.8 m in diameter. Environmental conditions within the MSC system were controlled independently for pressure (8.5 ± 0.2 mbar), gas composition (99.99% purity of CO₂), temperature ($-10 \pm 0.5^\circ\text{C}$), and UV irradiation. The temperature was controlled through the use of a liquid-nitrogen cold plate (model TP2555, Sigma Systems Corp., San Diego, CA) placed within the MSC. The UV irradiation (Fig. 3) was produced by two 450-W xenon-arc lamps (model 6262, Oriel Instruments, Stratford, CA) mounted on the exterior of the MSC. The UV-enriched light was brought into the MSC system via two sets of UV-transmitting fiber optic bundles (Optran UVNS nonsolarizing fibers, CeramOptec, East Longmeadow, MA). Samples of *Chroococcidiopsis* sp. 029 were placed within aluminum holders (Schuerger *et al.*, 2003), which were then mounted directly to the liquid-nitrogen cold plate.

In experiments 1 and 4, where a bench-top light source was used, a 150-W xenon arc lamp source provided the UV and visible light flux (Cairn Research Ltd., Faversham, UK). The UV flux emitted by the lamp was measured with an S-2000 spectroradiometer (Ocean Optics, San Diego), and the distance to the disc holder was altered so that the flux matched that described above. These experiments were conducted at the British Antarctic Survey in the UK.

The experiments on the effects of vacuum on viability were carried out at the German Aerospace Center in Germany. Cells were placed into an MSC, and vacuum was achieved using a turbomolecular pumping system. Cells were exposed to a 1×10^{-6} mbar vacuum for 20 days. Temperature for the first 13 days was 20°C. For the final 3 days, the temperature was 0°C with five peaks to 35°C that lasted for less than 5 min each.

To determine the UV flux on the surface of Mars, the irradiance models of Schuerger *et al.* (2003) and Cockell *et al.* (2000) were used to match the UV-visible radiation output of the xenon-arc lamps to simulate the Mars incident flux. The Mars UV models were developed to represent the UV flux on equatorial Mars at the mean orbital distance from the Sun and without a significant contribution of ozone to UV absorption (Fig. 3). The spectral characteristics of the Mars incident flux simulation used here yielded UVC (200–280 nm), UVB (280–315 nm), and UVA (315–400 nm) flux of 3.73, 8.27, and 37.67 W m⁻², respectively.

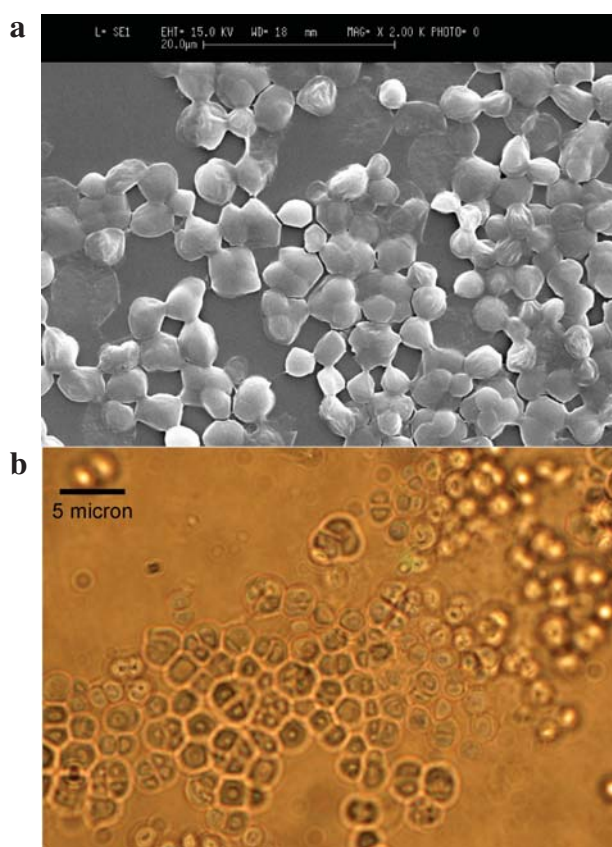


FIG. 1. Monolayers of *Chroococcidiopsis* sp. (strain 029, Negev) used in this study. **a:** Scanning electron micrograph of cells on borosilicate disc. **b:** Optical micrograph. $\times 100$.

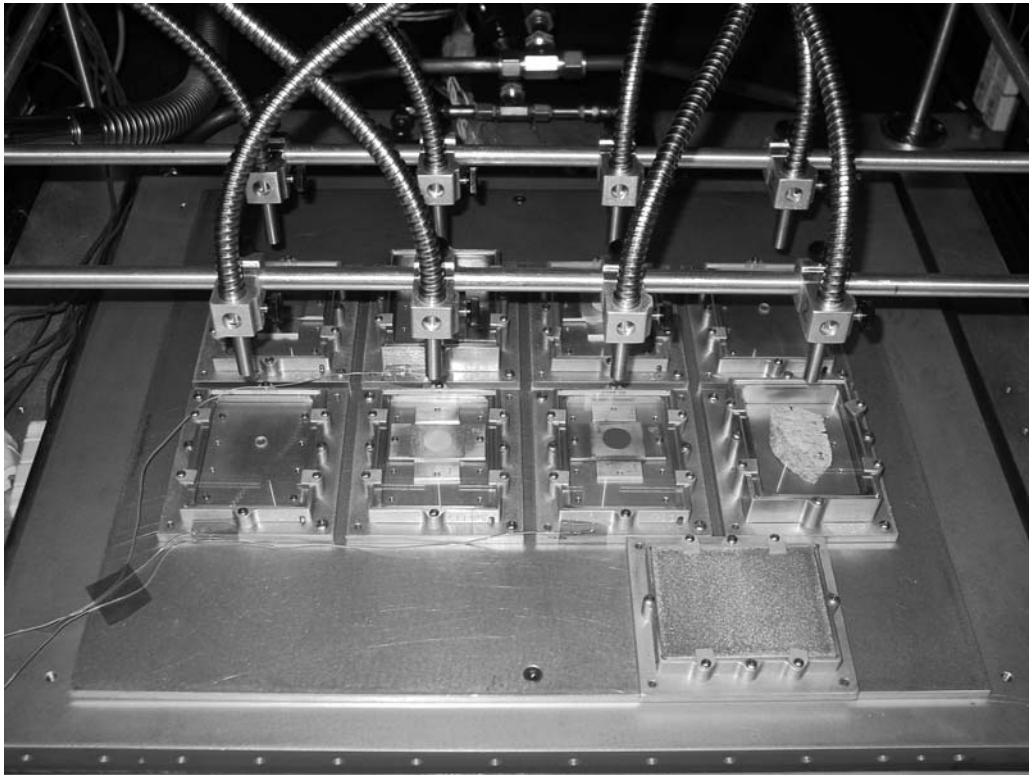


FIG. 2. Experimental set-up of *Chroococcidiopsis*-covered discs under simulated martian conditions under various substrates (from left to right: control disc, 1 mm ground gneiss, 1 mm Mars soil-simulant, 1 mm solid gneiss) using the Kennedy Space Center MSC. Details of this chamber are described in the text.

The values predicted by the model in Schuerger *et al.* (2003) were very close to the Mars solar flux for UV irradiation predicted by Kuhn and Atreya (1979), Appelbaum and Flood (1990), and Cockell *et al.* (2000). In cases where a cutoff at 295 and

400 nm was obtained with the benchtop system, cutoff filters were obtained from the Andover Corporation (Salem, NH). Cutoff filters were placed in front of the light source during the experiments.

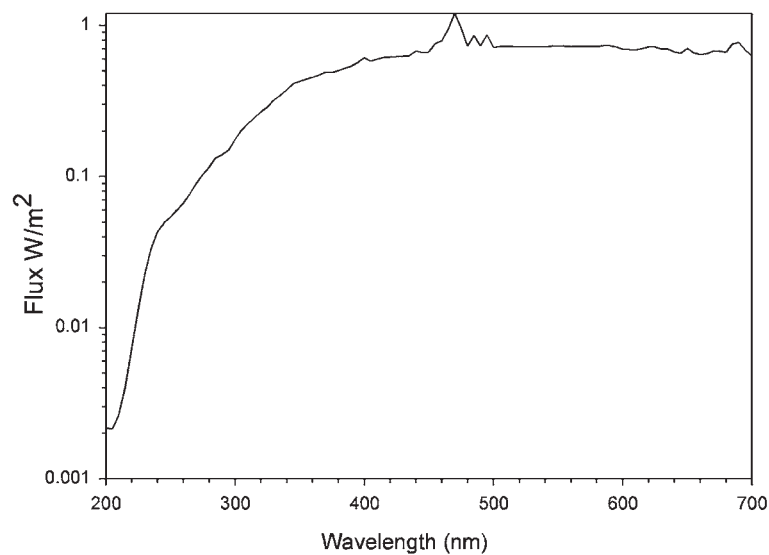


FIG. 3. Spectrum of simulated martian UV flux used in these studies.

Micro-habitat studies

A simulated Mars soil was derived from surficially altered ash of Pu'u Nene, a volcanic cone at 1,850 m elevation on Mauna Kea's south flank, and described elsewhere (Allen *et al.*, 1981, 1998). The palagonitic tephra was collected in 1985 by R. Singer and is very similar to the Johnson Space Center Mars-1 soil simulant described by Allen *et al.* (1998). The volcanic palagonite closely matches the reflectance spectrum of Mars regolith (Singer, 1982; Morris *et al.*, 1990) and is similar in chemical composition, particle size, density, porosity, and magnetic properties to surface soils on Mars (Allen *et al.*, 1981, 1998; Morris *et al.*, 1990, 1993). The palagonite is primarily composed of hawaiite, calcium feldspar, with traces of magnetite, hematite, pyroxene, olivine, and glasses composed of SiO₂, Fe₂O₃, and CaO (Allen *et al.*, 1998). The majority of the iron (64%) is present as nanophase (<20 nm) ferric oxide particles (Allen *et al.*, 1998).

In addition to the Mars analog soil, Precambrian gneiss obtained from the Haughton impact structure in Canada was also used in these experiments. This material was described by Metzler *et al.* (1988). It is a crystalline, feldspar-rich material shocked up to 60 GPa during the collision of the asteroid or comet within this region of the Arctic 23 million years ago. As this material contains endolithic *Chroococcidiopsis*-like microorganisms (Cockell *et al.*, 2002), it was considered a proxy to a plausible habitat on Mars.

The Mars analog soil and crushed gneiss were dry-sieved to pass a 140-mesh screen (45 μm) to better simulate the actual size distribution of Mars regolith and aeolian dust (Kahn *et al.*, 1992; Landis and Jenkins, 2000). The soil fractions were dry-heat-sterilized at 130°C for 24 h prior to use. Samples of *Chroococcidiopsis* sp. 029 on glass discs were covered with 1-mm-thick layers of either the Mars analog soil or crushed gneiss prior to placing the samples within the MSC for specific Mars simulations (see below). A 1-mm thickness was chosen as the depth that would be sufficient for visible light to penetrate to the organisms for photosynthesis, but where UV flux would be reduced compared with surface conditions (Cockell and Raven, 2004).

For studies on organisms in their natural habitat, sandstones containing *Chroococcidiopsis* were provided by E.I. Friedmann as described in Friedmann and Ocampo-Friedmann (1985). These

sandstones were stored in a desiccated state in the dark prior to use. These communities form a coherent band of organisms approximately 1–3 mm beneath the surface of the rock. Rocks were cut into 1 × 1 cm sections and exposed to the light source as described above. Following exposure, the samples were placed into sterile 10-ml tubes and incubated for 3 weeks in BG-11 as described for culture. The rocks were observed for growth, manifested by a darkening and thickening of the green layer, with cells also growing into the surrounding medium.

UV radiation and light exposures

The sets of exposure experiments were as follows:

1. Cells were exposed to a simulated Mars flux using the freestanding xenon-arc lamp source (described above) for 0.5, 1, 5, 10, 30, 60, or 240 min (equivalent to 1.5, 3, 15, 30, 90, 180, or 720 kJ of UV irradiation, respectively). Three sets of experiments were conducted: (a) exposure to full spectrum >200 nm (Mars simulation), (b) exposure to wavelengths >295 nm (terrestrial simulation) where the light source was also placed at a distance to increase the flux by 100% to take into account the smaller Sun–Earth distance, and (c) control exposure only to visible light (>400 nm). In this experiment, viability and autofluorescence of cells were examined, and three non-exposed dark controls were run. This experiment was conducted three times.
2. Cells were exposed to the simulated Mars solar spectrum (>200 nm) in the Mars simulation chamber (described above) for 0.5, 1, 5, 10, or 30 min and 1, 4, or 8 h under ambient Earth atmospheric conditions. In this experiment, esterase activity and 4',6-diamidino-2-phenylindole (DAPI) staining were examined after exposure. The experiment was conducted three times with two discs per experiment. Three non-exposed dark controls were run.
3. Survival of biomolecules was studied in micro-habitats under full simulated martian conditions (temperature, pressure, UV irradiation) as described above. Discs were covered in 1 mm of Mars simulation soil or crushed gneiss and exposed to 4 h of simulated martian conditions. The experiment was conducted three times. Three full spectrum (>200 nm) ex-

- posed control discs and dark, non-exposed control discs inside and outside the chamber were run.
- One experiment was run to test the viability of cells within a micro-habitat. Three pieces of sandstone from the Negev desert containing endolithic *Chroococcidiopsis* sp. communities were exposed to simulated martian UV and visible light flux for 8 h using the free-standing xenon-arc lamp source. The rocks were previously stored in a desiccated state at laboratory temperature (22°C).
 - A control experiment was run in which viability was assessed after exposure to 20 days of vacuum (1×10^{-6} mbar) to determine whether exposure to vacuum reduced the viability of the cells. Eight discs were exposed to vacuum, and eight non-exposed control discs were examined.

Cell viability

The Most Probable Number method was not used with *Chroococcidiopsis* sp. because of the slow doubling time (approximately 4 days). Instead, cell viability was determined by adding 30 μ l of sterile H₂O to the glass discs to rehydrate the cells following exposure to martian conditions. Some cells tended to clump together when rehydrated. We assumed that the mean number of cells clumped was the same for control and exposed cells, and this assumption was

verified by observations under bright-field microscopy. Thus, the fraction of viable cells calculated accounted for any clumping. Ten microliters of the cell suspension was evenly spread onto the surfaces of each of three BG-11 (2%) agar plates with 0.1 ml of sterile double deionized H₂O to bulk the sample so that it could be spread over the whole surface of the plate. The plates were incubated under the conditions described for cultures. The procedure was repeated for two discs for each independent experiment. An identical procedure was repeated for the dark, control discs (made from the same cell batch). After 1 month, the number of colonies was counted on plates of treatments and controls, and these colony counts were used to calculate the percent loss of viability for each treatment. Numbers are expressed as numbers of colonies in the treated cells over the number of colonies in the dark, untreated controls for each experiment (N_t/N_o).

Cell morphology and DNA staining

Cell morphology was first assessed under bright-field microscopy. Control discs and exposed discs were qualitatively compared to determine whether exposure to simulated martian conditions had caused cell disruption or lysis. Localization of nucleoids (DNA to the cell) was assessed using the DNA binding dye DAPI · 2HCl (Sigma Chemicals, Dorset, UK). Cells were rehy-

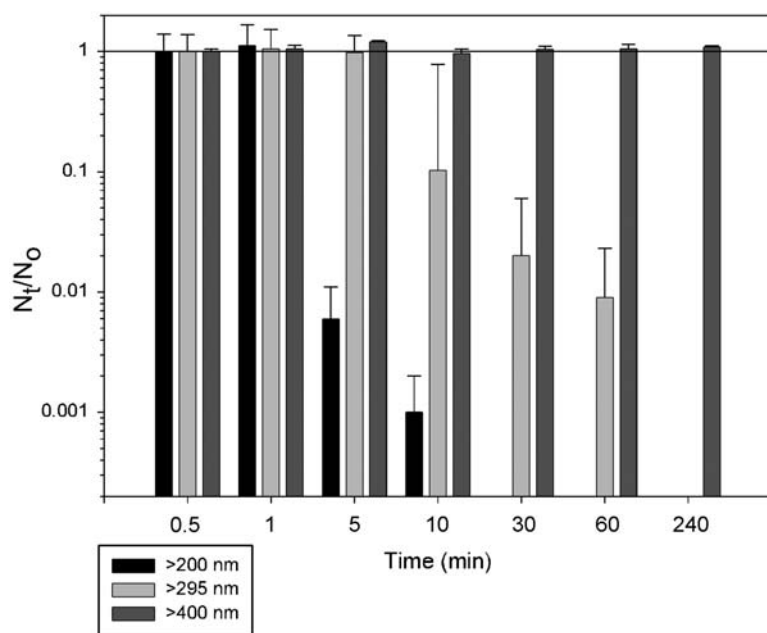


FIG. 4. Loss of viability under simulated martian light flux (>200 nm), simulated terrestrial flux (>295 nm), and all UV radiation removed (>400 nm). Numbers are expressed as colony counts in treated cells versus control cells (N_t/N_o).

drated with 30 μl of sterile H_2O , and 10 μl of cells was added to 10 μl of DAPI on a glass slide. DAPI was added at a concentration of 1 $\mu\text{g}/\text{ml}$, and cells were incubated at room temperature for 10 min in darkness. For DAPI visualization, cells were excited at 330 nm with a bandpass of 80nm and observed at the emission wavelength of 450 (bandpass 65 nm).

Autofluorescence

The fluorescence of the major light-harvesting pigments (contained in phycobilisomes) and chlorophyll a, found in cyanobacteria, was determined as a measure of the degradation of the photosynthetic apparatus. Measurements were made at the Multi-Imaging Center, University of Cambridge. Discs of cells were rehydrated with 30 μl of double-distilled H_2O and examined under a Leica TCS-SP-MP confocal laser microscope (Leica Microsystems, Heidelberg, Germany). Cells were irradiated with a 568 nm laser, and fluorescence at wavelengths greater than 590 nm was measured. Two discs were examined for each time period and each UV exposure. For each disc, 10 fields of view were chosen. Using the Leica LCS software, fluorescence was measured across the field of view along a transect between 10 and 30 μm in length. Fluorescence was then expressed in units per micrometer. Fluorescence is expressed as a ratio of the mean fluorescence for these fields of view in treated cells to the mean fluorescence in dark, untreated cells (F_t/F_o).

Esterase activity

Esterases are a group of intracellular enzymes present in all types of cells. They are involved in cell membrane formation. Esterase activity was assessed by the ability of the cells to intracellularly cleave fluorescein diacetate (FDA) to the fluorescent product, fluorescein (*e.g.*, Battin, 1997; Regel *et al.*, 2002). FDA (Sigma Chemicals) was made up to a final working concentration of 5 $\mu\text{g}/\text{ml}$. After exposure, cells were rehydrated with 30 μl of sterile H_2O , and 10 μl of cells was added to 10 μl of FDA on a glass slide. Cells were incubated at room temperature in the dark for 5 min. A coverslip was then placed over the cells. They were observed under a Zeiss Axioskop 2 Plus (Carl Zeiss, Jena, Germany) using an Omega (Omega Optica Inc., Brattleboro, VT) filter set. For FDA visualization cells were excited at 485 nm

with a bandpass of 22 nm and observed at the emission wavelength of 530 nm (bandpass 30 nm). Three samples from each of the two discs for each independent experiment were examined. The ratio of fluorescing and non-fluorescing cells in 20 different fields of view for each sample was determined as a measure of loss of esterase activity. Cells were scored as “fluorescing” when they showed a bright yellow/green coloration. Esterase activity was expressed as the ratio of the number of cells showing fluorescence in the treated cells to the number in the untreated control cells (E_t/E_o).

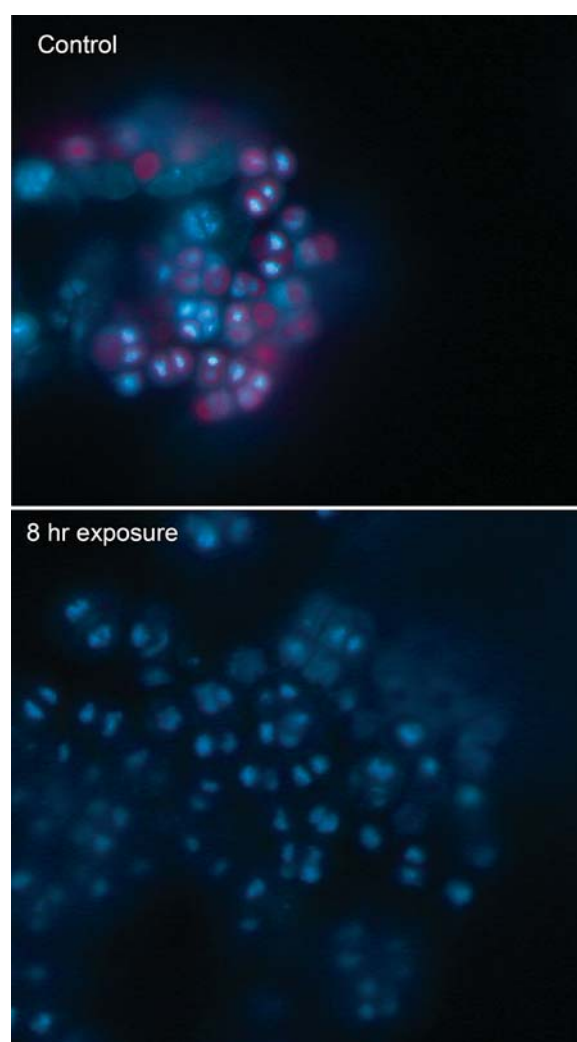


FIG. 5. Micrographs showing visualization of intact cell morphology and central localization of nucleic acids after an 8-h exposure to a simulated martian light flux. Note loss of background autofluorescence after the 8-h exposure, indicating degradation of phycobilisomes.

RESULTS

Loss of viability

Viability was rapidly lost under exposure to the simulated martian flux with wavelengths greater than 200 nm (Fig. 4). After 5 min, only 1% of the original population remained viable. After 30 min there were no remaining viable cells. The removal of UVC and part of the UVB spectrum to simulate the terrestrial surface flux (cutoff at 295 nm) greatly reduced the loss of viability. A 99% loss of viability required 60 min of exposure to UVB (>295 nm) and UVA radiation. The removal of all UV radiation (>400 nm) resulted in no significant loss of viability after 4 h (Fig. 4) (Student's *t* test, $P < 0.05$). In addition, no significant loss of viability was observed between control (laboratory) cells (mean colony counts 76 ± 38) and those exposed to vacuum for 20 days (mean colony counts 65 ± 48) in a separate, independent, experiment as assessed by Student's *t* test ($P < 0.05$) (data not shown).

Morphology and DNA staining

After a 8-h exposure to the full simulated martian UV flux, there was no obvious change in morphology of the cells as exhibited by DAPI staining. DAPI localized to the DNA within the cells after this time period (Fig. 5). Background fluorescence from phycobilisomes was markedly

quenched compared with control cells, consistent with the autofluorescence data.

Loss of autofluorescence

After a 1-h exposure to the unattenuated martian UV and visible spectrum, mean fluorescence was 14% of control cells (Figs. 6 and 7). Cells exposed to UV radiation without UVC radiation exhibited 19% of the mean fluorescence of control cells after a 4-h exposure, and when all of the UV radiation was removed (>400 nm), there was no measurable loss of fluorescence after a 4-h exposure (Fig. 6).

Loss of esterase activity

In this experiment, cells were only exposed to the unattenuated martian UV and visible spectrum with wavelengths >200 nm. A 99% reduction in the number of cells exhibiting esterase activity required at least 1 hr of exposure (Fig. 8) to the unattenuated spectrum. Esterase activity was completely lost after a 4-h exposure.

Effects of micro-habitat on biomolecule survival

After a 4-h exposure to unattenuated martian UV and visible flux under the simulated martian atmosphere and temperature regimens, there was no significant difference in either autofluorescence or esterase activity in cells under 1 mm of

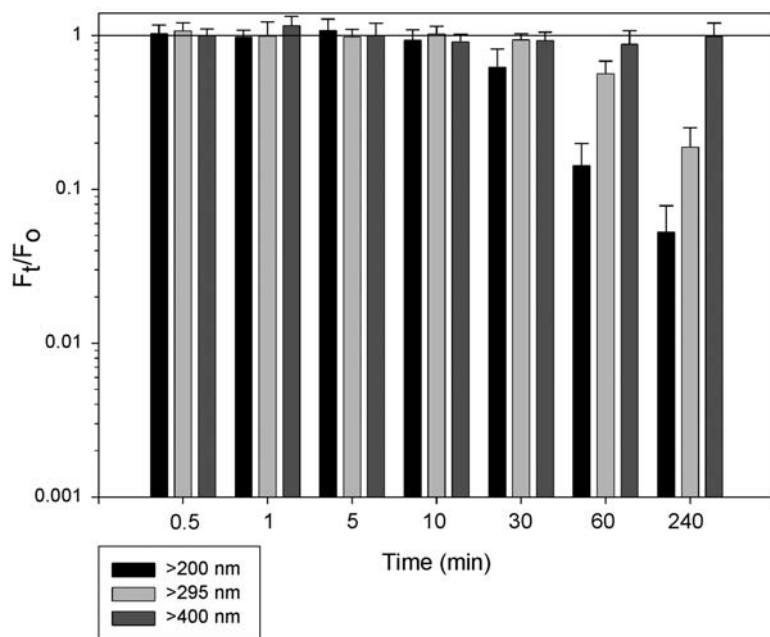


FIG. 6. Loss of autofluorescence of phycobilisomes under simulated martian light flux (>200 nm), simulated terrestrial flux (>295 nm), and all UV radiation removed (>400 nm). Numbers are expressed as autofluorescence in treated cells compared with control cells (F_t/F_0).

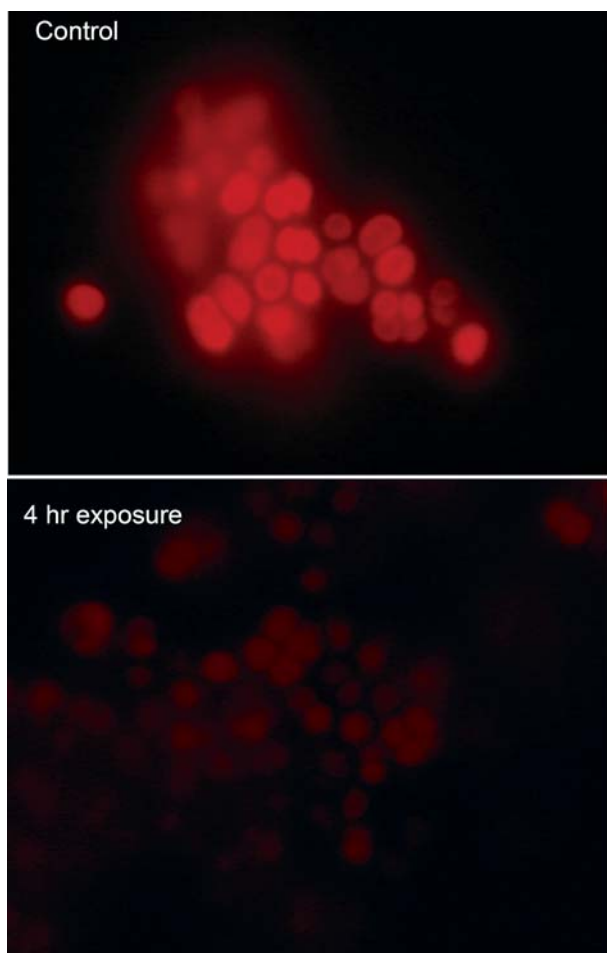


FIG. 7. Micrographs showing loss of autofluorescence after a 4-h exposure to a simulated martian UV and visible light flux compared with dark control cells.

crushed and intact gneiss or 1 mm of simulated martian soil (Fig. 9) compared with outside controls (Student's *t* test, $P < 0.05$). Cells fully exposed to unattenuated UV flux showed similar order of magnitude reductions in fluorescence and esterase activity as those reported in Figs. 6 and 8 under Earth-ambient conditions.

Cells within their natural sandstone habitat were viable after exposure to 8 h of martian UV flux at ambient Earth atmosphere, showing pronounced green growth around the edges of the rock after 3 weeks of immersion in BG-11 as described in Experimental Approach.

DISCUSSION

The surface of Mars, under clear sky conditions, experiences a surface UV flux with wavelengths

as low as 200 nm (Kuhn and Atreya, 1979). The surface of the early Earth prior to the formation of the ozone shield approximately 2.2 Ga ago may have experienced a similar UV flux (Kasting, 1987). Most biological action spectra, which describe the UV damage at different wavelengths, peak sharply at wavelengths in the UVC (200–280 nm) and UVB (280–315 nm) regions. The rate of kill of microorganisms directly exposed to the martian flux is therefore expected to be high. However, the loss of viability does not necessarily correlate to the destruction of biomolecules or cell morphology, meaning that although viable organisms might be killed rapidly after exposure to the unattenuated surface flux, their gross cell structure and some component biomolecules might survive much longer. We used the desiccation-tolerant, ionizing-radiation-resistant organism, *Chroococcidiopsis* sp. 029, as a model extremophilic phototrophic organism.

The organism we used in our experiments, though unlikely to be found on spacecraft, can be considered a model system for demonstrating how organisms transferred to Mars as contaminants may be killed quickly, while their structural components may survive for a long period of time and interfere with life-detection experiments.

Our experiments have shown that loss of viability occurs rapidly under a simulated martian UV flux. However, we obtained three surprising results. First, in comparison with organisms tested to date under a simulated martian UV flux, *Chroococcidiopsis* exhibited a greater longevity. We only observed reduction of viability to less than 1% after a 5-min exposure. However, studies by Schuerger *et al.* (2003) on spores of *B. subtilis* showed a reduction of viability to below 99.9% after just 15 s. Second, the martian UV flux was only 10 times more effective than the simulated terrestrial flux at killing the organisms, despite the fact that theoretically, using a DNA action spectrum, DNA-weighted irradiances might be up to 1,000 times greater on Mars than on Earth under clear skies and with the Sun directly overhead (Cockell *et al.*, 2000). *B. subtilis* does conform better to these theoretical calculations. Rettberg *et al.* (1998) showed, using orbital experiments, that spores exposed to UV radiation greater than 200 nm in Earth orbit were killed about 1,000 times more effectively than those on Earth. Third, we found that the cells did not lose any viability in the first few minutes of exposure, in contrast to spores of *B. subtilis*, which showed an initial rapid

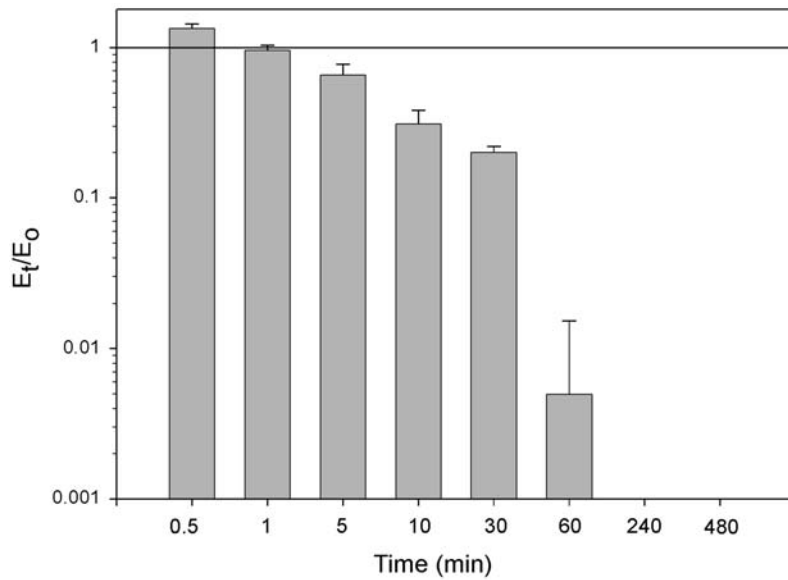


FIG. 8. Loss of esterase activity under a simulated martian light flux (>200 nm). Data are expressed as numbers of FDA-treated cells exhibiting fluorescein production compared with control cells (E_t/E_o).

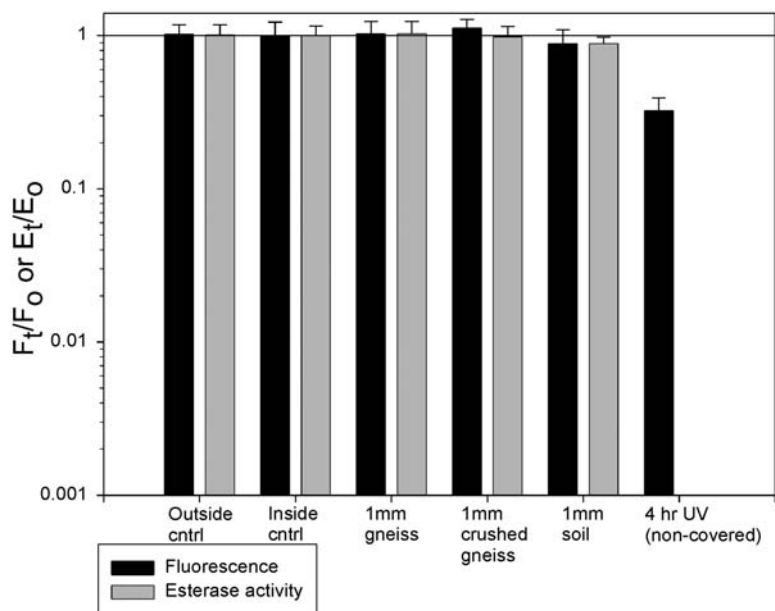
loss of viability proportional to the fluence received, followed by a slower decline in viability (Schuerger *et al.*, 2003).

There are a number of possible explanations for our data. *Chroococcidiopsis* cells are much larger than those of *B. subtilis* (approximately three times larger) and occur as single forms and two- to few-celled aggregates. This added light path length together with the organic-rich polysaccharide sheath that encapsulates cyanobacteria might provide protection against UVC radiation. Araújo and Häder (1997) suggest that the phyco-

biliproteins might act as a screen, absorbing environmental UV radiation before it reaches the DNA, although phycobilisomes do not themselves have a high UV absorbance peak when isolated. The data might also reflect differences in packaging of the DNA and the efficiency of repair processes.

Our data suggest that the calculations on the differences in the UV environments of Earth and Mars, using naked DNA action spectra, might not be an accurate indication of organismal response to the different UV radiation environments. For

FIG. 9. Effect of micro-habitat on loss of esterase activity and autofluorescence in *Chroococcidiopsis* sp. exposed to simulated martian environmental conditions for 4 h.



some organisms, the difference between the terrestrial and martian UV environment may not be as great as suggested by theory.

The lack of an effect of UV radiation on viability during the first 1 min of exposure to martian UV conditions and the first 5 min of exposure to the simulated terrestrial flux might be caused by efficient repair processes whereby damage at low exposures is completely repaired upon rehydration, but only until a threshold is reached where loss of viability begins to occur. Billi *et al.* (2000) suggest that redundant DNA may be involved in the high ionizing radiation resistance of this organism in a similar manner to that suggested for *Deinococcus radiodurans* (Englander *et al.*, 2004).

We did not find that exposure to vacuum caused any significant loss of viability for the time period measured here. Exposure to the UV flux was the most important factor affecting the loss of viability. This is consistent with previous results using *Bacillus* spp., which suggests that low pressures over several hours caused minimal loss of viability (Dose and Klein, 1996; Schuerger *et al.*, 2003). Stan-Lotter *et al.* (2003) reported high survival of *Haloarchaea* species following exposure to simulated martian atmospheric conditions. Longer exposures to low pressures might reduce viability in a similar way to that demonstrated for *B. subtilis* exposed to orbital space conditions for just under 6 years (Horneck, 1993).

Although our results suggest that *Chroococcidiopsis*-like organisms exposed to the martian UV flux will lose viability very rapidly, their component molecules can survive much longer. We found that esterases lasted long after viability was completely lost, with cells showing positive for the assay for this enzyme after a 1-h exposure to martian UV flux. Because esterases only require destruction of their active site to become inactive, it is not surprising that we found this enzyme to be degraded at a much faster rate than loss of autofluorescence, which requires the degradation of the more complex phycobilisomes and chlorophyll. The phycobilisomes are multi-subunit proteins that contain most of the light-harvesting antennae for cyanobacteria. After a 4-h exposure to a simulated martian UV flux, mean autofluorescence was almost 10% of that of the control cells.

As with the loss of viability, UV irradiation alone was responsible for loss of autofluorescence during the time period we examined. After 4 h under visible light (>400 nm), there was no loss of autofluorescence. However, visible light at

high intensity can bleach these proteins after sufficient exposure times (Sinha *et al.*, 2002). The martian UV flux is about four times more effective at bleaching these proteins than the terrestrial flux in the intact, desiccated organisms. UVB radiation has previously been shown to cause disassembly of the accessory pigment complexes, apparently partly resulting from the breakdown of linker polypeptides (Sinha *et al.*, 1995; Banerjee *et al.*, 1998). This prevents energy from being transferred from the light-harvesting complex to chlorophyll.

Although component molecules survive much longer than the period after which viability is lost, the loss of viability and degradation of biomolecules is still quite rapid. The exposure received in the 4-h period of our experiments is approximately half that received over the course of 1 day at the equator of Mars at vernal equinox under clear skies. Thus, complete loss of viability of these organisms and destruction of their biomolecules would occur in much less than a day if they were fully exposed to an unattenuated flux.

Biomolecules and organisms might well survive long enough to be mineralized and leave a fossil trace if protected inside lithic habitats. Our experiments show that after a 8-h exposure to the full unattenuated flux, cell morphology is not significantly altered, and DAPI staining shows that the cell membranes and localization of nucleic acid in the cells can still be visualized. Within rocks this potential survival will be even greater. If 1 mm of this particular gneiss, when intact, can reduce UV flux by about two orders of magnitude (Cockell *et al.*, 2002), then under 1 mm of this substrate on Mars, DAPI staining would show no significant alteration of cell morphology or nucleic acid localization after approximately 1 martian year. Our results on the importance of the micro-habitat in protecting organisms are consistent with others. Mancinelli and Klovstad (2000) showed that under 1 mm of martian analog soil a *B. subtilis* monolayer did not exhibit loss of viability after exposure to 12.3 kJ/m² of UV radiation from a deuterium source, and some protection was even afforded by a layer 12 μ m in thickness. Schuerger *et al.* (2003) also showed that a 0.5-mm layer of soil was sufficient to prevent loss of viability in *B. subtilis* after an 8-h exposure to a simulated martian UV flux from a xenon arc-lamp source.

Under 1 mm of gneiss or martian soil we found no significant loss of esterase activity or autoflu-

orescence after 4 h. After an 8-h exposure, cells within sandstone were still viable, growing out of the exposed rocks into the surrounding medium. Similar organisms on Mars or on a UV-irradiated early Earth with liquid water could repair damage that had accumulated during the day, allowing them to survive exposure on the next day. Thus, the experiments presented here suggest that under 1 mm of rock, *Chroococcidiopsis*-like organisms could potentially cope with the intense UV radiation environment on Mars, and although they have many other requirements for growth, such as nutrients, water, conducive temperature range etc., UV radiation alone would not be a limit to life in such habitats.

From the point of view of planetary protection and life detection, our data show that viability is not a reliable indicator of contamination. Biomolecules and cell structures can survive long after viability is lost, and these biomolecules and cell remnants could potentially affect life-detection experiments.

Our results also show that some live/dead viability tests, such as esterase activity, do not necessarily give reliable insights into the ability of a cell to grow. For example, after exposure to 30 min of martian UV flux, the FDA assay predicts that cells are still viable, but colony counts showed that they were not. Thus, biochemically based viability tests performed on organisms exposed to martian conditions may be open to questions of interpretation on Mars as they are on Earth (Villarino *et al.*, 2000).

The rapid loss of viability after just a few minutes of exposure to unattenuated UV flux suggests that aerobiological dispersal on Mars would be limited. If a hypothetical rock broke open and released *Chroococcidiopsis*-like organisms and they were removed and transported in a 50 kph wind on Mars, they would only achieve a distance of 4 km before they had all been killed. Thus, rocks certainly provide UV refugia for life on Mars, but the atmosphere may be a poor vehicle in which to colonize new regions, even for desiccation and radiation-tolerant organisms.

CONCLUSIONS

Chroococcidiopsis sp. 029 displays a greater tolerance to a simulated martian UV flux compared with other non-photosynthetic bacteria investigated previously. Although the unattenuated

martian UV flux rapidly kills these organisms when in an isolated state, potential biomarkers and cell morphology are retained long after loss of viability. Even modest coverings of rocks and soils are enough to prevent loss of viability after a simulated martian day. Our data suggest that even under the unattenuated martian UV flux, organisms could survive and perhaps grow within lithic habitats if there was a source of liquid water and essential nutrients.

ACKNOWLEDGMENTS

We thank Jeremy Skepper and the Multi-Imaging Center at the University of Cambridge for their help with the autofluorescence images. This work was partially supported by the British Council funding (Academic Research Collaboration no 1210) and by NASA grant NAS2-00087 from the Planetary Protection Office.

ABBREVIATIONS

BG, Blue-Green; DAPI, 4',6-diamidino-2-phenylindole; FDA, fluorescein diacetate; MSC, Mars Simulation Chamber.

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