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An analysis of potential photosynthetic life on Mars

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Abstract: This project researched the possibility of photosynthetic life on Mars. Cyanobacteria were used as potential analogs and were subjected to various Martian-simulated conditions. *Synechocystis* sp. PCC 6803 was exposed to low pressure, ultraviolet radiation and Martian-simulated atmospheric composition, and proved resistant to the combination of these stresses. However, this organism could neither grow within Martian Regolith Simulant, owing to the lack of soluble nitrogen, nor could it grow in cold temperatures. As a result, later research focused on psychrotolerant cyanobacteria capable of utilizing atmospheric nitrogen. These Antarctic nitrogen-fixing strains were able to grow in Martian Regolith Simulant at temperatures as low as 4 °C. In addition, they proved resistant to salinity, ultraviolet radiation and freeze/thaw conditions. These results suggest that Antarctic nitrogen-fixing cyanobacteria are good analogs for potential Martian life and should be considered in future exploratory missions for life on the red planet.

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Key words: astrobiology, cyanobacteria, extraterrestrial life, Mars, nitrogen fixation, photosynthesis.

Background

There are three requirements thought necessary for the existence of life: water, essential elements and an energy source (Arrieta 1997). Studies of Martian surface features (Christensen *et al*. 2000; Malin & Edgett 2000) and theoretical and experimental research indicate liquid water might exist on the present-day surface of Mars (Hecht 2002; Kuznetz & Gan 2002; Möhlmann 2005). There is also evidence that essential elements and, possibly, organic materials exist on Mars (McKay *et al*. 1999; Clifford *et al*. 2000). In addition, two potential sources of energy are present on or near the Martian surface: sunlight and chemical compounds (Arrieta 1997; Clark 1998; Clifford *et al*. 2000; Jakosky *et al*. 2003; Link *et al*. 2005). Therefore, Mars appears to have all the necessary requirements to sustain life. The difficulty is finding these three requirements together in a tolerant environment.

If liquid water exists on Mars, it is likely to be located beneath the surface (Christensen *et al*. 2000; Malin & Edgett 2000), possibly within the polar ice caps (Clifford *et al*. 2000; Gánti *et al*. 2003; Jakosky *et al*. 2003). It is theorized that photosynthetic organisms could exist within metres of the Martian surface, persisting in a dormant state that is punctuated by episodes of reproduction that occur during infrequent periods of thawing (Thomas & Schimel 1991; Arrieta 1997; Clark 1998; Clifford *et al*. 2000; Gánti *et al*. 2003; Jakosky *et al*. 2003; Vishnivetskaya *et al*. 2003). This theory is based upon examples found in extremophiles, many of them photosynthetic cyanobacteria, which currently live within the Arctic and Antarctic ice caps on Earth (Pearl & Priscu 1998; Tang & Vincent 1999; Clifford *et al*. 2000; Gilichinsky 2001; Vishnivetskaya *et al*. 2003). Many of these photosynthetic organisms remain dormant until the summer, when direct radiation is sufficient to create liquid water (McKay 1997; Pearl & Priscu 1998; Clifford *et al*. 2000). Such organisms remain viable within this permanent ice layer, where they perform photosynthesis and nitrogen fixation in small pockets of liquid water melted by increased and local radiant heat absorption by embedded dark sediments (Pearl & Priscu 1998).

Thus, it is possible that Martian photosynthetic organisms, living within a solute-rich aqueous milieu that effectively lowers the freezing point of water within the near-surface (Arrieta 1997; Gilichinsky 2001; Gilichinsky *et al*. 2003; Sears & Chittenden 2005), could receive enough sunlight at certain periods each year to thaw out a film of liquid water and reproduce (Arrieta 1997; McKay 1997; Clark 1998; Pearl & Priscu 1998; Clifford *et al*. 2000; Gilichinsky 2001; Gánti *et al*. 2003; Jakosky *et al*. 2003; Möhlmann 2005). Organisms on Earth remain viable within such cold, saline water (Gilichinsky 2001; Feller & Gerday 2003; Gilichinsky *et al*. 2003) and live through analogous perennial and cyclic thawing conditions (Pearl & Priscu 1998; Priscu *et al*. 1998; Priscu & Christner 2003). These periods of thawing could occur during high obliquity in the Martian orbit, when direct,

continuous sunlight might allow the surface temperature to temporarily rise above freezing (Clark 1998; Haberle 1998; Kuznetz & Gan 2002; Gánti *et al*. 2003). Other factors such as carbon dioxide (CO_2) greenhouse warming, the depth of maximum heating and dark dust absorption could also provide regions of elevated temperature for potential life on or near the Martian surface (Gánti *et al*. 2003; Jakosky *et al*. 2003).

Even if the Sun's radiation is not sufficient for a yearly thaw, it is believed that photosynthetic organisms can remain alive in a long-term hibernating state on Mars. On Earth, 3–4 Myr old organisms have been found in a state of suspended metabolism (Vorobyova *et al*. 1997); some life forms are possibly as old as 8 Myr (Gilichinsky 2001) or older (Jakosky *et al*. 2003). Such frozen organisms have proven to be particularly resistant to ultraviolet (UV) radiation, which is an essential attribute in the UV-transparent Martian atmosphere (Clark 1998; Gilichinsky 2001). Therefore, despite the more adverse conditions for dormant life on Mars owing to radiation, oxidizing and saline compounds on the surface and other potential environmental restraints, it is thought that life could still exist for thousands or even millions of years until the next period of climatic change permits a revival from this protracted dormant state (Thomas & Schimel 1991; Clark 1998; Vishnivetskaya *et al*. 2003). Such climatic changes, which are thought to coincide with periodic variations in Martian obliquity (Sagan *et al*. 1973), are projected to be of the order of $\sim 10^5$ yr (Ward 1973). Theoretically, this is a time period that organisms within the right environment could very well endure while waiting for the next Martian spring. Even if conditions are not conducive to net growth, at very low temperatures in the presence of water such Martian organisms could still metabolize and repair themselves – allowing for extreme long-term survival (Jakosky *et al*. 2003).

One potential environment in which organisms might be able to avert the number of Martian surface stresses is within minerals or rocks. On Earth, such endolithic (as well as hypolithic, organisms living on the underside of opaque rocks (Cockell & Stokes 2004)) communities are common – particularly in the Arctic and Antarctic (Friedmann 1982; Blackhurst *et al*. 2004; Parnell *et al*. 2004). These habitats allow organisms to withstand UV exposure (Mancinelli *et al*. (1998); reviewed in Wynn-Williams & Edwards (2000); Cockell *et al*. (2005)), wind (Parnell *et al*. 2004) and desiccation (Cockell *et al*. 2005). It is believed such an environment on Mars would provide sufficient protection for organisms to withstand many years of near-surface stresses (Thomas & Schimel 1991; Cockell *et al*. 2005).

Other factors that might limit potential Martian near-surface life include atmospheric composition, low pressure and desiccation. Cyanobacteria, which utilize CO_2 as part of their photosynthetic mechanism, have shown the ability to tolerate and even grow at CO_2 concentrations as high as 100% (Thomas *et al*. 2005). This is an essential attribute for potential life on a Martian surface with an atmosphere comprised of $\sim$95% CO_2 (Kanervo *et al*. 2005). Preliminary results have also shown a number of species of cyanobacteria to be resilient to low-pressure situations (0.1 atm) with sufficient CO_2 (Kanervo *et al*. 2005). While this value is still $\sim$10–20 times higher than the surface of Mars (Kanervo *et al*. 2005) this shows promise for potential Martian organisms being able to adapt to such a stress. In addition, some terrestrial cyanobacteria are extremely desiccation-tolerant, able to live off of only 0.02 g H_2O g^{-1} dry weight ($\sim$1% water) – an amount of water much less than can be tolerated by bacterial spores or cells under salt stress (Potts 1996).

A further reason to consider the possibility of photosynthetic life on Mars is the significant time potential ancient life had to adjust to the currently adverse Martian conditions. Current models predict early Mars as being warm and wet on the surface – indicating a suitable environment for the development of life (Clark 1998; Clifford *et al*. 2000). Over time, the Martian surface grew hostile to life as temperatures dropped well below freezing and liquid water turned solid (Clark 1998; Priscu & Christner 2003). However, it took millions of years for this transition to take place. As a result, it is theorized that any Martian life that developed in the planet's early stages had a large window of opportunity to evolve and adapt during the transition to the contemporary environment (Clark 1998).

As a combined result of these arguments, we theorize that photosynthetic organisms, under the right conditions and with properly adapted surroundings, have the necessary requisites for life and can still exist on Mars. We have analysed various cyanobacteria under a number of stresses simulating the Martian environment. Our first tests were done with *Synechocystis* sp. PCC WT 6803, a commonly used experimental model strain of cyanobacteria. We tested this strain to determine whether it could live within Martian Regolith Simulant (NASA-ARES, Johnson Space Center). We later placed *Synechocystis* under a number of Martian-simulated stresses within the Andromeda Chamber (Arkansas-Oklahoma Center for Space and Planetary Sciences). These stresses included low pressure, UV radiation and an atmosphere high in CO_2.

These initial results (see the 'Results' section) reaffirmed that Martian Regolith Simulant, which is an approximate replica based upon spectroscopic analysis of Martian soil done on the Viking and Pathfinder missions (Allen *et al*. 1998), is devoid of biologically available soluble nitrogen. As a result, assuming the Martian surface is indeed lacking in soluble nitrogen, it seems that potential near-surface organisms on Mars would be capable of using atmospheric nitrogen (N_2). Nitrogen-fixing bacteria on Earth have the ability to use such atmospheric nitrogen, even in extreme cold and barren conditions such as in the McMurdo Dry Valleys of Antarctica (Pearl & Priscu 1998) or under severely desiccating conditions (Potts 1996). As a result, we hypothesize that terrestrial psychrophilic (or psychrotolerant) nitrogen-fixing organisms provide a good model for potential Martian life owing to the planet's surface temperature as well as soil and atmospheric composition.

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Table 1. *Synechocystis was added to Martian Regolith Simulant Extract as well as a combination of this extract and nine stock solutions. Indicated growth was shown in each of the six samples tested per solution* (*two separate tests in triplicate*)

Solution (concentration)	Pure M.R.S.E.	$NaNO_3$ (10 ml l^{-1})	$CaCl_2$ (1 ml l^{-1})	$C_6H_8O_7$ (Citric acid) (1 ml l^{-1})	$FeNH_4$ Citrate (1 ml l^{-1})
Growth?	✘	✓	✘	✘	✘
Solution (concentration)	EDTA (1 ml l^{-1})	K_2HPO_4 (1 ml l^{-1})	$MgSO_4$ (1 ml l^{-1})	$NaCO_3$ (1 ml l^{-1})	Trace metals (1 ml l^{-1})
Growth?	✘	✘	✘	✘	✘

While extremophiles, such as these Antarctic strains, have been studied within their diverse, severe environments on Earth (see Pearl & Priscu 1998; Priscu *et al*. 1999; Quesada *et al*. 1999; Tang & Vincent 1999; Gordon *et al*. 2000; Feller & Gerday 2003; Cockell & Stokes 2004), there is no specific terrestrial environment that can properly simulate Martian conditions. Therefore, much remains to be learned about the possibility of such bacteria living on Mars by testing them under various Martian-simulated conditions. Low pressure, low temperature, large doses of UV radiation, wind, desiccation, atmospheric composition and limited minerals all provide distinct challenges for possible photosynthetic life to overcome. Consequently, the goal of this research became the study of the effects of various Martian-simulated stresses on strains of extremophile organisms found on Earth. Tests included inoculation in Martian Regolith Simulant, salinity, UV radiation and resuscitation from a frozen state.

Methods

Tests with Synechocystis sp. PCC 6803

The unicellular cyanobacterium *Synechocystis* sp. PCC 6803 was cultured in the standard mineral salts medium BG-11 (Rippka *et al*. 1979). The bacteria were grown in shaking incubators at 30 °C with constant illumination under fluorescent lamps at an incident intensity of approximately 50 μmol photon m^{-2} s^{-1}. Experiments were undertaken with cultures in the log phase of growth.

Synechocystis was tested within Martian Regolith Simulant by developing an extract for the bacteria to grow in. To create this Martian Regolith Simulant Extract, Martian Regolith Simulant was placed in de-ionized water and mixed thoroughly overnight. The mixture was then centrifuged and the heavy, non-transparent soil was removed. This created a transparent extract – essential for visually testing growth within the solution. After sterilizing the Martian Regolith Simulant Extract in an autoclave, 10 different mixtures were created (in triplicate) for the *Synechocystis* to grow in by adding various stock solutions (see Table 1). These were used to determine if any elemental deficiencies (for cyanobacterial growth) existed within the Martian Regolith Simulant. Each solution was inoculated with active cultures of *Synechocystis* and grown in the shaking incubator under fluorescent light at a flux of ~50 μmol photon m^{-2} s^{-1} of photosynthetically active radiation (PAR). The bacteria were washed repeatedly with de-ionized water prior to inoculation into the Martian Regolith Simulant Extract to ensure there was no media carry-over. In addition, the experiment was performed with multiple concentrations of Martian Regolith Simulant to ensure the bacteria were not inhibited or damaged by excessive mineral content. An aqueous extract created from 10% Martian Regolith Simulant (by volume) was found to provide a working concentration of mineral nutrients. To determine growth within the various solutions the samples were visually inspected for 20 days and the presence of macroscopic cell growth was assessed by the acquisition of the characteristic green colour of the cyanobacterium.

The *Synechocystis* was also tested under low pressure, UV radiation and an atmosphere high in CO_2 to work towards Martian conditions. This was performed within the Andromeda Chamber, which simultaneously dropped the pressure to 400 mbar, created an atmosphere of 95% CO_2 and 5% H and irradiated the sample with UV light. These values were made as first steps toward actual Martian values (5–10 mbar and ~95% CO_2, ~3% N_2 and ~2% Ar (Kanervo *et al*. 2005)). Active samples of *Synechocystis* were suspended in 400 ml of media within 1 l beakers (in triplicate) immediately before being placed in the chamber. A continuous discharge Xe lamp within the chamber provided ~600 μW cm^{-2} of UV radiation at the liquid surface within each beaker along with a PAR flux of ~10 μmol photon m^{-2} s^{-1}. The chamber was sealed and then slowly lowered to 400 mbar to avoid boiling of cell suspensions. Next, CO_2 was gradually added – ensuring the temperature within the chamber did not diverge far from room temperature – until the composition was 95% CO_2 and 5% H. The *Synechocystis* samples were kept within the Andromeda Chamber for 50 h under these conditions to provide time for changes in growth and any adaptations caused by the various stresses. As a control, three beakers of the same culture were put in an anaerobic glove box under approximately the same PAR flux over the same 50 h time frame. This glove box was kept at an identical atmosphere (95% CO_2 and 5% H) as the Andromeda Chamber.

Culture growth was estimated by measuring turbidity. A liquid sample was taken from each beaker directly after removing it from the chamber (and glove box) and a portion was immediately frozen with dry ice. The frozen samples were brought back to our laboratory, where the absorption by the various photosynthetic pigments within the *Synechocystis* could be measured with a spectrophotometer (Shimadzu UV-VIS 2100, Japan) as a means of following optical density at

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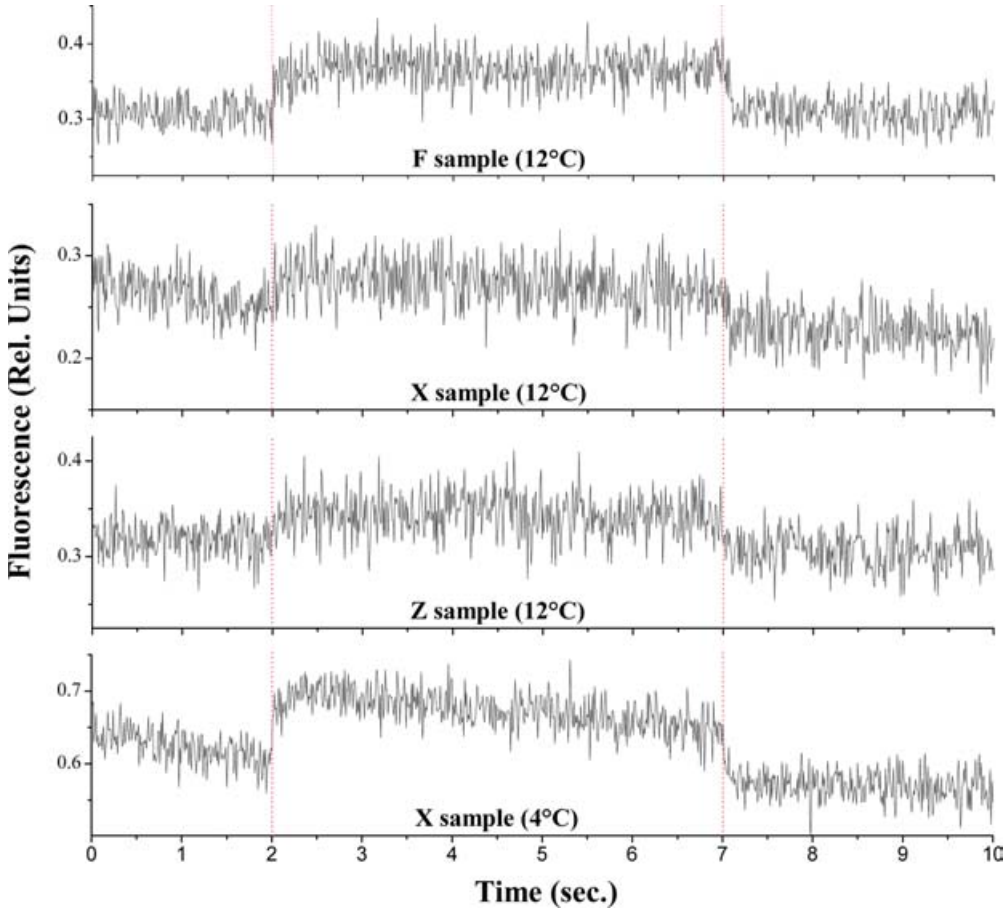

Fig. 1. Samples of three different cultures of nitrogen-fixing cyanobacteria were washed and inoculated in Martian Regolith Simulant Extract. Samples X and F are different cultures grown from samples from Lake Vida in the McMurdo Dry Valleys. Z samples are from Don Juan Pond. Active photosynthesis – used as an indicator of living cyanobacteria – is measured by fluorescence and indicated by a rise in absorbance between 2 and 7 s when an actinic light saturates the sample (see the 'Methods' section).

750 nm (OD_{750}). Cells do not have significant absorbance at this wavelength and OD_{750} is almost exclusively due to scattering (turbidity), which is proportional to cell density. The remaining liquid samples were immediately used in a serial dilution on agar plates. Samples were distributed in several tubes as a dilution series and aliquots were spread on solid media consisting of 1% w/v agar (Difco, Kansas City) in standard BG-11 media. Culture viability was evaluated by measuring colony-forming units (CFUs) that formed after the serial dilution. CFUs appeared after 7–10 days of growth at 30 °C under illumination from fluorescent lamps with an incident intensity of approximately 50 μmol photon m^{-2} s^{-1}.

Tests with Antarctic nitrogen-fixing strains

Strains of nitrogen-fixing cyanobacteria were isolated from samples of Antarctic material from various regions of the McMurdo Dry Valleys (provided by Dr John Priscu, Montana State University). This was accomplished by growing the samples in the mineral medium BG-11_o, which is the common formulation for BG-11 (Rippka *et al.* 1979) without combined nitrogen (no $NaNO_3$ is added). Under these conditions only autotrophic strains capable of fixing atmospheric nitrogen will grow. Indeed, strains grown under these conditions displayed clear heterocysts (specialized regions of nitrogen fixation within filamentous cyanobacteria (Fogg 1944), see Fig. 5). Samples were taken from Lake Vida (labelled X) and Don Juan Pond (labelled F and Z).

The methods for growing and experimenting with the Antarctic cyanobacteria were vastly different than those used for *Synechocystis* sp. PCC 6803. Bacteria were grown under lamps (~5 μmole photon m^{-2} s^{-1}) at 12 °C within a refrigerator or shaking cold-water bath. The cyanobacteria were also tested at 4 °C in a cold room where growth was still apparent (albeit slower).

Owing to better growth at 12 °C than 4 °C it appears the isolated bacteria were psychrotolerant and not psychrophilic. While isolating psychrophilic strains would likely provide a better analog for the Martian surface, the generation times are prohibitively slow for research purposes in such exploratory experiments. In addition, as mentioned, the

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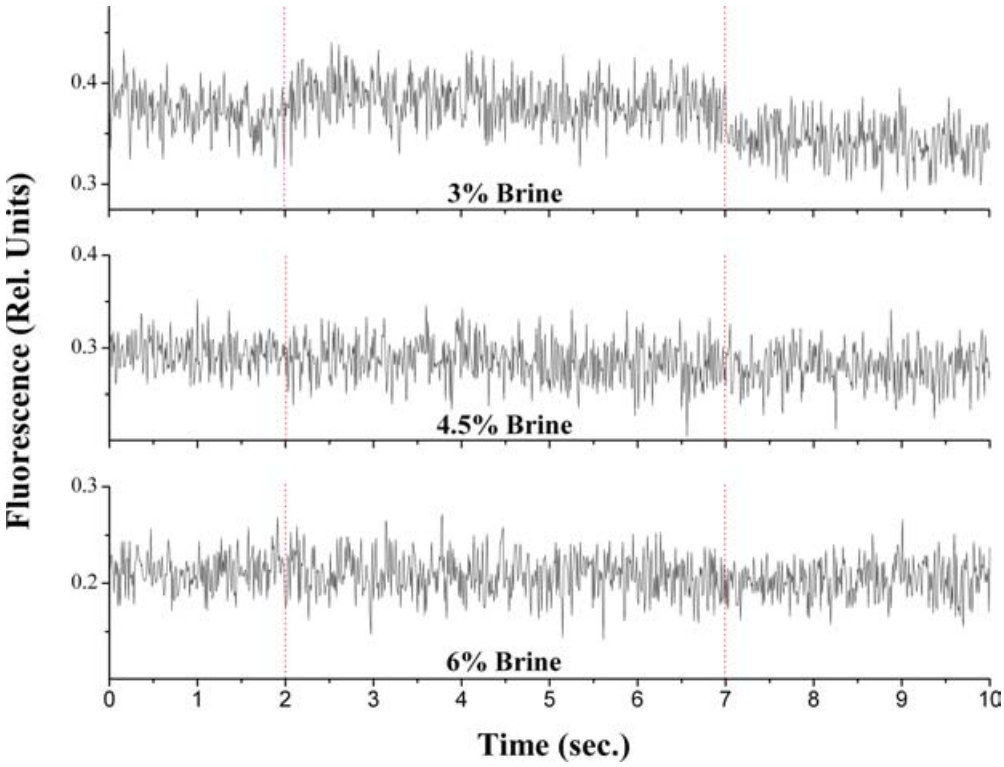

Fig. 2. Various concentrations of simulated ocean brine were used to determine the salinity that the Antarctic cyanobacteria (F, X and Z samples) could withstand.

psychrotolerant bacteria isolated for these studies were still able to grow at 4 °C.

The photosynthetic activity of the bacteria after exposure to various stresses was tested by kinetic fluorometry. A modulated fluorometer (PAM 101 chlorophyll fluorometer with PAM 103 flash attachment, Walz Inc., Germany) was used to test living cells by measuring the fluorescence originating from the photosynthetic apparatus, specifically photosystem II, within the bacteria.

This technique monitors changes in the yield of fluorescence, which is a function of the photochemical state of the photosystem II complex. The measuring apparatus employs a weak, non-actinic modulated beam of light. This measuring beam is used to interrogate the fluorescence yield of the sample, which varies according to the photochemical state of the photosystem II complex. In addition, the apparatus contains a strong, actinic broad visible spectrum light used to stimulate photochemistry within the photosynthetic mechanism. In samples with active photosystem II complexes, a significant increase in fluorescence yield occurs upon switching on the actinic light. This light saturates the sample and causes the photosystem II complexes of the bacteria to enter a charge-separated state that is maximally fluorescent. The actinic light is superimposed upon the measuring light for 5 s. This causes photosynthetically active cultures to maximally fluoresce, as detected by the fluorometer. Graphs were created to display this fluorescence yield versus time as a means of displaying active or inactive photosynthesis (Figs 1–4). Live cultures display a distinct rise in fluorescence between the 2 and 7 s time points (when the actinic light is on) above the baseline created by the initial light. This period of actinic light is indicated on the graph by the region between the dashed vertical lines. Some fluorescence graphs display a slope with a negative trend. This is caused by clumps of bacteria that are not fully soluble within the liquid sample and consequently settle during the 10 s interval in which they are being tested. This does not affect overall interpretations of the results for the fluorescence readings.

Antarctic cyanobacteria were tested within Martian Regolith Simulant by inoculating cultures into Martian Regolith Simulant Extract, which was created by the same process outlined above for *Synechocystis*. Cultures were washed to avoid nutrient carryover and were tested for growth in Martian Regolith Simulant Extract at 12 °C and 4 °C.

Cultures were inoculated in brine (Henley 2002) to determine their resistance to salinity. The cyanobacteria were washed as before, added to various concentrations of brine and kept in a shaking cold water bath at 12 °C.

UV tests were performed in the 4 °C cold room. Incident radiation was set at 100 μW cm^{-2} by placing a UV lamp (Mineralight lamp UVSL-58, Ultraviolet Products, Inc., San Gabriel, CA, USA) at a fixed distance of approximately 4 cm above the cultures along with a visible light lamp that provided ~5 μmol photon m^{-2} s^{-1}. In addition, a box was placed over the entire apparatus to avoid extraneous light exposure. Samples were removed at various times and tested for active photosynthesis using the fluorometer. Each sample was

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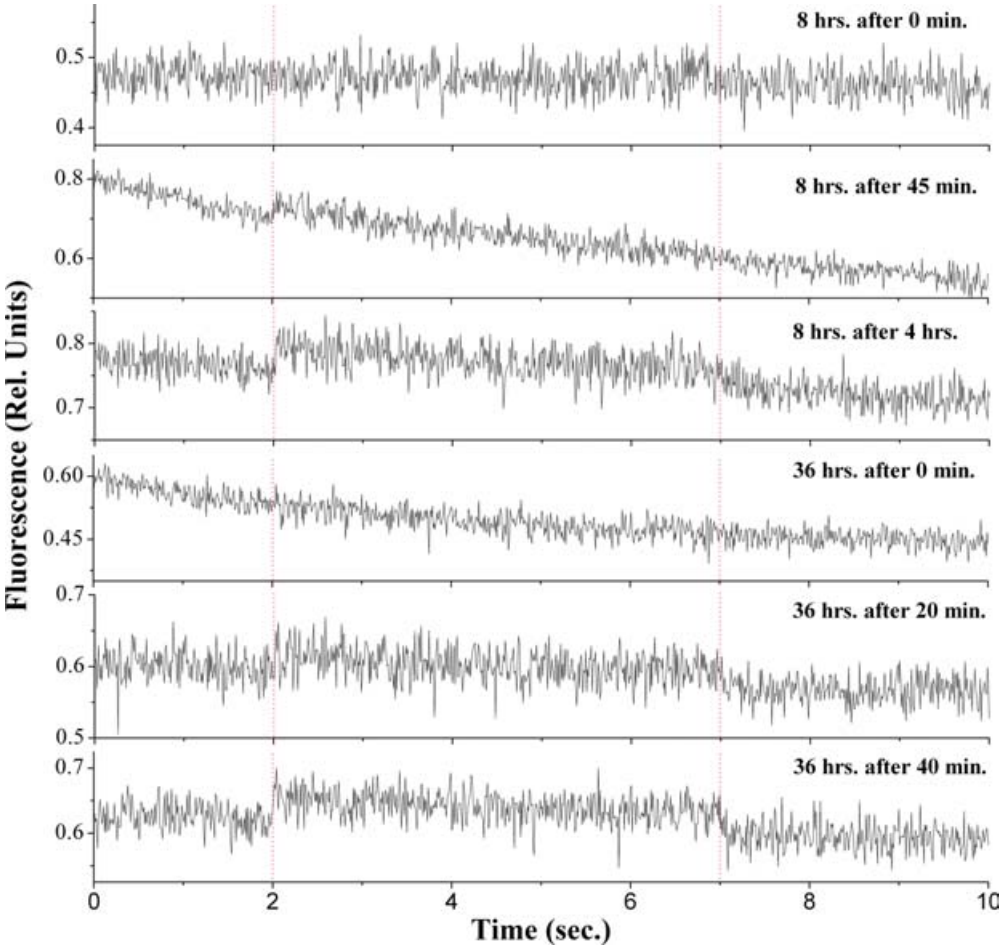

Fig. 3. A UV lamp was used to determine the length of exposure to radiation that the cyanobacteria (X sample) could endure. Subsequent measurements were taken after removal from the UV light to determine the length of time necessary for the bacteria to recover (e.g. '8 h after 45 min' indicates that the organisms were exposed to UV light for 8 h and then measured for fluorescence 45 min after removal).

subsequently placed in 12 °C under similar visible light after removal and tested at successive intervals afterwards to determine the time it took for recovery.

Resuscitation of organisms from a frozen state was tested by freezing the cultures at −20 °C overnight and then placing them in 12 °C with a constant light source (~5 μmol photon m^{-2} s^{-1}). Samples were then taken out after thawing and tested for photosynthetic activity. After these immediate measurements, samples were then placed on ice and tested for active photosynthesis at various time intervals afterward.

All experiments were performed using a sterile technique to avoid contamination. A laminar flow hood with a Bunsen burner flame was used for inoculations and all materials and media were autoclaved prior to inoculation.

Results

Of the various Martian Regolith Simulant Extract solutions inoculated with *Synechocystis*, only the sample with added sodium nitrate ($NaNO_3$) displayed growth (see Table 1). Considering the chemical composition of the other solutions it is apparent that biologically available soluble nitrogen was insufficient within the Martian Regolith Simulant for the cyanobacteria to grow. This concurs with compositional analysis of the Martian Regolith Simulant by the Johnson Space Center (Allen *et al*. 1998).

The samples of *Synechocystis* that were exposed to various stresses within the Andromeda Chamber remained viable as deduced from cell density by monitoring OD_{750} and, more directly, by the CFUs present after the serial dilution. It was clear from the virtually identical optical density values and numbers of colony forming units between the Andromeda and control samples (data not shown) that the conditions in the chamber had a minimal effect on the bacteria.

The Antarctic nitrogen-fixing strains displayed distinct visible growth in Martian Regolith Simulant Extract. Fluorometry was used for objective proof. These results are shown in Fig. 1. Samples F, X and Z all displayed clear signs of life at 12 °C (as shown by the clear rise in fluorescence between 2 and 7 s). The final graph in Fig. 1 indicates clear photosynthesis of the X bacteria in Martian Regolith Simulant Extract at 4 °C.

Kinetic fluorometry was also used to test the photosynthetic activity of the Antarctic cyanobacteria after being

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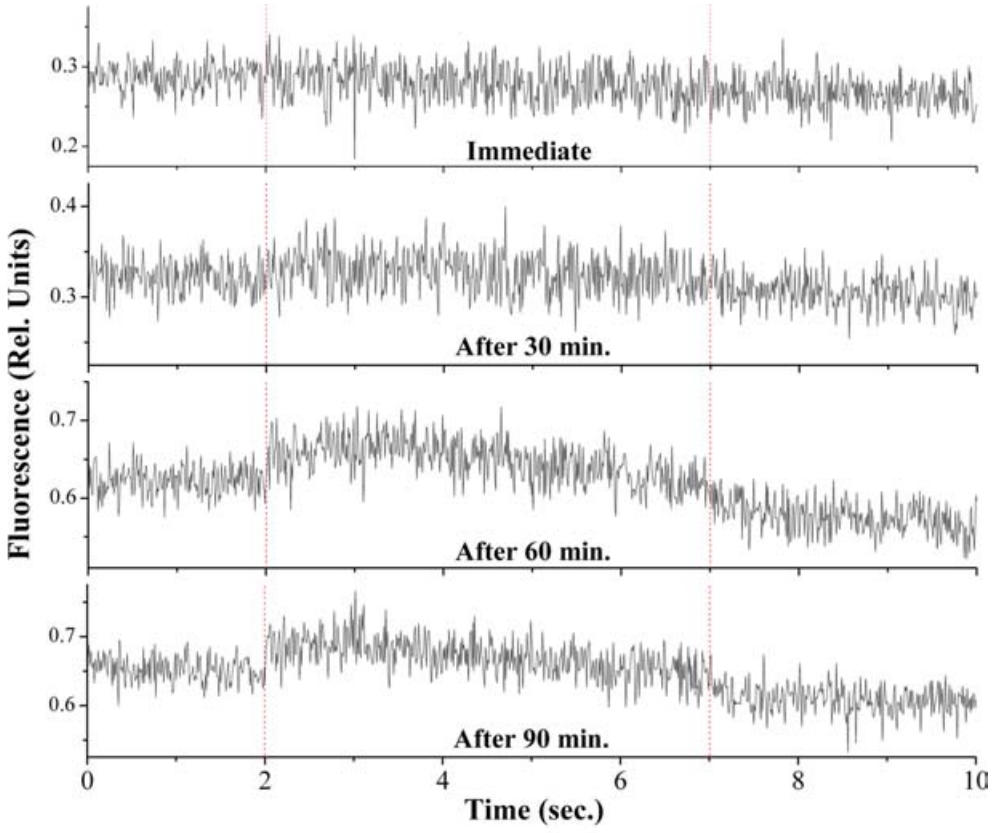

Fig. 4. Bacteria (F and X samples) were frozen overnight and then put in 12 °C under a lamp to simulate resuscitation from a frozen state. Measurements were taken at time intervals after thawing to determine when the bacteria regained photosynthetic activity.

tested under other various stresses. These included varying concentrations of salt and UV radiation, as well as resuscitation from a frozen state.

The first test displayed that the organisms were only able to withstand a concentration of 3% brine, approximately that of terrestrial oceans (Fig. 2). Anything over this concentration abolished photosynthetic activity in the samples, which eventually bleached and died.

The second test showed that the bacteria were resistant to UV radiation. After 8 h of UV exposure samples showed signs of photosynthetic activity 45 min after removal from the UV light (Fig. 3, graph 2) and clear photosynthesis 4 h after removal (Fig. 3, graph 3). After 36 h of UV exposure the bacteria showed initial photosynthetic activity only 20 min after removal (Fig. 3, graph 5) and clear photosynthesis 40 min after removal (Fig. 3, graph 6).

The organisms also displayed an ability to resuscitate from a frozen state. Initial photosynthetic activity from the cyanobacteria was measured 30 min after thawing (Fig. 4, graph 2) with increasing photosynthetic activity at 1 and 1.5 h afterwards (Fig. 4, graphs 3 and 4).

Implications for Mars

Considering the lack of nitrogen in analyses of Martian surface soil (Allen *et al.* 1998) it seems that nitrogen-fixing bacteria, which can obtain nitrogen from N_2 in the atmosphere, would be more likely to survive the Martian near-surface than other organisms. The Martian atmosphere (comprised of ~95% CO_2 and ~3% N_2 (Kanervo *et al.* 2005)) has available nitrogen, albeit in significantly lesser quantities than Earth. While this study demonstrated the ability of nitrogen-fixing cyanobacteria to grow in Martian Regolith Simulant (whereas common cyanobacteria could not), these tests were performed in a terrestrial atmosphere. A Martian-simulated atmospheric composition would provide a significantly better test for the nitrogen-fixing capabilities of these organisms.

The Antarctic cyanobacteria were also able to grow in Martian Regolith Simulant Extract, and consequently to harvest atmospheric nitrogen, at temperatures as low as 4 °C. This is of particular interest considering the harsh climate at the Martian surface, which might only rise above freezing for infrequent periods (Shuster & Weiss 2005). It should be noted, however, that limited Martian soil has been analysed thus far (Allen *et al.* 1998) and non-gaseous nitrogen sources are still possible (Clark 1998; Mancinelli & Banin 2003).

Various tests with each strain of cyanobacteria displayed their resilience to conditions approaching the Martian surface. *Synechocystis* samples exposed to the stresses within the Andromeda Chamber yielded identical spectrophotometric and plating results as the control. While this indicated little

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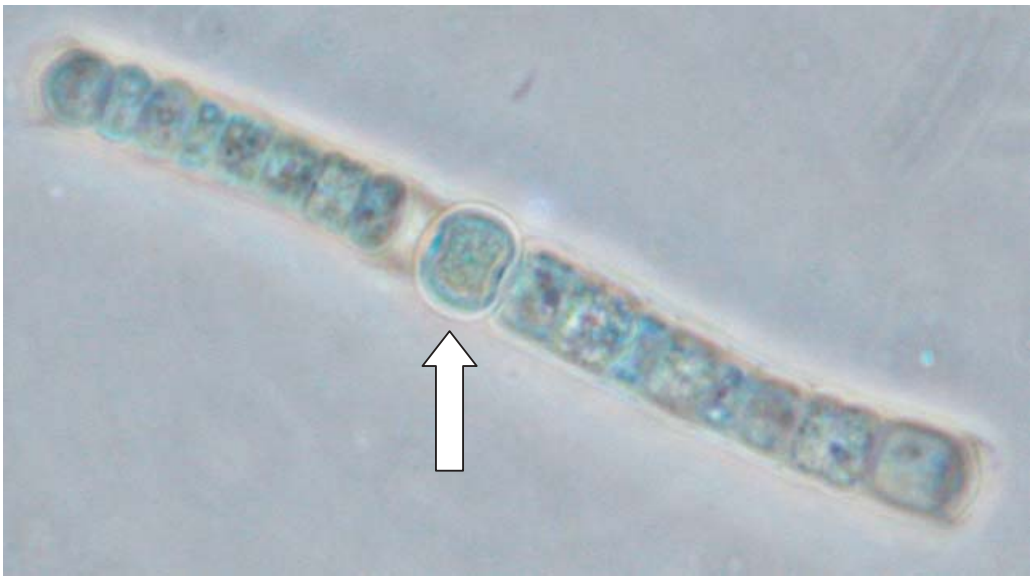

Fig. 5. A filament of Antarctic nitrogen-fixing cyanobacteria from the X sample with a clear heterocyst (arrow). The picture was taken with a Nikon Coolpix 5000 camera on a Nikon Eclipse E400 phase contrast microscope (Nikon, Melville, NY, USA) under ~1000× magnification.

about any potential adaptations to these stresses, it did show that the *Synechocystis* cultures were minimally affected by the various stresses within the Andromeda Chamber for the 50 h of exposure – including low pressure as well as Martian-simulated atmosphere and UV radiation. This attests to the robustness of this mesophilic and halotolerant bacteria in the face of immoderate conditions relative to its natural habitat (brackish waters). Kanervo *et al*. (2005) showed this same strain of cyanobacterium (*Synechocystis* sp. PCC 6803) survived 0.1 atm and 20% CO_2 over a span of 9 days, in accordance with the results presented here (0.4 atm and 95% CO_2 over 50 h).

In addition, the Antarctic nitrogen-fixing strains maintained activity under a number of stresses. The results indicate clear photosynthetic activity from these organisms in the Martian Regolith Simulant (at 12 and 4 °C), 3% brine and shortly after thawing out from a frozen state. Also, the Antarctic cyanobacteria were able to resume activity shortly after exposure to UV light. In fact, after hours of continuous exposure, the bacteria, if anything, became more resistant to the UV radiation over time (in the range of the tested timescales). This was indicated by the reduced time necessary for the recovery of photosynthetic activity in samples that were in UV light longer (36 h versus 8 h). Therefore, it appears these Antarctic cyanobacteria were able to employ mechanisms for adaptation to UV exposure.

Future tests on cyanobacteria under potential Martian conditions, in particular, incorporating atmospheric composition and surface pressure to the Antarctic strains, will provide important information on the growth restraints for these extremophiles. It will also be important to test these cyanobacteria under a combination of stresses, as performed by Hansen *et al*. (2005). While the Antarctic organisms might be able to survive low pressure and radiation separately, the ability to handle such stresses simultaneously remains to be assessed.

Such analyses of these Martian analogue will hopefully encourage researchers to consider the possibility for photosynthetic life on the red planet during its continued exploration, particularly considering that such a mission is seemingly within our current technological capabilities. Furthermore, studies such as these will help researchers learn the expected functionality of such organisms under Martian conditions. This kind of research will be essential for creating experiments to detect whether microbial life does exist on Mars. For example, instrumentation involving fluorometry (Weinstein *et al*. 2005) or Raman spectroscopy (Wynn-Williams & Edwards 2000; Ellery & Wynn-Williams 2003) could provide a straightforward way to test for such photosynthetic life on Mars. In addition, studies such as these may provide the methods necessary to enrich Martian soil with soluble nitrogen for use by future exploratory missions in the cultivation of food.

While little has been researched to this point, this first step towards simulating Martian conditions seems to have had minimal negative effect on cyanobacteria – making the possibility of potential photosynthetic life on Mars, within the right environment, still feasible.

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