Organics on Mars : Laboratory studies of organic material under simulated martian conditions

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Chapter 6

The behaviour of halophilic archaea under martian conditions

Mars is thought to have had liquid water at its surface for geologically long periods. The progressive desiccation of the surface would have led to an increase in the salt content of remaining bodies of water. If life had developed on Mars, then some of the mechanisms evolved in terrestrial halophilic bacteria to cope with high salt content may have been similar to those existing in martian organisms. We have exposed samples of the halophilic *Natronorubrum* sp. strain HG-1 (*Nr.* strain HG-1) to conditions of ultraviolet radiation (UV) similar to those of the present-day martian environment. Furthermore, the effects of low temperature and low pressure on *Nr.* strain HG-1 have been investigated. The results, obtained by monitoring growth curves, indicate that the present UV radiation at the surface of Mars is a significant hazard for this organism. Exposure of the cells to high vacuum inactivates ~50 % of the cells. Freezing to -20 °C and -80 °C kills ~80 % of the cells. When desiccated and embedded in a salt crust, cells are somewhat more resistant to UV radiation than when suspended in an aqueous solution. The cell inactivation by UV is wavelength dependent. Exposure to UV-A (> 300 nm) has no measurable effect on the cell viability. Comparing the inactivating effects of UV-B (250-300 nm) to UV-C (195-250 nm) indicates that UV-C is the most lethal to *Nr.* strain HG-1. Exposure to UV-B for a duration equivalent to ~80 hours of noontime equatorial illumination on the surface of Mars, inactivated the capability to proliferate of more than 95 % of the cells. It can be concluded that *Nr.* strain HG-1 would not be a good model organism to survive on the surface of Mars.

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in preparation

1. Introduction

Mars is the subject of numerous investigations concerning its planetary properties and the search for life by remote-sensing spacecraft and in-situ rovers (Rummel and Billings, 2004). Sample-return missions are being considered, and are likely to be included in a future round of missions that are expected to accommodate sophisticated (bio)chemically-oriented payloads. An important objective of those missions is to establish the presence and state of organic matter in the near-surface regolith of Mars. Almost thirty years ago the two Viking landers found no evidence (Biemann *et al.*, 1977; Klein, 1978) for the presence of native carbon-containing compounds in samples taken from two locations [1]. Given the meteoritic infall of organic matter that is expected at Mars (Flynn and McKay, 1990), the absence of organic material suggests that the martian near-surface harbours one or more chemically aggressive processes that degrade organic compounds. Laboratory experiments have been conducted to identify these processes (Oró and Holzer, 1979; Huguenin *et al.*, 1979; Stoker and Bullock, 1997; Yen *et al.*, 2000; ten Kate *et al.*, 2005; Garry *et al.*, 2005). It is therefore possible that molecular indications of a past martian biosphere will have been degraded or altered on Mars. Several possibilities can be envisaged.

1) Life has evolved on Mars but is now extinct due to the changed environmental conditions. Therefore robotic missions should look for compounds associated with life. If terrestrial geology and biology are taken as a guide, then isotope ratios and perhaps chirality measurements would be critical to unambiguously identify a biogenic origin of detected compounds. Indirect methods such as investigating biochemical remnants in geologic deposits, used on Earth (e.g. Russell, 1996; Hu Ming-An *et al.*, 1995) could be applied as well. However, this is a more complex method open to misinterpretation.

2) Life is still present on Mars but only in isolated niche areas. Some degree of adaptation would have been needed for the organisms to endure the changing climate of Mars. However, conditions in certain areas, such as those located deep in the crust, may have changed relatively little in the period during which the martian surface desiccated. The degree of environmental stress on an organism will therefore be a function of its location.

3) Life has never been present on Mars. All organic matter found in the martian environment is generated by purely abiotic processes, which may include meteoritic infall, chemical synthesis in volcanic processes and similar reactions.

In general, the conditions on the martian surface are hostile to terrestrial life. The main hazards are thought to be the lack of liquid water in the near-surface and the energetic UV radiation present in the sunlight. These conditions, combined with ionic reactions on or within mineral surfaces, may lead to aggressive oxidative agents (Yen *et al.*, 2000; Quinn, 2005d). On Earth, however, organisms exist that are able to adapt to extreme conditions and are therefore referred to as 'extremophiles'.

[1] Chryse Planitia and Utopia Planitia, landing sites of the Viking 1 and 2 landers, respectively.

Salt tolerant organisms, belonging to the domain of Archaea, evolved early in Earth's history (Oren, 2002). A class of these organisms is termed 'Halophiles', as they not only tolerate but also thrive in environments with salt concentrations up to ten times higher than that of present ocean water. Geological formations millions of years old still contain viable halophilic archaea and bacteria (e.g. the Permian-Triassic era, 290–206 Ma, Stan-Lotter *et al.*, 1999; McGenity *et al.*, 2000). In general, organisms can respond to environmental stress by the use of one or more protective mechanisms. Halophilic archaea appear to employ several protective mechanisms to provide tolerance to highly saline environments. It is possible that the diversity of such strategies is the result of multiple adaptations in ancient times. A possible connection between the origin of life on Earth and the ability of microorganisms to grow at high salt concentrations has been described by Dundas (1998).

Litchfield (1998) concluded that "the ability of halophilic archaea to survive within low-water activity environments such as 'evaporites' and their requirement for elevated salt concentrations make them likely candidates for life on Mars." Indeed, the ability of some halophilic Archaea to tolerate UV exposure and osmotic stress, is exemplified by their resistance to hard vacuum and unfiltered solar radiation during spaceflight (Mancinelli *et al.*, 1998).

Advances in the understanding of terrestrial biochemistry allows the evaluation of other life systems, either theoretically or practically, giving a better insight into how robust non-terrestrial life might be. In this light we have examined the response of the halophilic archaeon *Nr.* strain HG-1 to various Mars-like conditions. The rationale for using this species is the similarity of its natural growing environment to that of a martian setting as postulated from the results of the Mars Exploration Rovers (e.g., Squyres *et al.*, 2004 a,b). In this study we describe the effects of several environmental conditions on the survival of *Nr.* strain HG-1. The organism has been exposed to radiation in three different wavelength ranges, has been cooled to low temperatures, and was exposed to high vacuum. We have investigated the *Nr.* strain HG-1 both in suspension and after desiccation, which leads to dried cells, because *Nr.* strain HG-1 does not form spores. In section 2 details of the *Nr.* strain HG-1 are given along with its growth characteristics and the desiccation method used for the experiments. Experimental procedures concerning irradiation, cooling, and vacuum storage are discussed in section 3. Section 4 summarises the results of the behaviour of *Nr.* strain HG-1 to extreme conditions. In section 5 the implications of those results for future planetary exploration are discussed.

2. Materials and Methods

2.1 Strain and culture conditions

The archaeal strain *Natronorubrum* sp. strain HG-1 (*Nr.* strain HG-1) used in our studies has been isolated and described by Sorokin *et al.* (2005). It is an Euryarchaeon of the class Halobacteria and belongs to the order of Halobacteriales, in the

family of Halobacteriaceae. The cells are polymorphic rods with a diameter of 0.5-1 μm and a major axis length of 1.5-5 μm, which transform into irregular coccoids during stationary growth phase. 10 ml *Nr.* strain HG-1 cultures were grown in an agitated aqueous medium at 45 °C within closed 50 ml flasks. The growth medium included 240 g l^{-1} NaCl, 2 g l^{-1} K_2HPO_4, and 0.5 g l^{-1} $(NH_4)_2SO_4$, and the pH was adjusted to a value of 7.3 before the solution was sterilised by heating for 20 minutes at 120 °C. After sterilisation, the medium was supplemented with 2mM $MgCl_2$, 10 mM sodium acetate, 0.05 g l^{-1} yeast extract, and 1 ml l^{-1} of trace elements solution (Pfennig and Lippert, 1966).

2.2 Growth curves

Cells used for experiments were harvested in the exponential growth phase and their initial concentration was estimated by optical counting. After treatment, cultures of 10^7 cells ml^{-1} were incubated for up to 5 days. The concentration of cells within samples was estimated by optical counting, using a microscope, during the exponential growth phase. In this stage the number of dead or dying cells is assumed to be negligible in comparison to the number of living cells. Plating on solid medium could not be used, because *Nr. strain HG-1* does not form colonies on agar plates. At intervals of roughly 12 hours, the absorption spectra of the samples were measured over the 200-900 nm wavelength range using a UV-Vis diode-array spectrophotometer (Hewlett Packard, model 8453). Growth was monitored by plotting the absorbance at a wavelength of 600 nm (OD_{600}) as a function of incubation time. For each treated cell sample a new culture was prepared and incubated. Optical density, however, could only be detected at cell concentrations greater than 10^7 cells ml^{-1}. The number of living cells after treatment determines the time interval needed for a culture to reach a detectable optical density. From this delay in time the number of surviving cells can be calculated (see section 4.2).

2.3 Light sources

Two light sources have been used in our studies, a xenon solar simulator (ScienceTech, model 100150XUV, generating radiation between 200-2500 nm) and a deuterium discharge lamp (Heraeus-Noblelight, model DX 202, 190-400 nm). The xenon lamp is equipped with an AirMass 0 filter to remove strong xenon features that do not resemble the unfiltered solar spectrum. Intensity of the light was measured for both lamps in the UV and visible regions, using a Solartech UV-C sensor (model 8.0) and an Extech light meter (model EA30). The Solartech sensor measures irradiance over a Gaussian-shaped sensitivity profile centred on 250 nm, with a half-peak full-width value of 20 nm. The EA30 sensor is a broadband optical sensor and was used to ensure that the xenon lamp's output was stable over the experiment run.

Using the Solartech sensor, the intensity (in W m^{-2}) of both the xenon and the deuterium lamp in the wavelength range 245-265 nm was measured. From these values the irradiance of both spectra for our illumination scenarios could be derived.

Fig. 1 shows the spectrum from 190-400 nm of a generic xenon lamp of the same construction and type as the xenon lamp used in this work. Additionally, the spectrum of the deuterium lamp and the noontime illumination spectrum expected at equatorial regions on Mars, for a non-dusty atmosphere, as modelled by Patel *et al.* (2002) are shown.

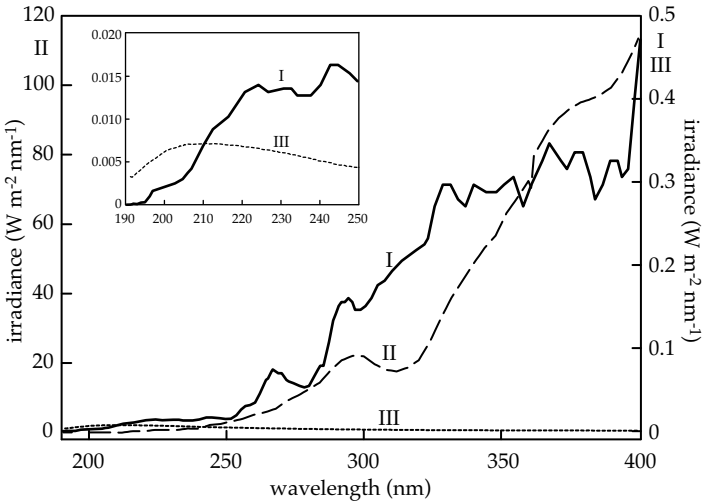

Fig 1. (I) The predicted noontime equatorial UV spectrum at the surface of Mars in the presence of a non-dusty atmosphere (continuous line) (Patel *et al.* 2002), with its irradiance plotted on the right ordinate. (II) The spectrum of the xenon lamp (dashed line), with its irradiance plotted on the left ordinate. (III) The spectrum of the deuterium lamp (dotted line), with its irradiance plotted on the right ordinate. The inset box shows the UV spectrum at the surface of Mars (I) and the spectrum of the deuterium lamp (III) from 190-250 nm.

When comparing the three spectra in this wavelength range, the xenon lamp gives an acceptable representation of the solar spectrum on Mars, although 250 times more intense. The deuterium lamp generates light with a spectrum that, when compared to the solar or xenon spectra, is enhanced in the shorter wavelengths (190-250 nm) and has a maximum output around 210 nm. At this wavelength the irradiance of the deuterium lamp is comparable to the solar irradiance at Mars. The inset box in Fig. 1 shows that, in the 190-250 nm range, the intensity of the deuterium lamp is of the same order as the solar intensity on Mars, and, as such, approximately 250 times lower than the intensity of the xenon lamp. The difference in UV output between the xenon and the deuterium lamp enables us to discriminate between the longer (250-300 nm) and shorter (195-250 nm) wavelength range of the UV spectrum.

3. EXPERIMENTAL PROCEDURES

The effect of various treatments has been examined using two types of cell samples, in liquid suspension and in desiccated state.

1) Cell suspensions have been irradiated using the xenon discharge lamp.
2) Desiccated cells have been irradiated using either a

xenon or a deuterium discharge lamp.

3) Desiccated cell samples have been placed in vacuum at room temperature, and, separately, have been exposed to low temperatures.

3.1 Irradiation of cell suspensions

Cell suspensions of 10^8 cells ml^{-1} were irradiated in sterile Petri dishes with the xenon lamp, while being constantly stirred. 1 ml samples were taken for analysis after defined periods of irradiation (30, 60, 120, 300, and 600 seconds). All samples, including a non-irradiated control, were diluted with 9 ml of growth medium, resulting in a concentration of 10^7 cells ml^{-1}. 10 ml Cultures were then incubated and growth curves were measured as described.

3.2 Irradiation of desiccated cells

Desiccated samples were prepared by heating 1 ml of cell suspension, containing 10^8 cells ml^{-1}, in Petri dishes for 1 h at 50 ^oC. These desiccated samples were exposed to three different types of irradiation. Firstly, desiccated samples were irradiated with the xenon lamp at the same intensity as the cell suspensions in section 3.2. To be able to discriminate between the effect of UV and visible light, the second set of desiccated samples was covered with a borosilicate glass plate during irradiation with the xenon lamp, thus filtering out light with wavelengths shortward of 300 nm. Both the first and second irradiation process took place for durations of 30, 60, 120, 300, and 600 s. The third set of desiccated samples was irradiated with the deuterium lamp for 21 h to measure the effect of UV only. The long exposure time was needed because the lamp's low intensity was expected to lead to a weak damaging effect on the cells. After irradiation, the dried cell samples were resuspended by adding 9 ml medium and 1 ml sterile water, resulting in a concentration of 10^7 cells ml^{-1}, equal to the concentration of the cells irradiated in suspension. The sample cultures were then incubated and growth curves were measured as previously described.

3.3 Desiccated cells in vacuum and in a cold environment

Three batches of desiccated samples were prepared as described in the previous subsection. The samples were separately exposed to one of three different environments: (1) -20 ^oC for 1 h, (2) -80 ^oC for 21 h, and (3) high vacuum (1.4×10^{-4} mbar) for 15 h. After freezing and exposure to vacuum, the samples were resuspended and incubated as described.

4. Results

We have measured the response of the archaea *Nr.* strain HG-1 to UV and visible radiation, both in suspension and in a desiccated state. Furthermore, the response of desiccated cells to hard vacuum and low temperatures has been investigated. In support of these experiments, growth curves have been measured, from which the growth rate at 45^oC could be derived.

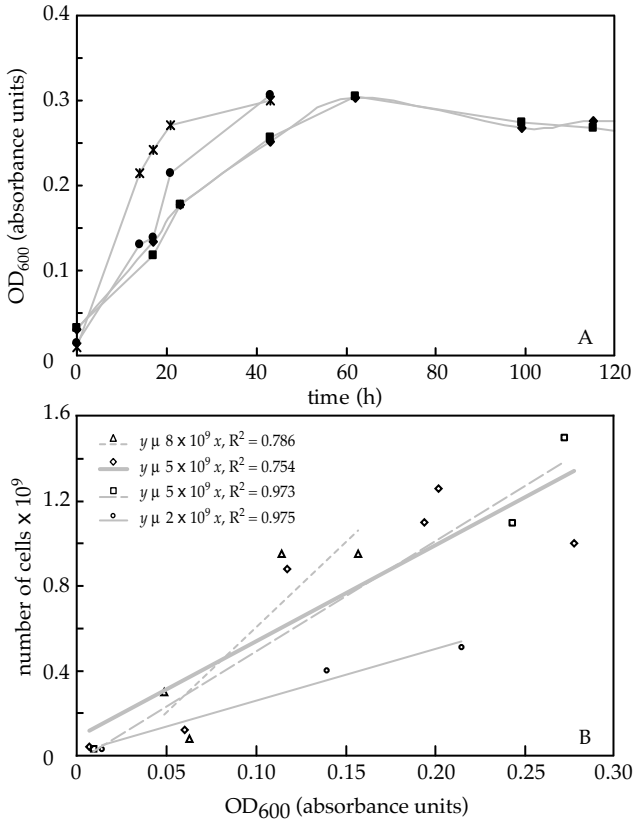

Fig 2. (A) Growth curves of *Nr.* strain HG-1 measured as OD_{600} versus incubation time. (B) Number of cells as functions of OD_{600}. The concentration is obtained by cell counting. The deviation in the data is caused by normal biological variation.

4.1 Growth curves

Several independent control cultures were been grown to obtain a reliable value of the growth rate at 45 °C. The optical density of the sample was followed by measuring the OD_{600} (absorbance at 600 nm). Fig. 2A shows growth curves of four representative *Nr.* strain HG-1 cultures, plotting the OD_{600} as a function of incubation time. The relation of the OD_{600} to the number of cells ml^{-1} counted by microscopy for the same set of four cultures is given in Fig. 2B, leading to an average concentration per OD of $5 \pm 2 \times 10^9$ cells ml^{-1} OD^{-1}. This number has been used to convert OD_{600} values into cell concentrations.

The concentration (cells ml^{-1}) in a culture after a certain incubation time t can be expressed by the following relation:

$$\frac{dC}{dt} = \mu C \qquad \text{Eq. 1}$$

Here, C is the concentration (cells ml^{-1}) as function of the incubation time t (h), and μ the growth rate (h^{-1}). Eq. 1 applies only to the exponential growth phase of a culture. It does neither account for the lag phase, where the cell has to adapt to the medium and start growing, nor for the stationary growth phase, where an equilibrium between growth and death is reached due to the limiting amount of nutrients.

Using the average concentration $5 \pm 2 \times 10^9$ cells ml^{-1} OD^{-1}, the available data yield an average growth rate of 0.12 ± 0.01 hr^{-1}. This is approximately three times faster than the rate reported

by Sorokin *et al.* (2005), a difference most probably caused by the different incubation temperature. Sorokin *et al.* (2005) incubated their cultures at 30 °C, in contrast to our incubation temperature of 45 °C.

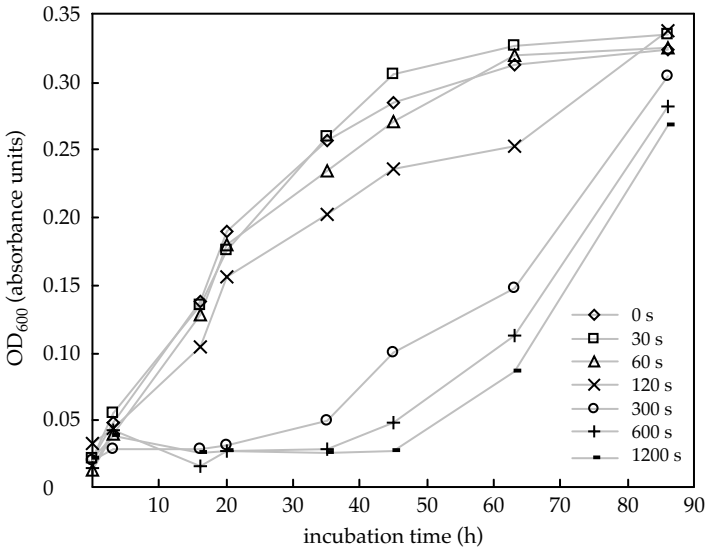

Fig. 3. Growth curves after different doses of irradiation with a xenon discharge lamp. The different curves correspond to different irradiation durations as described in the figure inset. The solid lines are a guide to the eye to show the trend of the growth, the markers represent the data points.

4.2 Irradiation of cell suspensions

Cell suspensions have been irradiated with UV and visible light from a xenon source. In the first and second experiment an amount (10ml) of the liquid cell suspension was irradiated. After exposure durations of 30, 60, 120, 300, and 600 seconds, 1 ml samples were taken out for analysis, using incubation and OD_{600} measurements. In the third experiment an extra sample was taken after an irradiation time of 1200 s. The results presented in Fig. 3 show the effects of the different irradiation intervals on the growth rate of the sample cultures.

From the growth curves shown in Fig. 3, the incubation time at which an OD_{600} of 0.15 was reached, $t_{0.15}$, can be calculated. The OD_{600} value of 0.15 was used, because it is around the point where maximum growth is expected and is sufficiently high above the detection limit of the optical density meter for that stage to be clearly detectable.

Integration of Eq. 1, leads to

$$C_{0.15} = C_{tr}\, e^{\mu\, t_{0.15}} \qquad \text{Eq. 2}$$

Here, $C_{0.15}$ is the concentration of 7.5×10^{8} cells ml^{-1}, corresponding to an OD_{600} of 0.15, $t_{0.15}$ is the incubation time at which this concentration is reached, μ is the growth rate of 0.12 generations h^{-1}, and C_{tr} is the initial concentration of viable cells *after* treatment (irradiation, vacuum, or cold). The number of cells still able to proliferate after radiation exposure is expressed by the ratio of C_{tr} to C_{untr}, where C_{untr} is the concentration of the sample *before* treatment. Table 1 shows

Table 1. $t_{0.15}$ and C_{tr}/C_{untr} corresponding to different irradiation time steps, for irradiation with the xenon lamp. The $t_{0.15}$ and C_{tr}/C_{untr} values are averages of three experiments, with their standard deviations. The 1200 s irradiation has only been performed once.

irradiation time (s)	$t_{0.15}$ (h)	C_{tr} / C_{untr}
0	19.4 ± 2.0	1.9 ± 0.8
30	21.3 ± 2.2	1.5 ± 0.4
60	21.2 ± 2.0	1.4 ± 0.5
120	26.7 ± 2.7	0.79 ± 0.3
300	58.4 ± 1.2	0.018 ± 0.007
600	60.4 ± 3.4	0.017 ± 0.01
1200	70.8	0.0019

$t_{0.15}$ and the C_{tr}/C_{untr} ratio as function of irradiation time. The results in Table 1 and in Fig. 3 show a delay in reaching the growth phase caused by progressive inactivation or death of a fraction of the cells.

4.3 Irradiation of desiccated cells

Desiccated cells embedded in a ~1 mm thick salt crust have been irradiated with UV and visible light from the xenon lamp. Subsequent experiments were performed with much of the UV (200-300 nm) filtered out. In both cases samples were irradiated for 30, 60, 120, 300 and 600 s. The trends in Fig. 4A (irradiation with the full xenon spectrum, with a wavelength range > 200 nm)) can be compared with the data in Fig. 4B (results from the filtered xenon spectrum, with a wavelength

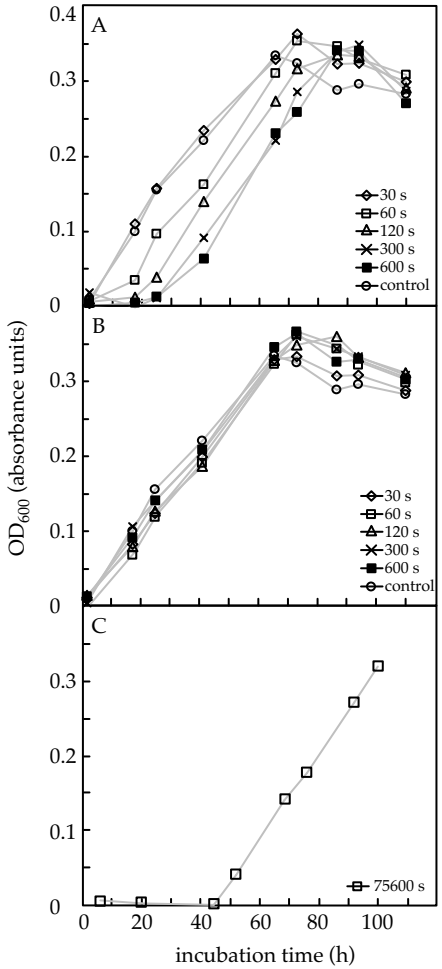

Fig. 4. The growth curves of resuspended irradiated desiccated samples, (A) after irradiation with the full xenon spectrum (> 200 nm), (B) after irradiation with the filtered xenon spectrum (> 300 nm), and (C) after irradiation with the deuterium lamp (190-400 nm). The keys show the irradiation times for the different conditions.

Table 2. $t_{0.15}$ and C_{tr}/C_{untr} corresponding to different irradiation times, for three different irradiation scenarios.

irradiation time	> 200 nm		> 300 nm		190-400 nm	
	$t_{0.15}$	C_{tr}/C_{untr}	$t_{0.15}$	C_{tr}/C_{untr}	$t_{0.15}$	C_{tr}/C_{untr}
(s)	(h)		(h)		(h)	
0	27.2	1.1	27.2	1.1	27.2	1.1
30	26.8	1.2	30.4	0.78		
60	36.9	0.36	32.5	0.61		
120	44.0	0.15	30.8	0.74		
300	50.6	0.07	28.6	0.97		
600	52.8	0.053	29.6	0.86		
75600					70.9	0.0061

range > 300 nm). Using the method described in section 4.2, the incubation time needed to reach an OD_{600} of 0.15 and the C_{tr}/C_{untr} ratio, representing the fraction of proliferating cells, were calculated. These values are given in Table 2 as function of irradiation time. The C_{tr}/C_{untr} ratio decreases after irradiation with > 200 nm light, indicating that a smaller fraction of cells has survived irradiation. This is in contrast to the C_{tr}/C_{untr} ratio after irradiation with > 300 nm light, which does not change and is independent of the irradiation duration, within the expected error. The results in Table 2 show that visible light has a negligible effect on survival and that the 200-300 nm range of the xenon spectrum causes much more lethal damage to the cells.

Fig. 4C shows the effect of 21-hour irradiation with UV only, using the deuterium lamp. The $t_{0.15}$ and C_{tr}/C_{untr} values can

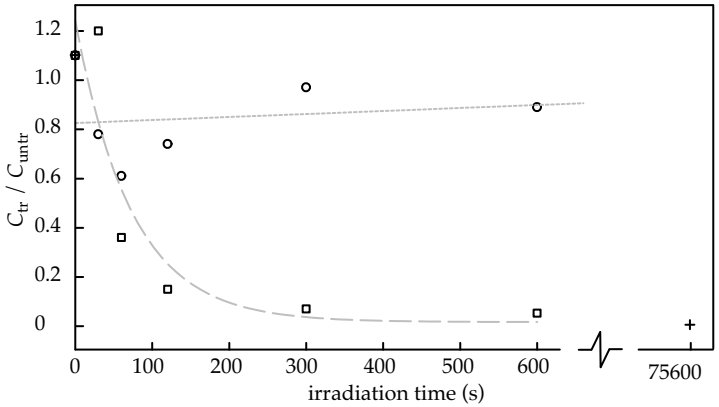

Fig. 5. Relative concentration of viable cells after irradiation (C_{tr} / C_{untr}) corresponding to different times of irradiation with (a) longward of 200 nm light (open squares), (b) longward of 300 nm light (open circles), and (c) 190-400 nm light, enhanced between 190-250 nm (plus signs). The trends (dotted for > 200 nm, dashed for > 300 nm) are plotted as a guide for the eye.

be found in the last column of Table 2. Irradiation by the deuterium lamp was for a period ~125 times longer than the irradiation by the xenon lamp (10 min.). Since the deuterium lamp is ~250 times weaker than the xenon lamp, as described in section 2.3, one would expect that the effect of the deuterium lamp would be approximately half the effect caused by the xenon lamp. However, the data in Table 2, plotted in Fig. 5 show that the effect of light from the deuterium lamp is ~10 times stronger than the effect of light from the xenon lamp, comparing the C_{tr}/C_{untr} ratios. This enhancement is probably

Table 3. $t_{0.15}$ and C_{tr}/C_{untr} after subjection to -20 °C, -80 °C, and hard vacuum (1.4×10^{-4} mbar). All collected data are given.

condition			$t_{0.15}$	C_{tr}/C_{untr}
temperature (°C)	pressure (mbar)	time[1] (h)	(h)	
+20	1.0×10^{3}	24	27.2	1.1
			24.5	1.6
+20	1.4×10^{-4}	15	32.0	0.6
			34.2	0.5
-20	1.0×10^{3}	1	45.0	0.1
-80	1.0×10^{3}	21	38.8	0.3
			42.0	0.2

[1] Time is the duration the samples have been exposed to the conditions given in the table. These samples have not been irradiated.

caused by the different spectral profiles of the UV and xenon lamps; the UV lamp generates light with a spectrum skewed toward short, more energetic, UV, while the xenon lamp produces light with a longer-wavelength bias.

4.4 Desiccated cells in vacuum and a cold environment

Two desiccated samples were placed in a -80 °C environment for 21 h, one sample was kept at -20 °C for one hour, and two samples were exposed in a vacuum system, at room temperature, to a pressure of 1.4×10^{-4} mbar for 15 h. These conditions have been applied without irradiation. Table 3 shows the effects of the different conditions on *Nr.* strain HG-1 (see section 4.2). The effects of cooling and exposure to vacuum are small compared to the effects of 5 and 10 minutes irradiation with the xenon lamp and 21 h with the deuterium lamp. However, exposing the samples to vacuum inactivates ~50 % of the cells. Freezing causes inactivation of 70-90 % of the cells, an effect that seems to be independent of the storage temperature.

5. Discussion

We do not know at present whether there is, or has been, any biological activity on Mars. Similarly, there are no compelling reasons to expect that the mechanisms evolved by terrestrial organisms to cope with stressful changes in their environment would be similar to those employed by any hypothetical martian biota. However, given the constraints imposed by biochemistry, there are relatively few steps available to an organism facing the loss of a vital fluid such as water.

It is widely thought that Mars may have shared many of the properties of the Earth in its past. Relict fluvial valleys and the presence of surface concretions resulting from aqueous alteration, point to the presence of bodies of water existing at some time under a warmer and denser martian atmosphere. Because the details of the terrestrial setting in which early life developed on Earth are not known, it is only possible to make a most speculative link to a hypothetical martian origin for life.

During crystallisation and desiccation caused by drought,

cells are often trapped within salt crystals in pockets of brine, where they can remain viable for very long time (Norton and Grant, 1988; McGenity *et al.*, 2000). Most halophilic microorganisms contain pigments that can protect them against damage by high radiation intensities (Oren, 2002). It has been suggested that brines may have existed on the surface of Mars in the past (Rothschild, 1990), which leads to the assumption that microorganisms similar to halophilic Archaea on Earth could have been present on Mars as well (Litchfield, 1998). If they still would be present in a desiccated state on the martian surface, their pigments could protect them against damage by the current high UV intensities at the surface.

In this work we have subjected *Natronorubrum* sp. strain HG-1 (*Nr*. strain HG-1) to various conditions, simulating several aspects of the current conditions on Mars. Sorokin *et al.* (2005) showed the presence of a pigment very similar to haloarchaeal bacterioruberins. Bacterioruberin pigments have several functions, including protection against DNA damage by high intensity UV and visible light and exposure to H_2O_2, and reinforcement of the cell membrane (Oren, 2002). The presence of this pigment in *Nr*. strain HG-1and the ability of the species to adapt to desiccation make *Nr*. strain HG-1 an interesting candidate to survive on the surface of Mars.

Comparing the results from irradiation of cell suspensions and of desiccated cell samples, both irradiated with a xenon lamp (> 200 nm), shows that desiccated cells are more resistant to irradiation. This suggests that the damage generated through UV exposure is more critical for non-desiccated cells. Nicholson *et al.* (2000) suggest UV-generated reactive oxygen species in the cell cytoplasm to act as damaging agents to cell DNA and other cellular components. UV interaction with water leads to the formation rate of damaging radicals. Low water-content cells, such as desiccated *Nr.* strain HG-1 samples, have little available water with which UV photons can interact. By contrast, cells in aqueous suspension would acquire higher levels of damaging radicals, a trend visualised by the data shown in Fig. 5. The results of our experiments show that desiccation itself has no measurable effect on the growth of *Nr*. strain HG-1 after resuspension. Placing desiccated cell samples in hard vacuum inactivates only ~50 % of the cells. Freezing desiccated cell samples inactivates around 80 % of the cells. These amounts of inactivation, however, are smaller than the inactivation caused by long duration UV irradiation, where only a few percent of an initial population survives. No conclusions can be drawn from these experiments about the protective of the pigment that is present in *Nr*. strain HG-1 to cell protection or recovery from UV-generated cell damage.

To be able to discriminate between higher and lower wavelength ranges in the UV spectrum, the irradiation results of the deuterium lamp are compared with those of the xenon lamp. The UV spectrum of the deuterium lamp is dominated by light in the short wavelength range (195-250 nm) when compared to the xenon lamp (see Fig. 1). Table 1 shows that irradiation with shorter wavelength light (195-250 nm) is more effective in inactivating the cells.

Scaling the results obtained from irradiation experiments with the xenon lamp to martian conditions, where the xenon lamp is ~250 times stronger than the illumination on the martian surface, would imply that approximately 5 % of a population *Nr*. strain HG-1 would survive ~80 hours continuous noontime irradiation, at a temperature of ~ 300 K. Although 80 hours continuous noontime irradiation will not occur, it seems still valid to conclude that *Nr*. strain HG-1 is not likely to survive the conditions at the surface. In desiccated state no water is available to the cells and no recovery process can take place during night time. Accumulation of shorter intervals of irradiation may therefore be as lethal as long continuous exposure. Furthermore, noontime equatorial temperatures are not always around 300 K, but may very well be colder. The results in Table 3 show that freezing also affects the viability of *Nr*. strain HG-1. Irradiation of *Nr*. strain HG-1 in colder environments than 300 K, for example during winter time or at polar regions, will have a higher inactivating effect on the cells than irradiation at higher temperatures.

From the reported experiments it can be concluded that the strain *Nr*. strain HG-1 would not be a good candidate organism to survive on the surface of Mars, even when embedded in salt crystals. On the other hand, the inability of *Nr*. strain HG-1 to survive these Mars-like conditions, makes it also an unlikely candidate to survive a trip from Earth to Mars on a spacecraft surface, as temperatures are even lower and UV radiation is even more energetic than the conditions on Mars. Halophiles, like *Nr*. strain HG-1, thriving in extreme environments on Earth, will not be able to survive unshielded on the surface of Mars even in a salt crust. It cannot be excluded that they can survive when embedded in the soil or buried underneath rocks, but further experiments are required to distinguish how soil activity (Garry *et al*., 2005) will effect the halophiles.

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