SURVIVAL OF MICROORGANISMS UNDER THE EXTREME CONDITIONS OF THE ATACAMA DESERT

KLAUS DOSE[1]*, ANGELIKA BIEGER-DOSE[1], BIRGIT ERNST[2], UWE FEISTER[3], BENITO GÓMEZ-SILVA[4], ANKE KLEIN[1], SERGIO RISI[1]* and CHRISTINE STRIDDE[1]

[1] *Institut für Biochemie, Fachbereich Chemie und Pharmazie, Johannes Gutenberg-Universität, 55099 Mainz, Germany*

[2] *Institut für Mikrobiologie, Johann Wolfgang Goethe-Universität, 60054 Frankfurt/M, Germany*

[3] *Deutscher Wetterdienst, Meteorologisches Observatorium Potsdam, Telegrafenberg, 14473 Potsdam, Germany*

[4] *Universidad de Antofagasta, Departamento Biomédico and Instituto del Desierto, Antofagasta, Chile*

(Authors for correspondence)*

(Received 1 March, 2000; accepted in revised form 30 June, 2000)

Abstract. Spores of *Bacillus subtilis*, conidia of *Aspergillus niger, versicolor* and *ochraceus* and cells of *Deinococcus radiodurans* have been exposed in the dark at two locations (at about 23°S and 24°S) in the Atacama Desert for up to 15 months. *B. subtilis* spores (survival ∼15%) and *A. niger* conidia (survival ∼30%) outlived the other species. The survival of the conidia and spores species was only slightly poorer than that of the corresponding laboratory controls. However, the *Deinococcus radiodurans* cells did not survive the desert exposure, because they are readily inactivated at relative humidities between 40 and 80% which typically occur during desert nights. Cellular monolayers of the dry spores and conidia have in addition been exposed to the full sun light for up to several hours. The solar fluences causing 63% loss in viability (F_{37}-values) have been determined. These F_{37}-values are compared with those determined at other global locations such as Punta Arenas (53°S), Key Largo (25°N) or Mainz (50°N) during the same season. The solar UVB radiation kills even the most resistant microorganisms within a few hours due to DNA damages. The data are also discussed with respect to possible similarities between the climatic conditions of the recent Atacama Desert and the deserts of early Mars.

Keywords: Atacama Desert, conidia, *Deinococcus radiodurans*, DNA lesions, dormant life, extreme dryness, Martian environment, solar UVB, spores, survival

1. Introduction

In northern Chile an extremely dry desert region stretches from north of the Río Copiapó (27°S) to north of the town of Arica (18°S). Being only 100 to 200 km wide it is bordered in the west by the Cordillera de la Costa (altitude ∼2000 m) and in the east by transitional high plateaus: the semidesert La Sierra (up to 3500 m) and the El Altiplano (3500 to 4500 m). The central part of this desert, also called Atacama Desert (Pampa), is located at 800 to 1200 m above sea level and stretches from the Río Salado (26.5°S) to the Río Loa (21°S). Most areas have been without

Origins of Life and Evolution of the Biosphere **31:** 287–303, 2001.

reported rainfalls for decades. A few areas may receive sporadic rainfalls (every ~4 yr) due to the recurrent climatic phenomenon called El Niño. Otherwise the desert is extremely dry. The relative humidity very seldom reaches the dew point. Because of some mining activities large parts of the desert are relatively easily accessible by motor vehicles. An essential north-south connection is the Panamerican Highway. One may drive here for hours without noticing any flora or fauna. Exceptions are a few oases (e.g. Pica) which typically receive water due to a flow of ground water from the Altiplano or eastern Cordillera, about 100 km away. Parts of the desert landscape strikingly resemble corresponding landscapes on Mars (see Figure 1). In fact, we suggest that the Atacama Desert may serve as a model for the extremely dry climatic conditions on early Mars, about 3.5 Gyr ago, when liquid surface water became rare (McKay, 1997). If active life existed on Mars more than 3.5 Gyr ago it was adapted to a CO_2-N_2 atmosphere. However, in order to survive dry periods without liquid water, possible Martian organisms, like terrestrial organisms, had to develop analogous strategies which especially involve the protection of biomembranes and macromolecules (Crowe and Crowe, 1992; Crowe *et al*., 1992; Leslie *et al*., 1995), while passing through a dormant, metabolically very largely inactive state. General strategies include the accumulation of non-reducing sugars, especially trehalose and sucrose, which help to prevent damages to membranes and proteins during dehydration. *Bacillus subtilis* spores, however, protect their integrity, especially of their DNA, by entirely different strategies (Setlow, 1992). These include the synthesis of a special class of DNA-binding proteins for DNA protection. All dry-resistant organisms have poor survival chances if their DNA is subjected to radiation-induced lesions while being in the dormant state: In a metabolically inactive state no DNA repair processes can operate. Thus all damages accumulate and if – after rehydration – the general conditions for growth become again favorable, the damaged organisms fail to recover and to replicate, if the number of DNA damages exceeds the repair capacity.

During the past decade we could demonstrate that DNA damages, especially double-strand breaks and covalent DNA-protein cross-links, play a crucial role among the molecular processes leading to the inactivation of microorganisms by dehydration during exposure to actual and simulated space vacuum at different temperatures (Dose *et al*., 1991, 1996; Dose and Gill, 1995; Dose and Klein, 1996). Some of these results have especially put further constraints on the panspermia thesis (Dose, 1994; Dose and Klein, 1996) and they have helped to explain the limited survival chances of microorganisms that are exposed to the extreme conditions of low water activities and/or solar radiation. Of critical importance is the efficient repair of dryness- and radiation-induced DNA damages during or after rehydration. This conclusion is also supported by the desiccation resistance of *Deinococcus radiodurans* (Dose *et al*., 1991). This vegetative bacterium has recently again drawn broader interest because of the analysis of its genome sequence (White *et al*., 1999) in connection with its long known resistance to a number of agents and conditions that damage DNA, including radiations and extreme desiccation (Mattimore and

Figure 1. View of the Atacama Desert near San Pedro de Atacama.

Battista, 1996). Especially well documented is also the desiccation resistance of spores of *Bacillus subtilis* (Setlow, 1995).

The aim of the present study is to evaluate the survival chances of various dryness-resistant microorganisms under the extreme conditions of the Atacama Desert, the driest desert on the Earth. We have focused in this study on those microorganisms that have earlier exhibited a good resistance to the conditions of open space: spores of *Bacillus subtilis*, cells of *Deinococcus radiodurans* and conidia of *Aspergillus niger*, *versicolor* and *ochraceus* (Horneck, 1993; Dose *et al.*, 1995, 1996).

2. Materials and Methods

2.1. Biological materials

The following samples have been exposed: Conidia of *Aspergillus ochraceus* strain 3174, *Aspergillus versicolor* strain 599, *Aspergillus niger* strain 737. *A. ochraceus* and *A. versicolor* were a gift from Dr. Röschentaler, Institute for Microbiology, University of Muenster, Germany. *A. niger* was obtained from the German Collection of Microorganisms at Braunschweig, Germany.

Spores of *Bacillus subtilis* strain TKJ 3412 (splB1 thyA thyb lys met). We are grateful to Dr. N. Munakata, National Cancer Research Institute, Tokyo, Japan, for

providing this strain. The vacuum-resistance of this strain is comparable to that of the wild type (Dose and Gill, 1995), although it is not capable of thymidine synthesis. The latter property, however, allows an efficient ^{14}C-labelling of DNA by incorporation of added ^{14}C-thymidine, an essential requirement for a detailed study of DNA lesions such as double-strand breaks and DNA-protein cross-links (Dose and Gill, 1995).

Cells of *Deinococcus radiodurans* strain R_1. We thank Dr. H. D. Mennigmann, Institute for Microbiology, University of Frankfurt/M, Germany, for providing this strain.

2.2. Preparation of conidia, spores and cells

The fungi were cultivated on Petri disks (diameter about 9 cm) containing 15 mL of an autoclaved medium consisting of 17 g L^{-1} malt extract bouillon from Merck, Darmstadt (Germany), 15 g L^{-1} agar-agar and 5 g L^{-1} peptone. About 10^4 conidia suspended in 100 μL were equally distributed over the surface of the medium. The fungi were cultivated for 10 to 14 days at room temperature (*A. ochraceus* 10 days, *A. versicolor* 12 days and *A. niger* 14 days). Thereafter the conidia were washed out under sterile conditions with a 1% solution of tween-80. The suspension was repeatedly sucked through sterile cotton wool filters to retain remains of the mycelium and residues from the medium. The purified conidia were collected at 10 °C by centrifugation for 10 min at 6000 rotations min^{-1}. The concentration of the conidia was determined with the help of a Thoma-counting chamber.

The spores of *Bacillus subtilis* were cultivated and prepared as described earlier (Dose and Gill, 1995). The cells of *Deinococcus radiodurans* were cultivated according to Krabbenhoft *et al*. (1967) as modified by Dose *et al*. (1991).

2.3. Exposure conditions

The exposure of the samples in the desert was performed in aluminum units that were constructed according to our experiences with the exposure units that had been used during the ERA/EURECA space mission (Dose *et al*., 1995). The unit for desert exposure is shown in Figures 2a and b. It consisted of a stack of disk-shaped compartments. Each compartment contained four glass disks (about 2 cm in diameter) each covered by a statistical monolayer of cells, as described for the above space mission. An essential feature of each compartment was the enclosure of the individual biological samples by gas-permeable, but germ-impermeable PTFE-Filters (polytetrafluoroethylene filters, obtained from Pall at Dreieich, Germany). Laboratory controls were kept in desiccators over silica gel in the presence of air at about 25 °C. After we became aware of the strong fluctuations of the relative humidity during day and night cycles in the desert, fractions of the samples were periodically kept for 12 hr in the open air (at about 60% relative humidity) and for the next 12 hr in a desiccator with silica gel (at about 5% relative humidity)

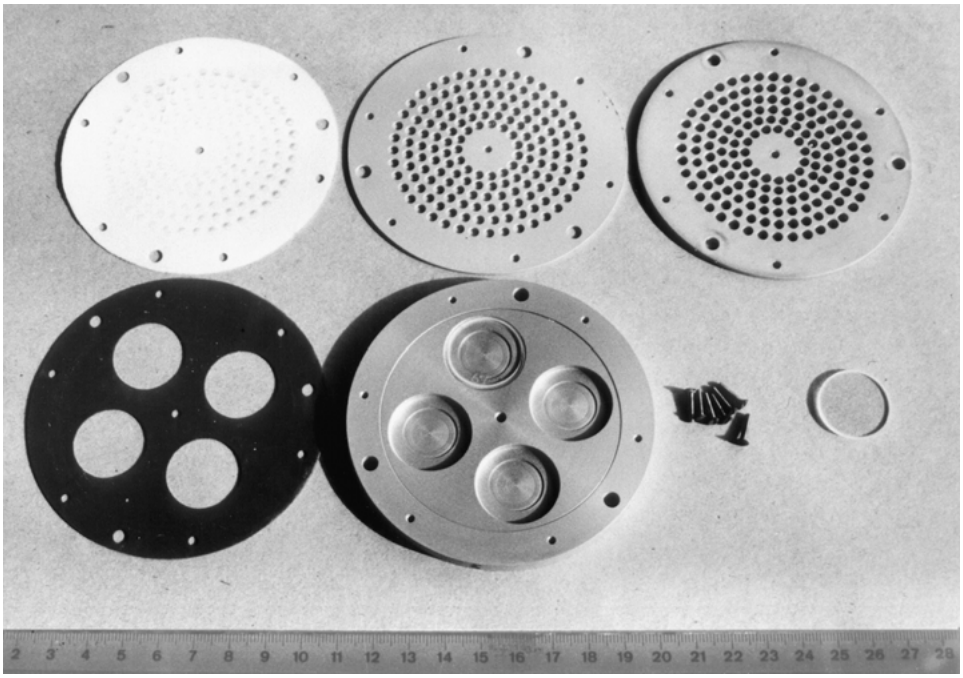

Figure 2a. Details of an exposure compartment. The gas-permeable membrane is shown at the top (left). On the bottom (right) is seen one of the glass discs that contained the biological materials.

during several weeks in the laboratory. The results of these experiments indicated that cells of *Deinococcus radiodurans* are rather inactivated by continuous exposure at 60% relative humidity than by subjecting them to cycles of changing relative humidities. For this reason *Deinococcus radiodurans* cells were exposed continuously to different relative humidities at 25 °C for 24 and 48 hr. The desired relative humidities were maintained by distinct concentrations of sulfuric acid as shown in Table I.

The geographical positions of the two exposure sites in the Atacama Desert were 23.0°S; 69.5°W (location Chacabuco, Oficina Florencia, CIMIN S.A.) and 24.1°S; 69.7°W (location Yungay). The exposure at Chacabuco lasted 15 months (from July 1993 to September 1994); that at Yungay for 13 months (from March 1995 to April 1996). The samples were kept in sealed containers for transportation from Germany to the exposure sites and *vice versa.* The exposure units were placed at shaded and dust-protected locations. The temperature varied between around 0 and 35 °C due to seasonal and diurnal changes. Laboratory simulations have shown that such temperature changes during exposure do not significantly influence the rate of survival.

Also the exposure to solar light was performed with samples that were distributed on glass disks in statistical monocellular layers. These samples had been dried for about one week under laboratory conditions and were then transported

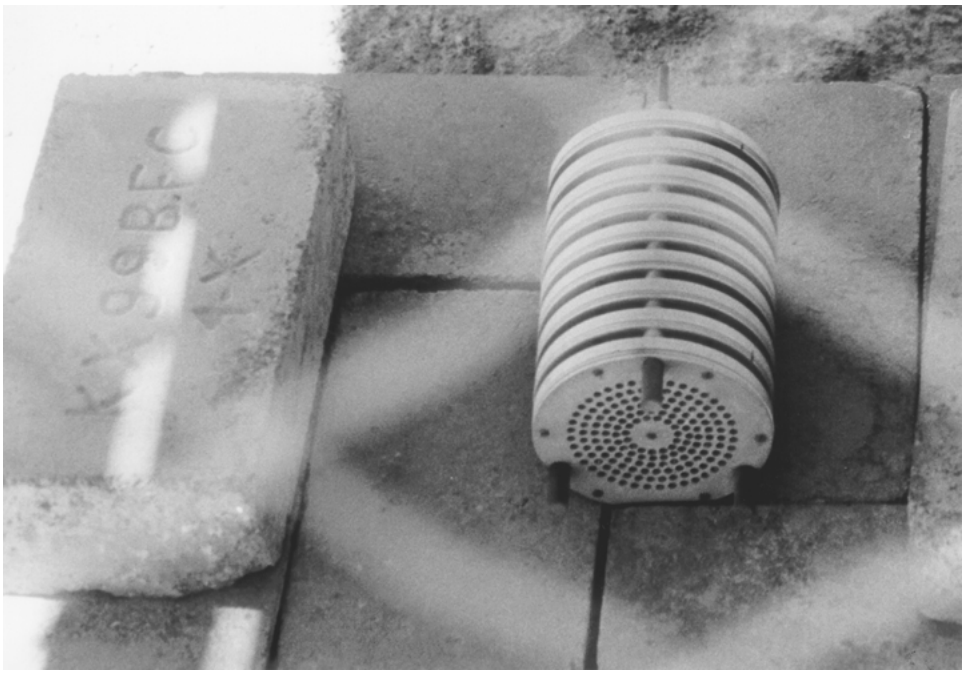

Figure 2b. View of the exposure unit. It consisted of a stack of disc-shaped compartments as shown in Figure 2a. The unit was exposed under a roof at a fenced location.

TABLE I

Concentrations of sulfuric acid used to maintain approximate relative humidities (Ruland, 1982) (Relative mean error ±2%)

Relative humidity (%)	Concentration of sulfuric acid (weight %)
90	18
80	28
60	38
45	45
20	58
<1	100

to the exposure sites. The samples were exposed to the full sunlight at noon time September 22, 1994 or March 3, 1996. In addition, a few samples were exposed to the full sunlight during 4 days (from September 22 to 26, 1994). Later in 1996 only *Bacillus subtilis* spores were exposed to sunlight as shown in Table VI. The solar fluences for the wavelength range 280 to 320 nm were obtained by model calculations according to Feister (1994). During the irradiation the maximal air temperature was about 25 °C, the glass plates were heated in the sun up to about 40 °C.

2.4. Viability tests

The survival of the various species was determined according to their colony-forming ability. The percentage values for survival have been determined according to $(N_t/N_o) \times 100\%$ (N_t is the number of colonies per aliquot after the exposure time t, N_o is the number of colonies per aliquot of the original culture.

2.5. Determination of DNA double strand breaks

The formation of DNA-double strand breaks was assayed by pulsed-field gel electrophoresis. The preparation of the DNA and its electrophoresis has been detailed earlier (Dose *et al*., 1991, 1996; Dose and Gill, 1995; Dose and Klein, 1996).

2.6. Determination of DNA-protein cross-links in spores

DNA-protein cross-links were analyzed as detailed earlier (Dose and Gill, 1995; Dose and Klein, 1996). The average error of this method is about 10%. Cross-linking of DNA to proteins is a typical UV-induced reaction in *B. subtilis* spores after irradiation with UV light of 254 nm.

2.7. Statistical significance

For each exposure (we had two different exposure sites and the corresponding laboratory controls) 5 samples from each set of experiments were analyzed. The error data in Tables III to V are given as standard deviations.

3. Results and Discussion

3.1. Survival in the desert (dark)

Table II shows the extreme variations of the temperature and of the relative humidity to which the surface of the Atacama Desert is exposed all over the year. Continuous measurements have been performed at Yungay since September 1994. The daily cycles of the relative humidity are caused by the temperature differences

TABLE II

Seasonal changes of the relative humidity and of the temperature in the Atacama Desert at Yungay (24.1°S; 69.7°W): The data are presented as mean values ± the maximal values measured during the indicated month. Actually the values have been recorded continuously during the whole year

Parameter	Summer (December)		Winter (June)	
	Maximum	Minimum	Maximum	Minimum
Temperature (°C)	33±3	10±2	28±3	1±3
Rel. humidity (%)	60±25	<10	40±35	<10

TABLE III

Survival of *Aspergillus* conidia, *Bacillus subtilis* spores and *Deinococcus radiodurans* cells after exposure for 13 to 15 months in the Atacama Desert (dark tray). No significant differences could be found between survival after 13 and 15 months of exposure

Sample	Laboratory (silica gel; 15 months) $(N_t/N_o) \times 100\%$	Chacabuco and Yungay (~24°S; 13–15 months) $(N_t/N_o) \times 100\%$
A. niger	35±5	28±5
A. versicolor	25±5	17±5
A. ochraceus	20±5	14±5
B. subtilis	17±3	15±3
D. radiodurans	30±10	0.1±0.05

between early afternoon and early morning hours: increase of relative humidity during the early morning hours, decrease to extremely low values during day time. The relative humidity values correspond to an average water vapor partial pressure of about 10 hPa. The mean maximum of the relative humidity is usually between 40 and 60%, the mean minimum is under 10% during the whole year. The daily variation of the surface temperature is between about 30 °C and –3 to 8 °C during the whole year. Only on 15 days in a summer month and on about 7 days in a winter month the relative humidity may reach about 70 to 80% for a few hours before sunrise.

The data on the survival of several species that were exposed in gas-permeable, but germ-impermeable compartments to desert conditions are summarized in Table III.

Cells of *Deinococcus radiodurans* show a significantly poorer survival under desert conditions than under laboratory conditions at constant temperature (25 °C)

TABLE IV

Inactivation of *Deinococcus radiodurans* strain R_1 in dependence on the increase of the relative humidity

Relative humidity	Exposure $(N_t/N_o) \times 100\%$	
(%)	24 hr	48 hr
0 (Vacuum)	99±5	99±5
<1 to 20	95±5	90±5
45	80±5	40±4
80	40±4	10±2
90	10±2	1±0.5

and relative humidity (5%). Whereas the stronger inactivation of conidia and spores under desert conditions in comparison to laboratory conditions is almost insignificant. After we became aware of the strong alterations of the relative humidity in the desert, we have simulated these alterations in the laboratory and could demonstrate that indeed the strong variation of the relative humidity (and not that of the temperature) causes a slightly increased loss in the viability of the conidia and spores. However, in the course of these experiments we realized that *Deinococcus radiodurans* cells are strongly inactivated if just exposed to 80% relative humidity. For this reason we have studied the inactivation of *Deinocoocus radiodurans* as a function of the relative humidity. Relevant data are summarized in Table IV.

Since the relative humidity in the Atacama Desert at night usually reaches values above 40%, the poor survival of *Deinococcus radiodurans* under desert conditions is unambiguously related to this increase in the relative humidity. However, an explanation for this unusual response can as yet not be given. Usually the killing rate during desiccation increases with the removal of cellular water. However, only the response of yeast cells and some bacteria has been studied in detail (Mazur, 1980). Asada *et al*. (1979) could only detect DNA strand-breaks when exposing *Escherichia coli* cells to relative humidities below 80%, nevertheless a significant killing rate was already observed at 85%. Koga *et al*. (1966) have found that partially dried yeast cells begin to take up oxygen at water contents above 20%. This value, however, is already reached if the relative humidity is reduced to about 90%. Most Microorganisms, including *Saccharomyces cerevisiae*, require at least 90% relative humidity for growth; only some molds (for an instance *Xeromyces bisporus*) are able to grow at 60% relative humidity and higher (Rose, 1976). The data available on the exposure of microorganism at 80% relative humidity do not support the view that DNA strand-breaks play a crucial role during inactivation. The nature of the injurious processes that operate in *Deinococcus radiodurans* (R_1) at increasing relative humidities thus requires further investigation. The effect is not

oxygen-dependent. In a different context we have earlier realized that *Deinococcus radiodurans* (R_1) is also inactivated when stored under wet Argon (above 20% relative humidity) for several months (Dose *et al*., 1995).

The loss of viability by exposure of anhydrobiotic conidia and *B. subtilis* spores to desert conditions was similarly related to the occurrence of DNA double-strand breaks as it had been shown earlier for their exposure to vacuum or extremely low relative humidities (Dose *et al*., 1991, 1995; Dose and Gill, 1995; Dose and Klein, 1996). This phenomenon of double strand-break formation due to incomplete repair of DNA damages has been discussed earlier (Dose, 1994). Under the present conditions the inactivation of *B. subtilis* spores (survival about 15%) was associated with the occurrence of 6 DNA-double strand-breaks per chromosome. This value is in agreement with earlier data on the correlation between loss in viability and the occurrence of DNA-double strand-breaks after exposure to vacuum or dry argon (Dose and Klein, 1996). Because of the difficulties in completely extracting the native DNA from (eukaryotic) conidia a corresponding relationship could so far only be demonstrated by semi quantitative data (Dose *et al*., 1995). The electrophoretic patterns (not shown) obtained in the course of the present investigations were similar to those published earlier (Dose *et al*., 1995). However, their exact quantitative evaluation is as yet not possible.

3.2. Inactivation by solar light

Spores of *B. subtilis* and conidia of *A. niger* and *versicolor* have also been exposed to solar light on glass disks in statistical monolayers. These samples were dried for about one week in a desiccator and then transported to the Atacama Desert in sealed containers for exposure to solar light. The exposure time during the midday hours of September 22, 1994 was 90 min. In addition, some samples were exposed without interruption for 4 days through September 26, 1994 (about 48 hrs at variable sunlight). *Deinococcus radiodurans* cells were not exposed to solar light because of their extremely poor survival under desert conditions. The results for 90 min exposure (corresponding to a fluence of about 16 kJ $m^{-2}\pm15\%$ for the wavelength range 280 nm to 320 nm) for conidia and spores are summarized in Table V. The fluence was estimated according to Feister (1994).

After exposure to sunlight for 4 days (fluence for the wavelength range 280 to 320 nm about 300 kJ m^{-2}) the viability of the microorganisms had decreased to zero.

In view of the thinning of the stratospheric ozone, especially on the southern hemisphere (Cabrera *et al*., 1995) we have also compared the biological effectiveness of the solar UVB in the Atacama Desert with that at other locations on the Earth. For this reason we have exposed to solar light *B. subtilis* spores within a period of a few weeks at 3 more global positions (see Table VI). *B. subtilis* spores are especially suitable for such studies because their photobiology and their photobiochemistry are relatively well known (Munakata, 1981, 1989; Munakata

TABLE V

Inactivation of spores and conidia by solar light in the Atacama Desert. The fluence for 90 min irradiation time was about 16 kJ m^{-2} (wavelength range 280 to 320 nm). N_o is the relative number of viable cells in the fresh preparation, N_d is the relative number of viable dried cells (monolayers on glass disks) prior to irradiation in the desert, N_{90} is the relative number of viable dried cells after additional exposure to solar light for 90 min

Sample (Conidia or spores)	Dark control $(N_d/N_o) \times 100\%$	Solar light $(N_{90}/N_o) \times 100\%$
A. niger	60±5	24±5
A. versicolor	25±5	2±1
B. subtilis	29±3	4±1

and Rupert, 1972; Dose and Klein, 1996). For a related reason these spores have frequently been used, though in different context, for the quantification of the biological effectiveness of solar UV radiation in space and on Earth (Horneck, 1993, 1995; Munakata, 1993). Relevant environmental details together with the F_{37}-values for the range 280 to 320 nm are presented in Table VI. The F_{37}-values have been obtained by evaluation of the fluence-effect curves shown in Figures 3 (survival) and 4 (DNA-double strand-breaks per chromosome).

The comparison of the fluence effect curves for the inactivation of the spores, the production of DNA double strand breaks (see Figures 3 and 4) and the resulting F_{37}-values (kJ m^{-2}) confirm that the solar light is slightly more efficient in the (rural) regions of the southern hemisphere due to a higher content in UVB (Madronich and De Gruijl, 1994; Cabrera *et al*., 1995). Unfortunately, the irradiations in a rural environment near Punta Arenas (southern Chile) had to be interrupted after 70 min because of increasing cloudiness. Therefore the values for this location are only approximate. The higher F_{37}-values for Mainz reflect a lower content in UVB due to air pollution. The relative effectiveness of the solar radiation (wavelength range 280 to 320 nm) towards *B. subtilis* strain TKJ 3412, if compared to UV light of 254 nm (Dose and Klein, 1996; F_{37}-value about 200 J m^{-2}), is about 2% on the basis of the F_{37}-values. This value is in good agreement with the decrease in DNA absorption between 250 and about 300 nm (Setlow, 1974) and also confirms that DNA is the main target. Besides DNA double-strand breaks UV light of 254 nm also produces large amounts of DNA-protein cross-links (Dose and Klein, 1996). However, no significant amount of DNA-protein cross linking has been observed after irradiation with solar light. This difference is likely due to different mechanisms of photophysical processes produced at the peak of DNA absorption and in

TABLE VI

Comparison of solar F_{37}-values, the fluences that leave 37% of the *B. subtilis* spores in a viable state. Dark controls are 100%. The fluence values (for the wavelength range 280 to 320 nm) which are presented here, have been obtained by model calculations (Feister, 1994). Average monthly ozone values (London *et al*., 1976) and ground albedo (Feister and Grewe, 1995) have been accounted for. DNA-DSB at F_{37} stands for the formation of DNA double-strand breaks per chromosome determined for the corresponding F_{37}-fluences of inactivation. The relative error was about 10% (obtained by analysis of 5 samples per experiment)

Place	Geographic location (°)	Altitude	Date	Local irradiation time	Total fluence (kJ m^{-2})	F_{37} (min)	F_{37} (kJ m^{-2})	DNA-DSB at F_{37}
Yungay, Atacama Desert, Chile	24.1°S, 69.7°W	800 m	18/3/1996	11.00–12.30	17.65	46	9.0	2.2
Key Largo, U.S.A.	25.0°N, 80.5°W	2 m	13/4/1996	13.20–14.50	20.75	43	10.0	2.2
Punta Arenas, Chile	53.2°S, 71.0°W	3 m	28/3/1996	11.30–12.40	6.59	∼160	∼14.0	∼2
Chile								
Mainz, Germany	50.0°N, 8.2°E	200 m	16/4/1996	14.30–16.00	8.42	165	15.5	1.8
Mainz, Germany	50.0°N, 8.2°E	200 m	19/8/1996	11.00–14.00	20.83	134	15.5	1.8

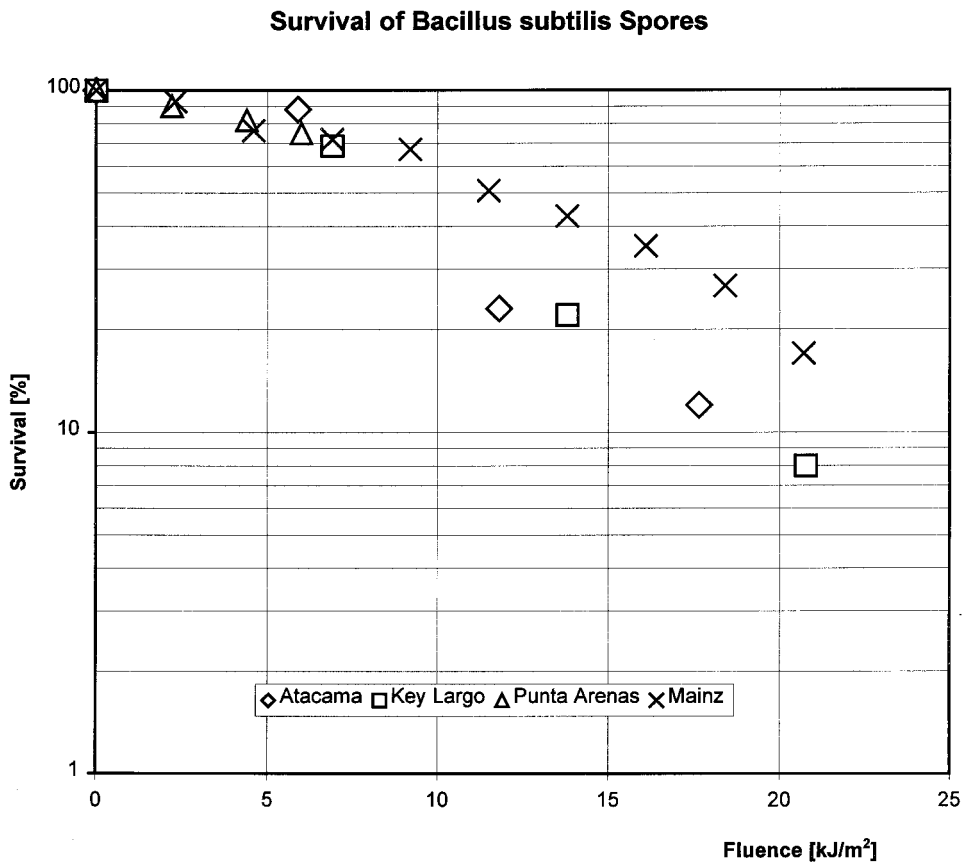

Figure 3. Fluence effect curves for the loss in viability by irradiation of *B. subtilis* spores with solar light at four different locations. The fluences (kJ m^{-2}) have been calculated for the wavelength range 280 to 320 nm.

the region above 300 nm. In the region above 300 nm the absorption of guanine prevails (Sutherland and Griffin, 1981).

4. Conclusions

The upper surface of the rocks in the Atacama Desert does not appear to be a habitat for any as yet known form of active life. Even though some fungi (*Saccharomyces* and *Xeromyces* species) may grow at about 60% relative humidity (Rose, 1976; Nealson, 1999), nutrient stress and a relatively strong UVB radiation pose additional restraints in the Atacama Desert. The relative humidity only occasionally exceeds 60%: Therefore, neither endolithic organisms (Friedmann, 1980; Friedmann and Ocampo-Friedmann, 1984; Friedmann and Weed, 1987) nor persistent

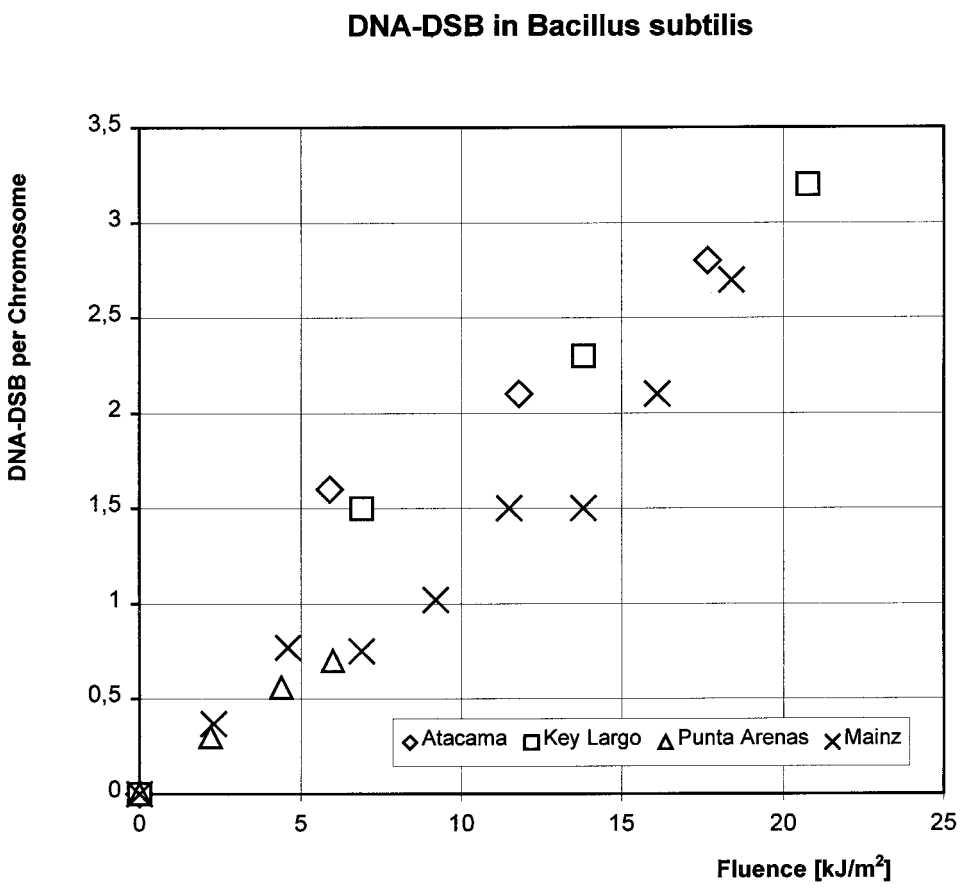

Figure 4. Fluence effect curves for the formation of DNA-double strand breaks by irradiation of *B. subtilis* spores with solar light at four different locations. The fluences (kJ m^{-2}) have been calculated for the wavelength range 280 to 320 nm.

lichens (Lange *et al.*, 1970) could be detected on the rocks in the central Atacama Desert.

Deinococcus radiodurans would at least temporarily resist the solar radiation and tolerate extreme dryness of less than 20% relative humidity. Unexpectedly, however, this microorganism is readily inactivated by exposure to relative humidities between 40 and 60% which occur at night in the Atacama Desert. Thus also *Deinococcus radiodurans* is a very poor survivor under extreme desert conditions. Good survivors, however, were spores of *B. subtilis* and conidia of *A. niger*. These dormant forms of life have survived for 15 months in shaded areas and even for hours in direct sunlight. There are chances that dormant life forms can survive in shaded areas for years (but not decades) until sporadic rainfalls may give them a new chance to grow and replicate. Under natural conditions spores and conidia could be brought to the desert surface by air transportation: The coastal areas or the

eastern Cordillera are generally less than 100 km away from the central parts of the Atacama Desert. At wind speeds above 10 km hr^{-1} and at night, airborne microorganisms, if dryness-resistant, could safely reach central parts of the desert from the peripheral areas. If they settle in shaded parts of the desert rocks a fraction of them could survive for a few years. On the surface of early Mars about 3.5 Gyr ago, when liquid water became rare (McKay, 1997), however, the conditions for living organisms were probably much less favorable than in the contemporary Atacama Desert, because no ozone shield filtered out most of solar UVB and all UVC. Therefore, on Mars solar light also puts tougher restrictions on airborne transportation than on Earth. Accordingly, surface areas inhabited by microorganisms may have been extremely scarce. We certainly do not know whether Martian life, if it ever existed, depended on DNA as genetic material. But if so, at least extensive shielding by pigments was required.

In the absence of any liquid surface water and at relative humidities under 60% terrestrial microorganisms cannot grow. However, microorganisms have likely found a refuge beneath the surface of the Atacama Desert, because of the presence of groundwater. A similar situation may have existed at special locations on Mars and may still exist deep underground. The planned search for life in the Martian soil could therefore gain valuable support by the corresponding search for microorganisms in the soil of the Atacama Desert.

Acknowledgements

We gratefully acknowledge the assistance provided by Oficina Florencia, CIMIN S.A., sector Chacabuco, II Region, Chile. We thank Prof. Dr. Mennigmann, Institute for Microbiology, University of Frankfurt/M, Germany, for his continuous intellectual and practical support.

References

Asada, S., Takano, M. and Shibasaki, I.: 1979, Desoxyribonucleic Acid Strand Breaks during Drying of *Escherichia coli* on a Hydrophobic Filter Membrane. *Applied and Environmental Microbiology* **37**, 266–273.

Cabrera, S., Bozzo, S. and Fuenzalida, H.: 1995, Variations in UV Radiation in Chile. *J. Photochem. Photobiol. B: Biology* **28**, 137–142.

Crowe, L. M. and Crowe, J. H.: 1992, Anhydrobiosis: A Strategy for Survival. *Adv. Space Res.* **12**(4), 239–247.

Crowe, J. H., Hoekstra, F. H. and Crowe, L. M.: 1992, Anhydrobiosis, *Ann. Rev. Physiology* **54**, 579–599.

Dose, K.: 1994, Life from Outer Space? *Viva Origino* **22**, 261–282.

Dose, K., Bieger-Dose, A., Dillmann, R., Gill, M., Kerz, O., Klein, A. and Stridde, C: 1996, UV Photochemistry under Space Conditions. *Adv. Space Res.* **18**(12), 51–60.

Dose, K., Bieger-Dose, A., Dillmann, R., Gill, M., Kerz, O., Meinert, H., Nawroth, T., Risi, S. and Stridde, C.: 1995, *Adv. Space Res.* **16**, 119–129.

Dose, K., Bieger-Dose, A., Kerz, O. and Gill, M.: 1991, DNA-Strand Breaks Limit Survival in Extreme Dryness. *Origins of Life and Evolution of the Biosphere* **21**, 177–187.

Dose, K. and Gill, M.: 1995, DNA Stability and Survival of *Bacillus subtilis* Spores in Extreme Dryness. Origins *of Life and Evolution of the Biosphere* **25**, 277–293.

Dose, K. and Klein, A.: 1996, Response of *Bacillus subtilis* Spores to Dehydration and UV Irradiation at Extremely Low Temperatures. *Origins of Life and Evolution of the Biosphere* **26**, 47–59.

Feister, U.: 1994, Model Calculations and Measurements of Chemically and Biologically Effective UV Radiation Reaching the Ground. *Ber. Dt. Wetterd.* **190**, 1–79.

Feister, U. and Grewe, R.: 1995, Spectral Albedo Measurements in the UV and Visible Region over Different Types of Surfaces. *Photochem. Photobiol.* **62**, 736–744.

Friedmann, E. I.: 1980, Endolithic Microbial Life in Hot and Cold Deserts. *Origins of Life and Evolution of the Biosphere* **10**, 223–235.

Friedmann, E. I. and Ocampo-Friedmann, R.: 1984, The Antarctic Cryptoendolithic Ecosystem: Relevance to Exobiology. *Origins of Life* **14**, 771–776.

Friedmann, E. I. and Weed, R.: 1987, Microbial Trace-Fossil Formation, Biogenous, and Abiotic Weathering in the Antarctic Cold Desert. *Science* **236**, 703–705.

Horneck, G.: 1993, Responses of *Bacillus subtilis* Spores to Space Environment: Results from Experiments in Space. *Origins of Life and Evolution of the Biosphere* **23**, 37–52.

Horneck, G.: 1995, Quantification of the Biological Effectiveness of Environmental UV Radiation. *J. Photochem. Photobiol. B: Biology* **31**, 43–49.

Koga, S., Echigo, A. and Nunomura, K.: 1966, Physical Properties of Cell Water in Partially Dried *Saccharomyces cerevisiae*. *Biophys. J.* **6**, 665–674.

Krabbenhoft, K., Anderson, A. W. and Elliker, P. R.: 1967, Influence of Culture Media on the Radiation Resistance of *Micrococcus Radiodurans. Appl. Microbiol.* **15**, 178–185.

Leslie, S. B., Israeli, E., Lighthart, B., Crowe, J. H. and Crowe, L. M.: 1995, Trehalose and Sucrose Protect both Membranes and Proteins in Intact Bacteria during Drying. *Appl. Environ. Microbiol.* **61**(10), 3592–3597.

Lange, O. L., Schulze, E.-D. and Koch, W.: 1970, Experimentell-ökologische Untersuchungen an Flechten der Negev-Wüste. *Flora* **159**, 38–62.

London, J., Bojkov, R. D. and Kelley, J. I.: 1976, *Atlas of the Global Distribution of Total Ozone, July 1957 to June 1967*, NCAR/TN 114+STR.

Madronich, S. and De Gruiji, F. R.: 1994, Stratospheric Ozone Depletion Between 1979 and 1992: Implications for Biologically Active Ultraviolet-B; Radiation and Non-Melanoma Skin Cancer Incidence. *Photochem. Photobiol.* **59**, 541–546.

Mattimore, V. and Battista, J. R.: 1996, Radioresistance of *Deinococcus Radiodurans:* Functions Necessary to Survive Ionizing Radiation Are also Necessary to Survive Prolonged Desiccation. *J. Bacteriol.* **178**, 633–637.

Mazur, P.: 1980, Limits of Life at Low Temperatures and at Reduced Water Contents and Water Activities. *Origins of Life* **10**, 137–159.

McKay, C. P.: 1997, The Search for Life on Mars. *Origins of Life and Evolution of the Biosphere* **27**, 263–289.

Munakata, N.: 1981, Killing and Mutagenic Action of Sunlight upon *Bacillus subtilis* Spores: A Dosimetric System. *Mutation Res.* **82**, 263–268.

Munakata, N.: 1989, Genotoxic Action of Sunlight upon *Bacillus Subtilis* Spores: Monitoring Studies at Tokyo, Japan. *J. Rad. Res.* **30**, 338–351.

Munakata, N.: 1993, Biologically Effective Dose of Solar Ultraviolet Radiation Estimated by Spore Dosimetry in Tokyo since 1980. *Photochem. Photobiol.* **58**, 386–352.

Munakata, N. and Rupert, C. S.: 1972, Genetically Controlled Removal 'Spore Photoproduct' from Desoxyribonucleic Acid of Ultraviole-Irradiated *Bacillus Subtilis* Spores. *J. Bacteriol.* **111**, 192–198.

Nealson, K. H.: 1999, Post-Viking Microbiology: New Approaches, New Data, New Insights. *Origins of Life and* Evolution *of the Biosphere* **29**, 73–93.

Rose, A. H.: 1976, in *The Survival of Vegetative Microbes*, Symp. 26. Soc. Gen. Microbiol., Cambridge University Press, pp. 155–182.

Ruland, A.: 1982, *Küster-Thiel, Rechentafeln für die Chemische Analytik*, 102 ed., Walter de Gruyter, Berlin and New York, pp. 126 and 260.

Setlow, P.: 1992, I Will Survive: Protecting and Repairing Spore DNA. *J. Bacteriology* **174**, 2737–2741.

Setlow, P.: 1995, Mechanisms for the Prevention of Damage to DNA in Spores of *Bacillus* Species. *Annu. Rev. Microbiol.* **49**, 29–54.

Setlow, R. B.: 1974, The Wavelengths in Sunlight Effective in Producing Skin Cancer: A Theoretical Analysis. *Proc. Nat. Acad. Sci. USA* **71**, 3363–3366.

Sutherland, J. C. and Griffin, K. P.: 1981, Absorption Spectrum of DNA for Wavelengths Greater than 300 nm. *Rad. Res.* **86**, 399–409.

White, O. *et al*.: 1999, Genome Sequence of the Radioresistant Bacterium *Deinococcus Radiodurans* R1. *Science* **286**, 1571–1577.