RESPONSES OF *BACILLUS SUBTILIS* SPORES TO SPACE ENVIRONMENT: RESULTS FROM EXPERIMENTS IN SPACE*

GERDA HORNECK

DLR, Institute of Aerospace Medicine, Biophysics Division, Köln, Germany

(Received 6 August, 1992)

Abstract. Onboard of several spacecrafts (Apollo 16, Spacelab 1, LDEF), spores of *Bacillus subtilis* were exposed to selected parameters of space, such as space vacuum, different spectral ranges of solar UV-radiation and cosmic rays, applied separately or in combination, and we have studied their survival and genetic changes after retrieval. The spores survive extended periods of time in space – up to several years –, if protected against the high influx of solar UV-radiation. Water desorption caused by the space vacuum leads to structural changes of the DNA; the consequences are an increased mutation frequency and altered photobiological properties of the spores. UV-effects, such as killing and mutagenesis, are augmented, if the spores are in space vacuum during irradiation. Vacuum-specific photoproducts which are different from the 'spore photoproduct' may cause the synergistic response of spores to the simultaneous action of UV and vacuum. The experiments provide an experimental test of certain steps of the panspermia hypothesis.

1. Introduction

The interplanetary space, characterized by a high vacuum, an intense radiation climate of solar and galactic origin and extreme temperatures (Table I), has been considered to be extremely hostile to all forms of life. Above all, it is the high vacuum of up to 10^{-14} Pa which is a definite barrier for life to grow and develop, since it will lead to complete dehydration of the organism exposed to it. However some living organisms have developed a strategy of surviving arid conditions in an anhydrobiotic state (Crowe and Crowe, 1992). Examples are bacterial spores. The high resistance of spores of the genus Bacilli is mainly due to a dehydrated protoplast, enclosed by a thick protective envelop, the cortex and coats (Gould and Hurst, 1959)

Space technologies have provided opportunities for *in situ* investigations on the responses of resistant forms of microorganisms to the harsh environment of space (reviewed by Mennigmann, 1989 and Horneck and Brack, 1992). Early experiments, carried out by use of balloons, rockets or spacecrafts have demonstrated that microorganisms will survive short periods of exposure in space – several hours or days –, provided they are shielded against the intense solar ultraviolet (UV) radiation (Lorenz *et al.*, 1968, 1969).

* Presented at the Session 'Water in the Solar System and Its Role in Exobiology' during the 26th General Assembly of the European Geophysical Society, 22–26 April 1991 in Wiesbaden, Germany

Origins of Life and Evolution of the Biosphere **23**: 37–52, 1993.
© 1993 *Kluwer Academic Publishers. Printed in the Netherlands.*

TABLE I

Environmental data of the experiments on Apollo 16, Spacelab 1 and LDEF

Parameter	Apollo 16	Spacelab 1	LDEF
Space vacuum			
Pressure (Pa)	app. 10^{-14}	app. 10^{-4}	app. 10^{-6}
Residual gas (cm^{-3})	1H	3×10^7 O	3×10^7 O
		2×10^6 He	2×10^6 He
		1×10^5 N	1×10^5 N
		Contaminants[a]	
Exposure time	1.3 h	10 d	2107 d
Radiation			
Solar ultraviolet			
Spectral range (nm)	254	> 170	full
		220	> 170
		240	
		260	
		280	
Fluence ($J\ m^{-2}$)	$\leqslant 150$	$\leqslant 1000$	app. 10^9
Exposure time	10 min	$\leqslant 317.5$ min	2107 d
Cosmic ionizing			
Dose (Gy)	4.8×10^{-3}	1.3×10^{-3}	4.8
HZE particles (cm^{-2})[b]	25.2 ± 10.4	5.4 ± 0.3	app. 60[c]
			2.5×10^4[d]
Exposure time (d)	11.1	10	2107
Temperature (K)	293	290–310	264–302

[a] Sources of contamination: waste dumping (H_2O, organics); thruster firing (H_2O, N_2O, NO) (Carignan and Miller, 1987).
[b] Linear Energy Transfer (LET) > 130 keV μm^{-1}.
[c] Primaries.
[d] Including secondaries.

In space, the spores have to cope with an interplay of various adverse environmental factors. To disentangle the network of potential interactions, we have pursued a strategy as follows. On the one hand, we have studied each space parameter separately, e.g. space vacuum, solar UV-radiation, HZE particles of cosmic radiation (for definition see Grahn, 1973), for its impact on the integrity of bacterial spores; on the other hand we have combined them one by one and investigated their combined action. This approach allows to identify types of interaction, whether additive, synergistic or antagonistic. In parallel to each space flight experiment, samples were kept in the laboratory at conditions simulating those of the space experiments as closely as possible. Survival, genetic changes and the efficiency of cellular repair processes were determined after retrieval of the flight samples.

2. Material and Methods

2.1. Biological samples

We have used spores of *B. subtilis* strains as follows:
- Marburg (microbial culture collection, Institute of Microbiology, University of Frankfurt)
- HA101 *hisB101 metB101 leuA8* (Okubo and Yanagida, 1968)
- HA101F *hisB101 met B101 leuA8 polA1* (Gass *et al.*, 1971)
- TKJ6312 *hisB101 metB101 leuA8 uvrA10 ssp-1* (Tanooka *et al.*, 1978)

Strains Marburg and HA101 are wild type in DNA repair capability (in the following called *w*), strains HA101F (in the following called *pol*$^-$) and TKJ6312 (in the following called *ssp*$^-$) are both deficient in excision repair and, the latter strain, also in spore photoproduct specific repair (Munakata and Rupert, 1974). Strains HA101 and HA101F were kindly provided by Dr. Spizizen and strain TKJ6312 by Dr. Munakata. The spores were prepared as described by Horneck *et al.* (1984b) and Baltschutkat and Horneck (1991). In brief, they were grown in liquid sporulation medium, treated with lysozyme and DNAse and purified by density centrifugation. They were stored in distilled water until used for the flight experiments.

2.2. Sample preparation

Dry layers of spores were prepared by transferring 20μl of spore suspension (in water, phosphate buffer or 5% glucose solution) in appropriate dilution onto square glass slides of 4 mm edge size (Apollo 16 flight) (Taylor *et al.*, 1974) or onto quartz discs of 7 mm diameter (Spacelab 1 and LDEF mission) (Horneck *et al.*, 1984b). The samples were dried overnight in laboratory air. For Apollo 16, in addition, liquid samples of 50 μl were contained in plastic cuvettes with quartz window (Taylor *et al.*, 1974).

2.3. Exposure to space environment

Samples were exposed to space vacuum for 1.3 h (Apollo 16), 10 d (Spacelab 1) or 2107 d (LDEF). For irradiation with solar ultraviolet radiation, full spectrum or selected spectral intervals, the samples were placed either beneath an optical filtering system (Apollo 16, Spacelab 1) or a perforated aluminium dome (LDEF). The perforation of this aluminium cover allowed access of space vacuum, solar electromagnetic radiation including UV and vacuum-UV, and cosmic radiation. The environmental data for the different missions are given in Table I. During the Spacelab 1 mission, the solar spectral irradiance was measured by Labs *et al.* (1987). The fluence at the sample site was determined by convolution of the solar spectrum with the transmission curves of the filters (Figure 1). For exposure to polychromatic solar UV-light, e.g. the UV-range transmitted by the quartz window, all wavelengths below 320 nm were taken into consideration. The hardware and flight protocol have been described by Talor *et al.* (1974) for Apollo 16, by Horneck *et al.* (1984b)

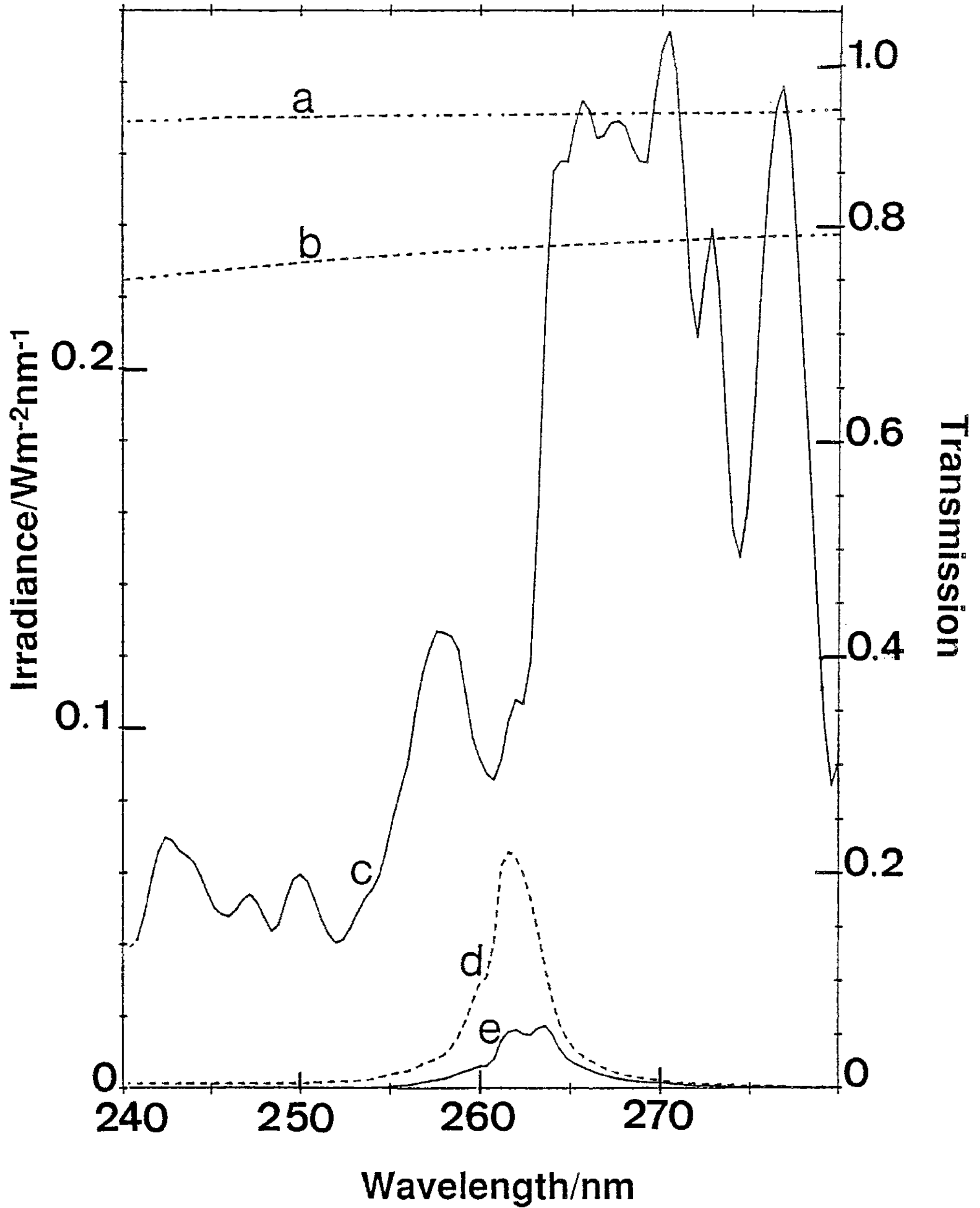

Fig. 1. Solar spectrum (c), transmission curves of the filters (a) quartz window, (b) neutral density filter, (d) band pass filter, and (e) UV-spectrum at the sample site; (a), (b) and (d) right ordinate, (c) and (e) left ordinate. The fluence at the sample site was obtained by convolution of the different curves.

for Spacelab 1 and by Horneck *et al.* (in preparation) for LDEF. In parallel to all space flight experiments, simultaneous ground control experiments were run in space simulation chambers. Most of the parameters of space, such as vacuum, temperature, UV-radiation have been duplicated to the extent possible.

2.4. Postflight assay

After retrieval, inactivation, mutagenesis to histidine reversion and the involvement of repair processes were tested by methods as described by Horneck *et al.* (1984b) and Baltschukat and Horneck (1991). The effects of each space parameter, such as space vacuum, solar UV-radiation, or cosmic radiation, on the spores were tested separately, as well as combined actions by solar UV-radiation and space vacuum as well as by the complete space environment. Survival from exposure to the space vacuum, S(V), indicated as colony forming ability, is given by

$$S(V) = (N/N_0) \times 100\ (\%) \qquad (1)$$

with
N = colony formers after the mission
N_0 = colony formers before the mission.
The frequency of histidine revertants, M, is given by

$$M = m/N \qquad (2)$$

with
m = his^+ revertants per sample
N = colony formers per sample
For UV-irradiated samples, the surviving fraction, S(UV), is given by

$$S(\mathrm{UV}) = N/N_0 \qquad (3)$$

with
N = number of colony formers after irradiation with a certain fluence
N_0 = number of colony formers at zero fluence.
Fluence effect curves were constructed by plotting the logarithm of the fraction of surviving spores versus fluence. The curves were fitted to the following equations by use of a computer program based on maximum likelihood model

$$\ln\ S(\mathrm{UV}) = -kF \qquad (4)$$

or

$$\ln\ (1\text{–}S(\mathrm{UV})) = n \times \ln[1\text{-exp})\text{–}kF) \qquad (5)$$

with
k = inactivation constant ($m^2\ J^{-1}$)
F = fluence ($J\ m^{-2}$)
n = extrapolation number which is the intercept with the ordinate of the extrapolated semi-log straight line.

The inactivation cross section, σ, was obtained from the reciprocal of the F_{10} values (UV fluence reducing survival to 10%) by multiplying it with the actual photon energy (quantum correction). Plotting σ versus wavelength gives the action spectrum of inactivation.

The frequency of UV-induced his^+ revertants, $M(i)$, is given by

$$M(i) = (m - m_0)/N \quad (6)$$

with
m = his^+ revertants per sample after UV-radiation
m_0 = his^+ revertants per sample without irradiation
N = colony formers per sample

3. Results

3.1. Space vacuum

From samples in vented compartments that were kept in the dark during the whole mission, the effects of space vacuum were investigated. In addition, these spores were exposed to cosmic radiation of doses as shown in Table I. Temperature was kept nearly at ambient, in the range of 264–310 K, depending on the kind of mission (Table I). The survival data are listed in Table II. It is noteworthy that, within experimental errors, N_0 of Equation 1 is identical with the actual number of spores per sample. This implies, that the preparation procedures, such as drying in laboratory air, did not affect the viability of the spores. In the flight control samples that were kept at atmospheric pressure during the mission, the number of colony formers was also in agreement with N_0, even after nearly 6 years in space.

Non-protected spores, i.e. in monolayers (10^5 – 10^6 spores/sample) prepared from an aqueous suspension, survive 10 days in space vacuum up to about 70% (wild type) or about 50% (repair deficient strains). After nearly six years in space, there is still a considerable amount of viable spores (1–2%), even if exposed in a monolayer without any protection against dehydration. Spores in a multilayer (10^8 spores/sample) survive even less (0.3%) than in a monolayer. The survival is significantly increased, if protecting substances are present, such as buffer salts or glucose. The protecting effect is especially pronounced for spores in a multilayer. In this case, e.g. multilayer with glucose present, nearly 70% of the wild type spores and 90% of the ssp^- spores survive a nearly 6 years exposure to space vacuum.

For the Apollo 16 and LDEF mission, within experimental errors, comparable survival data were obtained in both, flight experiment and ground control experiment (Table II). Concerning the Spacelab 1 ground control experiment, spores survived vacuum exposure to a higher extent than in space (Table II). During space flight, the presence of atomic oxygen or contaminants in the cargo bay of the Shuttle (Table I) might have stressed the spores, in addition to space vacuum.

The decisive step during vacuum exposure is water desorption (Bücker and

TABLE II

Survival S(V) (%) of B. subtilis spores from exposure to space vacuum

Strain	Spore layer	Additive	Apollo 16[a]		Spacelab 1[b]		LDEF	
			ground control	flight unit	ground control	flight unit	ground control	flight unit
Wild type[c]	monolayer	none	1.2 ± 0.7	0.7	85.3 ± 2.6	69.3 ± 15.8	5.4 ± 2.9	1.4 ± 0.8
		buffer salts	62.2 ± 27.0	81.0 ± 3.2			16.7 ± 8.3	11.0 ± 2.0
		glucose					30.5 ± 5.0	28.0 ± 3.5
	multilayer	none					1.0 ± 0.3	0.3 ± 0.1
		buffer salts					45.3 ± 6.0	40.0 ± 7.0
		glucose					77.0 ± 6.0	67.2 ± 10.1
pol⁻	monolayer	none			66.7 ± 7.0	49.8 ± 10.4		
ssp⁻	monolayer	none			99.9 ± 5.8	47.0 ± 9.4	0.5 ± 0.2	2.3 ± 1.9
		buffer salts					20.0 ± 6.7	32.3 ± 2.3
		glucose					45.8 ± 6.7	51.0 ± 5.3
	multilayer	none					4.2 ± 0.9	0.3 ± 0.2
		buffer salts						37.7 ± 5.0
		glucose					73.9 ± 13.6	86.0 ± 21.9

[a] Bücker *et al.* 1974.
[b] Horneck *et al.* 1984a.
[c] Strain Marburg (Apollo 16, LDEF), strain HA101 (Spacelab 1).

Horneck, 1969, 1970). It exerts a strong mechanical stress to the spore envelope – the membranes, cortex and spore walls –, and it affects the structural integrity of the macromolecules, such as nucleic acids, proteins and lipids. Protection by additives may be based on mechanisms as follows: (1) the substance binds additional water molecules thereby preventing complete dehydration of the cell, e.g. carbohydrates (Fry, 1966); (2) the substance replaces water molecules thereby stabilizing the structure of the macromolecules, e.g. polyalcohols (Webb, 1965); (3) the substance provides a cover layer less permeable for water molecules thereby allowing built up of an interior 'microclimate' of higher water pressure than outside (Fry, 1966). In most cases, protection degrades with time (Bücker *et al.*, 1972). During the LDEF mission, in multilayers of spores with the additive glucose, a combination of the three mechanisms may have led to the high survival.

After exposure of spores (strain HA101) to space vacuum for 10 days (Spacelab 1), the frequency of histidine revertants, M, is elevated by a factor of approximately 10, from $(1.04 \pm 0.09) \times 10^{-5}$ to $(1.18 \pm 0.08) \times 10^{-4}$ (Horneck *et al.*, 1984a). Since samples, kept at atmospheric pressure during the mission, did not show an increased mutation frequency, mutagenesis must be caused by space vacuum and not by cosmic radiation or other inevitably present space parameters. An increased mutation frequency was also observed in spores that were treated with vacuum during the ground control expetiment.

Vacuum-induced mutagenesis indicates that the DNA, the carrier of genetic information, is one of the critical targets. Water plays a decisive part in the maintenance of the native conformation of this biomacromolecule. With decreasing water activity, the hydration water from the grooves is removed, thereby changing the structure of the double helix. In vacuum-treated cells of *Escherichia coli*, less than 1 water molecule per nucleotide is available (Reitz, 1972, Schwager, 1973). The consequence of this nearly complete loss of hydration water is a partial denaturation of the DNA which is a reversible process (Falk, 1964). Dose *et al.* (1992) report of crosslinking between DNA and protein in vacuum-treated spores of *B. subtilis* which may finally lead to DNA strand breaks. As a consequence of the structural changes of the DNA molecule in vacuum-treated spores, their photochemical and photobiological properties are also altered. This will be discussed below.

3.2. Cosmic Radiation

In the deep space mission of Apollo to the Moon, the bacterial spores were exposed mainly to corpusculate radiation of galactic and solar origin, whereas in Earth's vicinity (Spacelab 1 and LDEF) a substantial fraction of dose is attributed to the geomagnetically trapped radiation of the belts (for review see e.g. Stassinopoulos, 1988). The doses received during these missions (Table I) were too low to allow the observation of a radiobiological effect by statistical analyses, even after nearly six years in space. Even for the most sensitive strain, about 30 times higher doses would be required for the D_{37} value (mean lethal dose) (Baltschukat and Horneck, 1991).

From the various types and sources of radiation in space, the HZE particles of cosmic radiation are of special radiobiological concern. Since they contribute to approximately 1% of the flux of particulate radiation in space only, methods have been required to precisely localize the trajectory of each HZE particle relative to a bacterial spore (Horneck *et al.*, 1974, Schäfer *et al.*, 1977). By use of the Biostack method (Bücker and Horneck, 1975), it was found that spores can survive even a central hit of a HZE particle and that the effective range of inactivation extends far beyond radial distances from the trajectory where inactivation by δ-ray dose would be effective (Facius *et al.*, 1978). Inactivation cross sections are about 20 times higher than those from comparable accelerator experiments. The biological effects of the HZE component of cosmic radiation have been recently reviewed by Horneck (1992).

3.3. Solar UV radiation

Fluence effect curves for inactivation of *B. subtilis* spores by solar UV-light of defined wavelength ranges were obtained for spores in suspension (Bücker *et al.*, 1974) or for dry layers of spores kept at atmospheric pressure (Figure 2, triangles). Wavelength selection was obtained by using band pass filters or cut off filters. Fluences were controlled by defined exposure periods of samples beneath different neutral density filters.

Shouldered survival curves are obtained for spores in aqueous suspension (Bücker *et al.*, 1974) and for dry layers of wild type or *pol*$^-$ spores (described by Equations(5), whereas the survival curve of dry *spp*$^-$ spores shows a straight line, intersecting the ordinate at 1 (described by Equation 4). The action spectrum of solar photons (220–280 nm) for inactivation of dry layers of wild type spores (Figure 3) roughly correlates with the absorption spectrum of DNA indicating the DNA being the critical chromophore for lethality. The response of the spores to solar UV of selected wavelengths is in agreement with results from ground control experiments (Figures 2 and 3).

The influence of the full spectrum of solar UV-light (> 190 nm) on dry layers of *B. subtilis* wild type spores, kept at atmospheric pressure, was investigated during the Spacelab 1 mission. The fluence effect curve of inactivation has no shoulder and is upward concave (Figure 4, triangles). The loss of the shoulder in the survival curve, compared e.g. to the response to 260 nm UV, may indicate that repair processes become less effective in restoring the UV-induced damage. The two components of the curve reflect two fractions of spores that are inactivated at different rates. We cannot exclude that the more resistant fraction which amounts to approximately 1% is due to shading effects. The inactivation by the full spectrum of solar UV-light is much higher, especially in the low fluence range (fraction 1), than it was expected from the ground control experiment, where a deuterium light source (Hanau D200F, line-free continuum between 170 and 370 nm) was used (Figure 4). This difference between space and ground control experiment is not surprising, since the spectrum of the deuterium lamp (data from the manufacturer) strongly differs

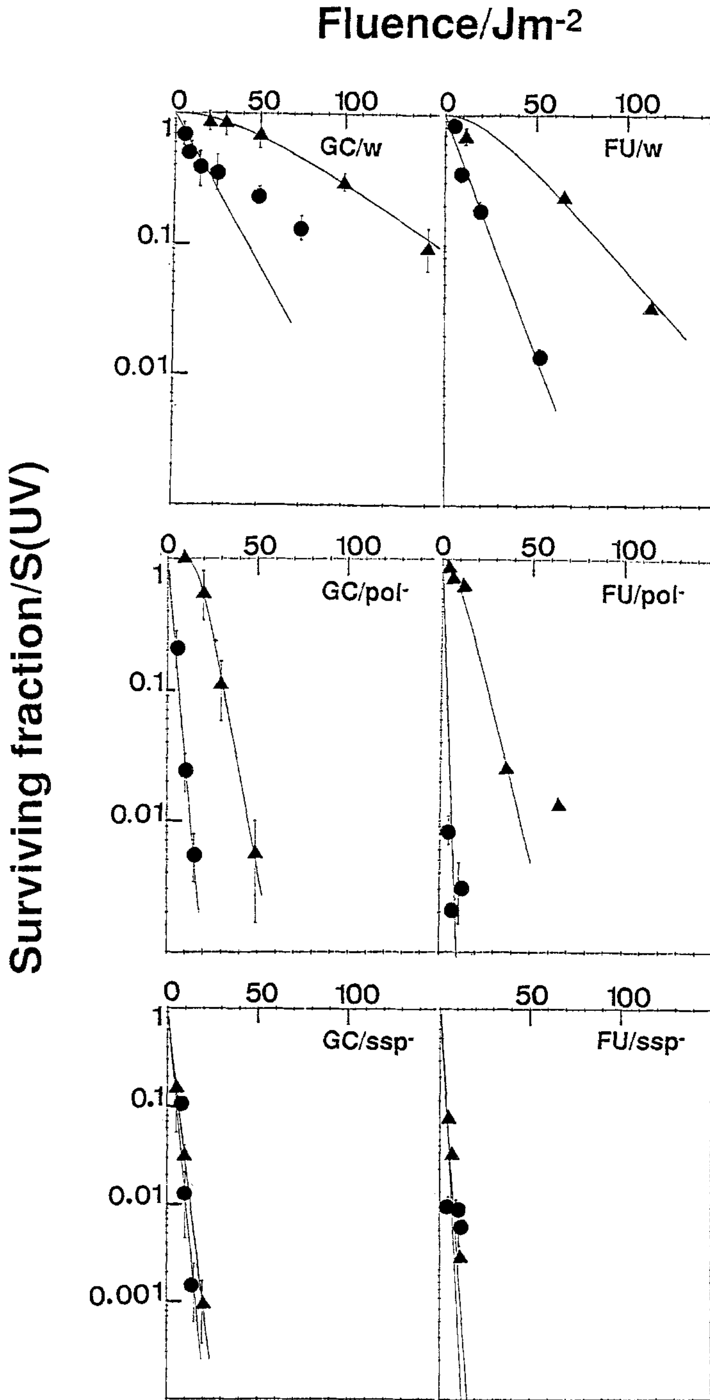

Fig. 2. Fluence effect curves for inactivation of dry layers of *B. subtilis* spores by solar UV-light in the range of 260 nm; the spores were kept at atmospheric pressure (triangles) or in space vacuum (circles) during irradiation; FU = flight experiment (Spacelab 1), GC = ground control; three strains of different repair capacity were used: w = wild type, *pol*$^-$ = deficient in excision repair, *ssp*$^-$ = deficient in excision and in spore photoproduct repair.

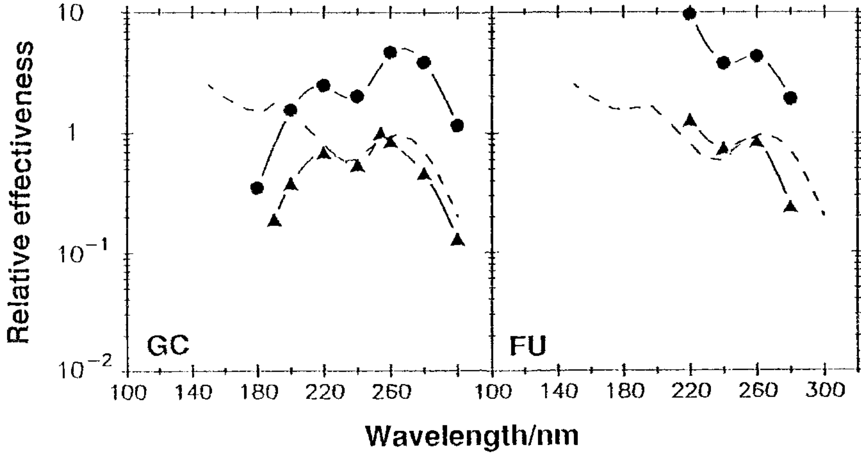

Fig. 3. Action spectrum for inactivation of spores of *B. subtilis* wild type, irradiated at atmospheric pressure (triangles) or in space vacuum (circles); FU = space experiment (Spacelab 1), GC = ground control; the dashed line gives the absorption spectrum of DNA.

from the extra-terrestrial solar UV-spectrum (Labs *et al.*, 1987).

From the response of spores to the extra-terrestrial solar UV-light of wavelengths above 190 nm, we have calculated that 10 seconds in space are sufficient to kill 95% of bacterial spores, if exposed without any shielding. To reach the same effect on Earth, an approximately 1000 times longer exposure to sunlight is required

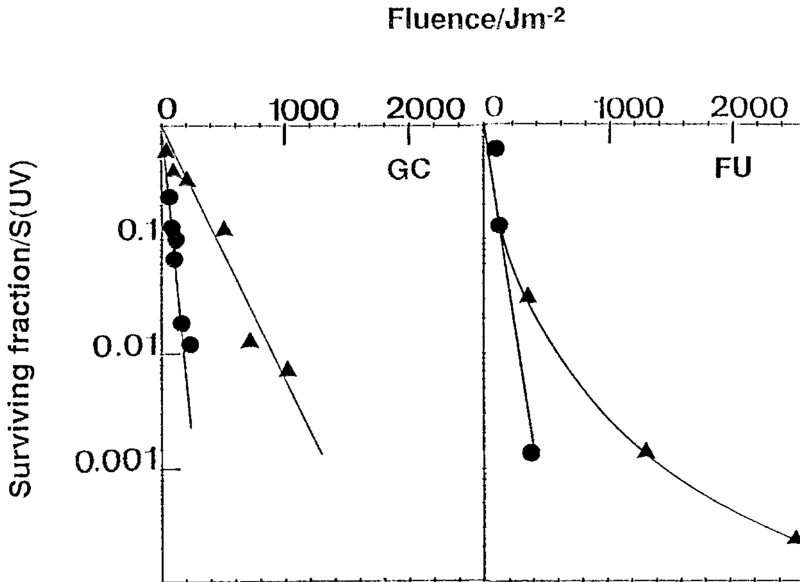

Fig. 4. Fluence effect curves for inactivation of dry layers of *B. subtilis* wild type spores by polychromatic solar UV-light above 190 nm, if irradiation at atmospheric pressure (triangles), or above 170 nm, if irradiation in space vacuum (circles); FU = space experiment (Spacelab 1), GC = ground control.

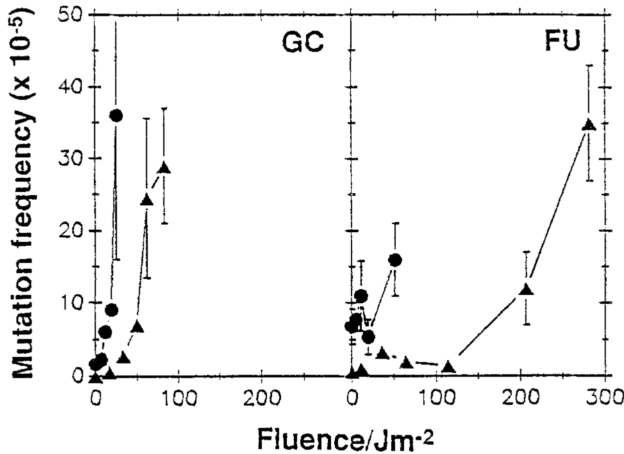

Fig. 5. Mutation induction curves (histidine revertants) of *B. subtilis* spores by solar UV-light of 260 nm; irradiation at atmospheric pressure (triangles) or in space vacuum (circles); FU = space experiment (Spacelab 1), GC = ground control (254 nm).

(Horneck and Brack, 1992). This difference is mainly attributed to the protective layer of ozone in our atmosphere that retains the most harmful fraction of solar UV-light (< 295 nm) from the Earth surface.

The mutation induction curve for solar UV-light in the range of 260 nm is nearly linear quadratic (Figure 5, triangles). To reach the same mutagenic effect as in the ground control experiment (254 nm), in space, much higher fluences are required. Very little mutants are induced by the full spectrum of solar UV-ligt above 190 nm (Figure 6, triangles).

3.4. Solar UV Radiation and Space Vacuum

Figures 2–4 show also the UV-sensitivity of spores that are exposed to space vacuum during irradiation by solar UV-light (circles). In this case, N_0 in Equation (3) gives the number of viable spores after vacuum treatment. For all three strains, the survival curves are purely exponential, following Equation (4). For wild type and *pol*⁻ spores, their slopes are higher than those of the exponential parts of the corresponding curves at atmospheric pressure. The disappearance of the shoulder and the increased slope of the survival curves reflect an increased UV-sensitivity of the wild type and *pol*⁻ spores in space vacuum compared to atmospheric conditions. The enhancement factor, derived from the ratio of the F_{10} values (Table III), ranks between 3.3 and 8.0 for solar UV of narrow spectral ranges between 220 and 280 nm (Horneck *et al.*, 1984a, b). For the full solar UV-spectrum, the UV-sensitization by space vacuum is less pronounced (enhancement factor of 1.4).

In Figure 2, the efficiency of repair processes after UV-irradiation in space is also illustrated. *ssp*⁻ spores which are deficient in excision as well as in spore photoproduct specific repair have the same UV-sensitivity at atmospheric pressure as at space vacuum conditions (F_{10}= 5.3 J m^{-2}, see also enhancement factor = 1

TABLE III

Enhancement factor (ratio of F_{10} values) for the response of B. subtilis spores to solar UV in combination with space vacuum

Strain	Wavelength (nm)	Apollo 16[a]	Spacelab 1[b]
Wild type[c]	220	–	7.9
	240	–	5.0
	254	5.0	–
	260	–	5.0
	280	–	8.0
Wild type[d]	260	–	3.3
	> 170	–	1.4
pol⁻	260	–	5.3
ssp⁻	260	–	1.0

[a] Bücker *et al.* 1974.
[b] Horneck *et al.* 1984a.
[c] Strain Marburg.
[d] Strain HA101.

in Table III). Their UV-sensitivity is also nearly the same as that of *pol⁻* spores in space vacuum (F_{10}=4.9 J m^{-2}) which are also deficient in excision repair but capable of spore photoproduct specific repair. Therefore, the spore photoproduct 5-thyminyl-5,6-dihydrothymine (TDHT) is probably not responsible for the increased UV-sensitivity of spores in space vacuum. Comparable results were obtained in the ground control experiments. The yield of TDHT formation is even reduced after UV-irradiation in vacuum compared to atmospheric conditions (Lindberg and Horneck, 1991). Since the UV damage to DNA strongly depends on the secondary structure of the double helix - which is further influenced by the amount of water bound to it –, it is conjectured that, in vacuum-exposed spores, UV-light induces 'vacuum-specific' photoproducts which are less amenable to the cellular repair processes and which may finally cause the synergistic effects of UV-radiation and vacuum. However, excision repair processes are still effective, since, in vacuum, the wild type spores are bout 5 times more UV-resistant (F_{10} = 27.5 J m^{-2}) than the two repair deficient strains.

Figures 5 and 6 (circles) show also an increased mutagenic efficiency of UV-light, at 260 nm as well as polychromatic light above 170 nm, if the spores are in space vacuum during irradiation.

3.5. Full environment of space

With the LDEF mission, for the first time, microorganisms were exposed to the full environment of space for an extended period of time, i.e. for nearly 6 years, and their survival was determined after retrieval. The samples were separated from free space by a perforated aluminium dome, only which allowed access of space vacuum,

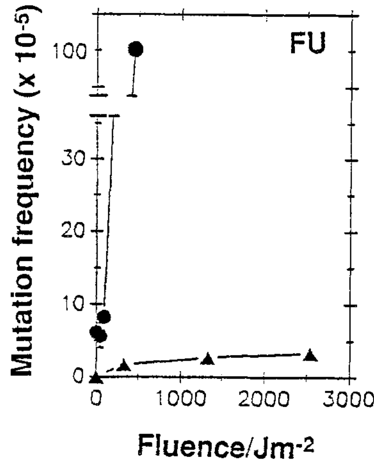

Fig. 6. Mutation induction curves (histidine revertants) of *B. subtilis* spores by polychromatic solar UV-light above 190 nm, if irradiation at atmospheric pressure (triangles) or above 170 nm, if irradiation in space vacuum (circles) (Spacelab 1 results).

solar UV-radiation and most of the components of cosmic radiation. Table IV gives viable counts of the different flight samples. Although there is a high variation between the different parallel samples, it is evident that, at least in some of the unprotected samples, thousands of spores have survived the space journey. All spores were exposed in multilayers. These had turned from white into yellow during the mission, a phenomenon which is probably due to photochemical processes. We suggest that all spores in the upper layers will be completely inactivated by the high influx of solar UV-light. With time, they will form a protective crust which considerably attenuates the solar UV-radiation for the spores located in layers

TABLE IV

Viable spores N, recovered after nearly 6 years in space (LDEF results)

Strain	Spore layer	Additive	N		
			before flight	dark control	full environment
Wild type	multilayer	none	6.7×10^7	2.4×10^4	4
				1.8×10^4	0
				2.3×10^4	
		buffer salts	1.0×10^8	4.5×10^7	1.5×10^3
				4.3×10^7	0
				2.9×10^7	
		glucose	1.0×10^8	5.4×10^7	9.6×10^3
				7.0×10^7	5.4×10^1
				2.9×10^7	
ssp^-	multilayer	glucose	8.8×10^7	2.1×10^7	9.5×10^1
				1.8×10^7	1.2×10^1

beneath. Therefore, the survivors probably originate from the innermost layers of the samples.

4. Discussion

When S. Arrhenius discussed the transport of life through space, e.g. in the form of spores, by means of the radiation pressure of the sun (1903), he was completely ignorant whether microorganisms will survive such an extreme journey. Today, space technology provides the tool for studying the chances of microbial survival in space in a direct approach. We have shown, that bacterial spores can survive over several years in space, provided they are protected against the high influx of solar UV-radiation. This can be achieved by a mantle of some material which attenuates solar UV-radiation by a factor up to several orders of magnitude, e.g. by dust, rock or clay material, or by a very thick layer of microorganisms where the outer layers form a protective crust for those organisms located beneath. However, for a hypothetical interplanetary transfer of life, the survival of microorganisms in space is only one of several requirements that have to be met. The different steps include (1) that life originates and evolves on a planet, (2) that dynamic processes exist that allow material to escape from the planet and that they are moderate enough to allow survival of microorganisms enclosed, (3) that microorganisms survive the interim state in space, (4) that space travelling microorganisms which are captured by the gravitational force of a planet or moon will survive entry and landing, and (5) that these microorganisms will find environmental conditions favourable for their growth and development (Horneck and Brack, 1992). Several of these steps can now be approached experimentally.

Although it will be difficult to prove that transport of life through our solar system will occur, it might be possible, by measurements, calculations and experiments to determine the chances for the different steps to occur. Recent discoveries have given new support to this idea of panspermia (reviewed by Dose, 1986). Examples are the detection of meteorites, some of them being of lunar and some probably of Martian origin (Kerr, 1987), the probability of rocks to reach escape velocities by the impact of a big meteorite on a planet (Melosh, 1984, 1988), the high UV-resistance of bacterial spores at deep space temperatures (Weber and Greenberg, 1985) and the likelihood of artificial or directed panspermia (Crick, 1981) by space missions to other planets. On the other hand, Nussinov and Lysenko (1992) have argued that the joint action of high temperature and vacuum will prevent spores migrating into outer space from a planetary surface.

Acknowledgements

I thank Prof. H. Bücker, Principal Investigator of the experiments on Apollo and LDEF, for the opportunity of exobiological experimentation in space, Drs. G. Reitz and J.U. Schott for providing the flight hardware of the Spacelab 1

experiment and U. Eschweiler for the skilful assistance in the microbiological experiment preparation and analysis.

References

Arrhenius, S.: 1903, *Die Umschau* **7**, 481.
Baltschukat, K. and Horneck, G.: 1991, *Radiat. Environ. Biphys.* **30**, 87.
Bücker, H. and Horneck, G.: 1969, *Biophysik* **6**, 69.
Bücker, H. and Horneck, G.: 1970, *Life Sci. Space Res.* **8**, 33.
Bücker, H., Horneck, G., Facius, R., Schwager, M., Thomas, C., Turcu, G., and Wollenhaupt, H.: 1972, *Life Sci. Space Res.* **10**, 191.
Bücker, H., Horneck, G., Wollenhaupt, H., Schwager, M., and Taylor, G. R.: 1974, *Life Sci. Space Res.* **12**, 209.
Bücker, H. and Horneck, G.: 1975, in *Radiation Research*, O. F. Nygaard, H. J. Adler, and W. K. Sinclair, E(eds.), Academic Press, New York, p. 1138.
Carignan, G. R. and Miller, E. R.: 1987, *Adv. Space Res.* **7**(5), 153.
Crick, F.: 1981, *Life itself, its Origin and Nature*, Simon and Schuster Publ., New York.
Crowe, L. M. and Crowe, J. H.: 1992, *Adv. Space Res.* **12**(4), 239.
Dose, K.: 1986, Adv. Space Res. **6**(12), 181.
Dose, K., Bieger-Dose, A., Labusch M., and Gill, M.: 1992, *Adv. Space res.* **12**(4), 221.
Facius, R., Bücker, H., Hildebrand, D., Horneck, G., Höltz, G., Reitz, G., Schäfer, M., and Toth, B.: 1978, *Life Sci. Space Res.* **16**, 151.
Falk, M.: 1964, *J. Am. Chem. Soc.* **86**, 1226.
Fry, R. M.: 1966, in *Cryobiology*, H. T., Meryman (ed.), Academic Press, p. 665.
Gass, K. G., Hill, T. C., Goulian, M., Strauss, B. S., and Cozzarelli, N. R.: 1971, *J. Bacteriol.* **108**, 364.
Gould, G. W. and Hurst, A. (eds.): 1959, *The Bacterial Spore*, Academic Press, London.
Grahn, D. (ed.): 1973, *HZE-particles in manned spaceflight*, Natl. Acad. Sci. Washington.
Horneck, G.: 1992, *Nucl. Tracks Radiat. Meas.* **20**, 185.
Horneck, G., Facius, R., Enge, W., Beaujean, R., and Bartholomä K.-P.: 1974, *Life Sci Space Res.* **12**, 75.
Horneck, G., Bücker, H., Reitz, G., Requardt, H., Dose, K., Martens, K. D., Mennigmann, H. D., and Weber, P.: 1984a, *Science* **225**, 226.
Horneck, G., Bücker, H., Reitz, G., Requardt, H., Dose, K., Martens, K. D., Mennigmann, H. D., and Weber, P.: 1984b, *Adv. Space Rev.* **4**(10), 19.
Horneck, G. and Brack, A.: 1992, in *Advances in Space Biology and Medicine*, Vol. **2**, S. L. Bonting (ed.), JAI Press, Greenwich, CT, p. 229.
Kerr, R. A.: 1987, *Science*, **237**, 721.
Labs, D., Neckel, H., Simon, P. C., and Thuillier, G.: 1987, *Solar Physics* **107**, 203.
Lorenz, P., Hotchin, J., Markusen, A. S., Orlob, G. B., Hemenway, C. L., and Hallgren, D. S.: 1968, *Space Life Sci.* **1**, 118.
Lorenz, P., Orlob G. B., and Hemenway, C. L.: 1969, *Space Life Sci.* **1**, 491.
Melosh, H. J.: 1984, *Icarus* **59**, 234.
Melosh, H. J.: 1988, *Nature* **332**, 687.
Munakata N. and Rupert, C. S.: 1974, *Molec. Gen. Genet.* **130**, 239.
Nussinov, M. D. and Lysenko, S. V.: 1992, *J. Brit. Interplanet. Soc.* **45**, 22.
Okubo, S. and Yanagida, T.: 1968, *J. Bacteriol.* **95**, 1187.
Reitz, G.: 1975, *Gravimetrische Messungen im Vakuum*, Thesis, University of Frankfurt.
Schäfer, M., Bücker, H., Facius, R., and Hildebrand, D.: 1977, *Radiat. Effects* **34**, 129.
Schwager, M.: 1973, *Untersuchungen über die Ursachen der Inaktivierung und der erhöhten UV-Empfindlichkeit von Bakterien durch Vakuumbehandlung*, Dissertation, University of Frankfurt.
Stassinopoulos, E. G.: 1988, in *Terrestrial Space Radiation and Its Biological Effects*. P. C. McCormack, C. E. Swenberg and H. Bücker (eds.), Plenum Press New York, p. 5.
Tanooka, H., Munakata, N., and Kataha, S.: 1978, *Mutation Res.* **49**, 179.
Taylor, G. R., Spizizen, J., Forster, B. G., Volz, P. A., Bücker, H., Simmonds, R. C., Heimpel, A. M., and Benton, E. V.: 1974, *BioScience* **24**, 505.
Webb, S. J.: 1965, *Bound Water in Biologicl Integrity*, C. C. Thomas, Springfield Ill.
Weber, P. and Greenberg, J. M.: 1985, *Nature* **316**, 403.