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LONG-TERM SURVIVAL OF BACTERIAL SPORES IN SPACE

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ABSTRACT

On board of the NASA Long Duration Exposure Facility (LDEF), spores of *Bacillus subtilis* in monolayers (10^6/sample) or multilayers (10^8/sample) were exposed to the space environment for nearly six years and their survival was analyzed after retrieval. The response to space parameters, such as vacuum (10^{-6} Pa), solar electromagnetic radiation up to the highly energetic vacuum-ultraviolet range (10^9 J/m^2) and/or cosmic radiation (4.8 Gy), was studied and compared to the results of a simultaneously running ground control experiment. If shielded against solar ultraviolet (UV)-radiation, up to 80 % of spores in multilayers survive in space. Solar UV-radiation, being the most deleterious parameter of space, reduces survival by 4 orders of magnitude or more. However, up to 10^4 viable spores were still recovered, even in completely unprotected samples. Substances, such as glucose or buffer salts serve as chemical protectants. With this 6 year study in space, experimental data are provided to the discussion on the likelihood of "Panspermia".

INTRODUCTION

The theory of panspermia which was originally put forward by Arrhenius /1/, has been recently revisited on the base of new findings /2, 3/. Several of the steps of a hypothetical interplanetary transfer of life can now be approached experimentally, e.g. the survival of microorganisms in space. Space has been generally viewed as extremely hostile to all forms of life, due to the high vacuum, the complex radiation field and extreme temperatures. This extreme environment is a definite barrier for the active biological processes of growth, metabolism and reproduction. However, some living organisms have the capacity to survive unfavourable conditions in a dormant state, examples being spores of bacteria and fungi. The high resistance of bacterial spores is mainly due to a dehydrated protoplast, enclosed in a thick protective envelope, the cortex and the spore coats /4/.

In space, the spores have to cope with an interplay of various adverse environmental factors. From *in-situ* experiments on the responses of microorganisms to the space environment it is known that the most deleterious factor is solar UV-radiation, especially that in the wavelength range around 260 nm and 220 nm /5, 6/, which is specifically absorbed by the DNA. Furthermore, space vacuum and solar UV act synergistically in reducing microbial viability /6, 7/. This phenomenon which is due to the formation of specific scarcely reparable DNA-lesions /8/ further decreases the chance for microorganisms to survive extended exposure in space. So far, microorganisms have been investigated after maximum exposure times in space of 10 days /6/. With the NASA mission of the Long Duration Exposure Facility (LDEF), we have obtained the opportunity to expose *Bacillus subtilis* spores for nearly 6 years to the space environment and to analyse their responses after retrieval.

MATERIAL AND METHODS

We have used *B. subtilis* spores of the strain Marburg (microbial culture collection, Institute of Microbiology, University of Frankfurt). The spores were prepared as described in /9, 10/. 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 experiment.

Dry layers of spores were prepared by transferring 20 µl of spore suspension (in water, phosphate buffer or 5 % glucose solution) in appropriate dilution onto quartz discs of 7 mm diameter either in monolayers (10^6 spores/ml) or multilayers (10^8 spores/ml). The samples were dried overnight in laboratory air.

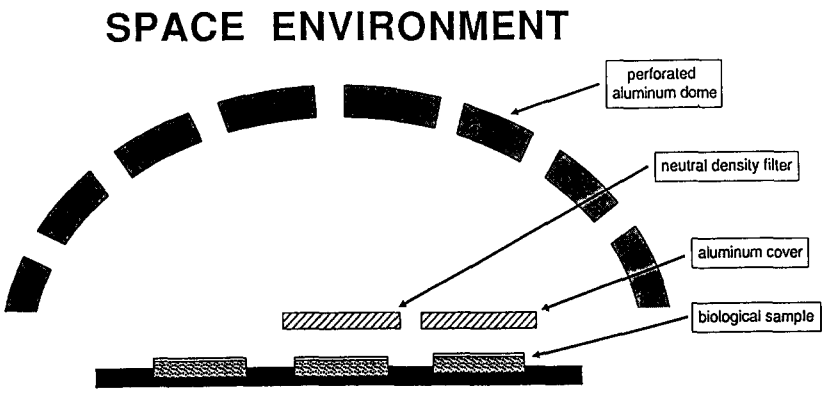

Fig. 1. Sketch of the exposure conditions of *B. subtilis* spores during the LDEF mission.

Table 1 Environmental Data of the LDEF Experiment during 2107 Days in Low Earth Orbit Compared with the Interplanetary Space

	LDEF experiment	Interplanetary space
Pressure (Pa)	app. 10^{-6}	10^{-14}
Residual gas (cm^{-3})	$2x10^6$ He $1x10^5$ N $3x10^7$ O	1 H
Radiation		
Solar UV (>110 nm)		
Fluence (J/m^2)	$1x10^9$	variable[1)]
Ionizing		
Sources	galactic solar belts (SAA)	galactic solar
Dose (Gy)	4.8	0.5
Heavy ions (cm^{-2}) [2)]	$2.5x10^4$ [3)] app. 60 [4)]	$3.6x10^4$
Temperature (K)	264-302	>4[1)]

(SAA)= South Atlantic Anomaly
1) depending on distance from the sun
2) atomic number >2, Linear Energy Tranfer (LET) >130 keV/µm
3) including secondaries
4) primaries

The samples were exposed to space vacuum for 2107 d. For irradiation with solar ultraviolet radiation, the samples were placed beneath a perforated aluminium dome. The perforation of this aluminium cover allowed access of space vacuum, solar electromagnetic radiation including UV and vacuum-UV, and cosmic radiation. Neutral density filters were used to reduced the UV transmission to 10 % and 30 % (Figure 1). The environmental data are given in Table 1. In parallel simultaneous ground control experiments were run in space simulation chambers. Most of the parameters of space, such as vacuum, temperature, and UV-radiation have been duplicated to the extent possible.

After retrieval, the effects of space vacuum, the combined action by solar UV-radiation and space vacuum as well as by the complete space environment on the viability of the spores were tested.

RESULTS

After nearly six years in space vacuum, there is still a considerable amount of viable spores (1 - 2 %), even if exposed in a monolayer without any protection against dehydration (Figure 2). Spores in a multilayer survive even less (0.3 %) than in a monolayer (Figure 3). The survival is significantly increased, if protecting substances are present, such as buffer salts or glucose (Figures 2 and 3). The protecting effect is especially pronounced for spores in a multilayer. In this case, e.g. multilayer with glucose present, nearly 70 % of the spores survive nearly 6 years exposure to space vacuum. This response to vacuum is confirmed in the ground controls, kept in simulated space conditions. Sugars and polyalcolhols are suggested to stabilize the structure of cellular macromolecules, especially during vacuum-induced dehydration.

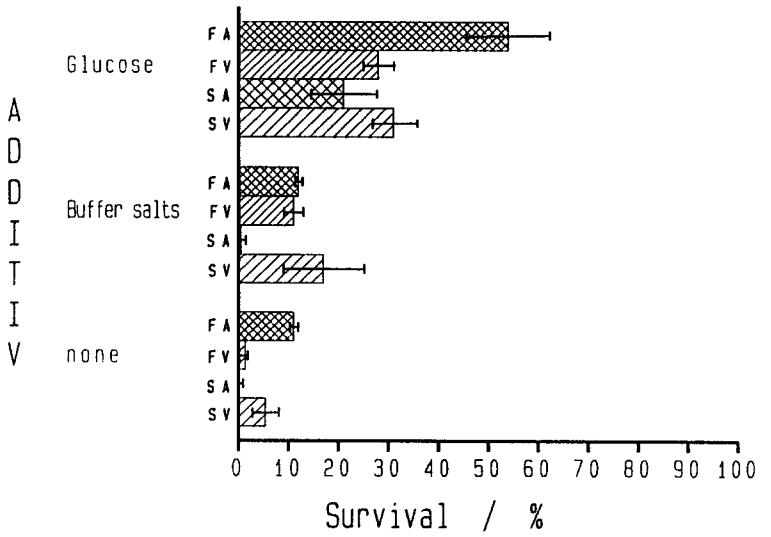

Fig. 2. Survival of *B. subtilis* spores in a monolayer after nearly 6 years in space during the LDEF mission (April 6, 1984 - January 20, 1990) and ground control data. The spores were shielded against solar optical radiation. Protective substances (5 % glucose or 1.4 % buffer salts) were added to some of the samples during preparation. F = flight samples; S = samples of the simulation experiment on ground; A = samples kept at atmospheric pressure; V = samples in vacuum.

From the in-flight controls, which were kept at atmospheric pressure during the LDEF mission up to 100 % of the spores initially sent into space survived.

To study the response to the full environment of space including solar UV-light, the samples were separated from free space by a perforated aluminium dome, only, which allowed access of space vacuum, solar UV-radiation and most of the components of cosmic radiation. Figure 4 shows the viable counts of the different 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 beneath. Therefore, the survivors probably originate from the innermost layers of the samples. A cover by neutral density filters of 10 % or 30 % transmission does not significantly change the response.

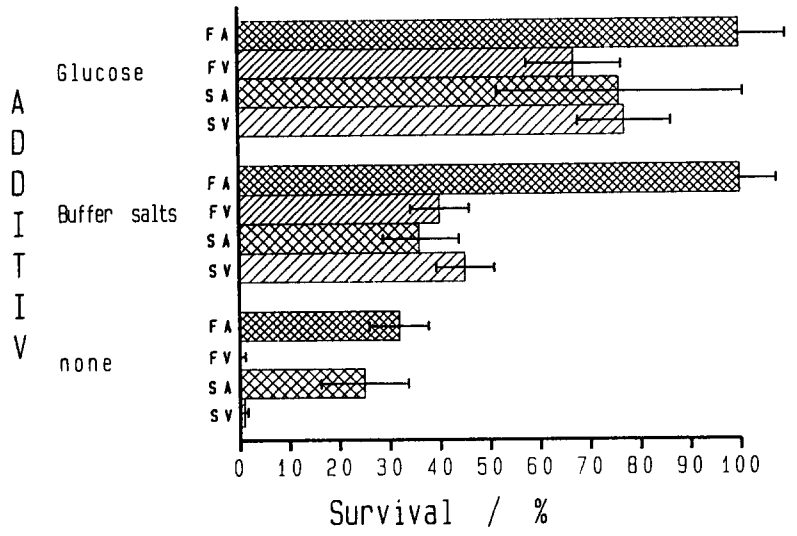

Fig. 3. Survival of *B. subtilis* spores in a multilayer after nearly 6 years in space during the LDEF mission and ground control data. The spores were shielded against solar optical radiation. Protective substances (5 % glucose or 1.4 % buffer salts) were added to some of the samples during preparation. F = flight samples; S = samples of the simulation experiment on ground; A = samples kept at atmospheric pressure; V = samples in vacuum.

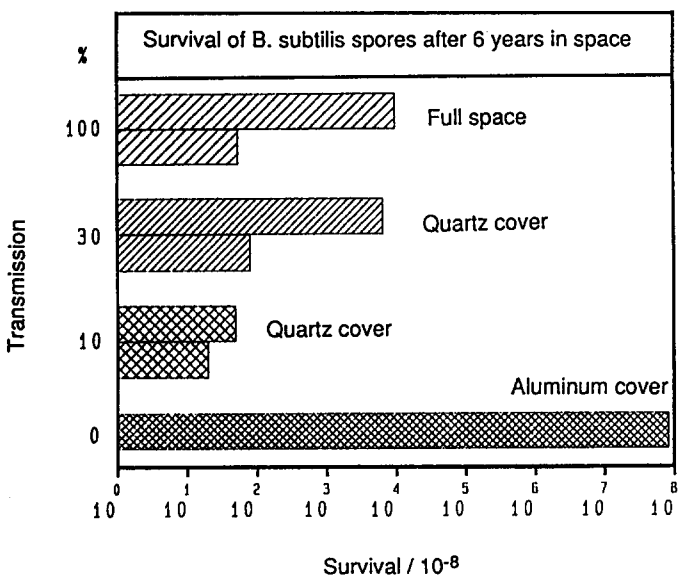

Fig. 4. Survival of *B. subtilis* spores exposed to the space environment for nearly 6 years during the LDEF mission. Spores were exposed in multilayers in the presence of 5 % glucose on quartz discs beneath a perforated dome without any further cover (100 % transmission), or beneath neutral density filters (30 % or 10 % transmission for solar UV-light of wavelengths > 170 nm), or beneatch an aluminum plate of 2 mm (0 % transmission). Non-covered samples received a solar UV-irradiation of 1 x 10^9 J/m^2.

DISCUSSION

With this 6 year *in-situ* study in space, we have contributed experimental data to the discussion on the possibility of interplanetary transfer of life. Since spores may withstand the dehydration process induced in space vacuum, they may survive for substantial time, provided they will be protected from solar UV. Such shielding could be reached by dust or soil particles, or by shadowing, or by thick layers as they occur in bacterial colonies, respectively. However, to travel from one planet of our solar system to another, e.g. from Mars to Earth by random motion, a mean time of 10^5 - 10^6 years has been estimated /11/. It might be precarious to extrapolate from this 6 year study to thousands or millions of years in space. In this large time span, radiation damage produced by cosmic radiation might accumulate, thereby setting the ultimate limit for survival of spores. However, considering the micron size of a bacterial spore and the low flux of cosmic ray heavy ions /12/, a spore will have a reasonable chance to escape a hit by a heavy ion even within very long time spans. Furthermore, we have shown that spores may survive a direct hit even of a cosmic ray iron particle /13/ being the most important component of cosmic radiation with respect to the amount of energy deposited.

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