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Review

Experimental methods for studying microbial survival in extraterrestrial environments

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ABSTRACT

Microorganisms can be used as model systems for studying biological responses to extraterrestrial conditions; however, the methods for studying their response are extremely challenging. Since the first high altitude microbiological experiment in 1935 a large number of facilities have been developed for short- and long-term microbial exposure experiments. Examples are the BIOPAN facility, used for short-term exposure, and the EXPOSE facility aboard the International Space Station, used for long-term exposure. Furthermore, simulation facilities have been developed to conduct microbiological experiments in the laboratory environment. A large number of microorganisms have been used for exposure experiments; these include pure cultures and microbial communities. Analyses of these experiments have involved both culture-dependent and independent methods. This review highlights and discusses the facilities available for microbiology experiments, both in space and in simulation environments. A description of the microorganisms and the techniques used to analyse survival is included. Finally we discuss the implications of microbiological studies for future missions and for space applications.

Contents

1. Introduction

The first recorded microbiological experiment into the effects of space and spaceflights on the survival of microorganisms was in 1935 aboard the high altitude balloon Explorer 2 (Stevens, 1936). The high altitude balloon permitted exposure of biological material to

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low temperature, decreased atmospheric pressure, and direct solar irradiation available at 150 km. Since then, the development of artificial Earth satellites and exposure facilities, such as BIOPAN and the EXPOSE facility on the International Space Station (ISS), has made it possible to carry out microbiological experiments in Low Earth Orbit (LEO), at over 300 km from Earth (Demets et al., 2005; Schulte et al., 1998). To date, representatives of all three domains of life have been exposed to the LEO environment (Zimmermann et al., 1994; Sancho et al., 2007; Olsson-Francis et al., 2009; Mancinelli et al., 1998; Horneck et al., 1994).

Studying microbial survival in space and in simulated extraterrestrial environments is important for space exploration. The results from the early microbial experiments were the foundation on which manned mission flights were developed (Antipov, 1967). They have also been vital for developing planetary protection procedures. Of particular concern are the Mars surface missions, as a number of terrestrial microorganisms can survive in a simulated Mars environment when protected from solar UV radiation (Nicholson and Schuerger, 2005; Cockell et al., 2005). Potential contamination could compromise future scientific studies for detection of past or present life (DeVincenzi et al., 1998; Crawford, 2005), or, more speculatively at the current time, have an irreversible impact on ecological systems on Mars, if they are present. Therefore it is imperative to determine the possible contaminants and investigate their ability to survive in space and in simulated planetary environments.

Furthermore, studying microbial survival is important for future space applications. Microorganisms have been suggested for use in a number of applications, such as life support systems, dust control and energy fuel cells (Hendrickx and Mergeay, 2007; Liu et al., 2008; Flinn, 2004). Extensive work has been conducted to develop a life support system that enables humans to live outside the Earth's atmosphere. An example is the MELiSSA loop, which is a system based on a number of microbial species and higher plants (Hendrickx and Mergeay, 2007). In addition, biotechnological processes that are successfully used on Earth could be employed in space. For example, bio-mining could be used to leach minerals from local materials such as basalt on Mars or the moon, which is rich in industrially useful elements such as iron and magnesium.

In this review, we will discuss the facilities that have allowed microbiologists to investigate microbial survival in extraterrestrial environments. This will include both facilities in space and simulation conditions. Emphasis will be placed on the process of selecting microorganisms for exposure studies and the techniques that are used to analyse microbial survival after exposure. In addition, we will discuss the importance of studying microbial survival in the context of planetary protection. Finally, we will discuss the relevance of microorganisms in space applications and their future in space exploration.

2. Microbiological experiments in space

Space, with its extreme temperatures, unfiltered solar radiation, solar wind, galactic radiation, space vacuum, and negligible gravity, is a hostile environment and is detrimental to biological systems, as seen in Table 1. Over the last sixty years, facilities have been developed that have allowed microbiologists to investigate the effect of the space environment on microorganisms.

Table 1
The parameters of the environment of interplanetary space and Low Earth Orbit (LEO).[a]

Space parameter	Earth[b]	Low Earth Orbit	Interplanetary space
Pressure (Pa)	10^3	10^{-4}–10^{-6}	10^{-14}
Solar spectra (nm)	>280	continuum	continuum
Cosmic ionizing radiation (Gy/yr)	$<10^{-4}$	400–10,000	≤0.1
Temperature (K)	Wide range[c]	Wide range[d]	>4
Microgravity (g)	1	10^{-3}–10^{-6}	$<10^{-6}$

[a] Table adapted from Horneck and Rettberg (2007).
[b] Values at sea level.
[c] Depending on location.
[d] Depending on orientation and distance from the sun.

The first microbiological experiments were conducted in 1935 by the stratospheric Explorer 2, which attained an altitude of 25 km 286 m (Stevens, 1936). Spores of several fungi were exposed to the low temperature, decreased atmospheric pressure, and direct solar irradiation. As space technology developed, microbiological specimens were used in Earth orbit flights to identify any detrimental biological effects that galactic radiation, weightlessness and other space flights factors could have on biological systems. A vast array of biological systems were evaluated, including viruses, bacteria, yeasts, fungi, plants, animals, and tissue cultures (Taylor, 1974). The information gathered on these early missions became the basis on which manned mission flights were developed (Antipov, 1967).

As the space race intensified, concerns were raised about the survival of biological contamination in space and the importance of spacecraft sterilisation. The first successful recovery of directly exposed unprotected terrestrial microorganisms in space was carried out in 1968 (Lorenz et al., 1969). In this experiment spores of *Bacillus subtilis*, type III poliovirus, and *Escherichia coli* bacteriophage T-1 were exposed for 500 s at an altitude of 155 km. This was followed by a series of experiments conducted in LEO that involved *Penicillium roqueforti*, T-1 coliphage, *B. subtilis* spores and the Tobacco Mosaic Virus. Each of the samples was exposed for 17 h, aboard the Gemini 9A and 12 crafts, and four months aboard the Agenda 8 rocket (Lorenz et al., 1969). Viable samples were recovered from the Gemini mission; however, due to problems with the launch of the Agenda 8 only the protected samples survived. Since then, facilities have been developed that allow both short- and long-term exposure of microbial experiments to LEO. A list of some of the key experiments conducted by NASA (National Aeronautics and Space Administration) and ESA (European Space Agency) is shown in Table 2.

3. Short-term exposure facility: BIOPAN

With an increase in demand for exposure facilities in space for microbiological experiments, ESA developed the short-term exposure facility BIOPAN, as seen in Fig. 1A. The facility was developed to provide the opportunity for various recoverable experiments to be exposed to LEO for periods of up to two weeks (Harboesorensen et al., 1994). The structure was based on the Russian exposure facility KNA and was designed to fly with a Russian spacecraft of the Foton class. It was launched with a Soyuz rocket into space and once in LEO, the lid would open 180° exposing the experiments to the space environment (Demets et al., 2005).

BIOPAN contains a temperature sensor AD590 and a thermoluminescence detector *Litho-Dose* to monitor radiation. There are two plates, a top and a bottom layer, where the samples are located, as seen in Fig. 1B. Samples on the lower layer are protected from UV radiation and on the top layer the samples are covered with optical long-pass filters which allow the following characteristics: (i) MgF_2, which is transparent to extraterrestrial solar UV radiation of $\lambda > 110$ nm; (ii) SQ0 synthetic quartz transmitting solar UV of $\lambda > 200$ nm, thereby simulating the UV radiation climate on the surface of Mars; (iii) long-pass filter for $\lambda > 290$ nm to simulate the terrestrial UV radiation climate (as a control) and (iv) for $\lambda > 400$ nm thereby cutting off all solar UV radiation (de la Torre et al., unpublished).

For each mission, BIOPAN was located on to the descent module of the satellite Foton, and was protected from re-entry by an ablative heat shield. After completion of the mission, the Foton satellite was de-orbited and landed within Russian territory or in Kazakhstan. BIOPAN was transported to the ESA ESTEC clean rooms where the experiments were removed and returned to the investigators. Equivalent samples, which had been stored at ESTEC for the duration of the mission, were returned to the investigators as controls (Horneck and Rettberg, 2007).

Table 2
Examples of microbiological exposure experiments conducted in LEO.

Payload	Mission	Exposure time	Experiment	Results	Reference
Short-term experiments					
Biostack I	Apollo 16	266 h	Biostack: Response of *B. subtilis* strain spores to HZE particles.	The viability of the control spores (not exposed to HZE) were the same as the ground-control samples. Spores were highly resistant to HZE particles.	Bucker et al. (1974), Taylor et al. (1974)
MEED			Response of *Aeromonas proteolytica*, *B. subtilis*, *B. thuringiensis* var. *thuringiensis*, the T-7 bacteriophage of *E. coli*, *Nematospiroides dubius*, *Trichophyton terrestre*, *Chaetomium globosum*, *Rhodotorula rubra* and *Saccharomyces cerevisiae* to the space environment.	Results indicated that microorganisms may survive exposure to space vacuum if shielded against solar irradiation.	
Biostack II	Apollo 17	304 h	Biostack: Response of *B. subtilis* spores to HZE[a] particles.	Dried spores of *B. subtilis* survived exposure, when protected against solar radiation.	Facius et al. (1978)
Biostack III	Apollo-Soyuz	218 h	Biostack: Response of *B. subtilis* spores to HZE[a] particles.	Dried spores of *B. subtilis* survived exposure, when protected against solar radiation.	Facius et al. (1979)
Advanced Biostack/ ES029	Spacelab I	9 d[b]	Biostack: Response of *B. subtilis* spores to HZE[a] particles. ES029: Response of *B. subtilis* spores to free space.	Dried spores of *B. subtilis* survived exposure when protected against solar radiation.	Bucker et al. (1984), Horneck et al. (1984)
UVRAD	Spacelab II	10 d	Biological response s of *B. subtilis* spores to defined extraterrestrial solar UV, to simulate different ozone column thicknesses, and space radiation.	A strong increase in biological effect of solar UV irradiance with decreasing (simulated) ozone concentrations.	Horneck et al. (1996) de Vera et al. (2004)
BIOPAN	Foton	–	Discussed in detail in the text.		
Long-term experiments					
Exobiology and radiation assembly	EURECA	302 d	Response of *Deinococcus radiodurans*, *B. subtilis* spores, *Aspergillus* sp. conidia, and cellular constituents to solar UV and/or vacuum.	Purple membranes, amino acids, and urea were not affected by space (when protected from solar radiation). However, plasmid DNA had a large number of breakages. Multi-layer microorganisms and spores survived the conditions of space, when protected by solar radiation.	Dose et al. (1995), Horneck et al. (1995) Mesland (1995)
Free Flyer Biostack	LDEF	2107 d	Response of *B. subtilis* spores to solar UV and/or vacuum.	Dried spores survived exposure, when protected from solar radiation.	Horneck et al. (1994)
Exobiologie	MIR	68 d	Response of *B. subtilis* spores to solar UV and/or vacuum whilst protected by inorganic such as artificial/real meteorites.	A thin layer of real or artificial meteorite did not protect spores against UV radiation to the expected level. However, layers of UV radiation inactivated spores served as a UV shield.	Rettberg et al. (2002)
EXPOSE		–	More details in Table 5		

[a] Component of cosmic radiation consisting of energetic heavy nuclei.
[b] Exposure to solar radiation varied between 17.5 min and 5 h.

The development of the BIOPAN facility has enabled scientists to carry out experimental procedures in space to determine the effect of the space environment on both biological and material/component samples. BIOPAN was designed to be used in multiple missions and there have been six missions since the facility was developed in 1992. These include: BIOPAN-1 (1994), BIOPAN-2 (1997), BIOPAN-3 (1999), BIOPAN-4 which was lost due to launch failure (2002), BIOPAN-5 (2005), and BIOPAN-6 (2007).

The BIOPAN facility has been employed in a number of microbiological experiments, such as Survival, Yeast, Marstox, Photo, Lichens, Permafrost, and Lithopanspermia. The aim of the Survival experiment was to provide an insight into the limiting factors of life in space. For this, spores of *B. subtilis* and two halophiles, *Synechococcus* and *Haloarcula-G*, were embedded in clay, meteorite powder, simulated Martian soil, or salt crystals and were exposed to the space environment (with/without UV). The unprotected spores were killed by solar light, but spores packed in clay survived. The experiment with the unprotected halophiles was lost; however, the protected samples survived (Mancinelli et al., 1998; Horneck et al., 2001a,b). Spores of *B. subtilis* were used in the Marstox experiment. The aim of this study was to investigate survivability under conditions as they exist on the surface of Mars. The results demonstrated that spores mixed directly with powdered clay, rock, or meteorites survived exposure (Rettberg et al., 2004).

In the Lichen experiment *Rhizocarpon geographicum* and *Xanthoria elegans* were exposed to the space environment. The lichens showed the same photosynthetic activity as the controls and there were no detectable ultrastructural changes in most of the algal and fungal cells of the lichen thalli (De la Torre et al., 2004). *Saccharomyces cerevisiae* was used in the Yeast experiment to measure the biological effects of radiation, with only a few tens of milligrams shielding. The experiment resulted in a loss of survivability (Kennedy and Volz, 1983).

The physiology role of the D1 protein, which is important for coping with radiation in photosynthetic organisms, was determined in the Photo experiment. *Chlamydomonas reinhardtii* (wildtype IL —a mutant without introns in the *psbA* gene encoding for the D1 protein and pools of several D1 mutants) was exposed to space radiation in combination with solar light, to test the effects on the turnover of D1 protein and photosystem II activity. It was observed that the effect of space stress on survival varied depending on the light conditions to which they were exposed. Photosythetically active cells were able to survive exposure to solar radiation (Bertalan et al., 2007).

The aim of the Lithopanspermia experiment was to investigate the ability of microorganisms to survive space travel. The epilithic lichen species *R. geographicum*, *X. elegans* and *Aspicilia fruticulosa*, on their natural rock substrate as well as their reproduction structures, microbial communities from Atacama halites, a cyanobacterial endolithic community from Beer, UK and akinetes from *Anabaena cylindrica* were exposed to space conditions. All of the lichens were resistant to the condition of LEO. One tenth of the microbial halite community survived; a cyanobacterium, from the endolithic community from Beer, was isolated after exposure to space and the akinetes survived exposure to space when protected from solar radiation (Olsson-Francis et al., 2009; de la Torre et al., unpublished). The Permafrost samples were destroyed (Novotoskaya-Vlassova et al., 2002).

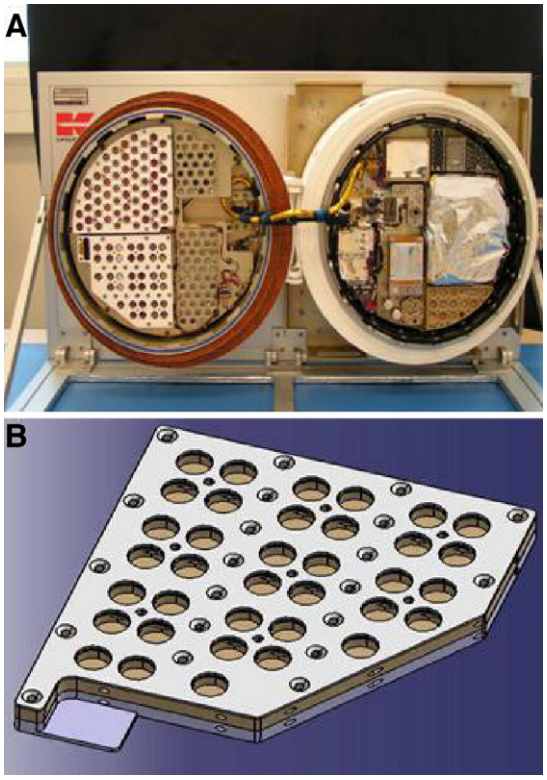

Fig. 1. (A) The inside lid of the BIOPAN module, which was opened in orbit to expose the biological samples to LEO conditions. The BIOPAN contained a temperature sensor AD590 and thermoluminescence detector *Litho-Dose*, to monitor the conditions. Two plates; a top (level-1) and a bottom (level-2) plate where the samples were located inside the BIOPAN. The facility measured 38 cm in diameter and 23 cm in height. (B) The cells of the top plate were covered by optical long-pass filters, as described in the text.

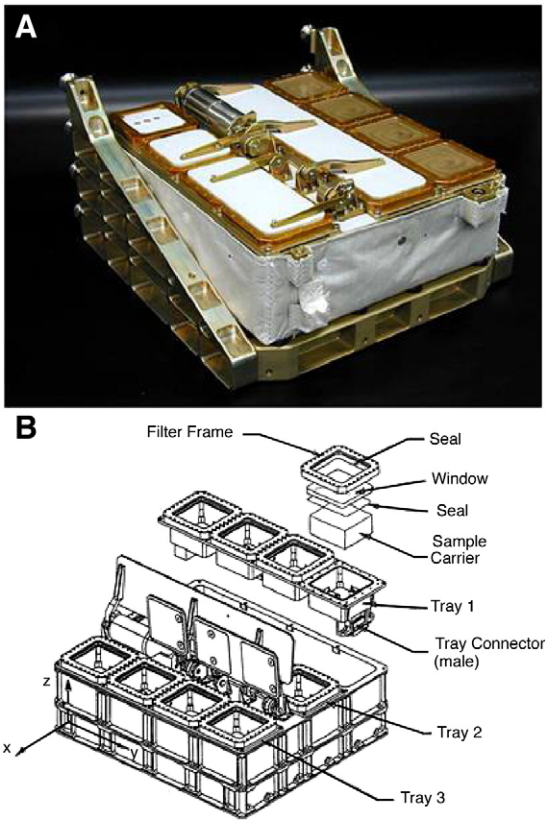

Fig. 2. The EXPOSE facility. (A) A photograph of EXPOSE-E. (B) A schematic drawing of the EXPOSE facility (images courtesy of ESA). The experiments are accommodated in three sample trays, each with four sample compartments of approximately 77 × 77 mm inner width and 36 mm inner depth.

4. Long-term exposure facility: EXPOSE

EXPOSE is the latest facility developed by ESA; it is designed for medium to long-term exposure experiments on the ISS, as seen in Fig. 2. Experiments are located in hundreds of tiny cells that are pressurized or vented, protected with windows and filters of various geometrics and materials (Schulte et al., 2007). The EXPOSE facility includes three experimental trays, each one contains four sample carriers, with either vented or sealed units. The sealed units can be pressurized and the gas composition can be defined. Each of the sample carriers has one or two layers, including a dark control. Biological samples are attached within the tray by a variety of methods, including attachment to quartz discs. The samples are kept open to the space environment or are covered with optical long-pass filters which allow for control of wavelength and the amount of light that the samples receive. There are a number of sensors attached to the facility that measure temperature, pressure, UV, and radiation dosimeters (Baglioni and von Heise-Rotenburg, 2004).

The EXPOSE assembly includes the facility and its supporting structure, interfacing with the EUTEF-CEPA (Columbus External Platform Adapter) for Expose-E or with the external platform of the Russian segment of the ISS for Expose-R. The biological experiments onboard the Expose-R facility were installed in March 2009 and are planned to be exposed for one and a half years. After this, the trays will be removed and stored in sealed containers within the ISS and returned to Earth aboard the Soyuz re-entry module. The facility will stay outside the ISS and more experiments will be carried out in the future. In contrast, Expose-E was only used for one mission for two years, as it has no removable experimental trays. It is therefore part of the EUTEF was dismounted with the samples (Horneck and Rettberg, 2007).

There are more than 1000 biological, chemical, and dosimetric samples from eight international scientific groups which are accommodated on the Expose facility. A list of the microbiological experiments is listed in Table 3.

5. Ground-based simulation facilities

Simulation facilities provide an opportunity for microbiologists to study the effects of extraterrestrial conditions and processes on microorganisms in a laboratory environment, without the major cost of a space mission. In sophisticated simulation facilities it is possible to carry out experiments in controlled environments where the parameters can be monitored and examined separately. These facilities are important in particular for preparation for missions and for understanding the requirements for life in space. Although simulation facilities are available for bodies such as Mars, Titan and the moon, in the following sections we will discuss Mars and space simulation facilities in detail.

5.1. Simulated Mars conditions

With future missions to Mars planned by both NASA and ESA, Mars simulation facilities have been used by microbiologists to study microbial survival on Mars and to identify environmental parameters that are critical for the survival of terrestrial life transferred to Mars. The microbiological studies are essential for selecting landing sites for life detection missions and for predicting biological sterilisation rates for instruments and landers (Hansen, 2007).

Mars simulation facilities have developed over the last sixty years from basic anaerobic jars to sophisticated chambers. Concomitantly, the information available about the atmosphere on Mars has developed from indirect measurements and modelling to direct information from the Mariner missions (Hansen, 2007). The original experiments were conducted in anaerobic jars that did not include solar radiation, simulated

Table 3
Microbiological experiments aboard the Expose facility, on the ISS.[a]

Experiment	Aim of research
ROSE (Responses of Organisms to the Space Environment)	
ROSE1/ENDO	To assess the survival of *Chroococcidiopsis* and algae from the Negev desert and the Arctic in impact shocked rocks and as isolated organisms. The experiment studies the effects of space conditions on cell viability and structure.
ROSE2/OSMO	To understand the response of *Synechococcus* and *Haloarcula-G*, to the space environment. This experiment assesses the role of gypsum–halite and halite salts as habitats, and high intracellular potassium concentration, for protecting halophiles from desiccation.
ROSE3/SPORES	To assess the protection of bacterial (*B. subtilis*), fungal (*Penicillium expansum*, *Thermomyces lanuginosus*, *Xeromyces bisporus*) and lycopodial (*Selaginella* sp.) spores by meteorite material against space conditions.
ROSE4/PHOTO	To measure the vacuum solar radiation-induced DNA damage within spores, by assessing the yield and kinetics of formation of photoproducts resulting. The samples are exposed naked, or within artificial meteorite materials, clays, and halites.
ROSE5/SUBTIL	To determine the mutational spectra of *B. subtilis* spores and plasmid DNA in the space environment. Also, the molecular differentiation between vacuum-induced and UV-induced mutations.
ROSE8/PUR	To determine the responses of phage T-7, phage DNA, and polycrystalline uracil to the space environment. The prime goal is to determine whether Phage T-7, T-7 DNA and poly-uracil may be used as biological dosimeters for measuring biologically effective UV dose in the space environment.
PROTECT	To investigate the extremely resistant spacecraft survivors to the environment of space. This will involve studying the degree of resistance, the types of cellular damage sustained; and the mechanism (s).
ADAPT	The hypothesis to be tested experimentally is whether longer-lasting selective pressure by UV radiation of different quality results in a higher UV resistance as well as in a higher resistance against the simultaneous action of further extreme environmental factors that exist in space or on other planets like vacuum or cosmic radiation.
LIFE	To determine the effect of the space environment on extremophilic lichens, fungi and symbionts under simulated space conditions. Antarctic communities, Antarctic strains of *C. antarcticus* and *C. minteri*, and the lichens *X. elegans and R. geographicum* are used to evaluate their survival in space conditions.

[a] The table was adapted from Baglioni and von Heise-Rotenburg (2004).

Martian gas composition, or altered pressure (Fulton, 1958). It was not until 1965 that the first microbiological experiment was carried out in simulated Martian temperature, pressure, atmosphere, and solar radiation, as seen in Table 4 (Zhukova and Kondratyev, 1965). In this study the authors used a xenon light source, which is considered to most closely simulate the present Martian UV environment in terms of fluence rates in the UV-C wavelength and it includes the full spectrum of visible and infrared light (700–2500 nm): On Mars the solar radiation that reaches the surface is >200 nm (Hansen, 2007).

Modern Mars simulation facilities are normally constructed from stainless steel and allow for the environmental parameters to be monitored and controlled independently, as seen in Fig. 3. Gas is constantly circulated through the chamber to eliminate any external air that has leaked into the chamber. Mars gas mix can be purchased commercially and consists of CO_2 (95.3%), N_2 (2.7%), Ar (1.7%), O_2 (0.2%), and H_2O (0.03%). The UV radiation is normally produced by a xenon-arc light which is mounted on the exterior of the chamber and the light is passed into the chamber by UV transmitting optical bundles (Schuerger et al., 2003).

Mars simulated experiments have been conducted with both pure cultures and communities of microorganisms, as seen in Tables 5 and 6. UV radiation has been shown to be the main factor in cell inactivation (Schuerger et al., 2003; Cockell et al., 2005; Zhukova and Kondratyev, 1965). To date, none of the prokaryotes that have been examined were able to withstand long periods of UV radiation. For example, endospores of *B. subtilis* and desiccated cells of *Chroococcidiopsis* were killed after 15 and 30 min, respectively (Schuerger et al., 2003; Cockell et al., 2005). Whereas, in experiments where the biological samples were exposed to simulated Mars conditions, but protected from the UV radiation, there was no major effect on their viability (Schuerger et al., 2003; Hawrylewicz et al., 1962; Cockell et al., 2005).

5.2. Simulated space conditions

Although exposure facilities such as EXPOSE and BIOPAN have enabled microbiological experiments to be carried out in space, the opportunities are restricted and their capacity limited. Therefore, ground-based simulation facilities are routinely used prior to space exposure studies and are essential for investigating the individual environmental parameters associated with space. For example, the simulation facilities at DLR in Germany were used prior to the launch of the EXPOSE missions. Ground-based experiments were conducted to ensure that the biological experiments were able to survive short-term exposure (Horneck et al., 2000; Onofri et al., 2008). For example, the parameters that were examined, as part of the LIFE experiment, are listed in Table 7.

Space simulation facilities are predominately constructed from stainless steel, like Mars simulation facilities, and allow for the environmental parameters to be monitored and controlled independently. The pressure is kept constant by a pump system that reaches a low final pressure, for example 10^{-5} Pa. A deuterium light source can be used for UV radiation (>160 nm), so the samples are exposed to UV-A, UV-B and UV-C (Beegle et al., 2007). The results from the space simulation experiments are generally in agreement with the experiments conducted in LEO. UV radiation was found to have the most detrimental effect on microorganisms (Beegle et al., 2007; Koike et al., 1992).

6. Experimental techniques to test the concept of lithopanspermia

Lithopanspermia, the transfer of organisms in rocks from one planet to another either through interplanetary or interstellar space, remains speculative (Thomson, 1871). Although there is no evidence that lithopanspermia has occurred in our own Solar System, the various stages have become amenable to experimental testing (Cockell, 2008).

6.1. Planetary ejection

For Lithopanspermia to occur, microorganisms must survive ejection from a planetary surface which involves extreme forces of acceleration and shock with associated temperature excursions. Hypothetical values of shock pressures experienced by ejected rocks are obtained with Martian meteorites, which suggest the shock pressures of approximately 5 to 55 GPa, acceleration of 3×10^6 m/s^2 and jerk of 6×10^9 m/s^2 and post-shock temperature increases of about 1 K to 1000 K (Cockell, 2008).

To determine the effect of acceleration during ejection on microorganisms, rifle and ultracentrifuge methods are used. Mastrapa et al. (2001) successfully used both methods to examine the effect of acceleration on *B. subtilis* and *Deinococcus radiodurans*. In the rifle experiment, freshly prepared samples were placed in the rear cavities of commercial 0.177 calibre gun pellets and fired from a compressed-air pellet rifle into plasticine modelling clay. The velocity of each pellet was measured and corresponded to an acceleration of between 1.5×10^6 m/s^2 and 4.5×10^6 m/s^2 and a jerk of 1.5×10^{10} m/s^2 and 1.5×10^{11} m/s^2. For longer term experiments an ultracentrifuge was used, as 100,000 rpm is the equivalent acceleration of 4.27×10^6 m/s^2 (Mastrapa et al., 2001).

The effect of shock pressure can be determined using a light gas gun or a plate-flyer apparatus. The two-stage light gas gun permits the study of millimetre to centimetre size projectiles accelerated to speeds above 5 km s^{-1} (Burchell et al., 1999). The projectiles are

Table 4
Examples of incubation conditions used to investigate biological response to simulated conditions.[a]

	Incubation method	Temp. (°C)	Pressure (mbar)	Atmospheric composition (%)				Solar radiation		Reference
				CO_2	N_2	Ar	O_2	(nm)	Lamp	
Present Mars	–	−123/25	7.6	95.3	2.7	1.6	0.13	>200	–	
1958	Anaerobic jar	−25/25	87	100	–	–	–	–	–	Fulton (1958)
1958	Anaerobic jar	−25/25	72	100	–	–	–	–	–	Kooistra et al. (1958)
1959	Anaerobic jar	−25/25	87	100	–	–	–	–	–	Davis and Fulton (1959)
1962	Anoxic tubes	−25/25	~0/87	100	–	–	–	–	–	Hawrylewicz et al. (1962)
1963	Anaerobic jar	−60/20	100	5	95	–	–	254	Mercury	Packer et al. (1963)
1963	Anoxic tubes	−75/25	1013	–	100	–	–	–	–	Young (1963)
1964	Anoxic tubes	−60/26	113	2.2	93.8	4	–	–	–	Hagen et al. (1964)
1965	Anoxic tubes	−65/25	113	2.2	93.8	4	–	–	–	Hawrylewicz et al. (1965)
1965	Mars facility	−60/25	100	0.25	95.5	0.25	–	200–2500	Xenon	Zhukova and Kondratyev (1965)
1967	Anoxic tubes	−65/28	113	2.2	93.8	4	±	–	–	Hagen et al. (1967)
1967	Tubes	−60/25	1013	0.03	78.1	0.93	20.9	254	Mercury	Imshenetsky et al. (1973)
1968	Mars facility	−64/28	100	–	100	–	–	240–280	Mercury	Belikova et al. (1968)
1968	Anoxic tubes	−65/30	10–40	37–100	13.27	21.30	–	–	–	Hawrylewicz, et al. (1968)
1969	Mars facility	18–20	7.1–60	–	99	–	<1	–	–	Lozina-Lozinsky and Bychenkova (1969)
1970	Mars facility	−65/30	20	67	30	3	–	200–300	Mercury	Hagen et al. (1970)
1971	Mars facility	−60/25	8	70	25	5	–	200–2500	Xenon	Green et al. (1971)
1971	Anoxic tubes	−25/25	13	99	–	–	–	–	–	Lozina-Lozinsky et al. (1971)
1973	Anoxic tubes	−60/28	7	80	–	20	–	–	–	Imshenetsky et al. (1973)
1974	–	−60/25	7	80	–	20	–	–	–	Forster and Winans (1974)
1978	Anoxic tubes	−65/24	7	99.9	–	–	0.01	–	–	Foster et al. (1978)
1979	Tubes	−10/25	0.001	–	100	–	±	+	Mercury/xenon	Oro and Holzer (1979)
1984	Mars facility	−80/25	7–9	95	2–3	1–2	<0.4	254	Mercury	Imshenetskii et al. (1984)
1992	Anoxic tubes	−70	13	95.52	2.73	1.62	0.13	–	–	Moll and Vestal (1992)
1995	Mars facility	−160/50	0.001	95.46	2.7	1.6	0.17	115–400	Hydrogen	Koike et al. (1995)
1996	Mars facility	60	10	95.46	2.7	1.6	0.17	115–400	Hydrogen	Koike et al. (1996)
1997	Mars facility	25	100	95.59	–	4.21	0.11	210–710	Xenon	Stoker and Bullock (1997)
1998	Tubes	−23/10	1013	–	–	–	–	–	–	McDonald et al. (1998)
2000	–	25	1013	0.03	78.1	0.93	20.9	200–400	Deuterium	Mancinelli and Klovstad (2000)
2003	Mars facility	−10	8.5	95.3	2.7	1.7	0.2	200–2500	Xenon	Schuerger et al. (2003)
2003	Mars facility	−60	6	98	–	–	–	–	–	Stan-Lotter et al. (2003)
2005	Mars facility	−10	8.5	100	–	–	–	200–2500	Xenon	Cockell et al. (2005)
2005	Mars facility	−95/12	9–13	77.5	8.7	–	1.3	200–2500	Xenon/Mercury	Hansen et al. (2005)
2005	Mars facility	20	12.5	100	–	–	–	120–180	Hydrogen	Nicholson and Schuerger (2005)
2006	Mars facility	−60 to 15	7	95.55	2.7	1.6	0.5	200–400	Halide	Pogoda de la Vega et al. (2006)
2006	Mars facility	25	7	95.96	–	–	–	200–400	Deuterium	Osman et al. (2008)
2007	Mars facility	−70/20	10^{-3}	95.3	–	–	–	–	–	Morozova et al. (2007)
2008	Mars facility	−41 to 22	7.6–9.7	91.4	4.8	2.8	0.24	200–1000	Mercury–Xenon	Hansen et al. (2009)
2008	Mars facility	<30	6	99.9				200–400	Polychromatic	Onofri et al. (2008)
2009	Mars facility	− 28	8	99				>200	Xenon	Olsson-Francis et al. (2009), Olsson-Francis (unpublished)

[a] The table is adapted from Hansen (2007).

soaked with microbial cultures and placed in the sabots of the gun before firing the projectile into a target such as glucose yeast extract plates or into ice. During the experiment a vacuum is maintained and the peak shock pressure varied between 1 and 70 GPa (Burchell et al., 2001). The plate-flyer apparatus can be used with two different plane wave impact techniques, the high explosive techniques, and the air gun wave impact technique (Horneck et al., 2001a,b). A plane metal plate is accelerated at velocities of 0.5–2.6 km s^{-1}, which impacts a metal container that holds a sample composed of a layer of microorganisms that are sandwiched between two thin discs of rock (Horneck et al., 2008).

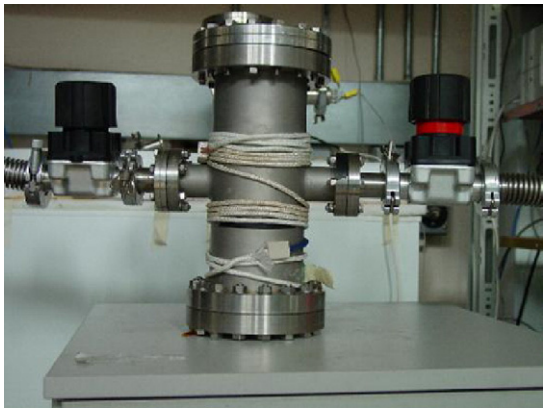

Fig. 3. The Mars simulated chamber at the Open University. The chamber measures 15 cm in diameter and 30 cm in height. The samples are placed inside the chamber, before tightly securing the lid and adding the Mars gas slowly. The pressure is adjusted until 8 mbar ± 2 by means of an Edward rotary pump.

6.2. Atmospheric entry

An important aspect of the lithopanspermia hypothesis to test is that microbes situated on or within rocks could survive hypervelocity entry from space through Earth's atmosphere (Cockell, 2008). As with planetary ejection, this has proven to experimentally tractable, with sounding rockets and orbital vehicles being used for microbiological experiments.

B. subtilis spores inoculated onto granite domes were subjected to hypervelocity atmospheric transit (twice) by launch to a ~120 km altitude on an Orion two-stage rocket. The spores were shown to have survived on the sides of the rock, but they did not survive on the forward-facing surface that was subjected to a maximum temperature of 145 °C (Fajardo-Cavazos et al., 2005a).

Furthermore, as part of the ESA STONE experiment, an experimental campaign designed to investigate the survival of artificial meteorites during atmospheric entry, microbiological experiments were performed on embedded organisms (Brack et al., 2002). For this

Table 5
Examples of pure cultures investigated in simulation environments and Low Earth Orbit.

	LEO[a]	Planetary ejection	Atmospheric reentry	MSE[a]	Reference
Bacillus cereus				x	Hagen et al. (1967), Hawrylewicz et al. (1962)
Bacillus megaterium				x	Imshenetskii et al. (1979)
Bacillus mycoides				x	Imshenetskii et al. (1984)
Bacillus pumilus				x	Imshenetskii et al. (1984)
Bacillus subtilis	x	x	x	x	Hotchin et al. (1965), Horneck et al. (1994), Fajardo-Cavazos et al. (2005a), Brandstatter et al. (2008)
Bacillus thuringiensis	x				Taylor et al. (1975)
Clostridium botulinum				x	Hawrylewicz et al. (1962)
Clostridium butyricum				x	Parefenov and Lukin (1973), Koike et al. (1996)
Clostridium celatum				x	Koike et al. (1996)
Clostridium mangenotii				x	Koike et al. (1996)
Clostridium roseum				x	Koike et al. (1996)
Lactobacillus plantarum				x	Hawrylewicz et al. (1968)
Staphylococcus aureus				x	Parefenov and Lukin (1973), Hawrylewicz et al. (1968)
Streptococcus mutans				x	Koike et al., (1995)
Kocuria rosea				x	Imshenetskii et al. (1979)
Luteococcus japonicus				x	Zhukova and Kondratyev (1965)
Micrococcus luteus				x	Zhukova and Kondratyev (1965)
Streptomyces albus				x	Hawrylewicz et al. (1968)
Streptomyces coelicolor				x	Koike et al. (1995)
Actinomyces erythreus				x	Dublin and Volz (1973)
Rhodospirillum rubrum				x	Robert (1963)
Azotobacter chroococcum				x	Moll and Vestal (1992)
Azotobacter vinelandii				x	Robert (1963)
Enterobacter aerogenes				x	Young (1963)
Escherichia coli	x	x		x	Grigoryev et al. (1972), Koike et al. (1996), Willis et al. (2006)
Klebsiella pneumoniae				x	Hawrylewicz et al. (1962)
Photobacterium sp.				x	Zhukova and Kondratyev (1965)
Pseudomonas aeruginosa				x	Hawrylewicz et al. (1968)
Pseudomonas fluorescens				x	Hawrylewicz et al. (1968)
Serratia marcescens				x	Hagen et al. (1967)
Serratia plymuthica		x			Roten et al. (1998)
Aeromonas proteolytica	x				Taylor et al. (1975)
Hydrogenomonas eutropha	x				Grigoryev et al. (1972)
Deinococcus radiodurans	x	x		x	Dose et al. (1995), Mastrapa et al. (2001), de La Vega and Rettberg (2006)
Rhodococcus erythropolis		x			Burchell et al. (2001)
Chroococcidiopsis sp.	x	x	x	x	Cockell et al. (2005), Horneck et al. (2008), Brandstatter et al. (2008)
Synechococcus (halite)	x				Mancinelli et al. (1998)
Haloarcula-G	x				Mancinelli et al. (1998)
Anabaena cylindrica (akinetes)				x	Olsson-Francis et al. (2009)
Archaea					
Halobacterium sp.				x	Stan-Lotter et al. (2003)
Halobacterium salinarum				x	Koike et al. (1995)
Halococcus dombrowskii				x	Stan-Lotter et al. (2003)
Methanosarcina sp. SA-21/16				x	Morozova et al. (2007)
Methanosarcina barkeri				x	Morozova et al. (2007)
Methanobacterium MC-20				x	Morozova et al. (2007)
Fungi					
Chaetomium globosum	x			x	Taylor et al. (1975)
Penicillium roqueforti					Hotchin et al. (1965)
Sordaria fimicola	x				Zimmermann et al. (1994)
Trichophyton terrestre	x				Taylor et al. (1975)
Aspergillus niger				x	Zhukova and Kondratyev (1965)
Aspergillus oryzae	x			x	Zhukova and Kondratyev (1965), Dose et al. (1995)
Mucor plumbeus				x	Zhukova and Kondratyev (1965)
Rhodotorula mucilaginosa				x	Zhukova and Kondratyev (1965)
Ulocladium atrum			x		Brandstatter et al. (2008)
Bacteriophage/virus					
T-7				x	Taylor et al. (1975)
Phage T-1	x				Hotchin (1968)
Tobacco mosaic virus					Hotchin (1968), Koike et al. (1995)
Canine hepatitis	x				Hotchin (1968)
Influenza PR8	x				Hotchin (1968)
Vaccinia virus	x				Hotchin (1968)

(continued on next page)

Table 5 (*continued*)

	LEO[a]	Planetary ejection	Atmospheric reentry	MSE[a]	Reference
Yeast					
Rhodotorula rubra	x				Taylor et al. (1975)
Saccharomyces cerevisiae	x			x	Taylor et al. (1975), Koike et al. (1994)
Saccharomyces mitis	x				Grigoryev et al. (1972)
Zygosaccharomyces bailii	x				Grigoryev et al. (1972)

[a] Mars simulation conditions.

experiment, different types of rock, loaded with microorganisms, were mounted in the heat shield of the Foton re-entry capsule. On re-entry, the rock samples were subjected to temperatures and pressure loads comparable to those experienced in meteorites.

The STONE experiments were accommodated on the heat shield of the re-entry capsule of the Russian retrievable carriers of the Foton class. They were placed around the descent modules hottest point, inserted into the heat shield with specially designed holders that were made of the same ablative material used for the heat shield (Horneck and Rettberg, 2007). The rock samples thermal conductivity was higher than that of the capsule shielding; therefore, a protective layer was placed underneath the exposed samples.

The first STONE experiment consisted of three rock types—basalt, dolomite, and simulated Martian regolith (Brack et al., 2002). Further experiments were prepared; however, STONE 2, STONE 3, STONE 4 were not completed. For the STONE 5 experiment additional rocks were incorporated—sandstone, dolerite, and gneiss from Arctic. Holes were drilled into the back of each of the rock samples and were loaded with *B. subtilis* and *Ulocladium atrum* (fungal spores) and with dried *Chroococcidiopsis*. The gneiss rock was soaked with *Chroococcidiopsis* cells to simulate an endolithic community (Cockell et al., 2007; Brandstatter et al., 2008). To date, there has been one additional experiment, STONE 6; however, the microbiological experiments did not survive the heat of re-entry.

7. Survival of microorganisms

The survival of microorganisms has been studied extensively using both simulated facilities and LEO. A large number of microorganisms have been selected for exposure experiments, as listed in Table 5. It is possible to separate these microorganisms into two groups, the human-borne, and the extremophiles. Studying the human-borne microorganisms is significant for human welfare and future manned missions; whilst the extremophiles are vital for studying the physiological requirements of survival in space.

Extremophiles have adapted to live in some of the most extreme environments on Earth. This includes hypersaline lakes, arid regions, deep sea, acidic sites, cold and dry polar regions and permafrost (Rothschild and Mancinelli, 2001). The existence of extremophiles has led to the speculation that microorganisms could survive the harsh conditions of extraterrestrial environments and be used as model organisms to understand the fate of biological systems in these environments. The focus of many of the experiments has been to investigate the possible survival of organisms on Mars for understanding the likelihood of past or present life on that planet. On early Mars, the environment was significantly different than it is today, as around 3.5 Ga ago the climate was warmer and wetter, similar to that on early Earth (McKay, 1997). However, the loss of some of the atmosphere and thus liquid water at the surface made the climate cold and dry. Putative life may have been destroyed on the surface; however, it may have survived in protected environments such as the matrix of rocks (cryptoendoliths), the polar ice caps, permafrost regions, submarine or sub-ice hydrothermal vents or evaporites. Therefore extremophiles that live in these environments on the Earth, such as halophiles (that live in high salt environments), endoevaporites (that live in evaporites, such as halite or gypsum), cryptoendoliths (that live inside the rock matrix), psychrophiles (that live in cold environments) and UV resistant microorganisms, have been used in exposure experiments (Friedmann and Koriem, 1989; Horneck, 2000).

In addition, with the discovery of methane in the Martian atmosphere it has been speculated that methanogenesis could be one explanation. Simulation studies have been conducted to investigate the effect of

Table 6
Example of communities investigated in simulation environments and Low Earth Orbit.

Sample	MSE[a]	LEO[b]/ simulation	Reference
Environmental			
Soils	x		Green et al. (1971), Fulton (1958), Hansen et al. (2005)
Colonised sandstone, Antarctica	x	x	Onofri et al. (2008)
Permafrost; Arctic, Siberia and Antarctica	x	x	Novotoskaya-Vlassova et al. (2002), Morozova et al. (2007)
Halite rock, Atacama Desert	x	x	Wierzchos et al. (2006), de la Torre et al. (unpublished)
Coastal limestone cliff, Beer, UK	x	x	Olsson-Francis (unpublished)
Lichens			
Rhizocarpon geographicum	x	x	de la Torre Noetzel et al. (2007)
Xanthoria elegans	x	x	Sancho et al. (2007), de Vera et al. (2004)
Aspicilia fruticulosa	x	x	de la Torre et al. (unpublished)
Fulgensia bracteata	x	x	de la Torre (unpublished), de Vera et al. (2004)
Xanthoria parietina	x	x	de la Torre (unpublished), de Vera et al. (2004)

[a] Mars Simulated Environment.
[b] Low Earth Orbit.

Table 7
The parameters examined as part of the preliminary work for the LIFE experiment.[a]

Experiment	Parameters	Duration	Exposure
E1	Vacuum 10^{-5} Pa	1 h	1.3×10^{-5} Pa
		1 wk	2.3×10^{-6} Pa
	Temperature oscillation 50 cycles −20 °C to +20 °C, 1 atm air	2 wk	
	UV-C irradiation monochromatic 254 nm, 1 atm air, 71.4 μW/cm^2	14 s	10 Jm^{-2}
		2 min 20 s	100 Jm^{-2}
		23 min 20 s	1000 Jm^{-2}
	UV irradiation polychromatic 200–400 nm, 1 atm air	3 s (SOL2000)	1.44 kJm^{-2}
		52 min (SOL2000)	1.5×10^{3} kJm^{-2}
		87 h (SOL2000)	1.5×10^{5} kJm^{-2}
E2	Vacuum 10^{-5} Pa (dark)	22 d	
	Vacuum 10^{-5} Pa + UV irradiation polychromatic (200–400 nm)	22 d	1.5×10^{5} kJm^{-2}
		244.5 h	
	Mars atm. 600 Pa (dark)	21 d	
	Simulated CO_2 Mars atm 600 Pa + UV irradiation polychromatic (200–400 nm)	21 d	1.5×10^{5} kJm^{-2}
		18 min (SOL2000)	
		10 d 3 h 40 min 48 s (SOL1000)	
Control	Room temperature, dark, 1 atm air		

[a] This table is from the paper of Onofri et al. (2008).

Martian conditions on methanogens, as seen in Table 5 (Formisano et al., 2004). Methanogens are chemolithotrophs that consume hydrogen and carbon dioxide and produce methane. On Earth, methanogens can be found in many anaerobic habitats, such as hot springs, deep sea ocean vents, freshwater and marine sediment (Liu and Whitman, 2008). On Mars, methanogens could potentially live in subsurface environments, protected from the adverse UV radiation, near geothermal regions where hydrogen is produced.

For exposure experiments, the microorganisms are either studied in pure culture or in a mixed community. Each of these procedures will be discussed in more detail below.

7.1. Pure culture studies

The majority of exposure experiments have been conducted with pure cultures rather than communities. The bulk of these experiments have been focused on prokaryotes; however, a few experiments have included eukaryotes and viruses, as seen in Table 5. Pure culture exposure experiments generally focus on microorganisms that are known to survive in extreme conditions. These experiments provide an opportunity to investigate survivability and also an insight into the physiological requirements. This is achieved by investigating specific characteristics such as biomolecular production and sporulation in characterised microorganisms (Nicholson et al., 2000). Although pure culture studies are important, it is difficult to relate the results to mixed cultures or environmental samples.

Endospore-producing bacteria, which are known to be resistant to extreme environmental conditions, such as, heat, UV radiation, and low pressure have been used as model organisms for many exposure experiments (Nicholson et al., 2000; Nicholson and Schuerger, 2005; Schuerger et al., 2003). The most intensively studied bacterium is the endospore-producing *B. subtilis* as it is highly resistant to harsh environments. Mutants of *B. subtilis*, such as HA101f (pol^{-}) which is deficient in excision repair and TKJ6312 which is deficient in excision repair and spore photoproduct specific repair, have been used to study physiological requirements in more detail (Horneck, 1993).

7.2. Community studies

For community studies, investigations are carried out by *in situ* exposure of the microbial population in environmental samples, for example rock, or soil. Prior to exposure, the community is characterised, this may involve constructing a 16S rRNA gene library and culturing. Environmental samples are generally diverse; therefore, the number of exposed species is much higher than that of pure cultures. Exposing the community selects microorganisms that are resistant, therefore providing information about the physiological requirements of microbial survival in the conditions examined. Furthermore, the incubation of the microbial community *in situ* does not involve culturing the microorganisms in the laboratory prior to exposure. Experimental work with *Chroococcidiopsis* demonstrated that culturing the cyanobacterium in the laboratory made it less resistance to UV radiation and desiccation (personal communication, C.S Cockell).

It is important to include in this section lichens, as they have been used in a number of exposure experiments, as seen in Table 6. Lichens are symbiotic associations between a heterotrophic fungal host and algal and/or cyanobacterial cells as the photoautotrophic partner (Van Haluwyn, 1999). Lichens have multiple protective mechanisms which allow them to grow in some of the most extreme environments on Earth (de Vera et al., 2004; de Vera et al., 2008).

Although community studies have been used for exposure studies, environmental samples may not be homogeneous, so it would be difficult to ensure exposure of the entire community to the same conditions (Hansen, 2007).

7.3. Novel approach

To date, most of the pure culture studies have involved microorganisms that have been selected for their known resistance to harsh environments or their importance for contamination studies. However, an ideal approach would be to use a combination of both community and pure culture techniques. Exposure to extreme conditions, such as LEO or a simulated Martian environment, could select for novel extremophilic microorganisms. The physiology of the isolates could then be characterised. This is a relatively unique method and has only been used in two studies. This includes the study of Davis and Fulton (1959) that involved isolating microorganisms from soil exposed to Mars simulated conditions and the work by Olsson-Francis et al. that involved isolating cyanobacteria from limestone rocks exposed to LEO and Mars simulated conditions (Davis and Fulton, 1959; Olsson-Francis and Cockell, unpublished).

8. Analysis of results

Analysis of microorganisms after exposure to LEO or simulated conditions is a crucial part of the experimental procedure. The use of traditional methods such as direct colony counts is still considered the most robust method to measure survivability in pure cultures (Horneck et al., 2008). However, in communities, the population is very complex and only a fraction of the total viable population can be cultured using standard techniques. The development of culture-independent methods has suggested that the size of culturable communities is several orders of magnitudes lower (Amann et al., 1995).

Culture-independent methods can also be used to quantify viable cells. It is possible to differentiate between live and dead cells by using a combination of fluorescent dyes, such as those available in the LIVE/DEAD viability assay kits. The kits are available commercially and are specific for bacteria or yeast/fungi. For bacterial cells, the fluorescent membrane-permeable label SYTO® 9 labels the live bacteria with green fluorescence whilst the membrane-impermeable stain propidium iodide labels the compromised bacterial membranes with red fluorescence (dead). For quantifying viable fungi and yeast cells there are two LIVE/DEAD viability assay kits available that use either the FUN-1 or the SYTO® 9 dye (Invitrogen, Molecular Probes). The plasma membrane integrity and metabolic function of fungi/yeast convert the yellow-green fluorescent intracellular staining of FUN 1 into red-orange fluorescent; whilst the dead cells are shown by a diffuse, green-yellow fluorescence that does not change colour. The SYTO® 9 dye labels the entire yeast/fungi population; whilst the propidium iodide penetrates only yeast with damaged membranes. This causes a reduction in the SYTO® 9 stain fluorescence resulting in yeast with intact membranes staining fluorescent green, whereas yeast with damaged membranes stain fluorescent red.

The LIVE/DEAD stains have also been used extensively for examining survival in microbial communities (Stan-Lotter et al., 2003). This method is particularly useful for studying environments where culturing is difficult. It must be noted that unlike pure culture, where a known number of cells can be used in each experiment and survivability can be measured as a percentage of the total population, environmental samples are heterogeneous. Therefore it is difficult to compare numbers with the control samples. A more informative method would be to isolate microorganisms after exposure, which selects for resistant isolates that can be further characterised.

The microbial population of a community can be identified using phylogenetic techniques; however, this method is not effective for examining survivability. Hansen et al. (2005), demonstrated, by using the molecular technique DGGE to examine survivability of soil communities in Mars simulation conditions, that there was no variation in the molecular finger-print, only in the intensity of the bands. Although there was no effect on the microbial diversity using DGGE, in the same study they demonstrated a change in the functional diversity after exposure. There are commercial systems, such as Biolog EcoPlate®,

available for functional analysis. The EcoPlate® is a 96-well plate that contains three identical sets of 31 freeze-dried substrates and tetrazolium (Weber et al., 2007). To examine functional diversity, the wells are inoculated with homogenised sample suspension and incubated and analysed at defined time intervals. The utilisation of the substrate is evaluated by the formation of a purple colour (reduction of tetrazolium). The functional activity of the community can be compared before and after exposure.

Metabolic activity has been used as a measure for microbial survivability. For example, analysis of the *Synechococcus* sp. samples exposed aboard BIOPAN II and soil samples after exposure to Mars simulated conditions (Finster et al., 2007; Mancinelli et al., 1998). Analysis of the BIOPAN II samples involved comparing carbon and nitrogen fixation rates with control samples. Although these were pure culture samples, the *Synechococcus* sp. produced extracellular material, making MPN determinations unreliable (Mancinelli et al., 1998). The carbon rates were calculated by measuring the production of carbon from $NaH^{14}CO_3$ (Rothschild et al., 1994). The nitrogen fixation was measured using the acetylene reduction assay (Postgate, 1972). Samples were placed in a 10 ml vial and acetylene was injected into the headspace of each chamber to yield an atmosphere containing not less than 20% acetylene. The vials were incubated in the light and the gas samples were collected to analyse for ethylene production.

9. Planetary protection

After the successful launch of Sputnik in 1957, concerns were raised about the potential contamination by terrestrial microorganisms on other planets. Furthermore experimental work using simulated conditions and LEO demonstrated that microorganisms, in particular spores, could be a major cause of contamination because of their ability to survive space conditions (Horneck, 1993; La Duc et al., 2004). Nowadays, there are strict procedures imposed by the major space agencies, as part of the Treaty of Principle Governing the Activities of States in the Exploration and Use of Outer Space, Including the Moon and Other Celestial Bodies and planetary protection measures are an important part of planning a space mission (United Nations, 1966).

Planetary protection can be divided to two areas of concern. The first involves protecting extraterrestrial environments from terrestrial contamination (forward contamination). The second involves protecting the Earth from contamination which may be brought by samples or hardware returning to Earth (back contamination) (Debus, 2006). Forward contamination is a major concern for planned mission to Mars as contamination will compromise future life sciences experiments and analysis. Concerns focus on both introducing organisms from Earth to the Martian environment, in which they might grow, and with introducing organisms or organic contamination originating on Earth into the environments in which they might be inadvertently mistaken for indigenous Martian life (Arnould and Debus, 2008).

The planetary protection recommendations for a mission depend on the risk of contaminating another planet (Debus, 2006). For example, a mission that involves flybys or orbiters that have no direct contact with the surface are designated as Category III and landers that have direct contact are designated as Category IV. Sterilisation and cleanliness depends on the category. Category III missions, including flybys and orbiters, require detailed documentation, implying procedures such as trajectory biasing, the use of clean rooms during assembly and testing and possible bioburden reduction. Missions searching for extant Martian life fall into Category IVb; whilst missions going to places where liquid water is present or where the presence of the space craft could cause liquid water to be present (special region) are termed Category IVc. Category IV are subjected to detailed documentation, including bioassays to enumerate the bioburden, a probability of contamination analysis, active decontamination methods, an inventory of the bulk constituent organic and increased number of implementation procedures (Debus, 2006).

Standard microbiological techniques, as stated in the NASA NPR 5340 document, are in place to monitor the microbiological contamination of spacecrafts/instruments (NASA, 1980). The bioload is measured using both cultured-dependent and independent methods. Cell counts are calculated for the clean room and the spacecraft by swabbing the surface and determining CFU numbers. The numbers of spores are calculated by heat-shocking the cells prior to plating. Non-cultivatable techniques can also be employed such as Live/Dead fluorescence analysis, ATP bioluminescence assay, or lipopolysaccharide analysis. However, each of these assays has limitations that make them unlikely to completely replace direct cell counts.

Planetary protection requires that the actual bioburden of a spacecraft/instrument is reduced. A number of sterilisation procedures, including biocleaning with IPA (isopropyl alcohol) or sporicides, dry heat sterilisation, H_2O_2 gas plasma, gamma or beta radiation can be used (Chen et al., 2008; Debus, 2006). The contamination requirements, for IV missions, are based on the bioburden levels obtained for the Viking landers. A twofold approach was employed to reduce the number of terrestrial microorganisms on the surface of the landers. This included assemble in a Class 100,000 clean room, and a sterilisation step involving dry heat (117 °C for 30 h). The total bioburden of the lander surface, pre-sterilisation, was 300,000; whilst sterilisation reduced the bioburden by a factor of 10^4 (Debus, 2006). Therefore, for Category IV missions, the level of contamination should be less than 300 bacterial spores per square meter and less than 300,000 bacterial spores per lander. For IVb missions, the contamination levels need to be reduced to 30 bacterial spores per lander (Debus, 2006; Horneck and Rettberg, 2007).

10. Space applications

The prospect of long-term manned missions and the development of planetary bases are reliant on self sufficiency for necessities such as food, water, and oxygen. Independency is crucial, as resupplying from Earth is prohibitive, in technical or economical terms. For successful manned missions, systems must be in place to recycle waste as it has been estimated that on a two year trip to Mars, a crew of six will generate more than six tons of unwanted solid organic material (Flinn, 2004). Microorganisms have been suggested for space applications as they can be used for the break-down of waste material, and the production of oxygen, energy, and food production (Madigan et al., 2002).

Micro Ecological Life Support System (MELiSSA) is a model system that is being developed by ESA as an advanced life support system based on different microbial species and higher plants, as seen in Fig. 4 (Hendrickx and Mergeay, 2007). The system consists of a number of bioreactors and a higher plant chamber. Compartment I contains a mixed culture of various microorganisms isolated from the human intestinal flora. The microorganisms break-down the organic waste from the crew and non-edible parts of the higher plants producing a mixture of volatile fatty acids, and CO_2. The volatile fatty acids are fed into Compartment II which contains the anaerobic photosynthetic bacterium *Rhodospirillum rubrum*. In this compartment the volatile fatty acids are transformed into biomass and are fed into Compartment III. Here, the nitrogen source is transformed into nitrate and fed into compartments IVa and IVb where oxygen and edible biomass are produced (Godia et al., 2002).

Furthermore, NASA is supporting research into the development of fuel cells that utilise the metabolic activity of the family Geobacteraceae to produce electricity from human waste (Flinn, 2004). This concept is based on the observation that when a slab of graphite was buried in an anaerobic marine sediment and connected to another piece of graphite electricity flowed between them. This can be recreated in the laboratory environment with pure cultures (Lovely, 2006). These anaerobic anode chambers contain organic fuel and a graphite chamber. The cathode chamber is similar but aerobic. The *Geobacter* transfers electrons released from the oxidised organic matter to the anode. The electrons flow from the anode to the cathode.

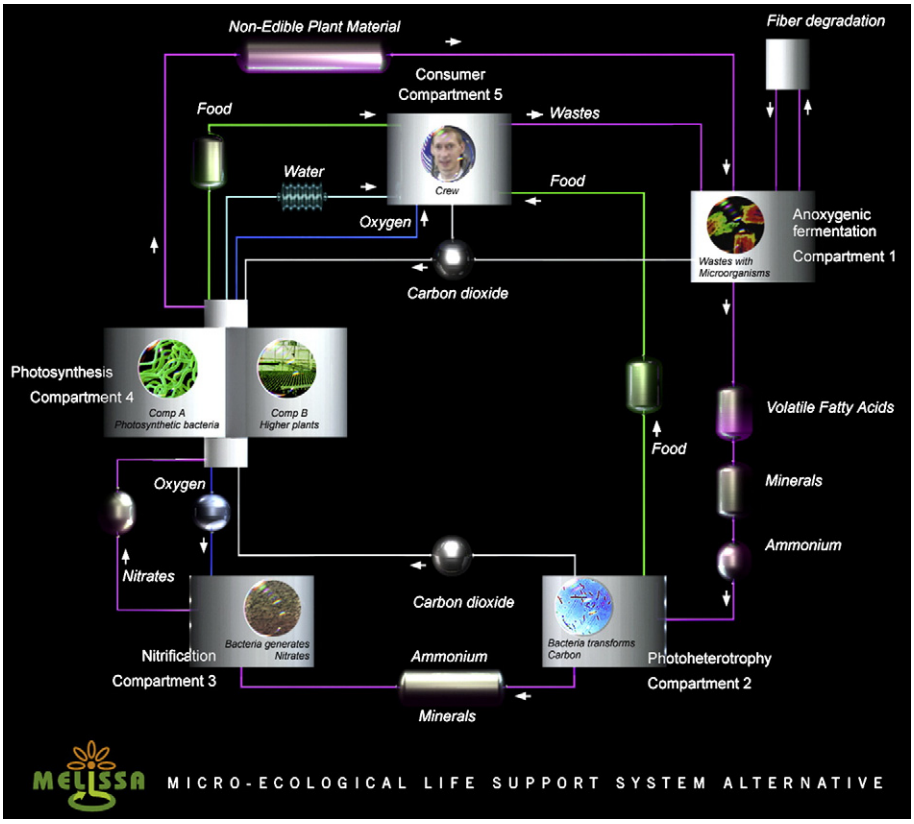

Fig. 4. A diagrammatic drawing of the MELiSSA loop (diagram courtesy of ESA).

The two chambers are separated by a cation-selective membrane that permits the protons that are released from the oxidised organic matter to migrate to the cathode side, where they combine with electrons and oxygen to produce water. The incorporation of an electrical circuit within the flow of electrons allows the energy to be harvested.

In addition to recycling, microorganisms, in particular cyanobacteria, have been suggested as a method for conditioning the nutrient poor soil on the surface of Mars or producing crusts that can be used to control surface dust (Liu et al., 2008). Experimental work with cyanobacteria has demonstrated that certain cyanobacteria can use basalt (analogous Mars basalt) as a growth substrate (Olsson-Francis, et al., 2009).

11. Conclusion and future work

Over the last sixty years, facilities have been developed for microbiologists to study the adverse effect of extraterrestrial conditions on microorganisms. Long and short-term facilities have been developed for exposure in LEO, for example BIOPAN and the EXPOSE facility aboard the ISS. In addition, simulation facilities have been developed to conduct microbiological experiments in the laboratory environment. A large number of microorganisms have been used for exposure experiments; these include pure culture and microbial communities. Characterisation of these organisms has furthered our understanding of the physiological requirements for survival.

Microbiological studies are essential for space exploration and developing life detecting instruments for other planets. The studies have been important for developing international regulations for planetary protection. Furthermore, studying microbial survival is important for future space applications. Microorganisms have been suggested for a number of applications, such as life support systems, dust control and energy fuel cells.

Although over the last fifty years a large number of microbial exposure experiments have been conducted to examine biological survival in extraterrestrial environments further work is required to develop more sophisticated instrumentation to monitor the physiological effect of the space environment *in situ*. Concomitantly, improved simulation facilities are required that enable microbial responses to be monitored. Molecular techniques are important for future analysis of resistant microorganisms, such as genomics for pure cultures and metagenomics, for community studies. Finally, additional microorganisms need to be examined, in particular microbial communities, as only a small selection have been examined to date.

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