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Space Microbiology

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INTRODUCTION 121
Scope of the Review 122
Space Environment 122
Earth's upper atmosphere 122
Outer space parameters 125
Microgravity 126
MICROBIOLOGICAL STUDIES IN THE SPACE ENVIRONMENT OR USING FACILITIES SIMULATING CONDITIONS OF OUTER SPACE 126
Upper Boundary of the Biosphere 126
Role of Gravity in Basic Biological Processes 127
Facilities for studying gravity effects 128
(i) Bioreactors inside the spacecraft habitat 128
(ii) On-orbit 1 × *g* flight controls 128
(iii) Ground-based spaceflight analogs 128
(iv) Numerical analyses of microgravity effects 129
Cause-and-effect theories and mechanisms 129
(i) Extracellular mass transfer 129
(ii) Cell mobility/motility influence 129
(iii) Membrane changes 130
(iv) Gene expression and exchange 130
Biological Effectiveness of Cosmic Radiation 130
Role of the Stratospheric Ozone Layer in Protecting Earth's Biosphere from Solar UV Radiation 132
Interactions of Microgravity and Radiation in Microorganisms 133
Survival of Microorganisms in Outer Space 135
Facilities for exposing microorganisms to outer space 135
Outer space as a test bed for assessing limits for survival of microorganisms 139
(i) Spectral effectiveness of solar extraterrestrial UV radiation 141
(ii) Desiccation effects by space vacuum 141
(iii) Interaction of space vacuum and solar extraterrestrial UV radiation in microorganisms 142
Likelihood of interplanetary transport of microorganisms by natural processes 143
Applied Aspects 145
Bioproduction of pharmaceutical compounds on orbit 145
(i) Secondary metabolite production 146
(ii) Vaccine development 146
Microbial virulence and drug resistance in space 146
(i) Microbial drug resistance 146
(ii) Virulence and pathogenicity 147
Microorganisms in the spacecraft environment 148
(i) Microflora composition and evolution 148
(ii) Crew health 148
(iii) The spacecraft environment 148
(iv) Biologically based life support systems 149
Outlook and Future Directions 149
ACKNOWLEDGMENTS 150
REFERENCES 150

INTRODUCTION

The vast, cold, and radiation-filled conditions of outer space present an environmental challenge for any form of life. Earth's biosphere has evolved for more than 3 billion years, shielded by the protective blanket of the atmosphere protecting terrestrial life from the hostile environment of outer space. Within the last 50 years, space technology has provided tools for transporting terrestrial life beyond this protective shield in order to study *in situ* responses to selected conditions of space (reviewed in reference 244 and, recently, references 26, 38, and 186). From a biological perspective applicable to organisms

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ranging from humans to microbes, the two most influential physical modifications experienced onboard an orbiting spacecraft are the state of near weightlessness created by the vehicle's free-fall trajectory and the increased radiation exposure incurred as a consequence of being outside Earth's protective atmosphere. Other environmental factors, such as space vacuum, thermal extremes, solar UV radiation, and the presence of high-velocity micrometeoroids and orbital debris, are mitigated by spacecraft design in order to provide internal conditions conducive to sustaining life. Alternatively, space technology provides the opportunity to expose microorganisms intentionally to the harsh external environment or selected parameters of it.

Scope of the Review

This review covers the primary aspects of space microbiology that have been studied to date. Emphasis is placed on recent findings that have not yet been dealt with in a critical review, especially those that are of relevance to future space exploration programs. The fields covered include (i) the use of the space environment for understanding basic biological mechanisms, such as the role of gravity at the cellular, subcellular, and extracellular levels, biological effects of the radiation field in space, survival factors in the upper boundary of Earth's biosphere, and the likelihood of interplanetary transport of microorganisms via meteorites; and (ii) application-oriented aspects, such as the use of microorganisms in bioregenerative life support systems, the monitoring, characterization, and control of spacecraft microflora, and associated microbial crew health concerns.

While all of these factors have scientific importance, the latter, applied topics will be of paramount importance in future space exploration activities and will pose high demands on the microbiological research community. By providing a comprehensive review of these somewhat disparate research disciplines, we hope to convey the complexity of characterizing and analyzing microbial responses to various space environment stressors and also to recognize that the potential for synergistic effects must be considered as well.

Experiments in space have also been complemented by studies using terrestrial laboratory facilities designed to simulate selected parameters of outer space, such as microgravity via clinorotation, space vacuum and thermal extremes in hypobaric chambers, and certain qualities of radiation in space, studied by use of heavy ion accelerators to simulate cosmic rays or polychromatic UV sources to simulate solar extraterrestrial UV radiation. In order to first familiarize the reader with the experimental conditions of relevance to space microbiology, this review starts with a short introduction describing the primary parameters encountered in the outer space environment that govern microbial growth and behavior or affect survival. A categorical review of the literature pertaining to microgravity, radiation, and atmospheric effects on microorganisms follows, including an overview of the novel types of facilities and payloads used to conduct the studies.

Space Environment

The majority of experiments on microorganisms in space were performed using Earth-orbiting robotic spacecraft, e.g., the Russian *Foton* satellites (50) and the *European Retrievable Carrier* (EURECA) (121), or human-tended spacecraft, such as space shuttles (106, 107) and space stations, e.g., *MIR* (220) and the *International Space Station* (ISS) (6). Only twice, during translunar trips of *Apollo 16* and *17* in the early 1970s, were microorganisms exposed to space conditions beyond Earth's magnetic shield, in the MEED (microbial ecology equipment device) facility and in the Biostack experiments (reviewed in reference 244). Arriving in space without any protection, microorganisms are confronted with an extremely hostile environment, characterized by an intense radiation field of galactic and solar origin, high vacuum, extreme temperatures, and microgravity (Table 1).

Earth's upper atmosphere. We first discuss the Earth's environment, from its surface, through the ozone layer, and up to interplanetary space. To understand airborne microbes and the extent to which they may be found viable, we must know the atmospheric environment. The atmosphere is a blanket of gases surrounding Earth that is held in by gravity. The atmosphere protects life on Earth's surface by absorbing ultraviolet solar radiation (Fig. 1), warming the surface through heat retention, and reducing temperature extremes between day and night. There is no definite boundary between the atmosphere and outer space. With increasing altitude, the atmosphere becomes thinner and eventually fades away into outer space. The Kármán line, at 100 km, is frequently regarded as the boundary between atmosphere and outer space. Three quarters of the atmosphere's mass is within 11 km of the surface. The five layers of the atmosphere are depicted in Fig. 2. Each layer possesses different characteristics. The temperature of the Earth's atmosphere varies with altitude; the mathematical relationship between temperature and altitude varies among the different atmospheric layers. The average temperature of the atmosphere at the surface of Earth is 15°C (154).

The troposphere is the lowest layer of the atmosphere; it begins at the surface and extends to between 7 km at the poles and 17 km at the equator. The troposphere contains approximately 80% of the total mass of the atmosphere. Fifty percent of the total mass of the atmosphere is located in the lower 5.6 km of the troposphere. Solar heating of the Earth's surface causes warm air masses to form, which cool as they rise and then fall to the surface to be warmed again. This leads to vertical mixing of not only the gases in the atmosphere but also any particles carried by those air masses, including microbes. Viable microbes have been isolated from the troposphere (see "Upper Boundary of the Biosphere"). The tropopause is the boundary between the troposphere and the stratosphere. Here the air stops cooling with height, remaining at approximately −56°C, and is nearly completely dry.

The stratosphere extends from the top of the troposphere to an altitude of approximately 50 km. Unlike the case in the troposphere, temperature increases with altitude in the stratosphere. The vast majority of the ozone layer is located in the stratosphere (Fig. 2). The stratopause, at an altitude of 50 to 55

TABLE 1. Environmental parameters in LEO and data obtained during space missions with microorganisms exposed to these parameters[a]

Space parameter	Value					
	LEO	SL1/D2	LDEF	EURECA	MIR-Perseus	Biopan 1, 2, 3, 5, and 6
Space vacuum						
Pressure (Pa)	10^{-7}–10^{-4}	≈10^{-4}	≈10^{-6}	≈10^{-5}	≈10^{-4}	≈10^{-6}
Residual gas (parts/cm^3)	10^4–10^5 H	10^5 H	10^5 H	10^5 H	10^5 H	10^5 H
	10^4–10^6 He	10^6 He	10^5 He	10^5 He	10^6 He	10^5 He
	10^3–10^6 N	10^6 N	10^4 N	10^4 N	10^6 N	10^4 N
	10^3–10^7 O	10^9 O	10^5 O	10^5 O	10^9 O	10^5 O
		H_2O, organics, N_2O, NO		H_2O, N_2O, NO	H_2O, organics, N_2O, NO	
Solar electromagnetic radiation						
Irradiance (W/m^2)	≈1,370	1,365	≈1,370	1,367	1,370	≈1,370
UV fluence (J/m^2) (>110 nm)		≤10^3	≈10^9	≤3 × 10^8	Not determined	≈10^7
Spectral range (nm)	Continuum from X-rays (0.01) to IR (10^6)	>110	>50	>110	>110	>110
		>170	>170	>170		>170
		>290		>280		>200
		>300		>295		>290
		210		220		>400
		220		230		
		230		260		
		260		290		
		290				
Cosmic ionizing radiation						
Dose (Gy)	1–10,000[b]	0.001	4.8	0.2–0.4	0.037–0.049	0.004–0.074
HZE particle fluence (parts/μm^2)	Low[c]	5 × 10^{-8}	6 × 10^{-5}	6 × 10^{-7}	Not determined	5 × 10^{-8}
Particle mass spectrum	Continuum of protons to Fe ions	Continuum of protons to Fe ions	Continuum of protons to Fe ions	Continuum of protons to Fe ions	Continuum of protons to Fe ions	Continuum of protons to Fe ions
Particle energy spectrum	Continuum up to 10^{20} eV	Continuum up to 10^{20} eV	Continuum up to 10^{20} eV	Continuum up to 10^{20} eV	Continuum up to 10^{20} eV	Continuum up to 10^{20} eV
Temperature (K)	Wide range (153–393)[d]	243–290	264–302	295–318	259–316	235–288
Gravity (g)	≈10^{-3}–10^{-6}	≈10^{-3}	≈10^{-6}	≈10^{-6}	≈10^{-3}	≈10^{-6}
Exposure time (days)		10	2,107	336	98	10–15

[a] Data are from references 47, 105, 107, 110, 115, and 216. Space mission dates are as follows: SL1 (Spacelab 1) with STS 9, 28 November to 8 December 1983; D2 (Spacelab D2) with STS 55, 26 April to 6 May 1993; LDEF, released with STS 41-C on 7 April 1984, retrieved by STS-32 on 20 January 1990, and returned to Earth; EURECA (European Retrievable Carrier), released with STS 46 on 31 July 1992, retrieved with STS 57 on 24 June 1993, and returned to Earth; MIR-Perseus French mission to *MIR*, 16 April to 23 July 1999; and Biopan missions attached to a *Foton* satellite: Biopan 1, 29 July to 17 August 1994; Biopan 2, 9 to 23 October 1997; Biopan 3, 9 to 24 September 1999; Biopan 5, 31 May to 15 June 2005; and Biopan 6, 14 to 26 September 2007.

[b] Dose per year, varying with altitude and shielding. The highest values were obtained at high altitudes (depending on crossings of radiation belts and polar horns) and with shielding of 0.15 g/cm^2.

[c] Annual fluence rates at peak energies of 200 to 700 MeV are about 0.2 proton/μm^2 and 6 × 10^{-5} Fe ions/μm^2.

[d] Varying with orientation to the sun and albedo of the spacecraft. Numbers give the tolerable limits for the ISS.

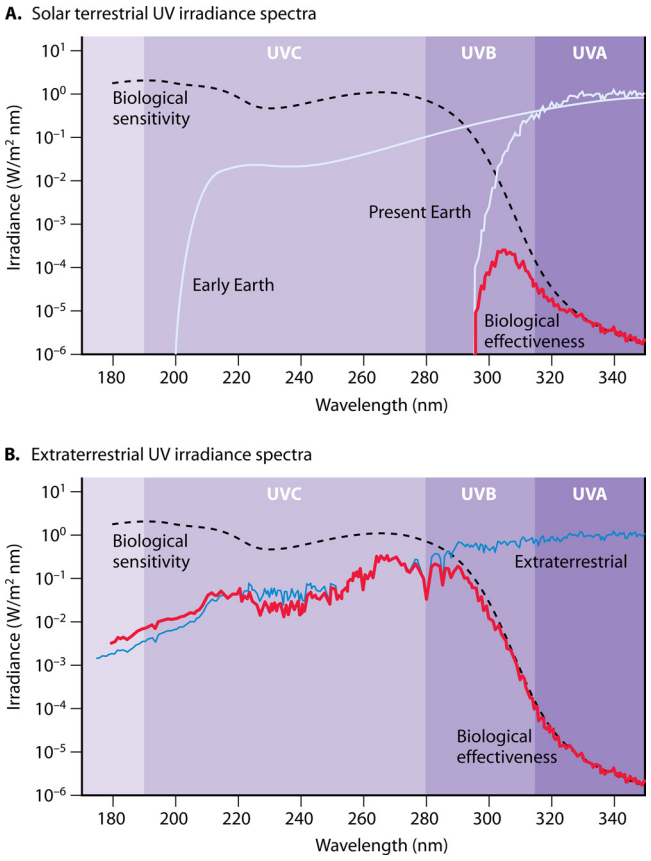

FIG. 1. Solar terrestrial (A) and extraterrestrial (B) UV irradiance spectra, action spectra for DNA damage as an example of biological sensitivity (dashed lines), and biological effectiveness spectra (bold red lines) for terrestrial and extraterrestrial conditions. (Modified from Fig. 1 in reference 111 with kind permission of Springer Science and Business Media.)

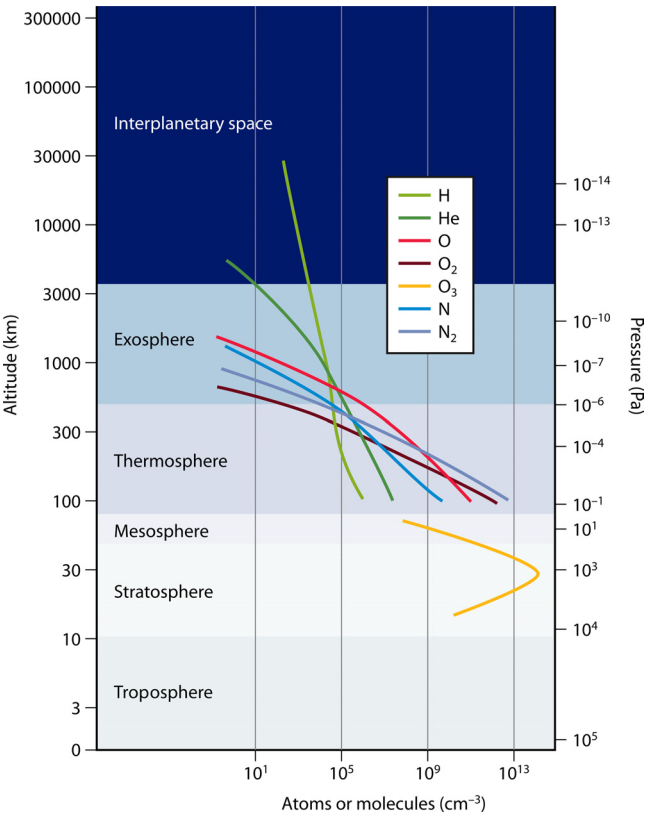

FIG. 2. Altitude profile of Earth's atmospheric components and pressure. (Modified from reference 86 with permission of the publisher.)

km and a pressure of 0.001% that at sea level, is the boundary between the stratosphere and the mesosphere. Temperature reaches a maximum in the stratopause.

The mesosphere, at an altitude of 50 to 90 km, is directly above the stratosphere and directly below the thermosphere. At this altitude, temperature decreases with increasing altitude due to decreasing solar heating and increasing cooling by CO_2 radiative emission. Temperatures in the upper mesosphere fall as low as −100°C (13). It is between the maximum altitude for aircraft and the minimum altitude for orbital spacecraft, and as a result, it is accessed by sounding rockets. The mesosphere is the highest altitude from which viable microbes have been isolated (120). The mesopause, at an altitude of 80 to 90 km, separates the mesosphere from the thermosphere. It is here that the temperature minimum occurs.

The thermosphere begins at an altitude of approximately 90 km and extends to 500 to 1,000 km. Thermospheric temperatures increase with altitude due to absorption of highly energetic solar radiation by the small amount of oxygen present. Temperatures are highly dependent on solar activity and can rise to more than 1,500°C in the upper thermosphere. Although the temperature is high, it would seem cold to microbes due to the scarcity of molecules of gas to transfer heat. The ISS has a stable orbit within the thermosphere, between 320 and 380 kilometers. It is within the thermosphere that UV and cosmic radiation causes some elements to ionize and create the ionosphere.

The exosphere is the uppermost layer of the atmosphere before the gasses dissipate into outer space (205). In the exosphere, an upward-travelling molecule will fall back to Earth due to gravity unless it is travelling at escape velocity (11.2 km/s) and flies off into space. The gases within the exosphere are primarily hydrogen, helium, carbon dioxide, and atomic oxygen.

The average atmospheric pressure at sea level is about 1×10^5 Pa (1,013 mbar). Atmospheric density decreases with height (Fig. 2), dropping by 50% at an altitude of about 5.6 km. This pressure drop is approximately exponential, so that pressure decreases by approximately half every 5.6 km and by 63.2% every 7.64 km, the average scale height of Earth's atmosphere below 70 km. For reference, 50% of the atmosphere by mass is below an altitude of 5.6 km, 90% of the atmosphere by mass is below an altitude of 16 km, and 99.99997% of the atmosphere by mass is below 100 km.

The chemical composition of the Earth's atmosphere to an altitude of about 100 km is presented in Table 2. The radiation that falls to the surface of the Earth ranges from approximately 290 nm (UVB) up through the visible spectrum (VIS) (~400 to 700 nm) and continues through to the thermal infrared (IR), to about 1,100 μm.

To demonstrate how the atmosphere affects incoming solar

TABLE 2. Composition of Earth's atmosphere[a]

Constituent	Vol fraction (ppmv [%])
Nitrogen (N_2)	780,840 (78.084)
Oxygen (O_2)	209,460 (20.946)
Argon (Ar)	9,340 (0.9340)
Carbon dioxide (CO_2)	383 (0.0383)
Neon (Ne)	18.18 (0.001818)
Helium (He)	5.24 (0.000524)
Methane (CH_4)	1.745 (0.0001745)
Krypton (Kr)	1.14 (0.000114)
Hydrogen (H_2)	0.55 (0.000055)

[a] From the NASA Earth Fact Sheet. Water vapor is not included in the dry atmosphere data; water vapor makes up ~0.40% of the volume over the full atmosphere and ~1% to 4% near Earth's surface.

radiation, Fig. 3 shows that ozone (O_3) absorbs UV (190 to 350 nm). O_2 absorbs UV (195 to 220 nm) as well as some VIS (~700 nm) and IR (10 μm). The UV absorption properties of O_3 and O_2 are central to the protective nature of the ozone layer.

Outer space parameters. In low Earth orbit (LEO), which reaches up to an altitude of 450 km, the radiation field is composed primarily of three types of radiation: (i) galactic cosmic radiation (GCR), (ii) solar cosmic radiation (SCR), and (iii) radiation belts composed of radiation trapped by the Earth's magnetosphere (183). GCR originates outside the solar system in cataclysmic astronomical events, such as supernova explosions. It consists of 98% baryons and 2% electrons. The baryonic component is composed of 85% protons, 14% α-particles (helium nuclei), and about 1% heavier nuclei. The latter component comprises the so-called HZE particles (particles of *h*igh charge *Z* and high *e*nergy), which are defined as cosmic ray primaries with charges Z of >2 and with energies high enough to penetrate at least 1 mm of shielding. Though they contribute only about 1% of the flux of GCR, they are of special interest to radiobiologists because of the inefficiency of adequate shielding and the highly localized damage caused by HZE particles. Along their trajectory, HZE particles interact with the atoms of the target, thereby causing a track of destruction that is a function of the energy deposition along their path. If the particle flux is weighted according to the energy deposition, Fe ions become the most important component of GCR, although their relative abundance is comparatively small (0.03% or 6×10^{-5} particles/year-μm^2). To catch such rare events, methods have been developed to precisely localize the trajectory of an HZE particle relative to the biological system and to correlate the physical data of the particle to the observed biological effects along its path (reviewed in references 94, 98, 100, 101, and 128). The fluence of GCR is isotropic, and energies of up to 10^{20} eV can be present. When GCR enters our solar system, it must overcome the magnetic fields carried along with the outward-flowing solar wind, whose intensity varies with the approximately 11-year cycle of solar activity. With increasing solar activity, the interplanetary magnetic field increases, resulting in a decrease of the intensity of GCR of low energies. Hence, the GCR fluxes vary with the solar cycle and differ by a factor of approximately 5 between the solar minimum and solar maximum, with a peak level during minimum solar activity and the lowest level during maximal solar activity.

SCR consists of the low-energy solar wind particles that flow constantly from the sun and the so-called solar particle events (SPEs) that originate from magnetically disturbed regions of the sun and sporadically emit bursts of charged particles with high energies (up to several GeV). These events are composed primarily of protons, with a minor component (5 to 10%) of α-particles and an even smaller component (1%) of heavy ions and electrons. SPEs develop rapidly and generally last no more than a few hours. However, for missions in LEO, the Earth's magnetic field provides a latitude-dependent shielding against SPE particles, so they are experienced only in high-inclination orbits.

The van Allen belts in the vicinity of Earth are a result of the interaction of GCR and SCR with the Earth's magnetic field and atmosphere. These van Allen belts consist of two radiation belts that are comprised of electrons and protons as well as some heavier particles trapped in closed orbits by the Earth's magnetic field. The main production process for the inner belt particles is the decay of neutrons produced in cosmic particle interactions with the atmosphere. The outer belt consists mainly of trapped solar particles. In each zone, the charged particles spiral around the geomagnetic field lines and are reflected back between the magnetic poles that act as mirrors. Electrons reach energies of up to 7 MeV, and protons reach energies of up to about 200 MeV. Of special importance for LEO missions is the so-called "South Atlantic anomaly" (SAA), a region over the coast of Brazil where the radiation belt reaches as low as 200 km above the Earth's surface. This behavior is due to an 11° offset of the Earth's magnetic dipole axis from its axis of rotation and a 500-km displacement towards the Western Pacific Ocean, with corresponding significant reduced field strength values. The inner fringes of the inner radiation belt come down to the altitude of LEO, which results in a 1,000 times higher proton flux than in other parts of the orbit. Almost all radiation received in LEO is due to passages through the SAA. This complex radiation field experienced in outer space cannot be simulated by any ground-based facility.

The spectrum of solar electromagnetic radiation spans sev-

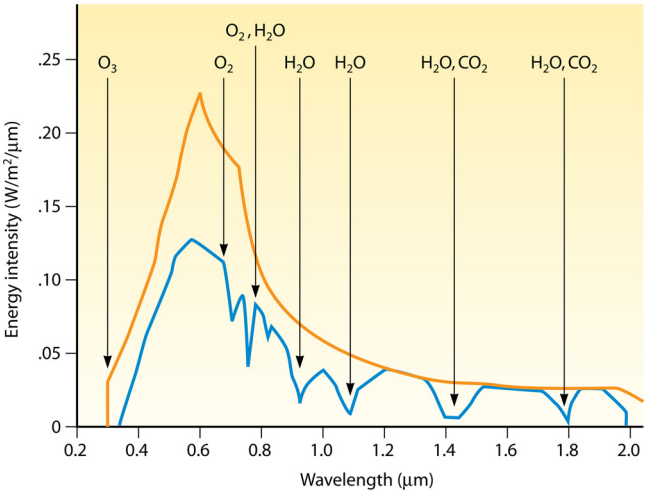

FIG. 3. Absorption spectra of Earth's atmosphere at the surface and at altitude. The solar radiation spectra are given for the top of the atmosphere (orange) and at sea level (blue).

eral orders of magnitude, from short-wavelength X-rays (<0.01 nm) to radio frequencies (several m). At 1 astronomical unit (AU), the mean distance of the Earth from the Sun, the solar irradiance equals 1,366 W m^{-2}, the solar constant. The spectrum of extraterrestrial solar UV radiation has been measured during several space missions, including Spacelab 1 (SL1) (140) and EURECA (261), and continuously for about 11 years by use of the Solar Spectral Irradiance Monitor (SUSIM) onboard an Earth-orbiting satellite (68). Solar UV radiation can be divided into three spectral ranges: UVC (200 to 280 nm), contributing 0.5% to the whole solar electromagnetic spectrum; UVB (280 to 315 nm), contributing 1.5%; and UVA (315 to 400 nm), contributing 6.3%. Although the UVC and UVB regions make up only 2% of the entire solar extraterrestrial irradiance, they are mainly responsible for the high lethality of extraterrestrial solar radiation to microorganisms exposed to it (41), due to the high absorption at those wavelengths by DNA, the decisive target for inactivation and mutation induction within that UV range.

On its way through the atmosphere, solar radiation is modified by scattering and absorption processes. Numerous lines of isotopic and geologic evidence suggest that the Archean atmosphere was essentially anoxic. As a result, the amount of ozone in the stratosphere, if any, would have been insufficient to affect the surface UV radiation environment. Thus, UVB and UVC radiation would have penetrated to the Earth's surface, with the associated biological consequences. It took more than 2 billion years, until about 2.1 billion years ago, when as a consequence of oxygenic photosynthesis the Earth's atmosphere was subjected to rapid oxidation, and hence a stratospheric ozone layer was photochemically formed. This UV screen allowed life to spread more easily over the continents and to colonize the surface of the Earth (39). Today, the stratospheric ozone layer effectively absorbs UV radiation at wavelengths shorter than 290 nm.

In order to determine the biological effectiveness of environmental UV radiation, E_{eff}, spectral data are multiplied with an action spectrum of a relevant photobiological reaction. For example, for DNA damage (233), the effectiveness of environmental UV radiation follows the equation

$$E_{eff} = \int E_{\lambda}(\lambda) \cdot S_{\lambda}(\lambda) d\lambda \quad (1)$$

where $E_{\lambda}(\lambda)$ = the solar spectral irradiance and $S_{\lambda}(\lambda)$ = the spectral sensitivity or action spectrum for a critical biological effect. The biologically effective irradiance E_{eff} is the given in the parameter $(W/m^2)_{eff}$. Figure 1 shows the solar UV irradiance spectrum, the action spectrum for DNA damage, and the biological effectiveness spectrum for terrestrial and extraterrestrial conditions.

In LEO, space vacuum reaches pressures down to 10^{-7} to 10^{-4} Pa (Table 1). The major constituents of this environment are molecular oxygen and nitrogen as well as highly reactive oxygen and nitrogen atoms. In the vicinity of a spacecraft, the pressure increases and varies depending upon the degree of outgassing from the spacecraft. If the pressure reaches values below the vapor pressure of a certain material, then the material's surface atoms or molecules vaporize. Vacuum desiccation is the main process affecting biological samples exposed to space vacuum.

The temperature of a body in space, determined by the absorption and emission of energy, depends on its position with respect to the sun and other orbiting bodies as well as on its surface, size, mass, and albedo. In LEO, the energy sources include solar radiation (1,366 W m^{-2}), the Earth's albedo (480 W m^{-2}), and terrestrial radiation (230 W m^{-2}). Periodically, an Earth-orbiting object is shaded from the sun as it passes on the Earth's night side. Within a 90-min orbit, which is typical for LEO, the spacecraft is exposed to the sun for about 60 min and moves into the Earth's shadow for the remaining 30 min. Therefore, in LEO, the temperature of a body can reach both extremely high and extremely low values within as little as 90 min. For the ISS, the tolerable temperature limits are defined, with +120°C as the highest value and −120°C as the lowest value (6).

In many space experiments, microorganisms were protected from most of the hostile parameters of space described above by containment within a space capsule, i.e., a pressurized module with an efficient life support system (LSS). Within the space capsule, mainly microgravity and/or cosmic radiation was the parameter of interest.

Microgravity. Gravity can produce two effects on an object as a function of its mass: displacement (motion) and/or deformation (weight). As long as gravity is present, one or both of these reactions will occur and can be analyzed primarily as follows. First, consider what it is that constitutes a gravity-driven effect. The gravitational constant ($G = 6.672 \times 10^{-11}$ $N \cdot m^2 \cdot kg^{-2}$) is neither a force nor an acceleration *per se*, but rather a physical constant used to dimensionally derive the force ($F_{1,2}$) resulting from the attraction by a particle of mass (m_1) on another mass particle (m_2) a distance (r) away. The magnitude of this attractive force is determined from Newton's law of gravitation, $F_{1,2} = (Gm_1m_2)r^{-2}$.

The familiar force (F) equation governing weight is derived from this relationship, taking into account the gravitational acceleration (a) at the surface of the Earth ($9.81 \text{ m} \cdot \text{s}^{-2}$ or $1 \times g$) acting on a given mass (m): $F = ma$.

The outcome of this relationship is so ubiquitous in our daily lives that weight is usually not considered a variable to be manipulated experimentally. Even in low Earth orbit, the force of gravity is not actually removed. Rather, without the significant equal and opposite resistance needed to impart weight, an orbiting object simply experiences a continuous state of gravity-induced free fall (i.e., accelerated motion) around the Earth. This state is what is commonly referred to as "weightlessness" or "microgravity," as attributed to a relative frame of reference in which an object appears to "float" inside the spacecraft (133, 254).

MICROBIOLOGICAL STUDIES IN THE SPACE ENVIRONMENT OR USING FACILITIES SIMULATING CONDITIONS OF OUTER SPACE

Upper Boundary of the Biosphere

The atmosphere, even up to a height of 30 km, presents a series of challenges for life (225). The absolute amount of solar radiation and the proportional contribution of UVB and UVC increase (Fig. 3), both of which are particularly hazardous to biomolecules, most notably nucleic acids and proteins, which

have peak irradiance absorptions at 260 and 280 nm, respectively (Fig. 1). Furthermore, the low temperature and pressure 29 km above the surface of the Earth are similar to those of Mars and create problems due to freezing and desiccation. Finally, nutrient availability and the gaseous composition of the atmosphere create additional challenges to life.

Essentially, the survival of airborne microbes depends on two independent factors: (i) the extent of damage inflicted on the microbe while airborne and (ii) the extent to which that damage can be repaired by the injured microbe (for a review, see reference 43).

The survival of airborne microbes should not be confused with growth and division while airborne. In fact, one of the critical questions that has yet to be answered unequivocally is the following: do microbes metabolize, grow, and divide while airborne? If they do, then the atmosphere may be considered a true habitat rather than just a place where they are transient interlopers. Although it was reported that *Serratia marcescens* could undergo cellular division while in a nutrient-containing aqueous droplet of 2 to 6 μm in diameter (52, 53, 54, 240), the results are not unequivocal. Glucose, a constituent of the medium, is a reducing sugar that can undergo nonenzymatic Maillard reactions that consume O_2 and liberate CO_2, confounding the results of the study, which relied on O_2 consumption and CO_2 production as indirect indicators of metabolism (44).

Given the apparent hostility of the environment, Earth's atmosphere just above the surface contains a variety of airborne microorganisms that are thought to originate from the soil, lakes, oceans (20, 75, 82, 127, 196, 204, 221, 271), animals (21), plants (151), sewage treatment plants (1, 168, 182), animal renderings (237), solid waste recovery systems (145), wastewater spray irrigation sites (25), and fermentation and other biotechnological processes (36, 43). The numbers of viable airborne microbes recovered from the atmosphere seem to vary seasonally, with the largest numbers obtained during the summer and fall and the lowest in the winter (124, 148, 234). Given the potential sources of airborne microbes listed previously that do change significantly with seasons, the changes observed may be related to climate, but it is uncertain. The distances that airborne organisms may travel have been analyzed for mid-latitudes, modeled (e.g., 138, 148, 149), and found to range from a few km to thousands of km. Temporal and spatial variations in numbers and types of microbes in the atmosphere have also been found (e.g., see references 67 and 234). For example, larger numbers of viable fungi were found in the western and southwest portion of the United States than in the northeast region (157, 234). Mancinelli and Shulls (157) showed a statistically significant positive correlation between the total number of viable bacteria isolated from urban air and the concentration of suspended particulate matter, and they suggested that the bacteria in the air may be protected from drying by adsorbed water on the surfaces of these suspended particles.

Studies of the biology of the upper atmosphere, that is, the upper troposphere and lower stratosphere (5 to 20 km), date back to the late 1800s. But these studies are few in number owing to few sampling opportunities. In most cases, balloons were used to reach these altitudes (223). The organisms collected included fungi and spore-forming bacteria (e.g., see references 46, 88, and 223). It should be noted that these early studies were not well controlled and that what was reported may not be an accurate representation of what was in the upper atmosphere. Later studies reported a larger variety of microbes, including species of *Micrococcus* and *Staphylococcus* and species related to *Deinococcus*, as well as a variety of pigmented bacteria (28, 74, 83, 84, 120, 258). Using meteorological rockets, fungi and pigmented bacteria have been isolated from as high as 77 km, the highest altitude from which microbes have been isolated (120). A recent study of the biology of the upper atmosphere was conducted using a balloon flying over India (235). Air samples were collected from 24, 28, and 41 km above the surface of the Earth, using a cryogenic sampler and Millipore filters. Only four species of *Bacillus* were isolated in this study. The previous studies, however, all used culturing methods to determine microbial counts. It has been estimated that culturing methods allow for study of only between 0.1 and 10% of the total microbial flora in any given environment (79a). Therefore, it is speculated that a number of microbes may exist in the upper atmosphere that we do not have the ability to culture and that therefore go unnoticed and uncounted in these studies.

Role of Gravity in Basic Biological Processes

Results from the "first microbiological experiments in space" are summarized by Zhukov-Verezhnikov et al. (269) as follows: "on flights similar to the orbit of the spaceship *Vostok I*, there is practically no effect from factors capable of primary action on isolated cells." Early theoretical analyses by Pollard (201) similarly concluded that the threshold for microgravity to produce an effect on cells was about 10 μm in diameter, which is larger than most bacterial cells. A review of the literature from the decades that followed, however, reveals that a variety of differences in microbial growth and behavior have in fact been observed as a result of spaceflight, with the results presumably attributable to some aspect of weightlessness (132, 146, 186, 200, 257).

While the majority of these experiments reported predominantly similar basic responses across a number of bacterial species, namely, a reduced lag phase and increased final cell population numbers in space, unexplained inconsistencies deviating from the typical findings were also occasionally reported by different investigators over the years. An interesting trend identified by a recent detailed analysis of the literature proposes that cell motility may be the key variable responsible for the seemingly disparate results (16). By categorizing the findings in terms of cell motility, a parameter not always clearly indicated and sometimes a function of growth medium, it was found that those experiments conducted with nonmotile bacterial cells reported the typically observed differences in growth kinetics, while those using motile strains tended to conclude that no effects from space occurred. This correlation gives insight into the underlying cause-and-effect mechanisms that can theoretically be traced to a gravity-initiated event. In the absence of motility, it is suggested that the fluid surrounding the cell remains quiescent, thereby reducing mass transfer between the suspended cell and its fluid environment (135). This, in turn, can lead to an altered chemical makeup of the fluid surrounding the cell, which then accordingly elicits a specific biological response. The flagellar action associated

with motility is presumed to be sufficient enough to mix the quiescent boundary layer around the cell, thus predictably eradicating the suspected cumulative effect that is caused by weightlessness. At least one earlier study tested this hypothesis directly (248), with the results corroborating the above explanation.

This altered biophysical relationship between the cell and its environment is often referred to as being an indirect effect of spaceflight. As such, it does not contradict earlier predictions suggesting that bacteria are too small to be affected directly by microgravity; rather, it extends the gravity-dependent phenomena outward to include the cell as well as its surrounding environment as a complex system. While the exact mechanisms of action have not yet been determined fully, the proposed gravity-driven cascade of events can be summed up as (i) starting with an altered physical force acting on the cell and its environment upon exposure to microgravity (the "gravity trigger"), resulting in (ii) reduced extracellular transfer of nutrients and metabolic by-products moving toward and away from the cell, which consequently (iii) exposes the cell to a modified chemical environment, the sum of which ultimately gives rise to (iv) an observed biological response that differs from what occurs under normal conditions ($1 \times g$). Results from studies published in the past decade or so are providing additional insights into the underlying physical phenomena as well as the genetic propagation of these effects. Furthermore, space research is increasingly becoming aimed toward commercial pharmaceutical applications, such as secondary metabolite (antibiotic) production, controlling the spread of multidrug-resistant pathogens, and most recently, vaccine development.

Facilities for studying gravity effects. (i) Bioreactors inside the spacecraft habitat. A wide variety of payloads have been developed and flown by numerous international teams to support cellular and molecular biology studies inside the pressurized environment of the spacecraft. Generally, the systems must attempt to mimic the conditions in a typical terrestrial laboratory as much as possible, while adhering to safety concerns of handling and mixing potentially hazardous biological samples and other reagents in the spacecraft habitat and doing so under considerable mass, power, and volume constraints (130). Summaries of biological and other, more comprehensive, current ISS experimental facilities are available at the following National Aeronautics and Space Administration (NASA) and European Space Agency (ESA) websites: http://generations.arc.nasa.gov/generations.php?pg=flt_hdw, http://www.nasa.gov/mission_pages/station/science/experiments/Facility_Cat.html, and http://www.esa.int/SPECIALS/Columbus/ESAAYI0VMOC_0.html.

(ii) On-orbit $1 \times g$ flight controls. In many cases, onboard centrifuges and matched flight-like ground control hardware are utilized in an attempt to enable researchers to more definitively isolate reduced gravity as the independent experimental variable. An example is the slow-rotating centrifuge microscope NIZEMI (*Ni*edergeschwindigkeits-*Ze*ntrifugen–*Mi*kroskop) that was used during Spacelab missions to determine the threshold of gravity perception in single-cell systems (72). The use of an on-orbit $1 \times g$ centrifuge as a control can provide an ideal method for ensuring that the experimental group is exposed to the same overall space environmental factors with the exception of microgravity. Even this simulation of $1 \times g$ while on orbit can introduce variables, however, such as vibration or inertial shear forces arising from constant-velocity rotation across a range of effective sample radius values when a flat-bottom culture vessel is used (255). To take this phenomenon into account, the on-orbit hardware must be designed with the $1 \times g$ control container "bottom" curved to match the arc of the centrifuged radius, which introduces yet another experimental variable that must be factored into the results in comparing them to a set of ground-based (true $1 \times g$) samples.

(iii) Ground-based spaceflight analogs. In addition to actual spaceflight, various ground-based methodologies are often employed to simulate different attributes of weightlessness. One of the most common devices used to provide a model for microgravity is the clinostat or a derivative called a rotating wall vessel (RWV) bioreactor (87, 131). Both devices employ rotation normal to Earth's gravitational pull to effectively nullify cumulative sedimentation of particles or cells suspended in a viscous medium. Neither, however, can fully reproduce the concurrent lack of structural deformation, displacement of intercellular components, or reduced mass transfer through the extracellular fluid that all occur in actual weightlessness. A state of relative "motionlessness" of a cell with respect to its surrounding bulk fluid, however, can theoretically be achieved through clinorotation as the fluid experiences rigid-body rotation and the cells remain constantly suspended by the continuous reorientation. The RWV bioreactor, on the other hand, while similarly maintaining cells in low-shear suspension as they continually fall through the medium under $1 \times g$ conditions, can also purposefully induce a perfusion of nutrients to and waste from the culture. A clinostat, therefore, is typically used in an attempt to reproduce the quiescent, unstirred fluid conditions achievable on orbit, while the RWV bioreactor creates a desirably mixed, fluid environment that is optimized for suspension culture and tissue growth without inducing shearing forces associated with shaking or stirring. Other techniques for exploring altered inertial environments while still on Earth, such as temporary free fall, neutral buoyancy, and diamagnetic levitation (79), can also provide additional insight into how gravity affects microbial systems.

While each of these spaceflight simulation techniques offers an opportunity for isolating gravity's role in the various biological processes, they also present complicating experimental design factors that must be taken into account when interpreting the results. For example, when using a clinostat or rotating bioreactor, the initial parameter that must be defined is an appropriate rotation rate. For suspension cultures, if the sample is rotated too quickly, the particles or cells in the medium will be centrifuged outward toward the container wall, and if it is rotated too slowly, they will sediment downward appreciably during the period of one rotation, and at extreme, they will simply roll around on the bottom of the container (136). Neither condition then represents the full quiescence achieved in microgravity. Therefore, considerable research has been aimed at defining an optimal rotational rate for maintaining a collection of suspended particles in a nearly "motionless" state, as would be experienced in actual microgravity. However, if the suspended particles or cells are of various sizes and/or densities, then the rotation rate cannot be tuned to a given sedimentation velocity as for a uniform mixture, and the resultant suspension will experience various degrees of relative motion

between the differing parts with respect to the fluid environment. In addition, living organisms add the complexity of metabolic reactions, which means that extracellular components excreted and absorbed to and from the surrounding environment must also be factored into the balance of forces acting on the system undergoing rotation. Begley and Kleis (12) characterized transport and mixing of cells and perfused oxygen in a rotating wall vessel by using numerical models. Results are presented for the transport of oxygen for cell densities and consumption rates typical of colon cancer cells. It was determined that increasing the differential rotation rate (microgravity) increased mixing and transport, while increasing the mean rotation rate (ground-based system) suppressed both. Mass transport was shown to increase comparably with an increasing perfusion rate under both conditions, with diminishing returns reached for ranges tested above 5 to 10 ml/min. Even when operating near the theoretical minimum perfusion rate, only a small fraction of the total volume was found to provide less than the required oxygen level.

It must be recognized that ground-based simulations, while generally yielding empirical results that tend to follow the trends of actual spaceflight microbial responses, do not fully replicate the same underlying mechanisms (7, 8, 15, 118, 126). Being aware of this difference, however, can actually be used to gain an advantage of more fully isolating gravity's independent principal actions of imparting weight and/or motion to a mass as a function of relative density. Carefully contrasting the physical conditions of actual microgravity, simulated microgravity, and $1 \times g$ controls therefore offers the possibility of more concisely identifying specific cause-and-effect pathways linking the influence of gravity with the observed experimental outcomes.

(iv) Numerical analyses of microgravity effects. As a complement to empirical studies, numerical analysis can also provide useful insight into defining the role that gravity plays at the subcellular level. A study conducted by Liu et al. (152) characterized the forces and trajectories that suspended particles experience within a rotating environment as a function of rotational velocity and particle size and density. Gao et al. (77) developed and validated computational models for estimating external mass transfer rates for a biophysical rather than a pure biological system, where reactions can more readily be predicted and monitored. Using different chemical species that react with the surfaces of bioactive glass particles suspended in liquid in a rotating bioreactor, they showed that simulated microgravity in a rotating bioreactor enhanced the surface modification rate of the suspended beads relative to those that were allowed to sediment to the bottom surface of a static vial. This study highlighted the importance of isolating the normally ($1 \times g$) concurrently occurring forces of convection, which is gravity dependent, and diffusion, which is independent of gravity, on net extracellular mass transfer efficacy. The subtle interplay between the cell and its environment becomes increasingly important, and more complicated, as effects of cell motility are introduced.

Cause-and-effect theories and mechanisms. Gravity induces density-driven weight and/or relative motion on a mass. If a given response is to be attributed to microgravity, therefore, it stands to reason that the initiating stimulus that ultimately gives rise to the observed altered biological outcome must stem from a physical basis involving weight or motion (253). As such, the effects of gravity on microorganisms must, in principle, be traceable to the removal of some normally present weight or motion causing a relative change to occur between components within the cell or between the cell and its environment. Hence, identifying a gravity trigger is, by definition, the first step in a complex cascade of cause-and-effect events propagated via mechanical or biochemical pathways that culminate in a measured biological response. For single-celled microbes, the intracellular components are of such uniform density and small size that they were theoretically shown early on to be unlikely to experience any sort of relative physical impact of sufficient magnitude to enable direct sensing of gravity (201, 202). In addition, the concurrent and significant influence of Brownian motion, which is not gravity dependent, also suggests that microbial cells are not likely to discern the lesser influence of gravity at any given instance, although the cumulative effect of sedimentation can result in altered environmental conditions, hence indirectly affecting microbial metabolism (137). Therefore, the manner in which microgravity alters the behavior of *in vitro* microbial suspension cultures is most likely attributable to the response of the cell to changes in the environment, including transport phenomena governing nutrient uptake, waste dispersion, and quorum-sensing processes (135). As cells increase in size beyond approximately 10 μm, such as paramecia, internal phenomena become plausible, and research is aimed at how the organism can perceive and respond to gravity (90).

(i) Extracellular mass transfer. Indirect effects of gravity acting on microbial metabolism are defined as those that are attributable to a cascade of cause-and-effect events in the extracellular environment that govern cellular behavior. Any number of physical phenomena can influence bacterial growth under unstirred, $1 \times g$ conditions (135). Suspension cultures sediment downward under gravity's ubiquitous pull, experiencing some level of shear force as they move through the resisting viscous fluid until reaching the container bottom, at which point they begin resting on other cells, consequently introducing a cumulative local environment of by-products and increasing competition for nutrients in the boundary layer above the cells.

In addition, the microenvironment surrounding a cell is comprised of a dynamic balance of nutrients being taken up from the bulk medium into the cell through its membrane and waste products excreted from the cell and diluted outward via extracellular mass transfer processes driven by diffusion and convection under $1 \times g$ conditions. The reduced-gravity environment of space essentially eliminates mass-driven convection, thereby limiting this extracellular transfer of molecules to and from the surface of the cell to diffusion only, and may alter membrane transport fluidity as well. As cells congregate on the container bottom at $1 \times g$, their cumulative action on the fluid boundary layer has been shown to create a density-driven upwelling of fluid as it becomes less dense and ultimately unstable due to nutrient consumption. The degree to which this reduction acts on single cells, however, has yet to be established fully (17, 123).

(ii) Cell mobility/motility influence. The environmental effects of microgravity can be examined on Earth, to a certain extent, by using various rotational microgravity simulation

techniques, as described above. Since under these terrestrial spaceflight analog conditions gravity remains a constant influence, the near-motionless state of the cell relative to the surrounding medium achieved from continuous reorientation is thought to be the primary factor causing the altered responses. A complementary approach for evaluating the effects of reduced cell sedimentation on growth behavior in a different manner was conducted using gas vesicle-producing *Escherichia coli* cultures that were genetically modified to be neutrally buoyant (147). In comparison with clinostat results relative to $1 \times g$ unstirred conditions, this experiment showed that comparable behavior could be achieved by partially immobilizing the cells through matched density with the medium, further suggesting that the dominating role of gravity at this scale is that of indirectly altering the extracellular environment, not action on the cells directly (15).

In addition to external forces acting on a cell and/or its environment, motility can also exert an influence on the local fluid surrounding a cell due to mixing resulting from flagellar action and removal of the cell from its otherwise quiescent location. Although most reports from space studies dating back to the 1960s indicate that bacterial growth is generally enhanced in space, several exceptions over the years have created controversy and complicated explanations of how, or even whether, microgravity affects microorganisms. As noted above, a recent detailed review of the literature showed a strong correlation between cell motility and the effect of space flight (including microgravity analogs) on the final cell numbers of bacterial suspension cultures. In general, for conditions conducive to cell motility, the typical differences observed in space, such as a shortened lag phase and increased final cell count, did not occur if motile strains were used in the experiment (16). For nonmotile cells, extracellular mass transfer of nutrients and waste in microgravity is reduced to diffusion only, so it is reasonable to envision that relative to $1 \times g$ controls, the space samples would experience a very different environment, thus altering their growth and behavior. If flagellar action is introduced, however, this difference is no longer present, since both groups experience similar mixing at the local environment level; hence, it stands to reason that no effect of space flight would be presented. Taken collectively with other findings apparently similarly governed by motility, this correlation provides additional insight into how microgravity dictates the relationship between the cell and its environment, further reinforcing the mechanistic explanation that the indirect altering of mass transfer is responsible for the changes observed in space. Identification of this subtle trend illustrates how confounding experimental factors, such as cell motility and growth medium, can complicate our understanding of the mechanisms by which reduced gravity profoundly affects biological systems. For completeness, future spaceflight (and microgravity analog) studies should thoroughly characterize the level of bacterial motility for the culture under investigation and draw conclusions about the results accordingly.

(iii) Membrane changes. Moving beyond the initial gravity trigger event, the cellular membrane, which isolates the internal components from the surrounding environment, is the next logical step to examine in the cascading cause-and-effect pathway. Goldermann and Hanke (81) showed that gravity can influence porin fraction opening in reconstituted membranes under conditions of free fall (in a drop tower) and hypergravity (in a centrifuge). This suggests that the membrane barrier between the biological and physical worlds may be affected as a function of gravity level, giving rise to altered uptake or excretion rates. Huitema et al. (118) reported an increase in *E. coli* membrane fluidity when cells were cultured under conditions of simulated microgravity, but England et al. (60) found no difference for a different species (*Pseudomonas aeruginosa*). It was also suggested that increased membrane fluidity in microgravity could be responsible for increased drug resistance.

(iv) Gene expression and exchange. Further upstream in the metabolic pathway, the initial gravity-dependent physical alterations to the cell may influence its genetics. Much current research is focused on differential gene expression in an attempt to correlate responses to weightlessness (or simulated weightlessness) to specific genes being up- or downregulated. Although this still maturing field has yet to positively identify which genes are responsible for the various gravity-dependent responses observed, a growing database of relationships is being documented (2, 242, 243). Wilson et al. (264) conducted a microarray analysis on *Salmonella* cells cultured under conditions of simulated microgravity and found that overexpression of 100 genes was significantly altered, including genes encoding transcriptional regulators, virulence factors, lipopolysaccharide (LPS) synthetic enzymes, and iron utilization enzymes. Advances in this field from recent spaceflight experiments are likely to greatly expand our understanding of how microgravity ultimately governs microbial behavior on a genetic basis.

Another contributing factor in this regard is that of genetic transfer. DeBoever et al. (48) observed that plasmid exchange between Gram-positive bacterial strains occurred in space flight and that plasmid exchange occurred more efficiently than that in the ground control experiment, but no significant differences were observed between space flight and ground control for a Gram-negative bacterial strain. In addition to understanding how genetic expression is altered in space, additional experiments are also needed to fully evaluate the occurrence and implication of microbial adaptation and evolution via mobile genetic elements such as phages, plasmids, and transposons, which play a crucial role in bacterial adaptation and evolution.

Biological Effectiveness of Cosmic Radiation

In-depth knowledge regarding the biological effects of the radiation field in space is required for assessing radiation risks to humans in space. To obtain this knowledge, microorganisms, plants, and animals have been studied as radiobiological model systems in space and at heavy ion accelerators on the ground (reviewed in references 94, 98, 100, 101, and 128).

Radiation interacts with matter primarily through ionization and excitation of electrons in atoms and molecules. Biological effects are induced either through direct energy absorption by key biomolecules, such as proteins and nucleic acids, or indirectly via interactions of those molecules with radiation-induced radicals, which are produced, for example, by radiolysis of cellular water (Fig. 4). With an increasing density of ionizations, the number and magnitude of local damages in cells increase. This is especially valid for HZE particles of GCR, which produce clusters of ions and radicals along their passage

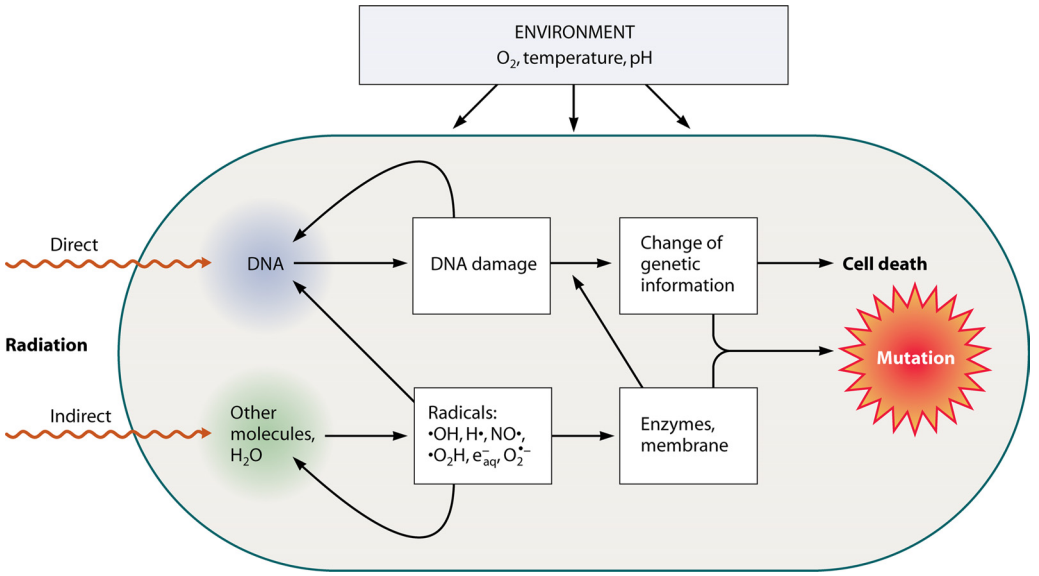

FIG. 4. Radiobiological chain of events that starts in a microbial cell after exposure to ionizing radiation, with two alternative pathways of interaction, resulting in either direct or indirect radiation damage. (Modified from Fig. 7-05 in reference 101 with kind permission of Springer Science and Business Media.)

through a cell. Microdosimetric concepts consider the radial distribution of energy around the particle's track core as a critical parameter (33). In this case, the action cross section, the track structure, and the energy deposited in the sensitive sites of the biological system must be known. Bacterial endospores having a cytoplasmic core with a geometric cross section of 0.2 to 0.3 μm^2 are good test organisms for microdosimetric studies.

In a variety of space experiments, spores of *Bacillus subtilis* have been used as biological dosimeters at the μm scale to determine radial biological efficiency along the trajectories of individual HZE particles. For this purpose, the Biostack experiments were developed. The Biostack experimental concept consisted of a sandwich of monolayers of bacterial spores mounted on cellulose nitrate foils as visual track detectors (29, 94). After return from space (Apollo Soyuz Test Project and Spacelab 1 mission), the viability of each spore in the vicinity of the trajectory of an HZE particle was analyzed separately by microscopy after one-side etching of the track detector, micromanipulation of the spores onto nutrient agar, and incubation. A daily fluence rate of 0.3 to 0.7 HZE particle/cm^2, with a linear energy transfer rate (LET) of ≥130 keV/μm, was measured by counting the tracks in the detectors. LET is a measure of the rate of energy loss per unit length of a particle track in matter. Figure 5 shows the frequency of inactivated spores as a function of the radial distance from the HZE particle path, that is, the impact parameter, and statistical analyses. The data suggested two complementary effects for the inactivation of spores by HZE particles: a short-range effect up to a radial distance of 0.2 μm from the HZE particle trajectory that can be traced back to effects by secondary electrons (δ-rays) and a long-ranging effect that extends to a distance of 3.8 μm, for which other mechanisms, such as shock waves or thermophysical events, were suggested (reviewed in references 98, 101, and 185). It should be noted that in those outer areas the spores,

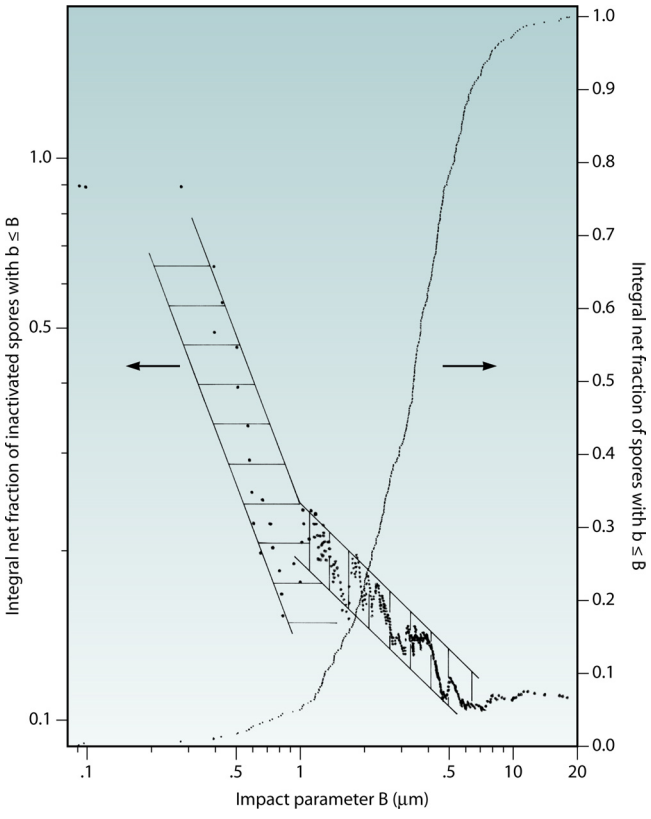

FIG. 5. Integral net fraction of inactivated spores of *B. subtilis* as a function of the impact parameter, i.e., the radial distance from the HZE particle trajectory, and integral fraction of all spores investigated in that area. Results are from Biostack III on the Apollo Soyuz test project (ASTP). (Modified from reference 61 with permission from Elsevier.)

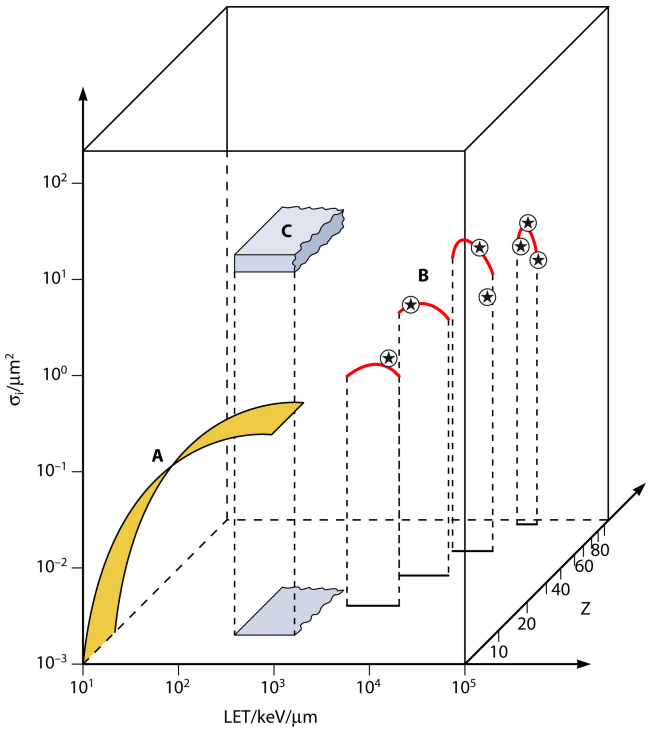

FIG. 6. Inactivation cross section, σ_i, of *B. subtilis* HA 101 spores as a function of the LET and atomic number Z, determined from fluence inactivation curves at heavy ion accelerators (Lawrence Berkeley Laboratory, Berkeley, CA [A] and Gesellschaft für Strahlenforschung, Darmstadt, Germany [B]) and from Biostack experiments in space (C). (Modified from Fig. 12 in reference 94 with kind permission of Springer Science and Business Media.)

being about 1 μm in diameter, were not directly hit by the HZE particle. Such a phenomenon, that a biological effect is induced in cells that are not directly traversed by a charged particle but are in close proximity to cells that are, known as the "bystander" effect, has since been observed for a variety of biological end points, such as inactivation, mutagenesis, and chromosomal aberrations in mammalian cells identified using narrow microbeams of particle radiation (reviewed in reference 176). Recently, bystander effects were also found *in vivo* in mice that were partially exposed to X-rays (160). Bystander effects may have severe consequences in assessing risks of radiation-induced adverse health effects for astronauts, because they may increase the risk of cancer induction (178, 198).

In order to compare the Biostack space experiment results with those obtained in irradiation experiments at heavy ion accelerators, the inactivation cross sections, σ_i, were determined (94). σ_i, which is a surface area, gives the probability for a single spore to be inactivated by a particle. σ_i is obtained from the slope of the exponential portion of fluence inactivation curves. Figure 6 shows that (i) σ_i values increased with the LET and Z of the particles, (ii) σ_i values for the space spores (Biostack experiments) were about 20 times higher than those found for spores irradiated at heavy ion accelerators with ions of comparable LET (from fluence inactivation curves), and (iii) σ_i values for the space spores (Biostack experiments) were about 20 times higher than the geometrical cross section of the spore core, which amounts to approximately 0.32 μm^2.

It must be noted that in the Biostack system very heavy and high-LET ions of GCR, such as Fe ions, with LET values of >100 keV/μm, produce long tracks through several detector layers. Those Fe ions were preferentially detected in the space experiments, whereas they were not available in the ground experiments. Therefore, the increased σ_i values for spores exposed to cosmic-ray HZE particles compared to those for ground controls may be a consequence of the high frequency of high-LET Fe ions recorded by the Biostack method.

Compared to spores of *B. subtilis*, the radiation-resistant bacterium *Deinococcus radiodurans* R1 is about 5 times more resistant to ionizing radiation, as inferred from their D_0 values (D_0 is defined as the dose of X-rays reducing survival by e^{-1}, as determined from the exponential slope of the survival curves). More important is the shape of the survival curve, which shows a pronounced shoulder for *D. radiodurans* R1, with the cells showing 100% survival when exposed to doses of up to 4 kGy. Because the survival curve for spores of *B. subtilis* is strictly exponential, the same high dose of ionizing radiation reduces spore survival by about 3 orders of magnitude (reviewed in reference 10).

DNA double-strand breaks (DSBs) are the most severe type of damage induced by HZE particles in microorganisms, as determined in cells of *E. coli* B/r (230), *D. radiodurans* R1 (270), and *B. subtilis* TKJ 8431 (166). The cross sections for DSB induction followed a similar dependence on the LET and Z of the ions to that found for inactivation of the cells (Fig. 6). In addition, oxidative base damage, such as 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo), has been found in *B. subtilis* spores exposed to HZE particles (C and Fe ions) (169), which may be caused by indirect effects of particulate radiation.

Microorganisms possess several mechanisms to repair DNA DSBs induced by HZE particles. These include rejoining the broken ends, by homologous recombination with a sister strand molecule (71) or by nonhomologous end joining (NHEJ) (22). In spores of *B. subtilis*, which contain a single chromosome arranged in a toroidal shape (70), NHEJ is the most efficient repair pathway during germination of spores exposed to ionizing radiation, such as X-rays (170) or HZE particles (173).

Mutagenicity of HZE particles is of special concern in assessing radiation risks to astronauts, because of their relation to cancer induction. Studies on the induction of mutations (e.g., histidine reversion in *B. subtilis* and *Salmonella enterica* serovar Typhimurium and azide resistance in *B. subtilis* spores) gave the following results: (i) few, if any, mutations were induced by light ions ($Z \leq 10$), and (ii) for heavier ions ($Z \geq 26$), the mutation cross section, σ_m, increased with energy up to a maximum or saturation point. From this dependence of σ_m on energy, a "mutagenic belt" inside the particle's track was suggested that is restricted to an area where the density of deparated energy is low enough not to kill the cell but high enough to induce mutations (139).

Role of the Stratospheric Ozone Layer in Protecting Earth's Biosphere from Solar UV Radiation

The full spectrum of solar UV radiation is experienced only in space. To gain a quantitative assessment of the implications of progressive ozone depletion for life on Earth, extraterrestrial solar radiation was used in a space experiment as a natural

UV source to irradiate spores of *Bacillus subtilis* 168 in a biological dosimeter "biofilm" (109). This biofilm technique directly weights the incident spectral components of the environmental UV radiation according to their biological effectiveness (179, 210, 211). During the German Spacelab mission D2 (Tables 1 and 3), precalibrated "biofilms" consisting of dry monolayers of immobilized spores of *B. subtilis* 168 were exposed for defined intervals to extraterrestrial solar radiation that was filtered through an optical filtering system to simulate different ozone column thicknesses down to very low values. After retrieval, the biologically effective irradiance E_{eff} was experimentally determined from the biofilm data for the different simulated ozone column thicknesses and compared with calculated data, using a radiative transfer model and the known biofilm action spectrum. Figure 7 shows the experimental and calculated data for an increase in biologically effective solar UV irradiance with decreasing (simulated) ozone concentrations. The unfiltered spectrum of extraterrestrial solar radiation led to an increment of E_{eff} of nearly 3 orders of magnitude compared to the solar spectrum at the surface of the Earth for average total ozone columns (Fig. 7C). The data demonstrate the value of space experiments in serving as a "time machine" for predicting the sensitivity of life to a shrinking ozone layer, i.e., assessing future trends, as well as for assessing the impact of the UV radiation climate on the early biosphere, before the stratospheric ozone screen was built up, i.e., looking back into Earth's history (41).

Spores of *B. subtilis* 168 in the biological dosimeter "biofilm" were also used on board the *MIR* station for quantifying the exposure of cosmonauts to harmful extraterrestrial UV radiation during "sunbathing" at a quartz window, as well as for assessing the sufficiency of this UV radiation for internal vitamin D production by the cosmonauts (218). It was found that the natural solar UV radiation penetrating through a quartz window of the *MIR* station was adequate for vitamin D synthesis during extended periods of "sunbathing"; however, it contained too much biologically harmful UVC and UVB radiation and was therefore a health hazard to the cosmonauts and should be avoided.

Interactions of Microgravity and Radiation in Microorganisms

In addition to health risks assessed for astronauts from exposure to radiation and microgravity, risks might arise from interactions of these spaceflight factors (97). Experimental support in favor of this hypothesis has been provided by Biostack space experiments on the embryonic development of the stick insect *Carausius morosus.* An increased number of embryos developed malformations after being hit by an HZE particle under microgravity conditions (215). It has been suggested that microgravity interferes with the operation of cellular repair processes of DNA damaged by radiation, leading to an increase in the radiation response during spaceflight (194; reviewed in reference 92). Experiments in the ESA Biorack facility (26) aboard Spacelab IML-1 (STS-42; 22 to 30 January 1992) with the temperature-conditional repair mutant *Saccharomyces cerevisiae* rad 54-3 provided further support for this hypothesis (208). Cells of *Saccharomyces cerevisiae* rad 54-3 repair radiation-induced DNA DSBs when incubated at 22°C; however, they fail to do so when grown at 36°C. Because the radiation dose of about 1 mGy received during the 8-day mission in LEO would be too low to detect any remarkable radiation-induced inactivation of the cells, stationary-phase cells were irradiated before the flight with X-rays at doses of up to 140 Gy and kept during the whole mission under nongrowth conditions to allow assessment of delayed plating recovery (69) After return, the cells were incubated at either 22°C or 36°C. It was found that in the flight samples that were kept in microgravity, the capacity to repair DNA DSBs was decreased by a factor of 2 compared to the $1 \times g$ ground control (208). These data, which suggest a synergistic interaction of microgravity and radiation, were not confirmed in a follow-up experiment during the Spacelab mission SMM-03 (STS-76; 22 to 31 March 1996) (206). Further experiments using an on-board radiation source are required to determine a possible impact of microgravity on DNA repair processes.

A more detailed investigation of the efficiency of different repair pathways in irradiated cells growing under microgravity was performed in the Biorack facility during the Spacelab IML-2 mission (STS-65; 8 to 23 July 1994), with different unicellular systems that were irradiated prior to the space mission. In this study, the following repair functions were investigated: (i) the kinetics of rejoining of radiation-induced DNA strand breaks in *E. coli* cells and human fibroblasts, (ii) the induction of the SOS response in cells of *E. coli*, and (iii) the inactivation kinetics in germinating spores of *Bacillus subtilis* with different repair capacities. For those studies, each Biorack-provided incubator was equipped with a $1 \times g$ reference centrifuge as well as with static compartments, with the latter exposing samples to microgravity conditions. Samples were collected periodically after 1 h to 5 h of incubation and stored in a −24°C freezer until analysis in the laboratory. In Fig. 8, the repair kinetics of the different microbial systems are depicted for the following gravity conditions during incubation: space ($0 \times g$ and $1 \times g$) and ground ($1 \times g$ and $1.4 \times g$). Comparison of cells that were allowed to repair in microgravity to those under gravity ($1 \times g$ reference centrifuge on board or corresponding ground controls) did not show any significant difference in their enzymatic repair reactions (108, 112). Using an on-board radiation source, Pross et al. (207) showed, using cells of *Saccharomyces cerevisiae* rad 54-3, that in microgravity both the number of radiation-induced DNA DSBs and the efficiency of their repair did not differ from those under terrestrial conditions. Therefore, the synergistic effects of microgravity and radiation in biological systems that have been observed in several instances, e.g., embryonic systems (reviewed in references 92 and 93), can probably not be explained by a disturbance of intracellular repair in microgravity. Other mechanisms conjectured include the following: (i) at the molecular level, the consequences of a convection-free environment (251); (ii) at the cellular level, an impact on signal transduction, on receptors, on the metabolic/physiological state, on the chromatin, or on the membrane structure; and (iii) at the tissue and organ level, modification of self-assembly, intercellular communication, cell migration, pattern formation, or differentiation. Further insight into a possible interaction of radiation and microgravity will be achieved with the Triple-Lux experiment, to be conducted within ESA's Biolab incubation facility on ISS (212). In this experiment, the bacterial biosensor

TABLE 3. Experiments in outer space to test survival of microorganisms

Year	Mission	Facility	Microbial assay system	Duration of exposure	Space parameter studied	GCR dose (mGy)	Phenomenon studied	Reference(s)
1965	Rockets; altitude of 150 km		Bacteriophage T1, *B. subtilis* spores, *Penicillium* spores	3 min	Space, solar UV		Inactivation	116
1966	Luster rocket; altitude of <149 km		Bacteriophage T1	204 s	Solar UV (163, 206, 254, 260–280, 306–320 nm)		UV action spectrum of inactivation	153
1966	Gemini 9; altitude of 300 km		Bacteriophage T1, TMV, *B. subtilis* spores, *Penicillium* spores	16 h 47 min	Space, solar UV		Inactivation	117
1966	Gemini 12; altitude of 300 km		Bacteriophage T1, TMV, *B. subtilis* spores, *Penicillium* spores	6 h 24 min	Space, solar UV		Inactivation	117
1972	Apollo 16 lunar mission	MEED	*B. subtilis* 168 spores, bacteriophage T7	Vacuum, 1.3 h; UV, 10 min	Space vacuum, solar UV (254, 280 nm)	4.8	Inactivation, repair	30, 238
1983	Spacelab 1; altitude of 240 km	ES029	*B. subtilis* HA 101, HA 101 F, and TKJ 6312 spores	Vacuum, 9 days; UV, 19 min to 5 h 17.5 min	Space vacuum, solar UV (>170, 220, 240, 260, 280 nm)	1.3	UV action spectrum of inactivation, photoproducts, repair	106, 107
1984–1990	LDEF; altitude of ~500 km	Exostack	*B. subtilis* spores	2,107 days	Space vacuum, solar UV	4,800	Long-term survival	105
1992–1993	EURECA	ERA	*B. subtilis* HA 101, HA 101F, TKJ 6312, and TKJ 8431 spores; *D. radiodurans* R1; plasmid pBR322; plasmid pUC19	327 days	Space vacuum, solar UV (>110, >170, >280, >295, 220, 230, 260, 290 nm)	240–410	UV action spectrum of inactivation, mutation, DNA strand breaks, shielding by dust	56, 115
1993	Spacelab D2	RD-UVRAD	*B. subtilis* 168 spores, *D. radiodurans* R1, plasmid pBR322, *Aspergillus ochraceus* condia, *Aspergillus niger* conidia	10 days (vacuum), 5–120 min (UV)	Space vacuum, solar UV (190, 210, 220, 230, 260, 280, >190, >304, >313, >314, >315, >316, >317 nm)	0.74	UV action spectrum of inactivation, photoproducts, repair, mutation, role of ozone layer	109, 114
1994	Foton 9	Biopan 1	*B. subtilis* HA 101, HA F, and TKJ 5312 spores, *Haloarcula* sp. cells, *Synechococcus* sp. cells	14.8 days	Space vacuum, solar UV	6–74	Survival, UV shielding by dust	110, 158
1997	Foton 11	Biopan 2	*B. subtilis* HA 101 spores, bacteriophage T1, *Haloarcula* sp. cells, *Synechococcus* sp. (Nägeli) cells	10 days	Space vacuum, solar UV	4–30	Survival, UV shielding by dust or salts	110
1999	Foton 12	Biopan 3	*B. subtilis* HA 101 spores	12.7 days	Space vacuum, solar UV	5–28	Survival, UV shielding by dust	110
1999	MIR-Perseus	Exobiologie	*B. subtilis* HA 101, TKJ 6312 spores	98 days	Space vacuum, solar UV	37–49	UV shielding by meteorite dust	220
1999	Terrier Black Brant rocket; altitude of <304 km	SERTIS	*D. radiodurans* R1, *Bacillus* sp. PS3D	395 s	Space vacuum, solar EUV (30.4 nm)		Survival	228
2004	Terrier Mark 70 improved rocket		*B. subtilis* spores, *B. amyloliquefaciens* spores	350 s	High-speed atmospheric entry		Survival, mutations	63
2005	Foton-M2	Biopan 5	*B. subtilis* spores, *Rhizocarpon geographicum*, *Xanthoria elegans*, permafrost microbial ecosystem	14.6 days	Space vacuum, solar UV (>170, >280, >320, >400 nm)	3.1	Survival, shielding by Martian regolith or permafrost soil	217, 229; D. Gilichinsky, personal communication
2005	Foton-M2	Stone 5	*B. subtilis* spores, *Ulocladium atrum* spores, *Chroococcidiopsis* sp.	14.6 days	Meteorite entry into Earth's atmosphere		Survival	24, 42

2007	Foton-M3	Biopan 6	*B. subtilis* spores, *D. radiodurans*, *Rhizocarpon geographicum*, *Xanthoria elegans*, *Aspicilia fruticulosa*, endolithic cyanobacteria, endoevaporites	10 days	Space vacuum, solar UV (>110, >200, >290, >400 nm)	3–80	Survival; shielding by Martian regolith, rock, and salt crystals; shielding by cortex and pigments	49, 219
2007	Foton M3	Stone 6	*Rhizocarpon geographicum*		Meteorite entry into Earth's atmosphere		Survival	262
2008–2009	ISS-EuTeF	EXPOSE-E	*B. subtilis* 168 spores, *B. pumilus* spores, *Halococcus dombrowskii*, *Anabaena cylindrica*, Antarctic cryptoendolithic communities, *Cryomyces antarcticus*, *Cryomyces minteri*	~1.5 yr	Space vacuum, solar UV (>110 nm), simulated Martian atmosphere and UV climate (>200 nm)		Survival, protection, DNA photoproducts, gene activation	192
2009-	ISS	EXPOSE-R	Bacteriophage T7, *B. subtilis* 168 spores, *B. pumilus*, *B. licheniformis*, *Halorubrum chaoviatoris*, *Chroococcidiopsis*, *Synechococcus* (Nägeli), *Penicillium italicum*, *Penicillium expansum*, *Penicillium aurantiogresium*, *Aspergillus sydowi*, *Aspergillus versicolor*, *Geomyces pannorum*, *Trichoderma koningii*	~1 yr	Space vacuum, solar UV (>110, >200 nm)		Survival, protection, DNA photoproducts, gene activation	102, 197

SOS-Lux-Lac-Fluoro toxicity test will be used to discriminate between radiation-induced and microgravity-induced damage in bacterial cells. It consists of a combination of the SOS-Lux test, i.e., recombinant *Salmonella enterica* serovar Typhimurium TA1535 cells transformed with the pBR322-derived plasmid pPLS-1 (209) and the similar, advanced plasmid SWITCH, carrying the promoterless *lux* operon of *Photobacterium leiognathi* as the reporter element, controlled by a DNA damage-dependent SOS promoter as the sensor element, with the plasmid pGFPuv to detect cytotoxic activity of chemicals or environmental agents (11). This combination biosensor has the potential for multiple applications in environmental toxicity monitoring systems (9).

Survival of Microorganisms in Outer Space

Since the advent of space flight, the ability of microorganisms to survive exposure to outer space conditions has been investigated to examine the following questions. What is the upper boundary of the biosphere? How far can we stretch the limits for life (metabolism and growth or survival)? Is interplanetary transport of microorganisms by natural processes feasible? To what extent does the space environment sterilize spacecraft during interplanetary travel? Can we use the space environment to simulate certain planetary environments to model and test the habitability of those planets?

Whereas the quest for the upper boundary of the biosphere has been investigated by using sampling devices on board meteorological rockets and high-altitude balloons (see "Upper Boundary of the Biosphere"), the other questions have been addressed by purposely exposing microorganisms to the space environment or to selected parameters of it and studying their responses after retrieval.

Facilities for exposing microorganisms to outer space. To expose microorganisms to outer space or to selected parameters of this extreme environment, several exposure facilities were developed for attachment to the outer shell of a spacecraft. Table 3 lists the experiments with microorganisms in outer space performed since 1965. The first sophisticated exposure device was built in 1972 by NASA; this was the MEED for the Apollo 16 mission (245, 246). The MEED was mounted to the distal end of the TV boom of the command module during the extravehicular activity phase of the *trans*-Earth coast. It was composed of 798 sample cuvettes with quartz windows as optical filters, with the optional provision of ventilation holes for access to space vacuum (Fig. 9). The MEED was exposed to space vacuum for 1.3 h and to three intensity levels of solar UV radiation for 10 min, with a peak wavelength of either 254 nm, 280 nm, or 300 nm. Using a solar positioning device, the MEED was oriented directly perpendicular to the sun.

The next opportunity for microbiological experiments in outer space was provided in 1983 by the SL1 mission (Table 3), with the ES029 German exposure experiment (106, 107). The exposure tray, partitioned in four square quartz-covered compartments, was accommodated in the cargo bay of SL1 and mounted on the cold plate of the pallet (Fig. 10). Two of the compartments were vented to the outside, allowing access to space vacuum; the other two compartments were hermetically sealed, with a constant pressure of 10^5 Pa. Each compartment

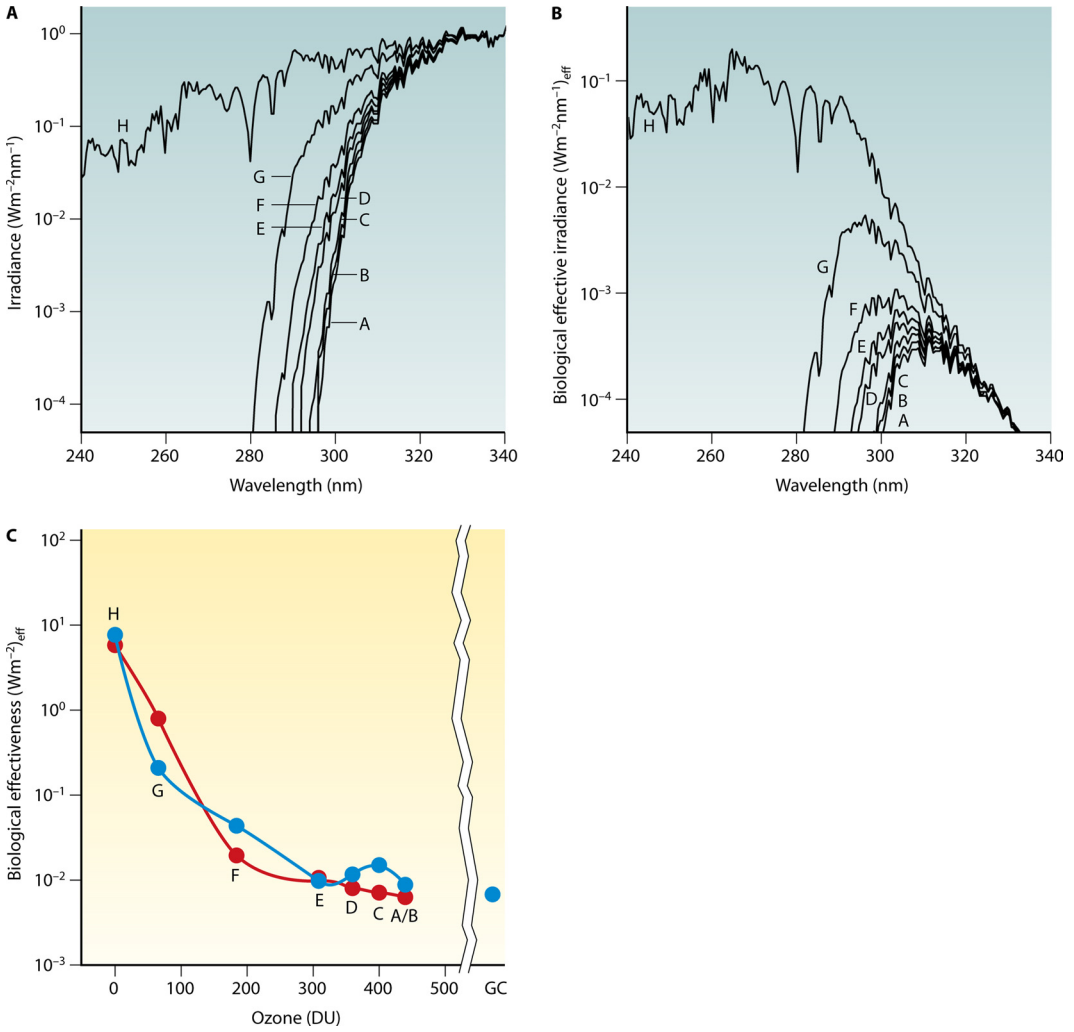

FIG. 7. (A) UV-irradiation conditions (short-wavelength cutoff by use of a filtering system, with corresponding simulated ozone column thickness) in the RD-UVRAD experiment during the German D2 mission. (B) Calculated biological effectiveness spectra for the different experimental conditions according to the sensitivity curve of the biofilm dosimeter. (C) Biological effectiveness of radiation, determined experimentally by use of the biofilm dosimeter (blue circles) and calculated by integrating the biological effectiveness spectra (B) over wavelengths (red circles). DU, Dobson unit, which measures the stratospheric ozone. 1 DU refers to a layer of ozone of 10 μm in thickness under standard temperature and pressure. GC, ground control data measured at noon in summer on the roof of the DLR in Cologne, Germany. (Modified from reference 109 with permission from Elsevier.)

accommodated 79 dry samples in the upper layer, allowing UV exposure, and the same number was kept in the bottom layer as dark flight controls. UV-irradiated samples were placed beneath an optical filtering system composed of interference filters for narrow wavebands (220 nm, 240 nm, 260 nm, and 280 nm) and neutral-density filters (Table 1). A nontransparent shutter with optical windows was used to achieve precise irradiation intervals during the "hot phase" of the mission, when during several orbits the cargo bay of the shuttle was perpendicularly pointing towards the sun. The samples were exposed to space vacuum for 10 days and to solar UV radiation for predefined periods (from 19 min to 5 h 17.5 min). The temperature ranged from 17°C to 35°C, with the highest values occurring during the "hot phase" of the mission. Two types of ground controls were performed, including a ground simulation experiment in a space simulation facility prior to the mission and a parallel ground control with an experimental setup identical to the flight unit that was kept at the Kennedy Space Center in a vacuum chamber (10^{-4} Pa) at temperatures mimicking the flight temperature profile, with a 1-day delay. The microbiological samples from the flight experiment and ground controls were analyzed after retrieval in the laboratories of the investigators. A similar device was flown in 1993 with the experiment UVRAD during the German SL D2 mission (Table 1), which provided a "hot phase" during two orbits at the end of the mission.

More advanced exposure facilities with up to four times the capacity of the ES029 experiment of SL1 were developed by ESA, with the exobiology radiation assembly (ERA) for the EURECA mission (Fig. 10) (115, 121) and the EXPOSE facilities attached to the ISS (6). EURECA was launched in 1992 for a 9-month sun-pointing mission and provided exposure to

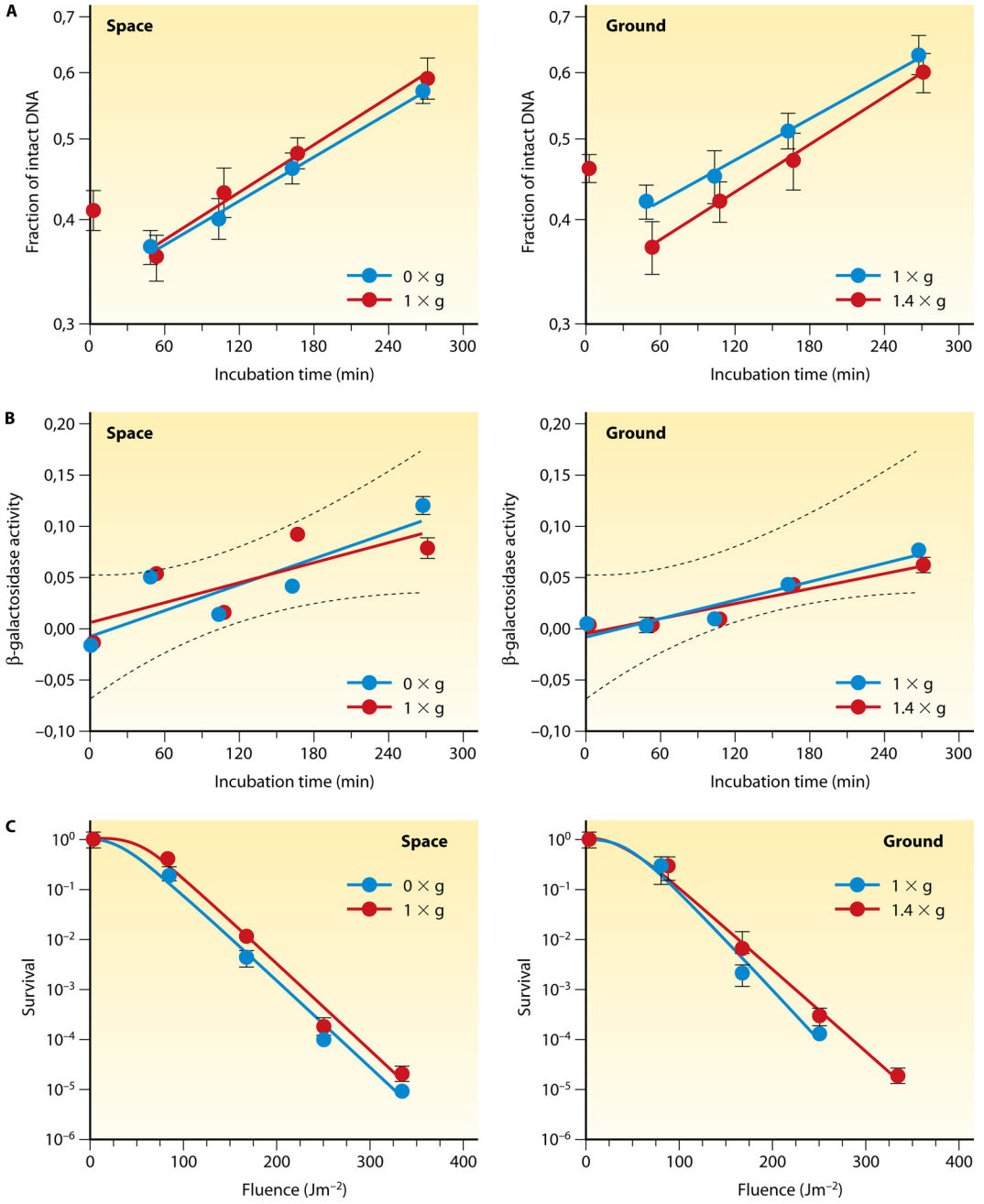

FIG. 8. Repair kinetics of radiation-induced DNA damage under microgravity conditions. (A) Rejoining of DNA strand breaks in X-irradiated cells of *E. coli* B/r. (B) Induction of SOS response in X-irradiated cells of *E. coli* PQ37. (C) Survival of spores of *B. subtilis* HA 101 after UV irradiation. (Modified from reference 112 with permission of the publisher.)

solar UV radiation for 6 months. Temperature was controlled by use of a cold plate and remained between 25°C and 40°C. The ERA facility consisted of two trays: one was covered by a shutter with optical windows, allowing UV irradiation at predefined intervals—similar to the SL facilities—and the other was thermally decoupled, thereby simulating the natural space travel of microorganisms encased in a meteorite. In the latter case, samples were exposed within so-called artificial meteorites, i.e., mixed with different soils, rocks, and meteorite powder, and solar UV radiation was filtered though different long-pass cutoff filters (>110 nm, >170 nm, >280 nm, and >295 nm) or was not attenuated at all. Temperatures of the latter tray ranged from 25°C to nearly 50°C (114, 115), and the radiation dose from GCR reached values of up to 0.4 Gy (216) (Table 1).

ESA's EXPOSE facilities of the ISS are the last developed entities in the series of exposure facilities. One EXPOSE unit consists of three trays, each housing four compartments similar to those of the SL exposure trays and of ERA (Fig. 11). The EXPOSE-E facility was launched with STS 122 on 7 February

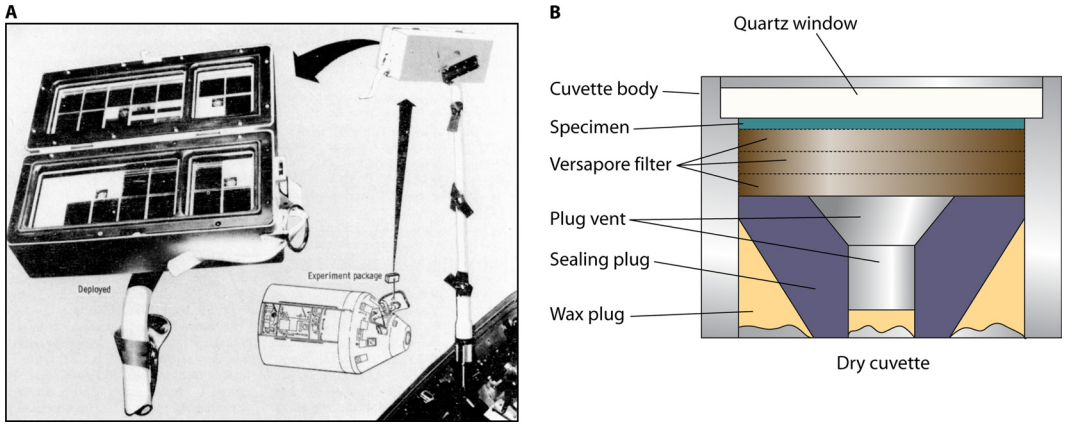

FIG. 9. MEED exposure facility mounted on the camera beam of the lunar orbiter of the Apollo 16 mission (A) and sample cuvette for exposing dry layers of microorganisms to solar UV radiation and space vacuum (B). (Reprinted from reference 245.)

2008 and mounted by extravehicular activity to the European Columbus Module of the ISS as part of the European Technology Facility (EuTeF) platform (6). One tray of EXPOSE-E has been reserved for experiments on prebiotic chemical evolution, the second tray provides outer space conditions (space vacuum and a solar UV spectrum of >110 nm), and the third tray provides conditions that simulate the Mars surface climate (600 Pa of pressure, 95% CO_2, and solar UV of >200 nm).

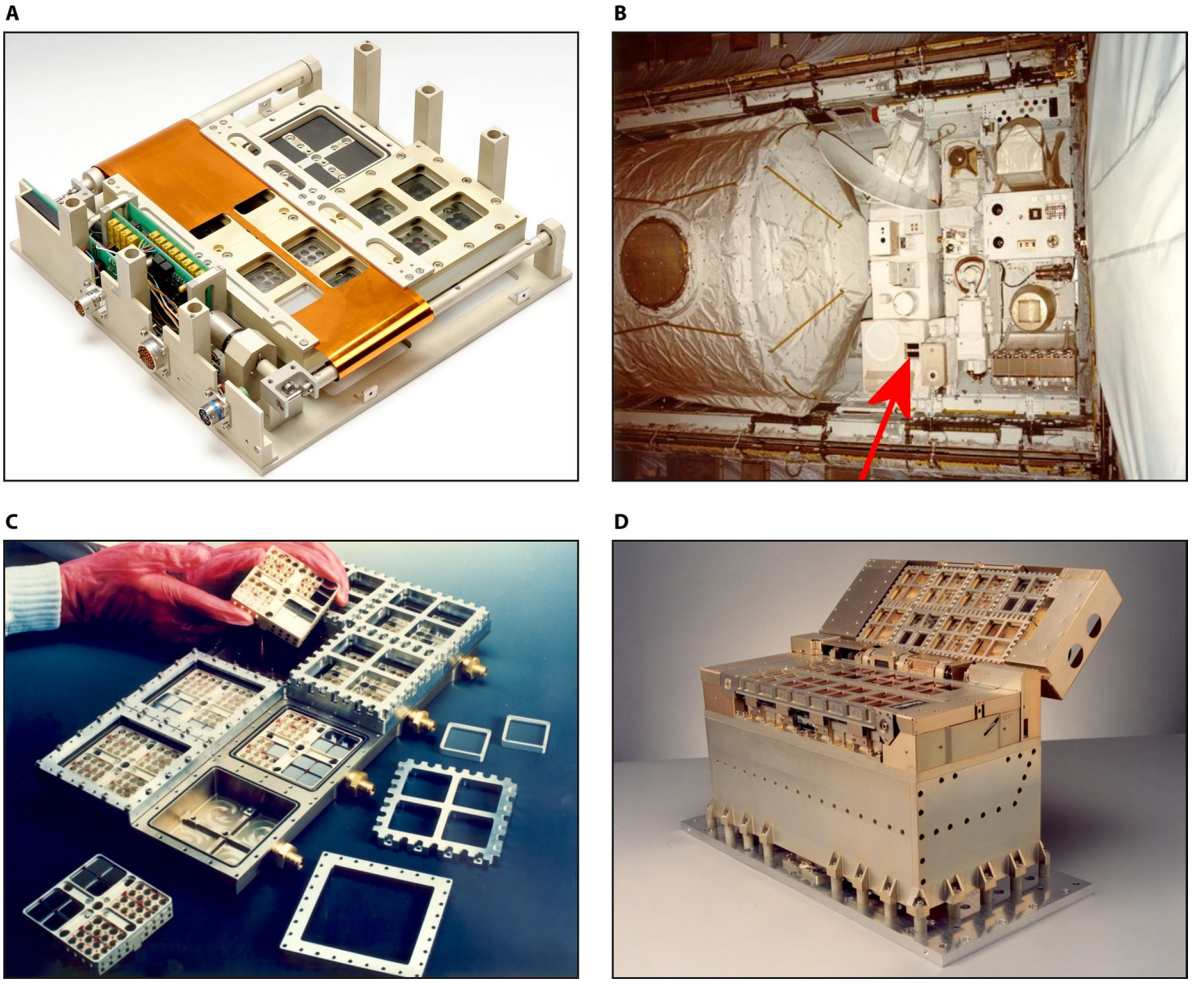

FIG. 10. Exposure tray of the ES029 experiment (A), which was mounted (arrow) on a pallet inside the cargo bay of SL1 (B), and exposure tray (C) of the ERA facility (D) on board the EURECA satellite. (Courtesy of DLR [A], NASA [B], and ESA [C and D].)

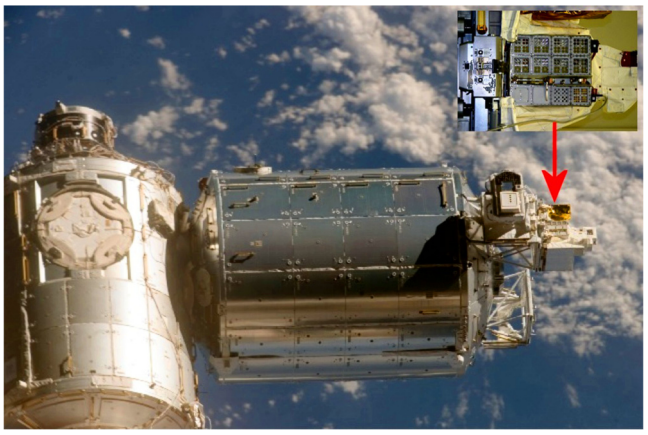

FIG. 11. EXPOSE-E facility mounted on the EuTeF platform of the European Columbus module of the ISS. The picture was taken by the crew of STS 122, when leaving the ISS. (Courtesy of ESA and NASA.)

EXPOSE-E will remain in space for more than 1 year. The second EXPOSE facility, EXPOSE-R, was launched in November 2008 and will remain attached to the URM-D platform, an external ISS facility at the Russian Svezda module, for about 1 year. EXPOSE-E and EXPOSE-R house a total of 13 different experiments that are performed in international cooperation (6, 102). Before being launched into space, all EXPOSE experiments were tested in carefully designed ground simulation experiments and in an experiment sequence test, using the Planetary and Space Simulation Facilities (PSI) at the German Aerospace Center DLR (213).

Long-term exposure (about 3 months) of organic chemical compounds and microorganisms to space was also conducted in 1999 by the French Perseus mission on the Russian *MIR* station (220). The insolation time was 1,045 h, and the temperature ranged from −14°C to +43°C. During this mission, a radiation dose of 48.7 mGy was received by the upper, sun-exposed layer, and 36.8 mGy was received by the bottom, dark layer (Table 1). The longest exposure of microorganisms to space, about 6 years (1984-1990), was achieved during the long-duration exposure facility (LDEF) NASA mission within the German Exostack experiment (105). LDEF was an Earth-pointing passive truss structure for stability testing of different materials in space (129). The biological samples were accommodated on a side pallet beneath a perforated dome, either without any cover, i.e., exposed to the full matrix of space parameters, or covered by quartz filters or aluminum foil (Fig. 12). A total UV (>100 nm) dose of 10^9 J/m^2 was estimated; a GCR dose of 4.8 Gy and a fluence of 60 HZE particles/cm^2 were measured within the Biostack experiments located on the same pallet (105) (Table 1). More advanced exposure facilities that keep the samples at very low temperatures, e.g., 10 K, during UV irradiation have been designed conceptually but have yet to be realized (89). An exposure and particle capture device for the Japanese KIBO module of the ISS has been developed and selected as a candidate for flight in 2010 (267).

Opportunities for short-duration exposure experiments (10 to 12 days) have been provided by the ESA Biopan facilities, cylindrical pan-shaped containers with a deployable lid mounted on the outer surface of the descent module of a Russian *Foton* satellite (Fig. 13) (6, 50). After attaining the proper orbit, the lid opens through 180°, exposing the experiments in the bottom and the lid to space. To monitor the exposure conditions, the Biopan facility is equipped with built-in solar UV, radiation, and temperature sensors. During ascent and reentry, the lid is hermetically closed and the whole facility is covered by an ablative heat shield. Since 1992, five Biopan missions have been completed successfully (Table 1). NASA is currently developing small astrobiology satellites which also foresee exposure of microorganisms to selected space parameters (226). Microorganisms have also been exposed to space for very short periods (several minutes) by using meteorological rockets (63, 153, 228).

Outer space as a test bed for assessing limits for survival of microorganisms. The question of whether certain microorganisms can survive in the harsh environment of outer space has intrigued scientists since the beginning of spaceflight, and opportunities were provided to expose samples to space. The first tests were made in 1966, during the Gemini IX and XII missions, when samples of bacteriophage T1 and spores of *Penicillium roqueforti* were exposed to outer space for 16.8 h and 6.5 h, respectively. Analyses after retrieval gave surviving frac-

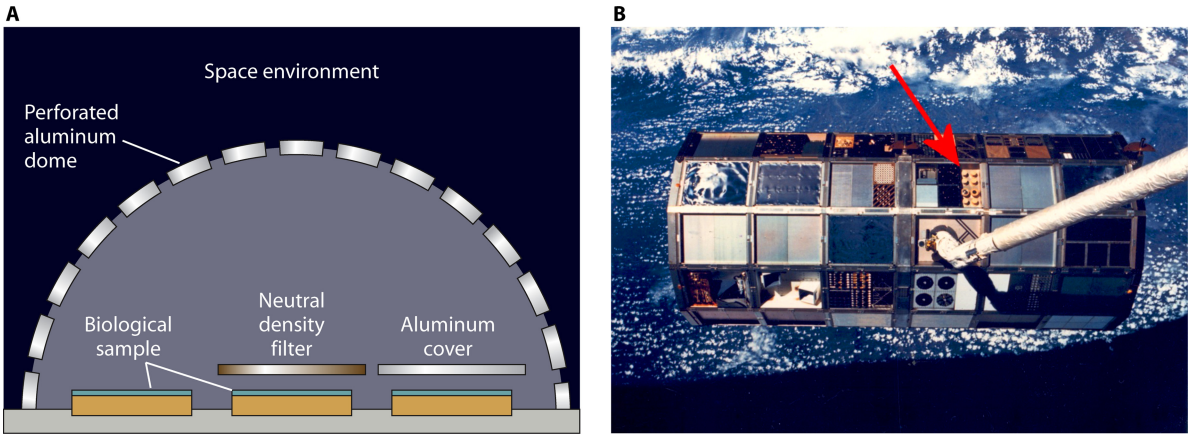

FIG. 12. Scheme of the exposure conditions in the Exostack experiment (A) on board the LDEF (B) (arrow). (Panel A modified from reference 96 with permission from Elsevier, panel B courtesy of NASA.)

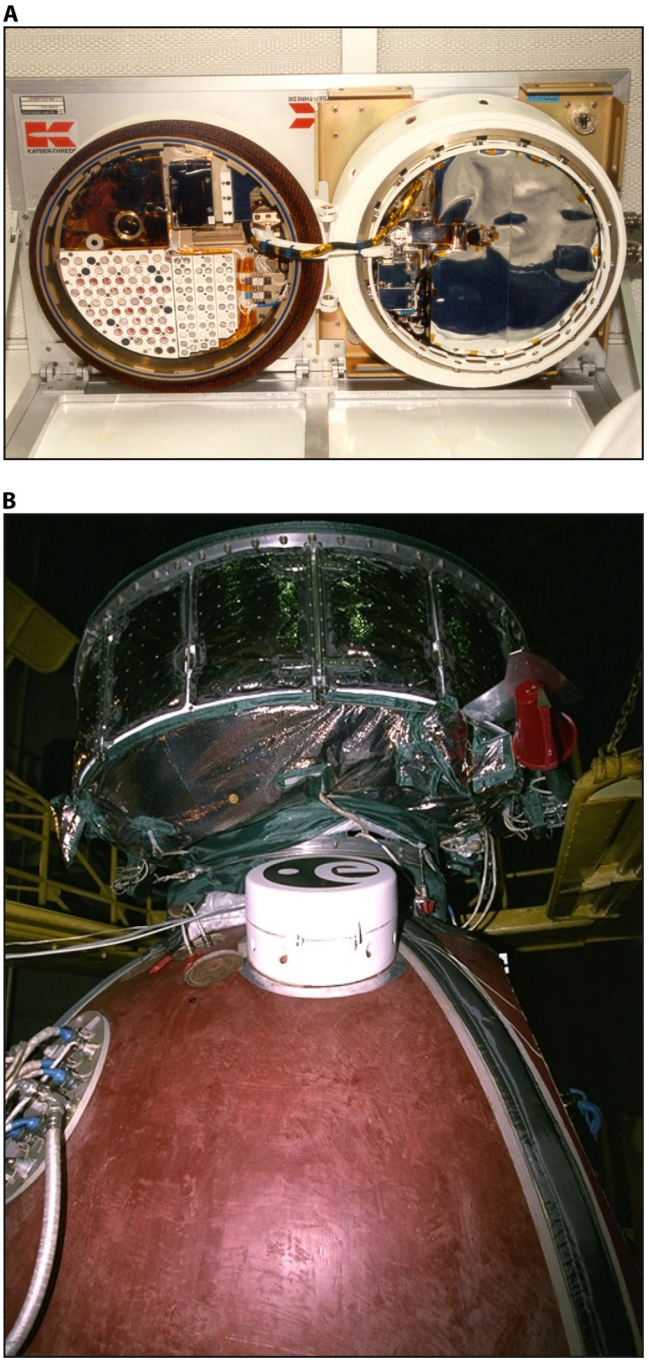

FIG. 13. Biopan facility open as in flight (A) and mounted on the *Foton* reentry capsule (B). (Courtesy of ESA.)

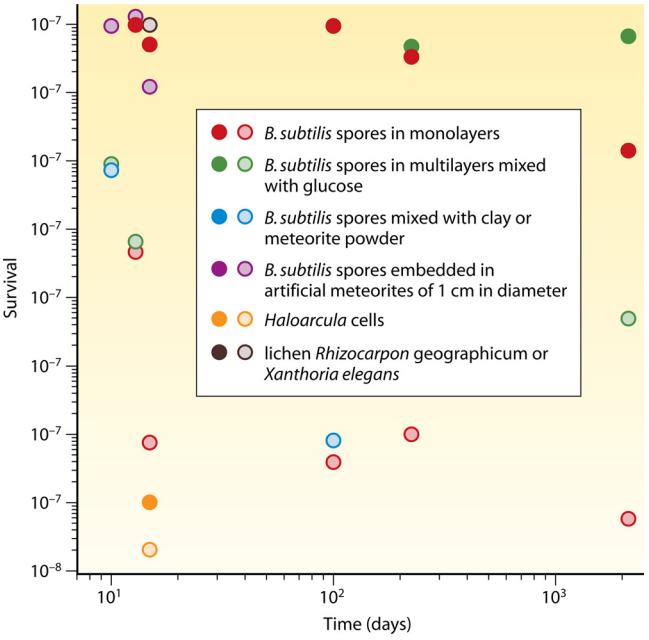

FIG. 14. Survival of microorganisms for extended periods in space vacuum (i.e., shielded against solar UV radiation) (filled symbols) or exposed to full outer space conditions (i.e., space vacuum, solar UV radiation, and cosmic radiation) (open symbols). The microbes examined were *B. subtilis* spores in monolayers, in multilayers mixed with glucose, mixed with clay or meteorite powder, or embedded in artificial meteorites of 1 cm in diameter; cells of *Haloarcula*; and the lichens *Rhizocarpon geographicum* and *Xanthoria elegans*. Data are from experiments on the *Foton* Biopan, *MIR*, EURECA, and LDEF.

tions of 3×10^{-5} (Gemini IX) and $<2 \times 10^{-6}$ (Gemini XII) for *P. roqueforti* and 2×10^{-6} (Gemini IX) and 3×10^{-5} (Gemini XII) for bacteriophage T1, demonstrating the strong killing power of the full space environment (117). However, covering the samples by a thin (0.4 mm) layer of aluminum caused a 3,000-fold higher survival of T1 and full survival of the fungal spores. This was the first indication that nonpenetrating radiation of space, probably solar UV radiation or soft X-rays, was mainly responsible for the inactivation of the test samples.

A similarly high lethality of microorganisms exposed to the full spectrum of space was later confirmed for a variety of microorganisms, including yeast cells, *Aspergillus ochraceous* conidia, cells of *Deinococcus radiodurans*, and spores of *Bacillus subtilis* with various DNA repair capacities (Fig. 14) (56, 105, 110, 115). Even if embedded in salt crystals, cells of the halophilic species *Halorubrum chaoviatoris* were inactivated, by more than 7 orders of magnitude, after exposure to full space (UV radiation dose, 10^4 kJ/m^2) during a 2-week flight aboard ESA's Biopan 1 facility (158). So far, only a few microbial systems are known that cope with the full space environment; these are the lichens *Rhizocarpon geographicum* and *Xanthoria elegans*, which had completely restored photosynthetic activity after retrieval from 2-week Biopan flights (Fig. 14) (49, 229), and cells of the marine cyanobacterium *Synechococcus* inhabiting gypsum-halite crystals, which maintained an almost normal carbon fixation capacity after a 2-week exposure to outer space (158). Further multimicroscopic analyses revealed no detectable ultrastructural changes in most of the algal and fungal cells of the lichens. Both systems—the lichens and the halophilic cyanobacteria—possess UV-screening capacities, namely, the thick and dense cortex, with UV-screening pigments rhizocarpic and parietin phenolic acids as an upper layer and as indigenous protection, for the lichen symbiotic system (51, 78), and the salt crystals as exogenous protection for the cyanobacteria.

To disentangle the biological effects of the different parameters of space requires special experimental hardware, as provided by the exposure experiments on board SL1, D2,

EURECA, and the ISS (Fig. 10 and 11). Most of the space parameters have been controlled sufficiently—solar UV radiation by using optical filters, temperature by using active cooling and/or heating, and access to space vacuum by using either vented or hermetically sealed compartments. However, shielding against GCR is nearly impossible, and it continuously impinges on all test samples in space. HZE particles are the biologically most effective component of GCR; however, as a consequence of their very low fluence of 6×10^{-5} particles/year-μm^2, as measured during several space missions, for instance, during the LDEF mission (105), not many microorganisms are hit by an HZE particle, and special technologies, such as the Biostack concept (29), are required to identify those microorganisms hit (see "Biological Effectiveness of Cosmic Radiation"). Microgravity is another space parameter that cells can escape only if hosted in an on-board $1 \times g$ reference centrifuge. The responses of microorganisms to microgravity at the molecular and cellular levels are discussed in "Role of Gravity in Basic Biological Processes." It should be noted that microgravity mostly interferes with growing or metabolizing cells; however, in the exposure experiments, dry layers of cells, fungal conidia, or bacterial spores were used that were not affected by the gravity environment.

(i) Spectral effectiveness of solar extraterrestrial UV radiation. From fluence effect curves, obtained within the ES029 experiment aboard Spacelab 1, it was found that 10 s of exposure to the full spectrum of solar extraterrestrial UV radiation (>190 nm) reduced the surviving fraction of spores of *B. subtilis* by almost 2 orders of magnitude (106, 107). Action spectra, first obtained for the inactivation of bacteriophage T1 during a rocket flight (153) and later for the inactivation of *B. subtilis* HA 101 spores in experiments aboard SL1, D2, and EURECA (95, 106, 107, 115, 185), correlated with the absorption spectrum of DNA, indicating that DNA is the sensitive target molecule for the inactivation of microorganisms in space (Fig. 15). This is due to the direct generation of bipyrimidine lesions by the absorption of photons and excitation. The primary DNA photoproducts in vegetative cells are cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone photoproducts that are formed between adjacent pyrimidine residues on the same DNA strand (35). In bacterial spores, UV radiation predominantly generates another bipyrimidine, 5,6-dihydro-5(α-thyminyl) thymine, the so-called spore photoproduct (SP) (174, 256). The preponderance of SP in DNA of UV-irradiated spores can be explained by different factors, including the dehydrated state of the spore core, the presence of large amounts of dipicolinic acid, and the binding of small, acid-soluble spore proteins (SASPs) of the α/β type to DNA (185). Binding of α/β-type SASPs to the DNA of spores, together with spore core dehydration, induces a change in the helical conformation of spore DNA from the B form to an A-like form, which in turn alters its UV photochemistry to favor the production of SP (58, 232). SP is extremely efficiently repaired during germination of spores, through an SP-specific repair pathway using the SP lyase (180, 214, 236).

Even extreme UV (EUV) (10 to 190 nm) efficiently killed microorganisms in space, as determined for cells of *B. subtilis* sp. strain PD3D and *D. radiodurans* after a short exposure to EUV at 30 nm during a rocket flight (228). In this EUV range, the spectral efficiency for inactivation sharply increases with

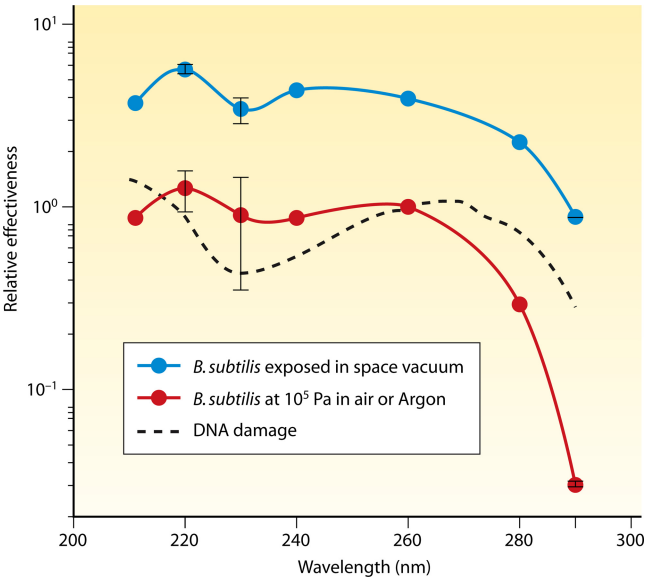

FIG. 15. Action spectra of inactivation of spores of *B. subtilis* HA 101 by extraterrestrial solar UV radiation, exposed to space vacuum (blue circles) or to 10^5 Pa in air or argon (red circles). The data are mean values from experiments on SL1, D2, and EURECA. The spectra are normalized to 1 at $\lambda = 260$ nm at 10^5 Pa. For comparison, the action spectrum of DNA damage is shown (dashed line). (Modified from reference 115 with permission from Elsevier.)

increasing energy of the photons (99). Because this increase is not reflected in the absorption spectrum of the DNA bases in the absorption curve of the sugar-phosphate moiety of DNA (122), it has been concluded that DNA strand breaks may be responsible for killing microorganisms by EUV. In addition to direct interaction with the DNA, EUV radiation can affect the DNA indirectly by generating reactive oxygen species, which can induce single- and double-strand breaks in DNA (Fig. 4). Actually, an increase in DNA strand breaks was found with the pUC19 plasmid after irradiation with EUV in space simulation facilities (99, 260). However, in microorganisms, EUV might be absorbed largely by the outer layers, resulting in damage to the membrane proteins rather than to the DNA (228). The biological consequences of that damage need to be investigated further. DNA strand breaks as well as DNA protein cross-linking were also detected in cells of *D. radiodurans* and *Halorubrum chaoviator* (formerly referred to as *Haloarcula* sp. and Halo-G), in conidia of *Aspergillus ochraceus*, in spores of *B. subtilis*, and in the plasmid pBR322, which were exposed to the full spectrum of extraterrestrial solar UV (>110 nm or >170 nm) during various space missions (56, 107, 158). They were generated in addition to the well-known pyrimidine UV photoproducts (34, 106, 107). UV-induced DNA damage may accumulate during exposure to space, with eventually severe consequences on the integrity of the genome when resuming cell growth and replication.

(ii) Desiccation effects by space vacuum. Space vacuum (10^{-7} to 10^{-4} Pa) (Table 1) is another harmful factor affecting microbial integrity. If cells are not protected by internal or external substances, dehydration will cause severe damage to the cell components: lipid membranes may change from planar bilayers to cylindrical bilayers and carbohydrates, proteins, and

nucleic acids may undergo amino-carbonyl reactions (Maillard reactions) that result in cross-linking and, finally, polymerization of the biomolecules (45). These structural changes can give rise to functional changes, such as inhibited or altered enzyme activity, changes in membrane permeability, and alteration of genetic information. The latter change is especially dramatic because it may lead to cell death or mutagenesis. In highly desiccation-resistant bacterial spores, the water content in the spore core is naturally reduced to 25 to 45% of their wet weight. As a consequence, proteins are immobile and enzymes are inactive during the spore phase (231).

Microorganisms exposed to space in vented compartments but shielded against solar UV radiation were studied to determine the effects of space vacuum. Although a variety of microbes are known to be desiccation resistant, not many were able to cope with the mechanical stress of the high vacuum of space. For example, cells of the desiccation-resistant bacterium *Deinococcus radiodurans* were killed after a 9-month exposure to space vacuum during the EURECA mission (56). In the same experimental series, conidia of *Aspergillus niger* and of *Aspergillus ochraceus* defied space vacuum and exhibited survival rates of about 30% and 5%, respectively. Spores of *B. subtilis* wild-type and repair-deficient strains dried in monolayers and exposed to space vacuum for 10 days showed 70% and 50% survival, respectively (95). After nearly 6 years in space vacuum—the record of space exposure reached so far—1 to 2% of *B. subtilis* spores in a monolayer survived the long-term exposure to space vacuum (Fig. 14) (105). Survival was significantly increased if protecting substances, such as sugars, or buffer salts were added to the spores. Seventy percent to 90% of spores predried in multilayers in the presence of 5% glucose survived the 6-year exposure to space vacuum on board the LDEF (Fig. 14) (95, 105).

The following mechanisms have been suggested for the observed increased survival of spores in space vacuum when exposed in the presence of protecting substances: (i) additives bind additional water molecules, thereby preventing complete desiccation of the cell (e.g., carbohydrates); (ii) additives replace water molecules, thereby stabilizing the structure of macromolecules (e.g., polyalcohols); and (iii) additives cover the spores by a layer less permeable to water, thereby building up a microclimate of higher water pressure beneath this layer (summarized in reference 95). During the LDEF mission, the combination of the three mechanisms may have led to the high survival of spores in dry multilayers in the presence of glucose.

The mutagenicity of space vacuum was first detected during the SL1 mission, when after the exposure of spores of *B. subtilis* to space vacuum the frequency of histidine revertants increased by a factor of 10 (106). This mutagenic effect by space vacuum, which has since been confirmed in other space and laboratory experiments, is probably based on a unique molecular signature of tandem double-base changes at restricted sites in the DNA (181). This was confirmed in studies with spores of *B. subtilis* strain TKJ6312 with double DNA repair defects (*uvrA10 spl-1*). Concerning mutations to nalidixic acid resistance, the majority of mutations induced by exposure to high vacuum belonged to one particular allele, *gyrA12*, carrying a tandem base change, 5′-CA to 5′-TT, at codon 84 of the *gyrA* gene, coding for DNA gyrase subunit A. Munakata et al. (181) reported that this allele has never been found among more than 500 mutants obtained by various treatments unless they were exposed to vacuum.

Vacuum-induced mutagenesis indicates that the DNA in spores is one of the critical molecules sensitive to exposure to vacuum. Increasing loss of water due to vacuum exposure leads to partial denaturation of the DNA (64). The consequences are DNA strand breaks, which have been identified in cells of *D. radiodurans* and Halo-G, now identified as *Halorubrum chaoviatoris* (159), as well as in spores of *B. subtilis*, after exposure to space vacuum (55, 56, 158). Spores of the triple mutant repair-deficient strain TKJ 8431 (*uvrA10 ssp-1 recA1*) of *B. subtilis*, which are deficient in recombination repair, were the most sensitive specimens under conditions of space vacuum (115). Using space simulation facilities, Moeller et al. (170) showed that NHEJ is a highly efficient repair pathway for DNA DSBs induced in spores of *B. subtilis* by high vacuum. They hypothesize that NHEJ is a key strategy used during spore germination to repair DSBs caused by ultra-high-vacuum-induced extreme desiccation, as well as by other extreme factors, such as UV and ionizing radiation, encountered during prolonged exposure to the harsh environment of space. These results indicate that forced dehydration of DNA in the microenvironment of the spore core might cause unique damage, with mutagenic and finally lethal consequences. Spore survival ultimately depends on the efficiency of DNA repair after rehydration and germination.

The high level of resistance of bacterial endospores to space vacuum and desiccation at large is due mainly to a dehydrated core enclosed in a thick protective envelope, the cortex and the spore coat layers, and the protection of their DNA by small proteins whose binding greatly alters the chemical and enzymatic reactivity of the DNA (185). However, the strategies by which *B. subtilis* spores protect their integrity, including that of the DNA, against vacuum damage are not yet fully understood. Nonreducing sugars, such as trehalose or sucrose, generally help to prevent damage to DNA, membranes, and proteins by replacing the water molecules during the desiccation process and thereby preserving the three-dimensional structure of the biomolecules. Although bacterial spores do not naturally accumulate these substances, the addition of glucose to the spores substantially increased the survival rate of spores in space vacuum.

(iii) Interaction of space vacuum and solar extraterrestrial UV radiation in microorganisms. When spores of *B. subtilis* were simultaneously exposed to solar UV radiation and space vacuum, they responded with a 5- to 10-fold increased sensitivity to a broad spectrum of solar UV (>170 nm), as well as to selected wavelengths, compared to UV irradiation in air or argon at atmospheric pressure (10^5 Pa) (Fig. 15). Upon dehydration, for example, in space vacuum, DNA undergoes substantial conformational changes, such as reversible denaturation (64). This conversion in the physical structure leads to altered DNA photochemistry. The photoproducts generated within the DNA of *B. subtilis* spores exposed to UV radiation in vacuum were different from those induced in wet spores. The predominant photoproduct induced by UV radiation in spores at atmospheric pressure is the dipyrimidine 5,6-dihydro-5(α-thyminyl)thymine, the so-called spore photoproduct (256). In spores that were UV irradiated in vacuum, two additional

thymine decomposition products, namely, the *cis-syn* and *trans-syn* cyclobutane thymine dimers, as well as DNA-protein cross-linking, were found (106, 107, 150) in addition to the spore photoproduct. The presence of *trans-syn* dimers is another hint to partially denatured DNA by vacuum (64), because they can only be formed if one thymine has rotated 180° with respect to an adjacent one. The predominant induction of 5,6-dihydro-5(α-thyminyl)thymine by UV (254 nm) in spores as well as dry samples of DNA was recently confirmed using high-performance liquid chromatography–mass spectrometry (57, 58, 171, 174). The application of this highly accurate technique for the analysis of photoproducts in the DNA of UV-irradiated spores in vacuum is currently under investigation (R. Moeller, personal communication). So far, the data suggest an altered conformation of the DNA of spores in space vacuum leading to different photoproducts.

Likelihood of interplanetary transport of microorganisms by natural processes. In 1903, Svante Arrhenius published his article "Die Verbreitung des Lebens im Weltenraum" ("the Distribution of Life in Space") in *Die Umschau*, thereby providing a scientific rationale for the theory of panspermia (4). The theory, now called radiopanspermia (184a), postulates that microscopic forms of life, for example, spores, can be propagated in space, driven by the radiation pressure from the sun, thereby seeding life from one planet to another or even between planets of different solar systems. Arrhenius based his considerations on the fact that the space between the planets of our solar system is teeming with micrometer-sized cosmic dust particles, which at a critical size below 1.5 μm would be blown away from the sun with high speed propagated by radiation pressure of the sun. However, because its effectiveness decreases with increasing size of the particle, this mechanism holds for very tiny particles only, such as single bacterial spores. In the end, panspermia was heavily criticized because it does not answer the question of the origin of life but merely places it on another celestial body without explanation. It was also criticized because it cannot be tested experimentally. Furthermore, it was suggested that single spores will not survive the physical forces imposed on them in space (191). As a result, panspermia fell into oblivion.

The concept of panspermia was revived when technology provided the opportunity to study the survival of bacterial spores ín the harsh environment of space. As described in "Outer space as a test bed for assessing limits for survival of microorganisms," it was found that isolated spores of *B. subtilis* were killed by several orders of magnitude if exposed to the full space environment for a mere few seconds. These results clearly negate the original panspermia hypothesis, which requires single spores as space travelers accelerated by the radiation pressure of the sun, requiring many years to travel between the planets.

The recent discovery of about 40 Martian meteorites on Earth (73; The Mars Meteorite Compendium [http://curator.jsc.nasa.gov/antmet/mmc/index.cfm]) provides evidence that rocks can be transferred naturally between the terrestrial planets. This was already envisaged in 1871 by both Hermann von Helmholtz and Lord Kelvin, who favored a version of panspermia in which fragments of extraterrestrial rocks carrying microbes as blind passengers within their cracks transported life from one planet to the other (184a, 249). However, during Lord Kelvin's lifetime, no mechanism was known to accelerate rocks to escape velocities in order to leave their planet of origin; as a result, Kelvin's idea was discarded.

We now know that Martian meteorites were ejected from Mars as a result of impacts of km-sized asteroids or comets. Petrographic studies of their shock metamorphism and numerical simulations of the impact-induced ejection of Martian rocks beyond the escape velocity for Mars demonstrated that a launch window for Martian meteorites exists between about 5 and 55 GPa, with postshock temperatures ranging from about 100°C to 600°C (5, 73). Although such impacts are very energetic, a certain fraction of ejecta are not heated above 100°C. These low-temperature fragments are ejected from the so-called spall zone, i.e., the surface layer of the target, where the resulting shock is considerably reduced by superimposition of the reflected shock wave on the direct shock wave (164). Estimates suggest that in the history of the solar system, more than a billion rock fragments were ejected from Mars at temperatures not exceeding 100°C, of which about 5% may have arrived on Earth (167). Hence, the 40 Martian meteorites so far detected on Earth represent only an infinitesimal fraction of those imports from Mars to Earth. The recognition of large numbers of microorganisms inhabiting the Earth's crust (14, 62, 193) provides support for the impact-driven, rock-mediated scenario of panspermia, now termed "lithopanspermia" (103).

The scenario of lithopanspermia involves three basic hypothetical steps: (i) the escape process, i.e., removal to space of biological material, with survival of being lifted from the surface to high altitudes and reaching escape velocities; (ii) an interim state in space, i.e., survival of the biological material over time scales comparable to interplanetary passage; and (iii) the entry process, i.e., nondestructive deposition of the biological material on another planet (103, 185). All three steps of lithopanspermia are now accessible to experimental testing with microorganisms, either in space experiments or using ground-based simulation facilities.

To tackle the question of whether endolithic microorganisms can survive the harsh conditions of a meteorite impact and ejection event, hypervelocity impacts were simulated in shock recovery experiments with a high-explosive setup (103, 104, 172, 239) or by accelerating microbe-laden projectiles by use of a rifle or gas gun (31, 32, 161). In systematic shock recovery experiments, pressure ranges of 5 to 50 GPa that mimicked those observed in Martian meteorites were applied on dry layers of microorganisms (spores of *Bacillus subtilis*, cells of the endolithic cyanobacterium *Chroococcidiopsis*, and the lichen *Xanthoria elegans*) that were sandwiched between discs of Martian analogue rock. Through such simulated hypervelocity impact processes, microorganisms are subjected to a complex matrix of physical stress parameters, including (i) defined extreme shock pressures of 5 to 50 GPa; (ii) peak shock temperature increases of up to about 1,000°C, lasting for nanoseconds; (iii) shock temperature increases of up to 200°C, lasting for fractions of μs; (iv) postshock temperature increases of up to 300°C, lasting for several seconds to a few minutes; and finally, (v) mechanical stress by friction and/or crushing. The magnitude of the temperature depends not only on the preshock temperature but also on the mineralogical composition, porosity, and water content of the host rock of the sample. Hypervelocity treatment of spores of *B. subtilis* resulted in a

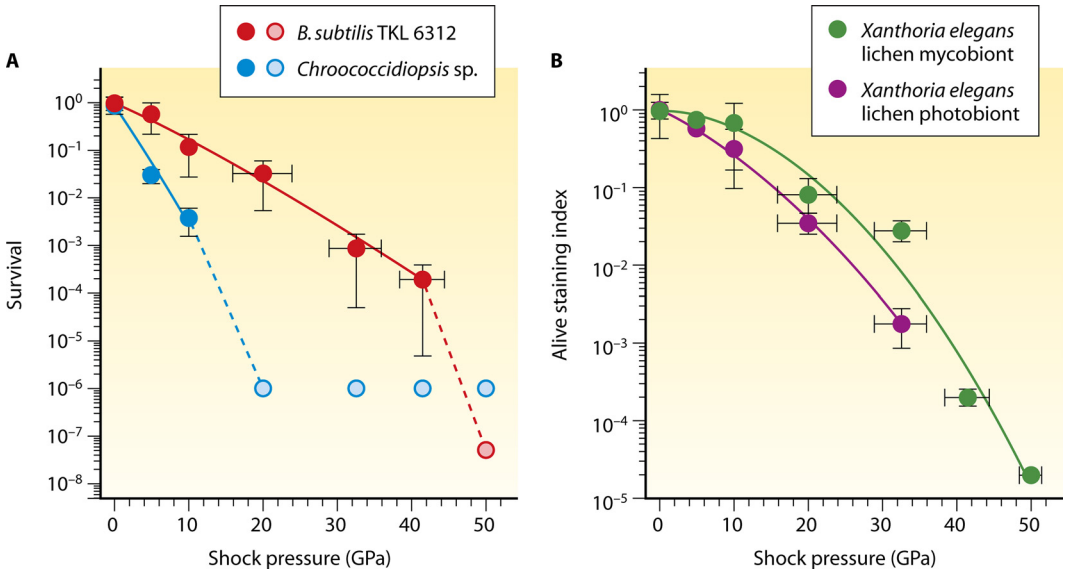

FIG. 16. Survival as a function of applied shock pressure during shock recovery experiments with spores of *B. subtilis* TKL 6312 and cells of *Chroococcidiopsis* sp. (A) and vitality of *Xanthoria elegans* lichen mycobionts and photobionts encased in gabbro rock plates (B). Open circles indicate survival below the threshold of detection. (Modified from reference 103 with permission of the publisher.)

nearly exponential survival curve (Fig. 16). Its slope decreased with decreasing preshock temperature, indicating that in addition to the potential mechanical stress exerted by the shock pressure, the accompanying high peak temperatures were a critical stress parameter for spores as well. In addition, applied pressure induced sporulation-defective mutants with a high frequency (up to 9%), in a linear manner (172). Spores of *B. subtilis* strains defective in major SASPs (α/β-type SASPs) that largely protect the spore DNA and of strains deficient in NHEJ DNA repair were significantly more sensitive to the applied shock pressure than were wild-type spores. These results suggest that DNA may be the predominant sensitive target of spores exposed to ultrahigh shock pressures (172). From pressure survival curves for the different organisms tested (Fig. 16), a vital launch window for the transport of rock-colonizing microorganisms from a Mars-like planet was inferred that encompasses shock pressures in the range of 5 to about 40 GPa for the bacterial endospores and lichens, with a more limited one for endolithic cyanobacteria (from 5 to 10 GPa). These vital launch windows for bacterial spores are large enough to support the concept of viable impact ejections from Mars-like planets (103, 239), although the limited dispersal filter of 5 to 10 GPa for cyanobacteria reveals possible barriers to cross-inoculation of photosynthesis between planets (40).

Transfer of viable microbes from one planet to another requires microorganisms to survive not only the escape process but also the journey through space within the time scales experienced by the Martian meteorites, i.e., between 1 and 20 million years. However, a small percentage of meteorites could travel within a few months or years between Earth and Mars or vice versa, as model calculations have shown (164a). To survive the journey in space, microorganisms must be shielded from solar UV radiation. This can be achieved by being covered with layers of dust of various thicknesses. Using ESA's Biopan facility for 2-week space experiments, it was demonstrated that mixing spores of *B. subtilis* with clay, rock, or meteorite powder increased their survival by 3 to 4 orders of magnitude compared to those without any additive (110). Embedding the spores in an "artificial meteorite," i.e., a sphere of 1 cm in diameter composed of clay or red sandstone, resulted in up to 100% survival (Fig. 14). However, it is questionable whether the UV shielding by dust demonstrated in the Biopan experiments holds for longer exposure times to space. Three-month exposure studies on board the *MIR* station showed only a slight increase in survival, by about 1 order of magnitude, if the spores were mixed with powder of clay or various meteorites (220). However, if completely shielded from solar extraterrestrial UV radiation, spores of *B. subtilis* can survive for years in space, as shown with the Exostack experiment aboard the nearly 6-year LDEF mission (Fig. 14) (105).

Finally, if not sufficiently shielded by meteorite material, microbes may be affected by the ionizing components of GCR. In particular, the HZE particles of galactic cosmic radiation cause the most damage to biological systems. However, because of their low flux (e.g., 1 Fe ion per μm^2 per 100,000 to 1 million years, which is at the lower time limit for Martian meteorites to travel to the Earth) (80), damage is localized, and few microorganisms suffer hits within time scales of an interplanetary journey. Additionally, radiation emanating from within a rock, from decay of the elements making up the minerals (e.g., potassium), would result in severe cell damage and death over the course of millions of years. Taking into account the probability of survival with respect to the complex mixture of hazards in space, that is, radiation damage, DNA decay by hydrolysis, and vacuum exposure, Mileikowsky et al. (167) have shown in a quantitative study that natural transfer of viable microbes from Mars to Earth and vice versa via Martian rocks of at least 1 m is a highly probable process that could have occurred many times during the history of our solar system. However, single bacterial spores, as suggested by the

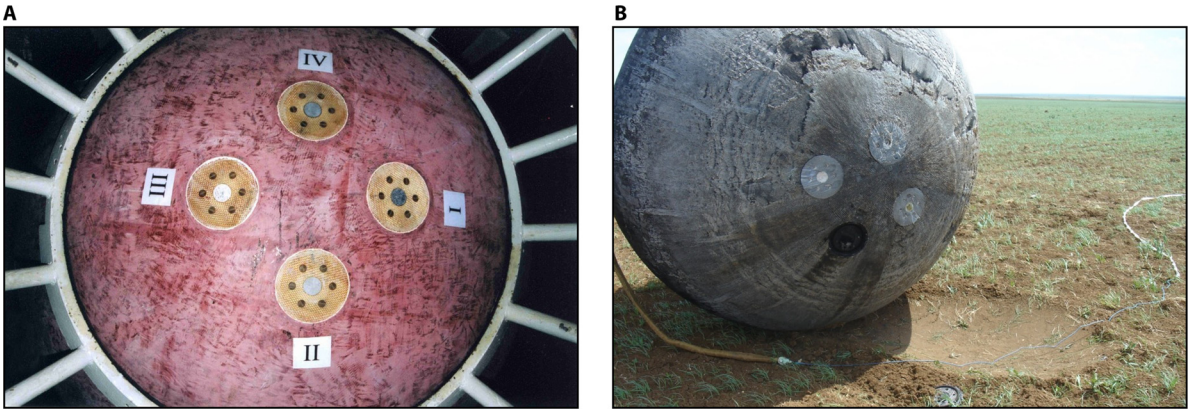

FIG. 17. STONE facility mounted on the stagnation point of the *Foton* reentry capsule, before launch (A) and after reeentry and landing (B). (Courtesy of ESA.)

original panspermia hypothesis (4), would be killed within a few minutes by the energetic solar UV radiation (110). Model calculations have shown that the transport of viable microorganisms via ejecta between planets of different solar systems seems to be very unlikely, even if one assumes that microbes can survive in space for tens of millions of years (253). However, if we consider that a stellar system forms within a star cluster, as assumed for our solar system, then one cannot rule out the possibility of transfer of life between the sister systems (253).

When captured by a planet with an atmosphere, most meteorites are subjected to very high temperatures during landing. Because the entire reentry process takes from only a few seconds to up to 1 minute, the outermost layers form a fusion crust, such that the heat does not reach the inner parts of the meteorite. Hence, except for a few mm or cm of ablation crust at the surface, the interior of the meteorite is not heated significantly above its in-space temperature (discussed in reference 63). To study the mineralogical changes in the rocks, the stability of enclosed microfossils, and the survival of endolithic microorganisms during the reentry process, ESA developed the STONE facility, which is attached to the heat shield of a Russian *Foton* satellite (Fig. 17) (23, 24, 42, 262). The objective was to simulate meteorite entry into the Earth's atmosphere. In igneous or sedimentary rocks of 2 cm in thickness or less, holes were drilled on the back side and loaded with microorganisms (spores of *B. subtilis* and the fungus *Ulocladium atrum* and cells of the radiation-resistant and desiccation-resistant endolithic cyanobacterium *Chroococcidiopsis* sp.) (24, 42). Additionally, the lichen *Rhizocarpon geographicum*, on its natural granite habitat, was mounted in the STONE facility, facing the backside. The *Foton* capsule with the STONE facility entered Earth's atmosphere with a velocity of 7.7 km/s, a velocity below the 12 to 20 km/s of medium-sized meteoroids. During the entry process, the samples experienced temperatures that were high enough to melt silica and basalt. These temperatures caused a fusion crust to develop on the samples. None of the biological samples survived this atmospheric entry (262). It has been argued that the 2-cm rock coverage was not thick enough to protect the microorganisms or that hot gases released during ablation pervaded the space between the sample and the sample holder, leading to intense local heating. This assumption was confirmed by surface melting observed at the nonexposed surfaces of the rock samples (24). In order to perform a more realistic simulation of the reentry of meteorites carrying endolithic microorganisms, the STONE facility needs to be modified so that larger rock samples can be accommodated.

In another approach to simulate hypervelocity entry of meteoroids from space, sounding rockets have been used, with granite samples permeated with spores of *B. subtilis* WN511 attached to various sites on the rocket surface (63). In this case, the entry velocity was 1.2 km/s and the temperature reached 145°C, a temperature far below that of an actual situation of meteoroid entry. One to 4% spore survivors were isolated from all surfaces, except from the forward-facing surface. It is interesting that among the survivors, about 4% developed spore-defective mutants, a phenomenon also observed after hypervelocity impacts acting on spores of *B. subtilis* (172).

Another example of a "safe entry" of microorganisms was reported after the tragic accident of the *Columbia* space shuttle STS-107, which disintegrated during reentry at an altitude of 61.2 km. *Columbia* accommodated a microbiological experiment using surface-adhering populations of *E. coli* ATCC 23848, *Chromobacterium violaceum* ATCC 12472, and *Pseudomonas aeruginosa* PAO1. None of these microorganisms survived the crash. However, a slow-growing, heat-resistant bacterium, identified as *Microbispora* sp., was recovered from the experiment hardware. It was assumed that *Microbispora* sp. was an environmental contaminant of the payload prior to launch (162). Another example is the nematode *Caenorhabditis elegans*, which was grown onboard STS-107 (the space shuttle *Columbia*). During the massive recovery effort, live organisms were recovered (241). These data demonstrate that animals can survive a relatively unprotected reentry into the Earth's atmosphere, which has implications with regard to the packaging of living material during space flight, planetary protection, and the interplanetary transfer of life.

Applied Aspects

Bioproduction of pharmaceutical compounds on orbit. A spaceflight biological research platform offers the potential for commercial applications beyond the previously described basic

studies, which were aimed at characterizing how gravity influences cellular phenomena. The focus in this case is not so much an attempt to elucidate how microgravity alters normal biological responses but rather to address applied research, with an emphasis on how microgravity can be used to manipulate processes at the molecular or cellular level to improve a desired outcome. One of the most distinctive attributes of bioprocessing in space may lie in the ability to keep cells suspended in a fluid medium without imparting the significant shear forces that often accompany stirred terrestrial systems (133). Space-based pharmaceutical research introduces an opportunity to improve our understanding of how bioprocesses occur by removing the ever-present influence of gravity from a cell and its surrounding environment. This unique research environment opens new horizons for exploring unconventional bioprocessing techniques, perhaps initially for gaining knowledge to be applied in terrestrial production facilities but also for future visions of space-based products with sufficient value added to warrant commercially viable, on-orbit production.

(i) Secondary metabolite production. Premised on basic research showing that space flight is generally conducive to bacterial growth, it was hypothesized that secondary metabolite production of commercial interest might likewise be enhanced. In the first of a series of experiments aimed at characterizing this response, Lam et al. (143) showed that monorden production by the fungus *Humicola fuscoatra* was increased when it was cultured on two different solid agar media in space. Interestingly, the increase was attributed to enhanced specific productivity, as fungal biomasses were not significantly different between the flight and ground cultures. A follow-up spaceflight experiment similarly showed specific productivity of actinomycin D by *Streptomyces plicatus* in suspension to be increased as well (142). In a related endeavor, Brown et al. (27) found that *E. coli* not only reached higher cell concentrations in space (and under clinorotation) but also did so without consuming more glucose, suggesting that a more efficient nutrient utilization process accompanied the gains in growth. These promising studies culminated in an experiment conducted using an automated fed-batch reactor placed onboard the ISS, which corroborated the findings that actinomycin D production by *Streptomyces plicatus* increased during the first 2 weeks of the mission. Samples subsequently taken over the remainder of the 72-day incubation, however, gave reversed results, with ground controls outperforming the flight cultures (18). These disparate findings suggest that longer-term studies in space may be needed to fully characterize how microorganisms ultimately behave over extended periods.

Another antibiotic production study, conducted using kanglemycin C (K-C), an immunosuppressant isolated from the culture broth of *Nocardia mediterranei* var. *kanglensis* 1747-64, was performed in 2002 aboard an unmanned Chinese vehicle, *Shenzhou III*. A variety of outcomes were reported, ranging from an observed yield increase of up to 12.5 ± 0.2 μg/ml to altered morphology and culture characteristics of the postflight mutant strain F-210 (268).

Additional ground-based research aimed at evaluating secondary metabolite production has been conducted using the RWV bioreactor microgravity simulation technique. Fang et al. (66) examined the effect of simulated microgravity on production of rapamycin by *Streptomyces hygroscopicus* and reported decreased dry cell weight, with growth occurring in pellet form, and inhibition of rapamycin production. The addition of beads decreased pelleting and increased dry cell weight without affecting rapamycin production, and interestingly, a proportionally greater quantity of rapamycin was extracellularly localized in the simulated microgravity environment, both with and without beads, than under normal gravity conditions (65, 76, 85).

(ii) Vaccine development. A series of commercially sponsored vaccine development experiments has been performed based on altered microbial virulence in spaceflight. These opportunities, now being carried out aboard the ISS, are part of the National Lab Pathfinder (NLP) missions. The goal of the NLP projects is to develop a vaccine against diarrhea-causing strains of *Salmonella*, for which no vaccine is currently available. The study is conducted by launching *S. enterica* and *Caenorhabditis elegans* worms in isolated containment, after which they are serially mixed, grown, and fixed in flight. More information is available on the following NASA websites: http://www.nasa.gov/mission_pages/station/science/experiments/NLP-Vaccine-1A.html, http://www.nasa.gov/mission_pages/station/science/experiments/NLP-Vaccine-1B.html, http://www.nasa.gov/mission_pages/station/science/experiments/NLP-Vaccine-2.html, and http://www.nasa.gov/mission_pages/station/science/experiments/NLP-Vaccine-3.html.

These promising pilot studies suggest potential beneficial commercial applications of enhanced antibiotic production efficiency and novel vaccine development from microbial space research; however, conclusive results from this research are not yet available.

Microbial virulence and drug resistance in space. Beyond the outcomes described above indicating that bacteria generally tend to fare well in space in terms of reduced lag phase, increased population growth, and potentially enhanced secondary metabolite production, other experiments further suggest that the effectiveness of antibiotics against microorganisms may be reduced and microbial virulence may be increased (186, 250). These factors, combined with the potential for immunosuppression suspected to occur in astronauts due to various spaceflight stressors, present increasing health-related concerns as human space missions become extended in duration and, more importantly, in distance from Earth (134).

(i) Microbial drug resistance. Leys et al. (146) reported that significantly greater concentrations of various antibiotics were needed to inhibit *in vitro* bacterial growth in space. Explanations for these observations, however, are still not fully known. Two somewhat competing hypotheses can be posed to address this observed outcome. Either bacterial resistance is increased in space or overall drug efficacy and/or uptake rate is reduced. Both scenarios can plausibly result in visible growth occurring in space (or a simulated space environment) on media containing an antibiotic MIC determined empirically under $1 \times g$ conditions.

Recent investigations have turned toward gene expression analysis in an attempt to characterize the responses of cells challenged by antibiotics in space and in clinostat analog facilities (2). The RpoS pathway is one primary regulator of *E. coli* and *Salmonella* stress responses. Wilson et al. (266) compared stress responses of wild-type and *rpoS* mutant strains. Since both strains were affected similarly, they concluded that the

stress response was *rpoS* independent under simulated microgravity. Resistance to acid stress, thermal stress, and osmotic stress and the ability to survive within macrophages were also reported to be increased under the simulated space conditions, while resistance to oxidative stress was decreased. It was suggested that the stress response pathway appears to be compensatory by allowing the *rpoS* mutant to exhibit responses similar to those of the wild type that are not observed in normal $1 \times g$ conditions. Although growth in the simulated space environment was not reported to have an effect on *rpoS* regulon gene expression, a number of other altered gene expression patterns were indicated.

Lynch et al. (155) also reported that *E. coli* cells grown under conditions of simulated microgravity were more resistant to hyperosmotic and acid stress, during both the exponential and stationary growth phases, than their matched $1 \times g$ controls. For both shock types, cells grown under clinorotation had a survival rate of approximately 50%, compared to an almost complete loss of viability in normal gravity. During the exponential growth phase, changes were found to be *rpoS* independent, leading to the conclusion that the increased stress response was due to changes in a previously undiscovered general stress response pathway. In stationary phase, however, the stress response was found to be dependent on *rpoS*, as wild-type strains exhibited greater resistance than *rpoS* mutants. Stationary-phase cells grown under clinorotation had greater resistance than cells in normal gravity, leading to the formation of superresistant cells. Transcriptional and translational characteristics of *rpoS* were also examined. Interestingly, the σ^s protein concentration in clinorotated cells was found to be 30% lower during exponential phase and 100% higher during stationary phase. Meanwhile, mRNA copy numbers were similar between the clinostat and normal gravity conditions for all growth phases. In exponential phase, mRNA stability was not affected, so the transcriptional rate was also unaffected. mRNA stability was increased in stationary phase, with a twofold difference noted between the clinostat samples and $1 \times g$ controls, so the transcription rate in stationary phase was apparently reduced under simulated spaceflight conditions. It was concluded that transcriptional regulation did not account for the differences in sigma protein concentration. Furthermore, it was found that protein stability was decreased under clinorotation during exponential growth, with a slight decrease persisting into the stationary phase. To account for the protein concentration differences, translational rate and efficiency were suggested to be increased in the clinostat compared to those in normal gravity. These observed differences provide insight toward identifying underlying gravity-dependent mechanisms associated with microbial responses to environmental stresses.

While increased drug resistance by the cell has generally been assumed for microgravity, at least one report has suggested that environmental conditions of space flight may also affect the stability of pharmaceuticals (59), and another study indicated that no clear residual drug resistance remained in bacterial cultures tested postflight (125). These findings, along with the reduced extracellular mass transfer factors in microgravity described earlier, warrant additional research into whether observations of bacteria growing under what are normally minimal inhibitory conditions (MIC) with an antibiotic in space flight have a physiological or physical (i.e., environmental) basis or some combination of the two.

(ii) Virulence and pathogenicity. In addition to the possibility that bacteria may become more difficult to treat with antibiotics in space (or simulated microgravity analogs), there is a growing body of evidence that they may also become more pathogenic. Nickerson et al. (188) showed increased virulence of *S. enterica* serovar Typhimurium following growth in simulated microgravity by infecting mice with cultures grown under simulated microgravity conditions and comparing them to mice infected with cultures grown under $1 \times g$ conditions. The simulated space cultures were also found to be more resistant to acidic conditions, suggesting the possibility of enhanced survival in the gastrointestinal tract. Bacterial protein expression was altered during growth, as determined by gel electrophoresis.

Another contributing factor was suggested by Poudrier (203), who reported that bacteria tended to adhere to human cells better in simulated microgravity than under normal $1 \times g$ conditions. Furthermore, gene and protein expression was found to be altered for the experimental group; in particular, an adhesion protein and a protein conveying resistance to trimethoprim-sulfamethoxazole were both upregulated.

While no definitive correlation has been established regarding how spaceflight induces these various altered genetic expression findings, Nickerson et al. (187) have proposed that a global regulatory signal may affect gene expression, physiological responses, and pathogenesis. Wilson et al. (265) have shown that spaceflight-induced increases in *Salmonella* virulence are regulated by the medium ion composition and that phosphate ions are sufficient to alter related pathogenesis responses in a spaceflight analogue model. Although it remains unclear exactly how bacteria sense spaceflight-associated changes to their growth environment and how these changes translate into altered phenotypes relevant to infection, the identification of evolutionarily conserved regulatory paradigms in these studies and other related phenomena describing common responses to space and simulated microgravity environments, along with potential mechanisms exhibiting global influences across microbial species, collectively provide an intriguing foundation that ongoing research continues to explore.

A recent 2008 space shuttle experiment conducted on STS-123, the Microbial Drug Resistance Virulence (MDRV) Study (http://www.nasa.gov/mission_pages/station/science/experiments/MDRV.html), supported four independent investigator teams (those of Niesel, McGinnis, Pyle, and Nickerson) in an effort to characterize gene expression and virulence potential of four model microorganisms: *Salmonella enterica* serovar Typhimurium, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Saccharomyces cerevisiae.* Wilson et al. (263) proposed that *hfq* may have a role as a global regulator in responding to spaceflight in altering bacterial gene expression and virulence. Spaceflight-induced alterations in *S. enterica* serovar Typhimurium virulence were characterized for cultures grown in nutrient-rich media and compared to growth in nutrient-limited media in order to examine physiological and virulence responses to varying nutritional status. In addition to reproducing earlier findings, Wilson et al. (265) also tested a new hypothesis, that modulating the ion concentration may counteract or inhibit the spaceflight-associated pathogenic responses in microorganisms. Bacterial gene expression of specific virulence factors for

S. pneumoniae and *P. aeruginosa* was similarly characterized under defined sets of experimental conditions for comparison across the different experiments. Resistance of *S. cerevisiae* to the antifungal agent voriconazole was also examined. Complete results are pending.

Microorganisms in the spacecraft environment. While this review has thus far focused primarily on different categories of *in vitro* research conducted by exposing microorganisms to various aspects of the space environment, it is important to note that their presence will inevitably accompany any mission with humans onboard; hence, it is critical to extend consideration of altered growth and behavior toward controlling undesired microbial outcomes within the overall spacecraft environment and toward their potential impact on crew health. As is true on Earth, however, microbes not only present cause for health and contamination concern but also may serve as a means for providing beneficial bioprocesses. In fact, many life support functions required in a human-occupied spacecraft can be performed by microorganisms (e.g., waste degradation, water recovery, and even food and oxygen production). Therefore, in addition to the potential detrimental crew health and environmental contamination concerns that microbes can present, long-term missions will ultimately demand that biologically based systems be incorporated into the spacecraft by design to enable regenerative, sustainable life support functions. This becomes increasingly likely and necessary as spacefarers move toward self-sufficiency, with decreased dependence on consumable resupply missions from Earth.

(i) Microflora composition and evolution. As observed under controlled experimental conditions, microbial behavior is affected in numerous ways as a consequence of exposure to spaceflight. The same holds true for microbes that inevitably accompany humans living in a closed spacecraft environment, both from the microflora consortia carried by the crew and from the resultant microbial contaminant presence in the air and water as well as on exposed surfaces inside the vehicle. In addition to matters involving crew health, microbial contaminants can also have adverse effects on avionics and spacecraft systems. Biofilm formation in space has been demonstrated under controlled *in vitro* conditions (163), and the potential for material damage to spacecraft components to occur as a consequence has been noted (189).

(ii) Crew health. The main concern regarding crew health is characterized by activation of opportunistic pathogens that have been noted to accumulate in the closed environments of long-duration spacecraft (119). As described earlier, *in vitro* studies suggest that the microbes may even become more pathogenic and also resistant to antibiotic treatment (134) as a result of the environmental conditions experienced during space flight. Further complicating the situation, spaceflight also appears to negatively impact the immune system (227), although conflicting reports indicate that there is a great deal of variation between individuals and missions; therefore, these effects are still not well understood, and better methodologies are needed to fully characterize the dynamics of immune response in flight (19). The risk of infectious disease occurrence increases with longer mission durations.

Other factors, such as living and working in relatively crowded conditions along with use of reclaimed water and air, contribute to this risk. Health concerns associated with permanently crewed outposts on the Moon or a Mars exploration mission may be even greater given the additional unknown variables associated with these environments. Finally, limitations in diagnostic and treatment technologies further increase the consequences of compromised immunity, and as missions extend in distance from Earth, crew return in the event of emergency is no longer a feasible option (113, 199).

Better collection of data *in vivo* during the flight without affecting the desired measured outcome is needed in order to fully understand the immune response process. To do so requires both identification of the appropriate biomarker(s) to measure and a device capable of conducting the analysis onboard the vehicle. Candidate markers such as cytokines, catecholamines, and other hormones can offer insight into immune system functionality; however, no single set of markers appears to provide sufficient information to characterize the overall immune system response, and no single space-qualified biosensor exists with multianalyte capability to fully characterize all possibilities (3). An alternate, RNA-based monitoring approach has been suggested by Larios-Sanz et al. (144). Using 16S rRNA-targeted molecular beacons, specific bacterial groupings were shown to be detectable with a methodology that lends itself to in-flight monitoring.

Finally, accepting that infection will occur at some point in a long-duration mission, regardless of risk mitigation measures taken, effective therapy for treatment must also be considered. A range of pharmaceutical agents intended to suppress the emergence of antibiotic-resistant pathogens is presented by Taylor and Sommer (247) to define on-board pharmacy needs and treatment protocols applicable to space flight. Insight such as this is a necessary component to the development of an integrated crew health care system for exploration missions that move beyond low Earth orbit.

(iii) The spacecraft environment. A variety of studies have been performed in an attempt to characterize, monitor, and control microbial contamination in the spacecraft such that a balanced state is achieved between deleterious, negligible, and desired effects (37, 141, 189, 199). An extensive database of environmental microbiological parameters has been cataloged for short-duration flights from over 100 space shuttle missions. Similarly, the joint NASA-*MIR* Program in the late 1990s provided data for long-duration missions. Interestingly, the major bacterial and fungal species found in the space shuttle are similar to those encountered after 15 years of operation in the *MIR* space station (199). Also of interest, the microbial loading on *MIR* was not found to progress linearly, but rather by a process of alternation between microflora activation and stabilization phases (189).

The focus for the ISS has been on establishing preventative measures through regular housekeeping practices, including visual inspections and microbiological monitoring. Novikova et al. (190) provided a survey of microbiological contamination found in potable water and air and on surfaces inside the ISS, including the presence of several opportunistic pathogens and strains involved in the biodegradation of structural materials.

In an environmentally closed, isolated system such as long-duration space stations, spacecraft, or planetary habitats, the potential for biofilms forming on all types of material must also be addressed. Compounding the general *in vitro* antibiotic resistance increases reported for space, the formation of biofilm

is also known, in and of itself, to increase resistance to antibiotics 10- to 1,000-fold over that of planktonic bacteria (156). Interestingly, some of the hypothesized mechanisms pertaining to biofilm resistance, such as the heterogeneous environment resulting from gradients of nutrients and waste in the community, are similar to those believed to cause spaceflight-related changes in antibiotic effectiveness. Morse and Jackson (177) described the potential for resistant strains to develop in a spacecraft water reclamation system as a result.

Similar to the needs for detecting crew health concerns, microbial monitoring is also needed to ascertain the dynamic microflora resident throughout the spacecraft. La Duc et al. (141) targeted several biomarkers, such as ATP, LPS, and DNA (ribosomal or spore specific), to quantify the total bioburden and specific types of microbial contamination on the surfaces inside the spacecraft and in drinking water reservoirs aboard the ISS. *Bacillus* species were found to be dominant among the spore formers by use of culture-dependent techniques. In contrast, culture-independent, rapid enumeration techniques revealed the presence of many Gram-positive and Gram-negative microorganisms, including actinomycetes and fungi. The presence of both cultivable and noncultivable microbes was further confirmed by DNA-based detection techniques. Although the ISS potable water was not found to contain cultivable microbes, molecular techniques did retrieve DNA sequences of numerous opportunistic pathogens.

Roman et al. (224) similarly investigated a flex hose assembly used to cool spacesuits and containing nonpotable aqueous coolant and iodinated water from the ISS. Fluid chemistry and pH changes were found to accompany an increase in planktonic microorganisms from <100 CFU per 100 ml to approximately 1 million CFU per 100 ml. A stable microbial population was noted as a precursor to biofilms forming on wetted materials in the system, including stainless steel, titanium, ethylene propylene rubber, and epoxy resins. Although not found here, biofilm formation in this context would impede coolant flow, reduce heat transfer, amplify degradation of system materials initiated by chemical corrosion, and enhance mineral scale formation. Preventative care may include the use of antimicrobials in fluid transport systems such as this.

The accumulated data offer evidence that control of microbial levels throughout the spacecraft is needed to maintain a desired sanitary, microbiological optimum to prevent the possibility of material biodestruction (189). Ott et al. (195) further described the presence of bacteria, fungi, and other organisms living in liquid condensate found trapped behind instrument panels aboard the *MIR* space station. These free-floating masses pose both crew and spacecraft system hazards. In a full circle of analysis, Baker and Leff (7, 8) examined the effects of simulated microgravity on bacterial cultures obtained from *MIR* and the ISS after return to Earth. Various growth responses were summarized, and the results were interpreted as indicating that selection in a microgravity, oligotrophic environment leads to bacteria that are better suited to the microgravity/ISS environmental conditions. Should this conclusion be found valid, additional complications will be introduced regarding the need to understand evolutionary pressures exerted on microorganisms by the spaceflight (or spacecraft) environment.

(iv) Biologically based life support systems. A final note is made regarding the beneficial use of microorganisms and other biological systems to provide regenerable life support functions for long-term space habitation. As missions move toward longer durations and greater distances, bioregenerative processes that mimic the Earth's natural biosphere offer an increasing potential for mass and cost savings compared to the need for periodic consumable resupply. While plants enable a primary means of reclaiming oxygen from carbon dioxide, purifying water through transpiration filtering, and providing edible biomass, bacteria, fungi, and cyanobacteria are also candidates for performing a variety of processes, including vitamin production, water recycling, air decontamination, and waste management (91, 175, 222). Many challenges exist in creating a stable mix of biological agents capable of providing sustainable, controlled life support functions in a closed system (184). Use of a lunar outpost as an operational test bed is one possibility for validating the inclusion and verifying the performance of a biologically based life support system intended for a future Mars habitat. Considerable work remains to bring such a system to fruition, and microorganisms will certainly play a role in the final design.

Outlook and Future Directions

With international plans being formulated for solar system exploration, either using robotic probes or with human crews, microbiologists are confronted with exciting new opportunities and challenging demands. The search for signatures of life forms on another planet or moon in our solar system is one of the most prominent goals of these enterprises. Our neighbor planet Mars and Jupiter's moon Europa are considered key targets for the search for life beyond Earth. By analogy, with terrestrial extremophilic microbial communities, e.g., those thriving in arid, cold, salty environments and/or those exposed to intense UV radiation, additional potential extraterrestrial habitats may be identified. Also, sulfur-rich subsurface areas for studying chemoautotrophic communities, rocks for endolithic communities, permafrost regions, hydrothermal vents, and soil or evaporite crusts are all of interest. Field studies with microbial communities in those extreme environments as well as microbiological studies under simulated planetary environments—in space as well as in the laboratory—will provide valuable information for preparing the correct "search-for-life" experiments on missions to those solar system bodies.

Another important role of microbiologists in space exploration concerns the planetary protection initiative. Spacecraft, whether robotic orbiters, entry probes, or landers, can unintentionally introduce terrestrial microorganisms to the planet or moon of concern. This may destroy the opportunity to examine these bodies in their pristine condition. To prevent the undesirable introduction and possible proliferation of terrestrial microorganisms on the target body, the concept of planetary protection has been introduced (COSPAR Planetary Protection Guidelines [http://cosparhq.cnes.fr/Scistr/PPPolicy%2820-July-08%29.pdf]). Depending on the target and type of mission, the planetary protection guidelines require cleaning and, in specific cases, sterilization of the spacecraft or components to avoid contamination with terrestrial organisms. The success of the cleaning and/or sterilization measures needs to

be controlled by establishing a thorough inventory of the bioload prior to launch. The development of guidelines for bioload measurements, sterilization procedures, and planetary protection control represent additional upcoming paramount tasks for microbiologists.

The presence of humans on the surface of the Moon or Mars will substantially increase the capabilities of space research and exploration; however, prior to any human exploratory mission, the critical microbial issues concerning human health and well-being need to be addressed, and effective planetary protection protocols must be established. Provision of metabolic consumables and removal of waste by-products from the closed, self-contained environment, whether constituting a human habitat or a cell culture bioreactor, represent the final necessities for life support. The closed cabin or habitat conditions also present added long-term challenges to their design with regard to crew health, due to the potential build-up of contaminants in the atmosphere and water systems and of biofilms on the surfaces of internal structures. Finally, in some cases, the life support functions themselves can be met by use of living systems acting through a variety of ecological pathways. In this sense, the living systems become an increasingly integral part of the spacecraft or habitat itself; therefore, analysis of space microbiological experiments should be done with a broad, systems-level point of view, taking into account the interaction between biological phenomena and physical influences associated with the overall environment both within and external to the space habitat.

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Continued next page

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