4 Viable Transfer of Microorganisms in the Solar System and Beyond

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It is now generally accepted that at the beginning of our solar system a considerable amount of organic molecules and water were imported to the early Earth as well as to the other terrestrial planets via asteroids and comets [1-3]. The period of heavy bombardment lasted until approximately 3.8 billion years (Ga) ago. These impactors would on the one hand have been delivering the volatiles as precursors of life, on the other hand, if sufficiently large and fast, would have eroded the atmosphere and perhaps sterilized the Earth and/or Mars, if life existed there [4, 5].

Impactors of sizes larger than 1 km lead to the ejection of a considerable amount of soil and rocks that are thrown up at high velocities, some fraction reaching escape velocity [6]. These ejecta leave the planet and orbit around the Sun, usually for time scales of a few hundred thousand or several million years until they either impact another celestial body or are expelled out of the solar system [7]. Meteorites of lunar and some of Martian origin detected within in the last decades are witnesses of these processes [8, 9]. The question arises whether such rock or soil ejecta could also be the vehicle for life to leave its planet of origin, or, in other words, whether spreading of life in the solar system via natural transfer of viable microbes is a feasible process.
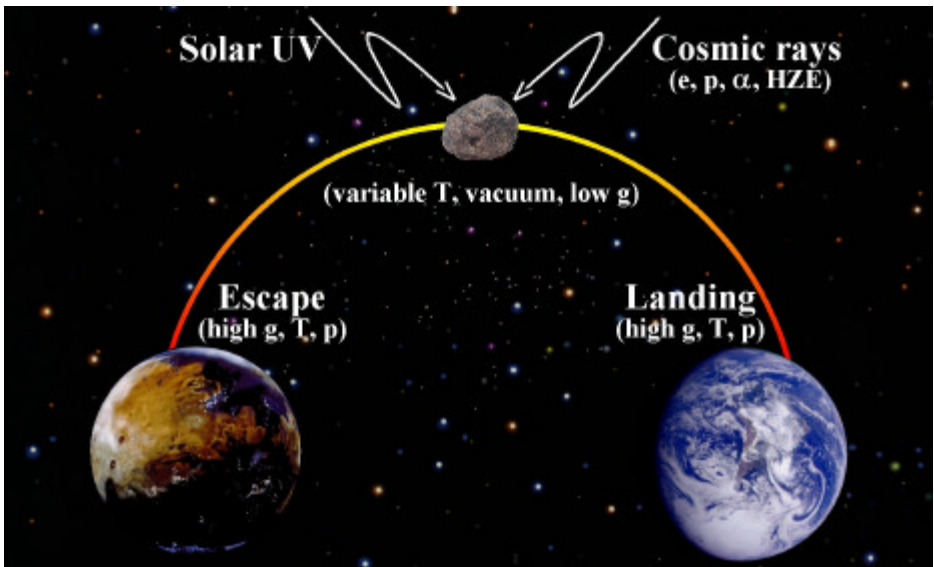
4.1 Scenario of Interplanetary Transfer of Life Within the Solar System

The supposition that life can be naturally transferred from one planet to another or even between solar systems goes back to the last century [10] and was formulated as hypothesis of Panspermia by S. Arrhenius in 1903 [11]. It postulates that microscopic forms of life, for example spores, can be dispersed in space by the radiation pressure from the Sun thereby seeding life from one planet to another, or one solar system to another, respectively. This hypothesis has been subjected to several criticisms with arguments, such as it cannot be experimentally tested and spores will not survive long-time exposure to the hostile environment of space, especially vacuum and radiation (reviewed in [12]). It has also been pointed out that Panspermia only shunts aside the question of the origin of life to another celestial body (see Chap. 1, Ehrenfreund and Menten).

However, a variety of recent discoveries have shed new light on the likelihood of viable transfer in space such as (i) the detection of meteorites, some of lunar and some

of Martian origin [9]; (ii) the detection of organics and the still highly debated suppo-
sition of microbial fossils in one of the Martian meteorites [13]; (iii) the probability of
small particles of diameters between 0.5 μm and 1 cm [14], or even boulder-sized
rocks reaching escape velocities by the impact of large comets or asteroids on a planet,
e.g., on Earth [6] or Mars [15, 16]; (iv) the ability of bacterial spores to survive to a
certain extent the shock waves of such a simulated impact [17]; (v) the high UV-
resistance of microorganisms at the low temperatures of deep space, tested at tem-
peratures down to 10 K [18]; (vi) the reported survival of bacterial spores over mil-
lions of years, if enclosed in amber or salt stocks [19, 20], or in space over periods
extending up to 6 years [21]; (vii) the paleogeochemical evidence of a very early ap-
pearance of life on Earth in the form of metabolically advanced microbial prokaryotic
ecosystems leaving not more than approximately 0.4 Ga for the evolution of life from
the simple precursor molecules to the level of a prokaryotic, photoautotrophic cell
[22]; (viii) the biochemical evidence of a common ancestor for all life forms on Earth
[23].

   Viable transfer from one planet to another requires that life, probably of microbial
nature, survives the following three steps: (i) the escape process, i.e. ejection into
space, e.g., caused by a large impact on the parent planet; (ii) the journey through space,
i.e. time scales in space comparable with those experienced by the Martian meteorites
(approximately 1-15 Ma); and (iii) the landing process, i.e. non-destructive deposition
of the biological material on another planet (Fig. 4.1).

Fig. 4.1 Scenario of an interplanetary transfer of life in the solar system

4.2   Survival of the Escape Process

The most plausible process capable of ejecting microbe-bearing surface material from a planet or moon into space is the hypervelocity impact of a large object such as an asteroid or comet, under strong or moderate shock metamorphism of the ejected rock fragments. The peak shock pressure estimates for the presently studied 15 Martian meteorites range from about 20 GPa to about 45 GPa and estimates of associated post-shock temperature range from about 100 °C at 20 GPa to about 600 °C at 45 GPa [24]. Although these impacts are very energetic, a certain fraction of ejecta is 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 one [25]. Estimates suggest that within the last 4 Ga, more than 10^9 fragments of a diameter of ≥ 2 m and temperatures ≤ 100 °C were ejected from Mars of which about 5% arrived on Earth after a journey in space of ≤ 8 Ma (Fig. 4.2). The corresponding numbers for a transfer from Earth to Mars are about 10^8 fragments ejected from the Earth with about 0.1% arriving on Mars within 8 Ma [26]. During the preceding period of "heavy bombardment" even 10 times higher numbers are estimated. Hence, the 15 Martian meteorites, so far detected on Earth, represent probably only an infinitesimal fraction of those imported from Mars within Earth's history.
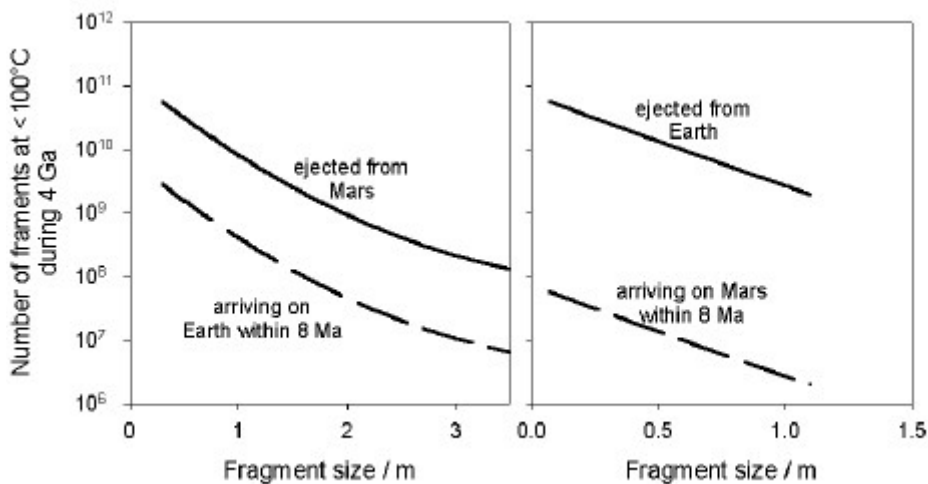
Fig. 4.2 Number of fragments ejected within the last 4 Ga at temperatures ≤ 100 °C from Mars or from the Earth and arriving within 8 Ma on Earth or on Mars (data based on calculations in [26]).

In experiments simulating impacts comparable to those experienced by the Martian meteorites, the survival of microbes was tested after subjecting spores of *Bacillus subtilis* to accelerations, jerks or shock waves. Accelerations as they occur during planetary ejections are apparently not a barrier to interplanetary transfer of life. Bacteria are routinely treated with even higher values of acceleration in normal microbiological separation techniques, although with much slower rise times. Ballistic experiments provide rise times equivalent to those estimated for an object receiving escape velocity during an impact. It has been shown that bacterial spores as well as cells of *Deinococcus radiodurans* survived gun shots with accelerations up to 4500 km s^{-2} (460 000 × g) with a rise time of <1 ms, which are equivalent or even higher than the acceleration and jerk values calculated for an object ejected from Mars [27, 28].

It is important to note that during a meteorite impact, the total duration of the pressure pulse is very short, i.e. on the order of 1 ms [26]. At impacts forming craters of a diameter of >10 km (as possibly required for the Martian meteorites studied) the pressure pulse can last up to 20 ms. In shock recovery experiments with an explosive set-up, the survival of spores of *B. subtilis* was studied after a shock treatment in the pressure range, which some Martian meteorites have experienced [17]. It was found that the a substantial fraction of spores (up to 10^{-4}) was able to survive a peak shock pressure of 32 ± 1 GPa and a post-shock temperature of about 250 °C. These data support the hypothesis that bacterial spores may survive an impact-induced escape process in a scenario of interplanetary transfer of life. Assuming a mean spore density of 10^8 spores/g in e.g., desert soil or rock, a 1 kg rock would accommodate approximately 10^{11} spores, of which up to 10^7 could survive even extremely high shock pressures occurring during a meteorite impact. At more moderate shock waves as they would occur in the spall zone of an impact, even substantial higher survival rates are expected than in these simulation studies at shock pressures of 32 GPa. To prove this, further survival studies using such moderate shock waves are required.

4.3   Survival of the Interplanetary Transfer Phase

4.3.1   Space Environment of Interest

Once rocks have been ejected from the surface of their home planet, microbial passengers have to cope with an entirely new set of problems affecting their survival, namely exposure to the space environment (reviewed in [29-32]). This environment is characterized by a high vacuum, an intense radiation of galactic and solar origin and extreme temperatures (Table 4.1.).

In interplanetary space, pressures down to 10^{-14} Pa prevail. Within the vicinity of a body, the pressure may significantly increase due to outgassing. In a low Earth orbit, pressure reaches values of 10^{-6} to 10^{-4} Pa. The major constituents of this environment are molecular oxygen and nitrogen as well as highly reactive oxygen and nitrogen atoms.

Table 4.1. The environment in Earth orbit and of interplanetary space (modified from [34])

Space parameter	Earth orbit (£500km)	Interplanetary space
Space vacuum		
Pressure (Pa)*)	10^{-6}-10^{-4}	10^{-14}
Residual gas (part/cm^{-3})	10^5 H, 2×10^6 He, 10^5 N, 3×10^7 O	1 H
Solar electromagnetic radiation		
Irradiance (W/m²)	1360	Different values[a]
Spectral range (nm)	Continuum from 2×10^{-12} to 10^2 m	Continuum from 2×10^{-12} to 10^2 m
Cosmic ionizing radiation		
Dose (Gy/a) *)	0.1-3000[b]	≤ 0.25[c]
Temperature (K)	100-400[a]	> 4[a]
Microgravity (*g*)	10^{-3}-10^{-6}	< 10^{-6}

*) 1 Pa = 10^{-5} bar, 1 Gy = 100 rad; [a] depending on orientation and distance to Sun; [b] depending on altitude and shielding, highest values at high altitudes and in the radiation belts; [c] depending on shielding.

The radiation environment of our solar system is governed by components of galactic and solar origin. The galactic cosmic radiation entering our solar system is composed of protons (85%), electrons, α-particles (14%) and heavy ions (1%) of charge Z>2, the so-called HZE particles (high charge Z and high energy E). The solar particle radiation, emitted in solar wind and during solar particle events, is composed of 90-95% protons, 5-10% α-particles and a relatively small number of heavier ions. In interplanetary space, the annual radiation dose amounts to $\le$0.25 Gy/a, depending on mass shielding with the highest dose at 30 g/cm² shielding due to built up secondary radiation. In the vicinity of the Earth, the radiation dose can increase due to the radiation belts where protons and electrons are trapped by the geomagnetic field.

The spectrum of solar electromagnetic radiation spans several orders of magnitude, from short wavelength X-rays to radio frequencies. At the distance of the Earth from the Sun (1 AU), solar irradiance amounts to 1360 W m^{-2}, the solar constant. Of this radiation, 45% is attributed to the infrared fraction, 48% to the visible fraction and only 7% to the ultraviolet range. The extraterrestrial solar spectral UV irradiance has been measured during several space missions, such as Spacelab 1 and EURECA [33].

The temperature of a body in space which is determined by the absorption and emission of energy, depends on its position with respect to the Sun and other orbiting bodies, and also on its surface, size, mass, and albedo (reflectivity). In Earth orbit, the energy sources include solar radiation (1360 W m^{-2}), the Earth's albedo (480 W m^{-2}) and terrestrial radiation (230 W m^{-2}). When orbiting a planet, an object can be shaded from the Sun as it passes on the planet's night side. Therefore, the temperature of a

body in space can reach both extremely high and low values. In experiments in Earth orbit, temperatures between 240 K and 320 K were measured.

4.3.2   Approaches to Studying the Biological Effects of Space

In order to study the survival of resistant microbial forms in the upper atmosphere or in free space, microbial samples have been exposed *in situ* by use of balloons, rockets or space crafts and their responses were investigated after recovery (reviewed in [29, 31]). For this purpose, several facilities were developed such as the Exposure Device on Gemini, MEED (microbal ecology exposure device) on Apollo, ES029 on Spacelab 1, ERA (exobiology radiative Assembly) on EURECA, UV-RAD on Spacelab D2, BIOPAN on FOTON, and EXPOSE for the International Space Station (ISS) [30, 34] (see also Fig. 4.6). These investigations were supported by studies in the laboratory, in which certain parameters of space (high and ultrahigh vacuum, extreme temperature, UV-radiation of different wavelengths, ionizing radiation) were simulated. The microbial responses (physiological, genetic and biochemical changes) to selected factors applied separately or in combination were determined.

Many spore-forming bacteria are found in terrestrial soils and their spores have been recognized as the hardiest known forms of life on Earth. The developmental pathway from a vegetatively growing bacterial cell to a spore, i.e. the dormant state, is triggered by depletion of nutrients in the bacterial cell's environment [35]. In the dormant stage, spores undergo no detectable metabolism and exhibit a high degree of resistance to inactivation by various physical insults such as cycles of extreme heat and cold, extreme desiccation including vacuum, UV and ionizing radiation, as well as oxidizing agents or corrosive chemicals (recently reviewed by Nicholson et al. [32]). The high resistance of *Bacillus* endospores is mainly due to two factors: (i) a dehydrated, highly mineralized core enclosed in a thick protective envelop, the cortex and the spore coat layers (Fig. 4.3), and (ii) the saturation of their DNA with small, acid-
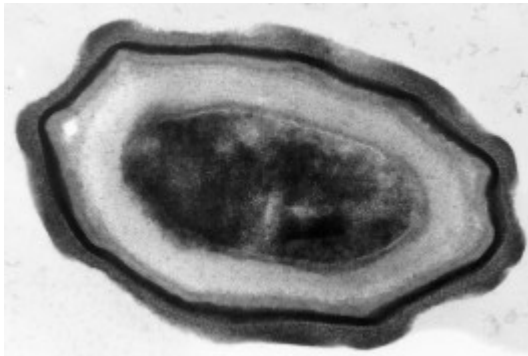
Fig. 4.3 Electonmicrograph of a spore of *B. subtilis* with the inner core containing the DNA surrounded by protective layers, the long axis of the spore is 1.2 μm, the core area 0.25 μm² (courtesy of S. Pankratz).

soluble proteins whose binding greatly alters the chemical and enzymatic reactivity of the DNA [36]. In the presence of appropriate nutrients, spores respond rapidly by germination and outgrowth, resuming vegetative growth. Hence, spore formation represents a strategy, by which a bacterium escapes temporally and/or spatially from unfavorable conditions: spores exhibit incredible longevity and can be relocated e.g., by wind and water, to remote areas. Among the bacterial spores, the endospores of the genus *Bacillus* are the best investigated ones [32].

In addition, a variety of microorganisms exist that are adapted to grow or survive in extreme conditions of our biosphere. Some of them may be suitable candidates for studies on microorganisms in space. Examples are endo- or epilithic communities, consisting of cyanobacteria, algae, fungi and/or lichens, which represent a simple microbial ecosystem living inside or on rocks [37, 38], or osmophilic microbial assemblages that are trapped in evaporite deposits [39, 40], or extremely radiation-resistant microorganisms, like bacteria of the species *D. radiodurans* as the most radiation-resistant bacteria known to exist on Earth today. Although *D. radiodurans* is non-sporulating, it can go into a kind of dormancy under certain environmental adverse conditions such as lack of food, desiccation or low temperatures [41, 42].

4.3.3   Biological Effects of the Vacuum of Space

Because of its extremely dehydrating effect, space vacuum has been considered to be one of the factors that may prevent interplanetary transfer of life [43]. However, space experiments have shown that up to 70% of bacterial and fungal spores survive short-term (e.g., 10 days) exposure to space vacuum, even without any protection [29]. The chances of survival in space are increased, if the spores are embedded in chemical protectants such as sugars, or salt crystals, or if they are exposed in thick layers. For example, 30% of *B. subtilis* spores survived nearly 6 years of exposure to space vacuum, if embedded in salt crystals, whereas approximately 70% survived in the presence of glucose [21] (Table 4.2.). Sugars and polyalcohols stabilize the structure of

Table 4.2. Survival of spores of *B. subtilis* after exposure to space vacuum (10^{-6} to 10^{-4} Pa) during different space missions.

Mission	Duration of vacuum exposure	Survival fraction at end of exposure in thin layers (%)		Survival fraction at end of exposure in thick layers and presence of protective sugars (%)		References
		in space	ground control	in space	ground control	
SL 1	10 d	69.3 ± 15.8	85.3 ± 2.6	n.d.	n.d.	[29]
EURECA	327 d	32.1 ± 16.3	32.7 ± 5.6	45.5 ± 0.01	62.7 ± 8.2	[44]
LDEF	2 107 d	1.4 ± 0.8	5.4 ± 2.9	67.2 ± 10.2	77.0 ± 6.0	[21,29]
n.d. = not determined						

the cellular macromolecules during vacuum-induced dehydration, leading to increased rates of survival.

To determine the protective effects of different meteorite materials, "artificial meteorites" were constructed by embedding *B. subtilis* spores in clay, meteorite dust or simulated Martian soil [44] and exposing them to the space environment. Crystalline salt provided sufficient protection for osmophilic microbes in the vegetative state to survive at least 2 weeks in space [40]. For example, a species of the cyanobacterium *Synechococcus* that inhabits gypsum-halite crystals was capable of nitrogen and carbon fixation and about 5% of a species of the extreme halophile *Haloarcula* survived after exposure to the space environment for 2 weeks in connection with a FOTON space flight.

The mechanisms of damage due to vacuum exposure are based on the extreme desiccation. If not protected by internal or external substances, cells in a vacuum experience dramatic changes in lipids, carbohydrates, proteins and nucleic acids. Upon desiccation the lipid membranes of cells undergo dramatic phase changes from planar bilayers to cylindrical bilayers [45]. The carbohydrates, proteins and nucleic acids undergo so-called Maillard reactions, i.e. amino-carbonyl-reactions, to give products that become cross-linked eventually leading to irreversible polymerization of the biomolecules [45]. Concomitant with these structural changes are functional changes, including altered selective membrane permeability, inhibited or altered enzyme activity, decreased energy production, alteration of genetic information, etc.

Vacuum-induced damage to the DNA is especially dramatic, because it may be lethal or mutagenic. The mutagenic potential of space vacuum was first demonstrated during the Spacelab I mission in spores of *B. subtilis*, which showed an up to tenfold increased mutation rate over the spontaneous rate [46]. This vacuum-induced mutagenicity is accompanied by a unique molecular signature of tandem-double base changes at restricted sites in the DNA [47]. In addition, DNA strand breaks have been observed to be induced by exposure to space vacuum [48, 49]. Such damage would accumulate during long-term exposure to space vacuum, because DNA repair is not active during this state of anhydrobiosis. Survival ultimately depends on the efficiency of the repair systems after germination.

4.3.4   Biological Effects of Galactic Cosmic Radiation

If not sufficiently shielded by meteorite material, microbes may be affected by the ionizing components of radiation in space. Especially the heavy primaries of galactic cosmic radiation, the so-called HZE particles, are the most biologically effective species (reviewed in [50, 51]). Because of their low flux (they contribute to approximately 1% of the flux of particulate radiation in space), methods have been developed to localize precisely the trajectory of an HZE particle relative to the biological object and to correlate the physical data of the particle relative to the observed biological effects along its path. In the Biostack method visual track detectors are sandwiched between layers of biological objects in a resting state, e.g., *B. subtilis* spores [52] (Fig. 4.4). This method allows (i) to localize each HZE particle's trajectory in relation to the biological specimens; (ii) to investigate the responses of each biological individual hit separately, in regard to its radiation effects; (iii) to measure the impact parameter b (i.e.

the distance between the particle track and the sensitive target); (iv) to determine the physical parameters [charge (Z), energy (E) and linear energy transfer (LET)]; and finally (v) to correlate the biological effects with each HZE particle parameters.

The small size of bacterial spores (their cytoplasmic core has a geometrical cross section of 0.2 to 0.3 μm^2) requires to develop special techniques in connection with the Biostack method. In this case, spores in monolayers are directly mounted on the track detector. After exposure, the track detector with the spores is etched under microscopical control on the spore-free side only and spores located around a track of an HZE particle (at a radius of ≤ 5 μm) are removed by micromanipulation and incubated each individually in special incubation chambers [53] (Fig. 4.4). Using this microscopical method, the accuracy in determining the impact parameter was ≥ 0.2 μm, depending on the dip angle of the trajectory. Figure 4.4 shows the frequency of inactivated spores as a function of the impact parameter b. Spores within b ≤ 0.2 μm were inactivated by 73%. The frequency of inactivated spores dropped abruptly at b >0.2 μm. However, 15-30% of spores located within $0.2 < b < 3.8$ μm were still inactivated. A statistical analysis showed that all data at b ≤ 3.8 μm are significantly different from the control value (at b >10 μm) (95% confidence) [54].
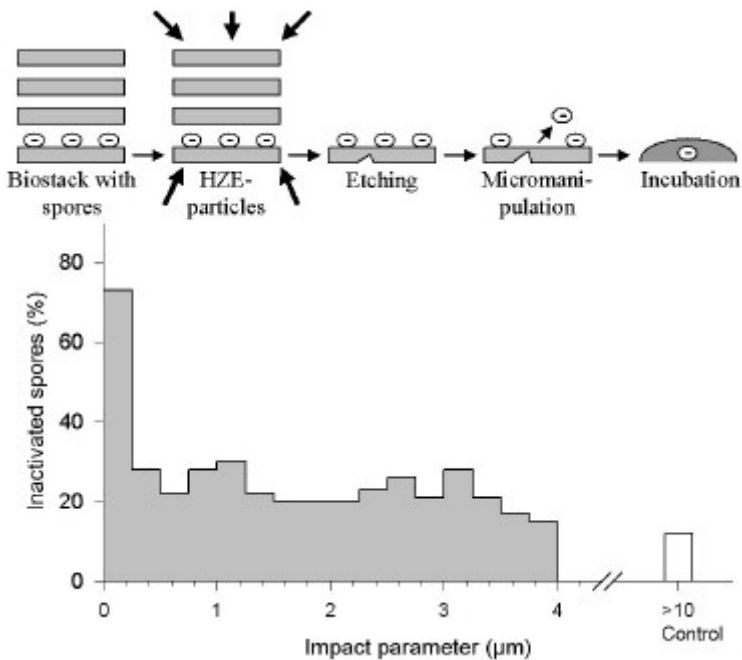
Fig. 4.4 Biostack method to localize the effect of single particles (HZE particles) of cosmic radiation (biological layers are sandwiched between track detectors) and results on the inactivation probability of spores of *B. subtilis* as a function of their distance from the particles trajectory, the impact parameter (data from space experiments [54]).

As shown above, *B. subtilis* spores can survive even a central hit of an HZE particle of cosmic radiation. Such HZE particles of cosmic radiation are conjectured to set the ultimate limit on the survival of spores in space, because they penetrate even thick shielding. However, since the flux of HZE particles is relatively low, it may last up to several hundred thousand to one million of years in space until a spore might be hit by an HZE particle (e.g., iron of LET >100 keV/μm).

With increasing shielding thickness, e.g., by the outer layers of the meteorite, the dose rate caused by cosmic radiation goes through a maximum, because the heavy ions interact with the shielding material and create secondary radiation. Based on experimental data from accelerator experiments with *B. subtilis* spores [55] and biophysical models, relating the structure of HZE tracks to the probability of inactivating the spores [56], an estimated density of the meteorite of 3 g/cm³ (taken from data on Martian meteorites) and a NASA model on an HZE transport code for cosmic radiation [57], the dose rates and probabilities for inactivating spores have been calculated behind different shielding thicknesses: the physical dose rates reach a maximum behind a shielding layer of about 10 cm (30 g/cm² shield thickness); behind 30 cm (90 g/cm²) the value is approximately the same as obtained without any shielding and only for higher shielding thicknesses the dose rate reduces significantly (Fig. 4.5) [26]. The calculations also show that even after 25 Ma in space, a substantial fraction of a spore population (10^{-6}) would survive the exposure to cosmic radiation if shielded by 2 to 3 m of meteorite material. The calculations are based on the assumption that the rock may accommodate about 10^8 spores/g, of which at least 100 spores/g would survive. The same surviving fraction would be reached after about 600 000 years without
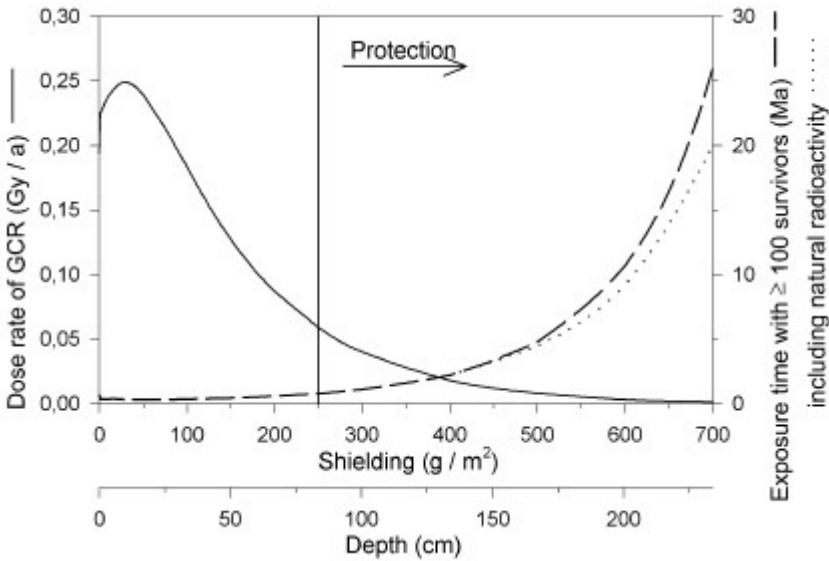
Fig. 4.5 Shielding of spores of *B. subtilis* against galactic cosmic radiation (GCR) by meteorite material and survival times ≥ 10^{-6} survivors) at different depths of the meteorite due to GCR (dashed line) or to GCR plus natural radioactivity of 0.8 mGy/a (dotted line) (data from [26]).

any shielding, after about 300 000 years behind 10 cm of shielding (maximum dose rate) and after about 1 Ma behind 1 m of shielding.

4.3.5   Biological Effects of Extraterrestrial Solar UV Radiation

Solar UV radiation has been found to be the most deleterious factor of space, as tested with dried preparations of viruses, and of bacterial and fungal spores [29]. The full spectrum of extraterrestrial UV radiation kills unprotected spores of *B. subtilis* within seconds [2] and is about thousand times more efficient than the UV radiation at the Earth's surface due to the effective protection of our biosphere by the stratospheric ozone layer [58, 59] (see also Chap. 14, Cockell; Chap. 15, Rettberg and Rothschild). The reason for this high biological efficiency of extraterrestrial UV is the highly energetic UV-C (190-280 nm) and vacuum UV region (<190 nm) that is directly absorbed by the genetic material of the cells, the DNA [44].

This high biological efficiency of extraterrestrial UV is even increased, if bacterial spores are simultaneously exposed to solar UV-radiation and space vacuum [29, 46]. Upon dehydration (e.g., in space vacuum) DNA undergoes substantial conformational changes. This conversion in the physical structure leads to an altered DNA photochemistry. The following photoproducts are generated within the DNA of *B. subtilis* spores exposed to UV radiation in vacuum: (i) two thymine decomposition products, namely the *cis-syn* and *trans-syn* cyclobutadithymine (Thy<>Thy); (ii) 5,6-dihydro-5(α-thyminyl) thymine (TDHT); (iii) DNA protein cross-linking [46, 60]. From the efficiency of repair processes (photoenzymatic repair, spore photoproduct specific repair) it is concluded that photoproducts other than *cis-syn* Thy<>Thy and TDHT seem to be responsible for the UV supersensitivity of spores, if irradiated under vacuum conditions [29].

However, one has to bear in mind that a few micrometers of meteorite material may be sufficient to protect the microorganisms enclosed against UV radiation. Therefore, microorganisms travelling through space inside a meteorite are probably not under a serious threat of being killed by solar UV radiation.

4.3.6   Bacterial Survival During Long-Term Dormancy

According to the Martian meteorites, so far detected, travelling times in space may span over several millions of years. Because of the enormous number of launched ejecta (Fig. 4.2), much shorter transfer times should be feasible. Simulations of Mars meteorite transfer show that some ejecta may arrive in tens of thousands of years and even time spans of less than a century are possible [61]. These relatively fast arriving meteorites are the most interesting ones with regard to viable transfer. The extreme environment of space obviously does not support active metabolism and growth of the microorganisms enclosed; however, a variety of organisms exist that are adapted to survive in extreme conditions when in the dormant state [62]. The question arises whether microorganisms in the dormant state, e.g., as bacterial spores, could survive the extreme conditions of space over extended periods of time.

Besides cosmic radiation and vacuum, threats to the stability of the DNA of spores inside meteorites may arise from various sources such as natural radioactivity of the meteorite, hydrolysis, chemicals, and temperature extremes. The natural radioactivity of the known Martian meteorites reaches values up to 0.8 mGy/a. It has been calculated by Mileikowsky et al. [26] that for smaller Martian ejecta (<2-3 m in diameter) the effects of cosmic radiation dominate over those of natural radioactivity. With increasing depth, however, in larger ejecta, the galactic cosmic radiation (GCR) dose rate decreases and finally the effects of natural radioactivity become more important than those of cosmic radiation (Fig. 4.5). The effects of natural radioactivity may be more serious for terrestrial ejecta, which on the average are about 5 times higher in natural radioactivity than Martian rocks [26].

So far, most data dealing with the instability and decay of DNA by hydrolysis, chemicals or temperature were obtained with moist biological systems at atmospheric pressure [63]. However, bacterial endospores are especially resistant to such stresses. Their cytoplasm is partially dehydrated and mineralized, causing enzymes to become inactive and the DNA stabilized [32]. Recent reports suggest that spores of the genus *Bacillus* can remain intact over millions of years, if preserved in amber [19] or in brine inclusions in salt crystals [20]. In these two latter studies, which report revitalization of bacterial spores in 25-40 Ma old amber [19] or even in 250 Ma old buried salt crystals [20], thorough sterilization procedures of the sample surface were applied to avoid contamination from contemporary microbes. However, the phylogenetic analysis revealed their very close relationship to contemporary species, which initiated a controversial discussion on the real age of the spores. Hence, several open questions have still to be solved, before assuming that bacterial spores are nearly immortal.

4.3.7   Combined Effects of the Complex Matrix of Space Parameters

During the Long Duration Exposure Facility (LDEF) mission, for the first time, spores of *B. subtilis* were exposed to the full environment of space, i.e. space vacuum, solar UV-radiation and most of the components of cosmic radiation, for an extended period of time, namely nearly 6 years, and their survival was determined after retrieval. The spores were exposed in multilayers and predried in the presence of glucose as chemical protectant. After retrieval, the spore samples had turned from white into yellow, a phenomenon, which is probably due to photochemical processes of the outer layers. However, in each sample thousands of spores survived the space journey, from an initial sample size of 10^8 spores [21]. One possible explanation is that all spores in the upper layers were completely inactivated by the high flux of solar UV-radiation, thereby forming a protective crust, which considerably attenuated the solar UV-radiation for the spores located beneath this layer. Therefore, the survivors probably originated from the innermost part of the samples. Of spores, covered by an aluminum foil ,which protected them against UV radiation, up to 70% survived the 6 years lasting space journey (Table 4.2.). These results are the first experimental proof that a very high percentage of spores survive at least 6 years in space, if efficiently shielded against solar UV radiation. The shielding could be achieved by the outer layers of rocks as used by those microbial communities that inhabit rocks. However, calculations show that most ejecta would require thousands or millions of years before reaching another

planet [6, 7, 14]. Therefore, 6 years seem to be by far too short for an interplanetary transfer of life.

## 4.4 Survival of the Landing Process

When captured by a planet with an atmosphere, most meteorites are subjected to very high temperatures during landing. However, because the fall through the atmosphere takes a few seconds only, the outermost layers form a kind of heat shield and the heat does not reach the inner parts of the meteorite. During entry, the fate of the meteorite strongly depends on its size: large meteorites may brake into pieces, however, these may be still large enough to remain cool inside until hitting the surface of the planet; medium sized meteorites may obtain a melted crust, whereas the inner part still remains cool; micrometeorites of a few μm in size may tumble through the atmosphere without being heated at all above 100 °C. Therefore, it is quite possible that a substantial number of microbes can survive the landing process on a planet. However, no experiments have been done so far to investigate the effects of the landing process experimentally. Recently, the European Space Agency (ESA) has developed a facility, called STONE which is attached to the heat shield of a FOTON satellite to test mineral degradations during landing [64]. This facility might be an ideal tool to study the effects of landing of bacterial spores embedded in an artificial meteorite.

4.5 Conclusions: On the Likelihood of Interplanetary Transfer of Life as a Mode of Distribution of Life Throughout the Solar System

Although it is difficult at present to prove definitely that life has been transported through our solar system, model calculations and experiments at simulation facilities and in space allow to estimate the chances of resistant microbial forms to survive the different steps of such a scenario. Experiments in space which were performed on free platforms since the Apollo era and more sophisticated on the external platform of Spacelab and on free flying satellites such as LDEF, EURECA, and FOTON, have given some insight into responses of bacterial spores and other extremophile microorganisms to the parameters of space. The most interesting results are summarized in the following: (i) extraterrestrial solar UV radiation is thousand times more efficient than UV at the surface of the Earth and kills within a few seconds 99% of *B. subtilis* spores; (ii) space vacuum increases the UV sensitivity of the spores; (iii) although spores survive extended periods of time in space vacuum (up to 6 years), genetic changes occur such as increased mutation rates; (iv) after 6 years in space, up to 70% of bacterial spores survive, if protected against solar UV radiation and dehydration; (v) spores could escape a hit of a cosmic HZE particle (e.g., iron ion) for up to 1 Ma. Calculations using radiative transfer models for cosmic rays and biological data from accelerator experiments have shown that a meteorite layer of 1 m or more effectively protects bacterial spores against galactic cosmic radiation.

The data obtained so far on the responses of resistant microorganisms to the environment of space support the supposition that space – although it is very hostile to terrestrial life – is not a barrier for cross-fertilization in the solar system. Ejection of microbes inside rocks and their transport through the solar system is a feasible process. If protected against solar UV and galactic cosmic radiation, spores may survive inside meteorites over extended periods of time.

However, several aspects justify continued research, e.g., on the survival rate during the ejection and landing process, on the shelf-life of spores in vacuum, as well as on the best mechanisms to effectively protect the microorganisms enclosed against the three steps of interplanetary transfer. Relevant studies to tackle these questions will be performed in future studies in space and at ground based facilities [34, 65, 66]. For future research on bacterial spores and other microorganisms in space, ESA is developing the EXPOSE facility that is to be accomodated outside of the ISS for 1.5 years (Fig. 4.6). EXPOSE will support long-term *in situ* studies of microbes in artificial meteorites, as well as of microbial communities from special ecological niches such as endolithic and endoevaporitic ecosystems [34]. These experiments on the Responses of Organisms to the Space Environment (ROSE) include the study of photobiological processes in simulated radiation climates of planets (e.g., early Earth, early and present Mars, and the role of the ozone layer in protecting the biosphere from harmful
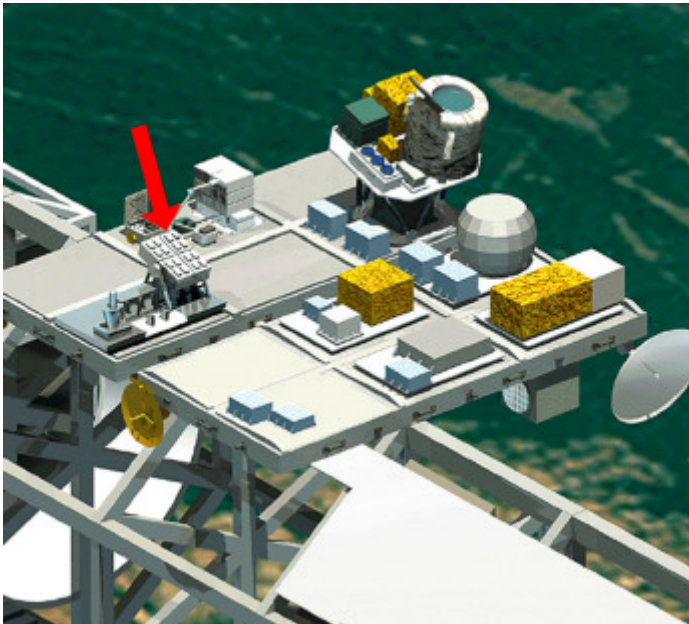
Fig. 4.6 EXPOSE facility to be mounted on the truss structure of the ISS to study the sensitivity of organics and microorganisms to space environment.

Table 4.3. Experiments of the ROSE consortium to study the responses of organisms to space environment on the EXPOSE facility of the ISS [34]

Code	Objective	Assay system
ENDO	Impact of ozone depletion on microbial primary producers from sites under the "ozone hole"	1. Endolithic microbial communities 2. Mats of cyanobacteria 3. Mats of algae
OSMO	Protection in evaporites against solar UV and anhydrobiosis	1. Synechococcus in and beneath gypsum-halite 2. Haloarcula (pigmented and non-pigmented) in and beneath NaCl 3. Haloarcula DNA in KCl
SPORES	Protection of spores by meteorite material against space (UV, vacuum, and ionizing radiation)	1. *B. subtilis* spores 2. Fungal spores 3. Lycopod spores All in or beneath meteorite material
PHOTO	Main photoproducts in dry DNA and DNA from dry spores	1. DNA 2. Bacterial spores
PUR	Sensitivity of the biologically effective UV radiation to ozone	1. T7 bacteriophage 2. Phage-DNA 3. Uracil
SUBTIL	Mutational spectra induced by space vacuum and solar UV	1. *B. subtilis* spores 2. Plasmid DNA

UV-B radiation), as well as studies of the probabilities and limitations for life to be distributed beyond its planet of origin (Table 4.3.). Here-to-fore, the results from the EXPOSE experiments will eventually provide clues to a better understanding of the processes regulating the interactions of life with its environment.

4.6   Outlook: On the Likelihood of Transport of Viable Microorganisms Between Solar Systems

Today more than 50 extrasolar planets have been detected, all of the size of Saturn or Jupiter (see Chap. 2, Udry and Mayor). The present detection methods – Doppler effect, astrometry and transit photometry - do not possess sufficient sensitivity to discover Earth-like planets nor giant planets, orbiting at distances larger than 3 AU from their sun. For that we have to wait for future planned space missions, e.g., Darwin of ESA (see Chap. 24, Foing) which are especially designed to detect Earth-like planets by searching for signatures indicative of a biosphere on a planet, such as atmospheric oxygen as a suggested biomarker for photosynthetic activity. Nevertheless, the information on the existence of extrasolar planets has corroborated the interest in the question whether life forms can be transported between the planets or moons of different solar systems.

In a follow-on study, Mileikowsky et al. [67] have estimated the likelihood of viable transfer of life from planets or moons of extrasolar planetary systems to the Earth where the source planet is located in either the general galactic star field or in a possible temporary "sibling" cluster born together with the Sun. To leave the planet, an impact ejection mechanism has been assumed similar as described in the studies of viable transfer within our solar system [26]. Because the information on the existence and frequency of Earth-like planets is still missing – and probably for many years to come -, they have based their calculations on specific estimations of the unknown circumstances as described below.

Major parameters of the calculations are (i) the star density and (ii) the relative speed between the emitting and receiving planetary system. In the case of the general galactic field, the present numerical density of stars in the solar neighborhood was used, namely 1 star per 10 $pc^3 = 0.1$ pc^{-3} (1pc = parsec = 3.26 light years). This is based on the generally held opinion of astronomers that the general star field during the youth of our solar system was probably quite similar to today's. The distribution of speeds of the stars in our galaxy is a very good Maxwellian approximation with about 20 km/s as the most common speed and 0.5 km/s being orders of magnitude less frequent. In the case of a possible cluster of "sibling" stars born together with our Sun, the cluster would last only limited time after its formation from the molecular cloud, before the stars disperse in all directions into the general galactic star field. However, the distances between the stars within the cluster would be much smaller than in the general galactic star field. Furthermore, the relative speed between the members of a cluster is less than 1 km/s which is much lower than the most frequent one between stars in the general galactic star field. To calculate the number n of hits on Earth by ejecta from extrasolar systems, the following formula was used [67]:

$$n \approx 2 \cdot 10^{-15} \; T \; R \; t \; \frac{N}{\boldsymbol{s}} \left(1 - e^{-\frac{\boldsymbol{n}_0^2}{2\boldsymbol{s}^2}} \right)$$

(4.1)

With N = $N_d \times f_{ps} \times f_{TP} \times f_{HZM} \times f_i$,

N_d = numerical density of stars in pc^{-3}; f_{ps} = fraction of stars with planetary systems; f_{TP} = fraction of planetary systems with terrestrial-like planets; f_{HZM} = fraction of terrestrial-like planets orbiting in zones habitable for microbes; f_i = fraction of other factors; T = time period studied comprising escape from a planet, expulsion into interstellar space, capture by our solar system, orbiting in our solar system, capture by the Earth, and hitting the Earth (Ma); t = time period for microbial survival inside ejecta in space (Ma); R = rate of expulsion from extrasolar systems into interstellar space (Ma^{-1}); $\boldsymbol{s}$ = dispersion in the Maxwellian distribution of the velocities of the stars (km/s); $v_0 = 0.5$ km/s.

For establishing the formula, it is not necessary to know either anything about the complete planetary configuration of the extrasolar systems or about the terrestrial-like planets. But, for each open entity, selected values have to be given. $N_d = 0.1$ pc^{-3} and $\boldsymbol{s}$ = 20 pc/Ma are the values known for our galaxy. For a possible temporary "sibling" cluster around our Sun, N_d and $\boldsymbol{s}$ are of course unknown; therefore, the estimations are based on observations of relatively new-born clusters such as Hyades. t is deter-

mined by biology as discussed in 4.3.4 and in [26]. However, several unknowns render the calculations difficult, such as (i) the fraction of stars in our galaxy with planetary systems; (ii) the fraction of those with habitable zones (see Chap. 3, Franck et al.); (iii) the fraction of those with Earth-like planets; and (iv) the fraction of those that have developed life (e.g., microorganisms, based on DNA/RNA/proteins with high resistance to the hostile environment of space). To overcome this problem, Mileikowsky et al. [67] have undertaken the following steps: (i) they have chosen for all fractions mentioned above the most favorable and overoptimistic value for viable transfer, namely 1; (ii) they have chosen a heavy bombardment in the early stage of the extrasolar system being orders of magnitude more intense than that in the early stage of our solar system; (iii) they have chosen very long survival times (e.g., 1-100 Ma) of microbes in ejecta in space. Table 4.4. shows that the transfer of ejecta from an extrasolar system to the Earth calculated by use of Eq. (4.1), is strongly enhanced by high densities, i.e. short distances (e.g., less than 1 pc) and/or by a very low relative speed between the emitting planetary system and the receiving system (e.g., less than 0.5 km/s) [67, 68].

In case of the conditions of the general galactic star field, n, the number of ejecta from all planetary systems of the galaxy, reaching the Earth within the early 500 Ma is utterly small, namely about 10^{-9}. Even if the bombardment by comets and asteroids would be thousand times more intense (given by term R), no ejecta would reach the Earth either, with n being about 10^{-5}. In the case of clusters of "sibling" stars, assuming the conditions as observed in the Hyades cluster, which has at its present age of 625 Ma $N = 2$ and $\sigma = 0.25$ km/s, n would be about 10^{-4}. Even assuming a 20 times higher star density than observed in Hyades, the number of extrasolar ejecta hitting the Earth is still far below 1. Hence, assuming impact ejecta as the mode of transportation of putative life from an extrasolar planet to the Earth, the data show that from the general galactic star field no life-bearing ejecta have reached the Earth within the first 05-0.6 Ga. If the sun was part of a group of stars in a "sibling" cluster, viable transfer from one of the sister systems cannot be completely ruled out, however, the probability remains very low.

The longest survival time assumed is $t=100$ Ma (Table 4.4.). Recently, revival of bacterial spores from a 250 Ma old salt deposit has been claimed [20]. However, as discussed above, this finding is still controversial: it has been argued that the spores may be the result of recent contamination, which have penetrated the salt through small invisible cracks. This objection is mainly based on the high genetic similarity of the revived spores with contemporary species of *Bacillus maremortis*, which live in the very saline Dead Sea. However, it can be deduced from Eq. (4.1) that the number of viable transfers from planets of the general galactic field to the Earth would remain low even on the base of a survival time in space of 250 Ma.

From this example calculated for the case of extrasolar ejecta reaching the Earth, it can be concluded that the probabilities are too low to allow transport of viable microorganisms from one solar system to another by impact ejecta. The chances for such interstellar exchange of life may be increased for very close sister solar systems which are born from one parent molecular cloud, where they form a cluster of "sibling" stars all with planetary bodies, which all – in these calculations – were overoptimistically assumed to be populated by microbes.

Table 4.4. Number of impacts n on Earth during time interval T assuming maximum allowed microbial travelling time t from ejection to impact, expulsion rate R, density if expelling extrasolar systems N, and stellar velocity dispersion σ from the general galactic star field and from clusters of "sibling"stars

Case	T (Ma)	t (Ma)	R (Ma^{-1})	N (pc^{-3})	σ (km/s)	n
From all planetary systems in the general galactic star field	500	10	10^8	0.1	20	1.6×10^{-9}
	500	100	10^{11}	0.1	20	1.6×10^{-5}
Our Sun within a cluster of "sibling" stars	100	1	10^8	40	0.2	3.8×10^{-3}
	100	10	10^8	40	0.2	3.8×10^{-2}
Our Sun within cluster like Hyades	625	1	10^8	2	0.2	8.6×10^{-4}

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