



Note

Experimental evidence for the potential impact ejection of viable microorganisms from Mars and Mars-like planets

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Abstract

Bacterial spores (*Bacillus subtilis*), cyanobacteria (*Chroococcidiopsis* sp.), and lichen (*Xanthoria elegans*) embedded in martian analogue rock (gabbro) were exposed to shock pressures between 5 and 50 GPa which is the range of pressures observed in martian meteorites. The survival of *Bacillus subtilis* and *Xanthoria elegans* up to 45 GPa and of *Chroococcidiopsis* sp. up to 10 GPa supports the possibility of transfer of life inside meteoroids between Mars and Earth and it implies the potential for the transfer of life from any Mars-like planet to other habitable planets in the same stellar system.

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The hypothesis of “Panspermia” (Arrhenius, 1903) postulates propagation of microscopic forms of life between planets by solar radiation pressure. Although the feasibility of this mechanism has been questioned, several recent discoveries make it worthwhile to revisit “Panspermia”: (1) martian meteorites have provided evidence for the transfer of rock fragments from Mars to Earth in the solid state at moderate shock pressures and temperatures (Melosh, 1984; Vickery and Melosh, 1987; Warren, 1994; Gladman, 1997; Head et al., 2002; Artemieva and Ivanov, 2004; Fritz et al., 2005); (2) microbial communities have been found inhabiting rocks down to great depths (Pedersen, 2000); (3) space exposure experiments have demonstrated a high survival capacity of bacterial spores in space, if protected against solar UV radiation (Horneck et al., 1994, 2001a; Nicholson et al., 2000). Based on these observations, we investigated experimentally a scenario of “lithopanspermia” that considers the transport of viable microorganisms between terrestrial planets by means of meteorites. In this scenario, microorganisms have to cope with three major phases of stress: (i) escape from the home planet by impact ejection, (ii) journey through space over extended time periods, and (iii) landing on another planet.

A frequent transfer of early life between Mars and Earth during the period of heavy bombardment has been proposed (Wells et al., 2003; Mileikowsky et al., 2000). This view is supported by the recent discoveries of extended occurrences of water on the ancient surface of Mars suggesting a habitable early Mars (Squyres et al., 2004), and by the transfer of rocks between Mars and Earth, confirmed by some 40 martian meteorites found on Earth so far. The transfer of rock fragments from Mars to Earth is possible in the solid state at moderate shock pressures and temperatures induced in the ejected target rocks by impacting projectiles on Mars (Melosh, 1984; Vickery and Melosh, 1987; Head et al., 2002; Artemieva and Ivanov, 2004; Fritz et al., 2005). Petrographic studies on shock metamorphism of martian meteorites and numerical simulations of the impact-induced ejection of martian rocks beyond the escape velocity of Mars have demonstrated a launch window for martian meteorites between about 5–10 GPa and about 55 GPa (Nyquist et al., 2001; Artemieva and Ivanov, 2004; Fritz et al., 2005). So far, little was known about whether rock-inhabiting microbial communities would survive the stress accompanying impact ejection. Except for some provisional studies on *Bacillus subtilis* (Horneck et al., 2001b; Mastrapa et al., 2001; Burchell et al., 2004), no systematic shock recovery experiments have been done with rock-inhabiting microbial communities hosted in appropriate martian analogue rocks.

We report here on a realistic and high-precision simulation of impacts on martian analogue rocks by subjecting microbial rock inhabitants sandwiched between thin layers of gabbro, to shock pressures in the range of 5–50 GPa which corresponds to the shock pressures determined for martian meteorites.

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Gabbro was used as an analogue for the relatively coarse-grained basaltic martian shergottites (Fig. 1; Table 1). Biological representatives of natural rock inhabitants were the endolithic cyanobacterium *Chroococcidiopsis* sp. which colonizes rocks in hot and cold deserts and is highly resistant to desiccation and radiation (e.g., Billi and Grilli-Caiola, 1996; Cockell et al., 2005), the lichen *Xanthoria elegans*, an epilithic symbiotic organism of a fungus and the

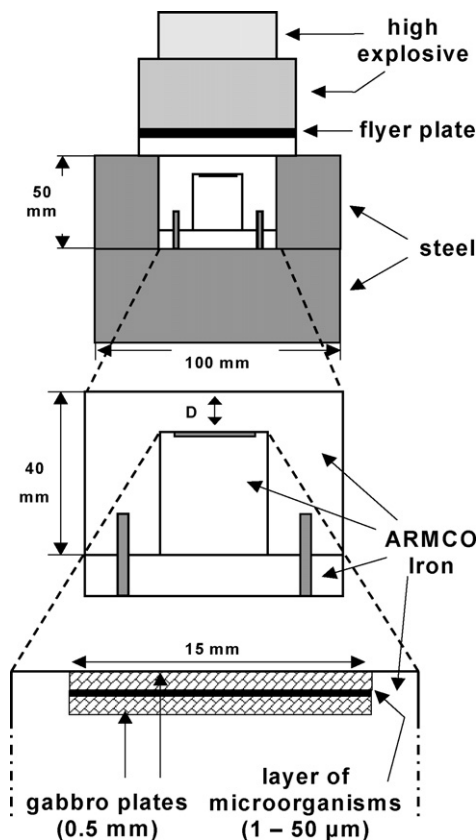


Fig. 1. Experimental set-up for the shock recovery experiments performed at the Ernst-Mach-Institut für Kurzzeitdynamik, Freiburg, Germany. Thin dry layers of microorganisms were sandwiched between two plates of gabbro and subjected to shock pressures between 5 and 50 GPa using a shock reverberation technique with an accuracy of $\pm 3\%$ (Stöffler and Langenhorst, 1994). Gabbro samples were obtained from Bushveld, South Africa.

alga *Trebouxia*, showing high resistance to the parameters of outer space (de Vera et al., 2003), and endospores of the bacterium *B. subtilis* (Benardini et al., 2003), recognized as the hardiest known forms of life on Earth (Nicholson et al., 2000). They exhibit a high degree of resistance to various physical and chemical stresses, such as desiccation, extreme temperatures including dry heat, UV and ionizing radiation. Spores of *B. subtilis* survived for nearly 6 years in the space environment (Nicholson et al., 2000).

In the shock recovery experiments using a high-explosive plane wave set-up (Stöffler and Langenhorst, 1994; Horneck et al., 2001b, Fig. 1) a suite of defined shock pressures of 5, 10, 20, 30, 41.5, and 50 GPa was applied on two gabbro plates separated by a few μm thick layer of viable microorganisms (Table 1). The nominal shock pressures were calculated on the basis of the shock reverberation technique (Stöffler and Langenhorst, 1994) and checked by refractive index measurements of plagioclase grains from the shocked and recovered gabbro plates (Stöffler et al., 1986, Table 1). In the reverberation technique used in our experiments, the maximum shock pressure is achieved by a sequence of increasingly stronger shock waves reflected at the iron–gabbro interfaces whereas the temperature is induced predominantly by the first “low pressure” shock wave in the gabbro. Therefore, the calculated shock temperatures prevailing in the gabbro sample for less than 1.4 μs are relatively low in our experiments ($\Delta T = 1\text{--}100^\circ\text{C}$ for 5–50 GPa; Table 1). The post-shock temperature increase ($\Delta T = 1\text{--}60^\circ\text{C}$; Table 1) lasted for a few minutes and probably increased to a final temperature ($\Delta T = 1\text{--}304^\circ\text{C}$) which is determined by heat exchange with the iron container. Moreover, the few μm thick microbial layer experienced maximum temperature spikes (e.g., $\Delta T = 500$ and 1000°C at 18 and 43 GPa, respectively, as calculated by B.A. Ivanov, pers. commun.) for less than 1 μs due to its low shock impedance. In reality, this temperature is probably much lower since the layer is generally much thinner than 50 μm in our experiments, e.g., about 1–7 μm for the bacteria.

Bacillus subtilis spores exhibited an exponential dose-response to impact-induced shock, determined as colony forming ability (Fig. 2). The spores survived up to a shock pressure of 42 GPa, where survival rates were reduced to 10^{-4} . Contamination of our samples was excluded by using a genetic marker test (Horneck et al., 2001b). At a shock pressure of 50 GPa, survival rates dropped below the threshold of detection of 10^{-8} . The “detection threshold” for the survival rate is defined by the initial microbe population in the rock material and the colony count method used. Our results are in distinct contrast to a light gas gun experiment at 78 GPa where limited survival of *B. subtilis* was observed (Burchell et al., 2004). Such experiments (Burchell et al., 2004) are not applicable to the expected martian lithologies, e.g., 78 GPa would lead to complete or partial melting of basalts and plagioclase-bearing ultramafic rocks, respectively (Stöffler, 1984).

The vegetative *Chroococcidiopsis* cells did not survive shock pressures >10 GPa (Fig. 2) where survival rates were reduced to less than 10^{-3} . At shock pressures higher than 10 GPa, no survivors were found with a detectable threshold of 10^{-6} . By contrast, the photo- and mycobiont of *Xanthoria elegans*,

Table 1
Pressure and temperature conditions in shock recovery experiments with microbes embedded in gabbro (see also text)

Multiple shock		First wave in gabbro			Single shock	Multiple shock	
P_N (GPa)	P_{RI} (GPa)	P_{FW} (GPa)	ΔT_S ($^\circ\text{C}$)	ΔT_{PS} ($^\circ\text{C}$)	ΔT_{ST} ($^\circ\text{C}$)	ΔT_{EQ} ($^\circ\text{C}$)	ΔT_{SM} ($^\circ\text{C}$) ^a
5			<1	<1	1	<1	
10		6.6	1	1	8	7	
(18) ^a							480
20	20 ± 4	12.2	7	6	45	39	
30	32 ± 3		23	17	126	109	
41.5	42 ± 3	22.7	53	35	244	210	
(43) ^a							1200
50		26.8	99	56	352	304	

P = shock pressure; ΔT = temperature increase relative to pre-shock ambient temperature;

^a Calculation by B.A. Ivanov (personal communication); P_N = nominal shock pressure, P_{RI} = shock pressure in gabbro based on measured refractive indices of plagioclase, P_{FW} , ΔT_S , ΔT_{PS} = shock pressure, shock temperature increase, and post-shock temperature increase, respectively, achieved by first shock wave in gabbro, ΔT_{ST} = post-shock temperature increase in iron, ΔT_{EQ} = temperature increase (equilibration temperature) achieved in gabbro after heat exchange with iron container, ΔT_{SM} = shock temperature increase in a 50 μm thick layer of water substituted for the microbe layer; all pressures and temperatures except for P_{RI} are based on calculations (see text).

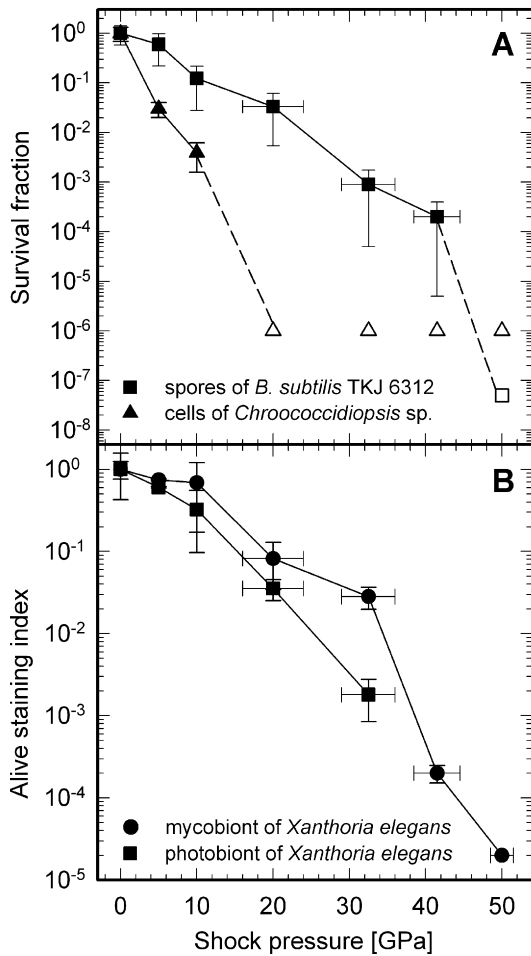


Fig. 2. (A) Survival rates of spores of *Bacillus subtilis* TKJ 6312 and cells of *Chroococcidiopsis* sp. and (B) vitality of the photobiont and mycobiont of the lichen *Xanthoria elegans* encased in gabbro plates plotted against the shock pressures to which they were exposed in 6 different shock recovery experiments with high-explosive plane wave set-ups (Fig. 1). The open symbols indicate survival below the threshold of detection. Data points represent averages and error bars correspond to 1σ standard deviation.

despite being vegetative cells, exhibited vitality dose-response effects similar to those of *B. subtilis* spores (Fig. 2). However, we have to stress that the vitality tests of the lichens indicated the integrity of the membranes as well as the physiological processes of the cells determined by LIVE/DEAD staining using confocal laser scanning microscopy (CLSM) (de Vera et al., 2003), which is different from the colony counts of actively dividing cells. The physiological activity is reflected by color changes of the plasma and is caused by transport processes from the cell plasma into vacuoles and vesicles where the reaction products with the staining substances (red crystals) are deposited. CLSM imaging showed that at a shock pressure of 10 GPa the structure of the lichen thallus was mainly preserved. Higher shock pressures showed increased disintegration of the thalli and fruiting bodies. At shock pressures above 42 GPa the thalli were completely destroyed and only few ascospores, the generative part of the lichen, survived the treatment.

We attribute the observed differential biological effects of shock waves, involving compression and heating, to the physiological states of the microorganisms. *Bacillus subtilis* spores are dormant and exhibit incredible longevity and resistance to environmental extremes as a consequence of specific structures: protective spore coats; a tough outer wall, the cortex; a relatively impermeable and desiccated cell, the spore core, with high levels of minerals; and saturation of the DNA by small proteins (Nicholson et al., 2000). During the process of shock compression the spore coatings would protect the core. The mineralized spore core with the well protected DNA enables bacterial spores to survive ex-

remely high temperatures, e.g., 150 °C for 1 h (Nicholson et al., 2000), and may have allowed them to cope with the even higher temperature peaks lasting for microseconds only. By contrast, *Chroococcidiopsis* has an outer sheath made from polysaccharides and no known resting states. The shear and compression caused by shock waves were observed by bright field microscopy to have ruptured the relatively soft cell wall, accounting for the observed low survival rates. Although the algal photobiont of *Xanthoria elegans* is also vegetative, it is embedded within a fungal matrix and protected by a thick cortex. This symbiosis by the photo- and mycobiont protects against extreme desiccation or temperature extremes due to their poikilohydric capacity. During dry periods the whole lichen passes into anabiosis, a latent form, and it revitalizes again under wet conditions. We attribute the high vitality of the lichen following high shock pressures to this poikilohydric capacity.

Our experimental data provide evidence of a well-defined but limited launch window for the transfer of rock-inhabiting microorganism from Mars to Earth by impact ejection involving shock pressures in the range from 5–10 to 45 GPa, which is in accordance to the launch window of 5–10 to 50–55 GPa determined for martian meteorites (Artemieva and Ivanov, 2004; Fritz et al., 2005). Shock and post-shock temperatures in the host rocks and microbial layers have to be considered in relation to the low pre-shock temperatures at the surface and subsurface of Mars prevailing currently and probably over most of its history. Assuming a surface temperature of –65 °C post-shock temperatures in martian basalt would rise to no more than 0 °C at 10 GPa and to the 550–950 °C range at 50 GPa (Fritz et al., 2005). Ultramafic rocks, such as the nakhlites which represent 7% of the martian meteorites, are considered most favorable and efficient for the transfer of life from Mars to Earth, because they display the lowest degree of shock and post-shock heating. However, our experiments have demonstrated that *Bacillus subtilis* spores and the lichen *Xanthoria elegans* are capable of surviving in all types of martian host rocks. These results strongly confirm the possibility of a “direct transfer” scenario of “lithopanspermia” for the route from Mars to Earth or from any Mars-like planet to other habitable planets in the same stellar system.

“Lithopanspermia” also includes a potential transfer of microorganisms in the opposite direction, i.e., from Earth to Mars. A direct transfer scenario is severely limited because very high ejection velocities in the solid state are required to escape the Earth’s gravity field and to pass its dense atmosphere. Favorable transfer conditions may be only achieved by very large impact events, which blow out at least part of the atmosphere. Such impact events happened frequently during the “early heavy bombardment phase,” i.e., before 3.75 billion years ago (Stöffler and Ryder, 2001; Grieve, 1987; Wells et al., 2003; Gladman et al., 2005; Cockell et al., 2002). After the period of the “early heavy bombardment” such impacts became rare and their frequency dropped by orders of magnitude. An additional effect of megaimpacts on existing microbial life on habitable planets, however, is that they lead to the sterilization of the whole planetary surface (Maher and Stevenson, 1988; Oberbeck and Fogleman, 1989; Sleep et al., 1989). This problem may be overcome in a wider concept of “lithopanspermia” by adopting an “indirect transfer” scenario where life may have avoided annihilation by escaping its home planet via impact-ejected rocks (Sleep and Zahnle, 1998) to either return to its home planet after conditions had changed again (Wells et al., 2003), or travel through space until captured by another near-by habitable planet (Mileikowsky et al., 2000; Gladman et al., 2005). In conclusion, it can be speculated that “lithopanspermia” from Earth to Mars had a high probability during the early history of the Earth; however, during the past 3.8 Ga the probability has been very low.

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