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Survivability of bare, individual *Bacillus subtilis* spores to high-velocity surface impact: Implications for microbial transfer through space

Brandon L. Barney [1], Sara N. Pratt [2], Daniel E. Austin *

Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602, USA

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ABSTRACT

Laboratory experiments show that endospores of *Bacillus subtilis* survive impact against a solid surface at velocities as high as 299 ± 28 m/s. During impact, spores experience and survive accelerations of at least 10^{10} m/s^2. The spores were introduced into a vacuum chamber using an electrospray source and accelerated to a narrow velocity distribution by entrainment in a differentially pumped gas flow. Different velocity ranges were studied by modifying the gas flow parameters. The spores were electrically charged, allowing direct measurement of the velocity of each spore as it passed through an image charge detector prior to surface impact. Spores impacted a glass surface and were collected for subsequent analysis by culturing. Most spores survived impact at all measured velocities. These experiments differ fundamentally from other studies that show either shock or impact survivability of bacteria embedded within or on the surface of a projectile. Bacteria in the present experiments undergo a single interaction with a solid surface at the full impact velocity, in the absence of any other effects such as cushioning due to microbe agglomerations, deceleration due to air or vapor, or transfer of impact shock through solid or liquid media. During these full-velocity impact events, the spores experience extremely high decelerations. This study is the first reported instance of accelerations of this magnitude experienced during a bacteria impact event. These results are discussed in the context of potential transfer of viable microbes in space and other scenarios involving surface impacts at high velocities.

1. Introduction

The potential transfer of life from one planetary body to another within our solar system depends upon the survival of an organism to the various stresses encountered during transfer, including exposure to vacuum, exposure to (or shielding from) extreme radiation, and survival through dynamic events that remove the organism from one body and deposit it on the other (Fajardo-Cavazos et al., 2007). This last factor may involve impact at high velocity of an organism contained within a larger projectile (such as contained within a meteorite). Bacteria exhibit significant natural resistance to a wide variety of environmental stresses and are therefore excellent candidates for studies of the possibility of survivability between planetary bodies. In general, bacterial resistance is increased when bacteria are in spore form rather than in vegetative form (Nicholson et al., 2000), when bacteria are grouped together such as when they are part of a biofilm (Kubota et al., 2008), or are enclosed in some other solid or liquid material that offers protection from environmental stress.

Potential transfer of life between planetary bodies is of interest both in the context of panspermia (including lithopanspermia) and planetary protection. Panspermia focuses on the potential transfer of life from one body in our solar system to another through natural means, such as a within a meteorite (Valtonen et al., 2009). For instance, a meteorite from Mars to Earth, containing viable microbes, may have introduced life to Earth (Gualtieri, 1977; Mileikowsky et al., 2000). The scenario dynamics are slightly different if the meteorite has an icy surface (Burchell et al., 2003). On the other hand, planetary protection arises from the concern that the search for signs of life elsewhere in our solar system could be compromised by terrestrial microbe contaminants; or that microbes, if present elsewhere in the solar system, could disrupt the ecosystem when introduced to another body, particularly the Earth. Planetary protection is of particular concern as we consider sample return missions to Phobos, or as we consider using Phobos as a staging area for exploratory missions to Mars (Murchie et al., 2014). For instance, Ramsley and Head (2013) have shown that some fraction of ejecta from Mars will impact and remain on the surface of Phobos, and suggested that as much as 250 ppm of the

* Corresponding author.
E-mail address: austin@chem.byu.edu (D.E. Austin).
[1] Present address: NAWCWD, 1 Administrative Circle, China Lake, CA 93555, USA.
[2] Present address: Intel, 2501 NW 229th Avenue, RA2-285, Hillsboro, OR 97124, USA.

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Phobos regolith consists of material that originated recently from Mars. Both panspermia and planetary protection therefore require knowledge of the limitations of microbes to the various types of stress that would be encountered during such a transfer.

Bacterial spores have been shown to survive a number of harsh conditions and stresses, including those expected during a planet-to-planet transfer event. Spores of *B. subtilis* were demonstrated to survive extreme dessication as a result of exposure to vacuum. Surviving spores were found after an exposure of almost six years on the Long Duration Exposure Facility in low-earth orbit (Horneck, 1993). In interplanetary space, unprotected spores would also be exposed to high amounts of UV radiation. Spores of *B. subtilis* and *B. pumilus* were subjected to 254 nm UV radiation and LD_{90} values of 210–551 J/m^2 were reported, depending on the specific strain (Benardini et al., 2003). In order to survive atmospheric entry, spores must resist extreme heat. *B. subtilis* spores were shown to survive atmospheric entry while they were embedded in the surface of granite targets that had been attached to the exterior of a sounding rocket (Fajardo-Cavazos et al., 2005).

In order to survive an interplanetary transfer, organisms must be able to survive an impact at some (presumably high) velocity. Several prior experimental studies have explored this question, as summarized in Table 1. Mastrapa and Bernardini embedded spores of *Bacillus subtilis* and cells of *Deinococcus radiodurans* into lead pellets and fired them into plasticene targets using a rifle (Benardini et al., 2003; Mastrapa et al., 2001). They found surviving spores at impact velocities up to 300 m/s. Burchell used a light gas gun to fire spores of the *Rhodococcus* genus that had been adsorbed onto the surface of ceramic fragments and loaded into a firing pellet into nutrient agar plates at 5.1 km/s and found surviving bacteria in approximately half of the experimental runs (Burchell et al., 2001). Fajardo-Cavazos embedded spores of *B. subtilis* into granite samples then placed these samples into the telemetry module of a sounding rocket (Fajardo-Cavazos et al., 2005). They found that spores survived atmospheric re-entry at a velocity of 1.2 km/s. The same research team also embedded spores onto the surface of a granite target and fired aluminum projectiles at them. These projectiles were fired at 5.4 km/s and surviving spores were found (Fajardo-Cavazos et al., 2009). Price enclosed yeast spores and agar in nylon cylinders and fired them into water using a light gas gun. They studied cylinder velocities between 1 and 7.4 km/s and found surviving spores at all velocities tested (Price et al., 2013). All of these studies investigated the question of impact survivability for spores under conditions where the specific forces on individual spores are not well-controlled or known. For instance, for a given velocity, spores suspended in agar within a nylon cylinder will experience a significantly different acceleration during the impact process (which may take many microseconds to milliseconds) than bare spores impacting a surface (which takes a few nanoseconds). The agar may also alter the impact process experienced by the spores in a way that is not well characterized. Further, the organisms used in some of these studies may have formed agglomerates that might act to cushion bacteria at the center of the agglomerates.

The question of impact survivability has also been approached in a slightly different way. Several studies have been done to determine the shock pressures that bacterial spores can withstand. Horneck embedded spores of *B. subtilis* between two quartz plates (Horneck et al., 2001) and subjected them to 32 GPa explosive shock pressures and recorded bacterial survival rates of up to 10^{-4}. Burchell subjected spores of *B. subtilis* as well as *Rhodococcus erythropolis* to shock pressures between 1 and 78 GPa by loading them into a sabot which was then loaded into a light gas gun and fired into nutrient agar or ice targets and found that the survival rate of tested bacteria decreased from 10^{-4} to 10^{-7} as the shock pressure was increased (Burchell et al., 2004). Willis subjected

Table 1
Overview of microbial survival experiments to high-velocity impacts and extreme shock pressures.

Researcher	Year	Species	Impact velocities (km/s)	Shock pressures (GPa)	Impact conditions	Survival rate
Burchell	2001	*R. erythropolis*	5.1	–	Spores embedded in ceramic, fired into agar	1 in 10^7
Mastrapa	2001	*B. subtilis, D. radiodurans*	0.1, 0.3	–	Spores encased in lead pellets, fired with a gun into hard surface	40–100%
Horneck	2001	*B. subtilis*	–	32	Spores between 2 colliding quartz plates	10^{-4}
Burchell	2003	*R. erythropolis*	5	–	Spores embedded in ice, fired on with light gas gun	$< 10^{-6}$
Burchell	2004	*R. erythropolis, B. subtilis*	0.35–5.4	3–78	Spores encased in projectile, fired from light gas gun into agar or ice	10^{-4}–10^{-8}
Fajardo-Cavazos	2005	*B. subtilis*	1.2	–	Spores embedded in granite, attached to rocket, atmospheric entry	1.2–4.4%
Willis	2006	*E. coli*	–	0.22–0.26	Cells suspended in liquid, encased in steel, and fired on using powder gun	10^{-2}–10^{-4}
Stoeffler	2007	*B. subtilis, Chroococcidiopsis* sp., *X. elegans*	–	5–50	Spores sandwiched between layers of gabbro, struck with flyer plate	10^{-1}–10^{-7}
Horneck	2008	*B. subtilis, Chroococcidiopsis* sp., *X. elegans*	–	5–40	Spores between layers of rock, struck with flyer plate	10^{-1}–10^{-7}
Moeller	2008	*B. subtilis*	–	5–50	Spores between layers of different types of rock, struck with flyer plate	10^{-3}–10^{-4}
Fajardo-Cavazos	2009	*B. subtilis*	5.4	57.1	Spores embedded in granite, aluminum projectiles fired at granite	10^{-5}
Hazell	2010	*E. coli, E. faecalis, Z. bailii*	0.3–0.4	1.2	Cell liquid suspension between two colliding flyer plates	10–100%
Meyer	2011	*B. subtilis, Chroococcidiopsis* sp., *X. elegans*	2	5–50	Spores in iron container next to explosive plane wave generator	0.002–60%
Price	2013	*S. cerevisiae*	1–7.4	2–45	Spores in agar-filled nylon cylinders, fired into water	10^{-4}–0.2
Barney (current work)	2015	*B. subtilis*	0.3	–	Bare, individual spores impact glass surface	75%