

BACTERIAL SPORES SURVIVE HYPERVELOCITY LAUNCH BY SPALLATION: IMPLICATIONS FOR LITHOPANSPERMIA.

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Introduction: The existence on Earth of meteorites originating from Mars has sparked renewed interest in testing the theory of interplanetary transport of viable endolithic microbes by natural impact processes, i.e., lithopanspermia [1]. In this scenario, the transfer process is initiated by launch of crust material from the donor planet into space by a hypervelocity impact event. Considerable theoretical and experimental support has accumulated favoring a spallation mechanism for impact ejection [1-3]. In this mechanism, a spallation zone forms around an impact site, where the reflected shock wave of the impact is directly translated into acceleration of surface rocks to escape velocity with relatively mild compression and heating. Mathematical modeling of the physics of spallation and analyses of the martian meteorite collection have resulted in estimates of acceleration, jerk, shock, and heating to which martian meteorites were subjected (summarized in Table 1).

Table 1. Estimates of physical parameters for martian meteorite ejection.

Parameter	Predicted (P) or Measured (M) Value	Ref.
Acceleration	$3.8 \times 10^6 \text{ m/s}^2$ (P)	3
Jerk	$6.0 \times 10^9 \text{ m/s}^3$ (P)	3
Shock	5-55 GPa (P, M)	4-6
Heating	10-1000°C (P, M)	4-6

To test the hypothesis that microbial cells could survive an impact-generated ejection event, bacterial cells and spores have been subjected to acceleration, jerk, shock pressure, and heating applied singly or in combination [3, 7-9]. However, to date none of these experiments have directly tested survival of microbes launched by spallation. In this study we used the 2-stage light gas gun at the NASA-Ames Vertical Gun Range (AVGR) to investigate the survival of bacterial spores embedded within granite targets to launch by spallation initiated by a hypervelocity (5.4 km/sec) impact.

Methods: The current collection of martian meteorites are of igneous composition, mostly basaltic [4]; however, we chose granite as target material so that the shock history of spalled

fragments could be more easily determined [10]. Targets consisting of two weathered Catalina granite blocks were dry-heat sterilized (132°C, 48 h) and cooled to room temperature. An aqueous suspension of spores of *Bacillus subtilis* strain WN511, a prototrophic, amylase-negative (Amy⁻), chloramphenicol resistant (Cm^R) laboratory strain [3], was infused into the surface of one target and air-dried. Each target was covered with a recovery fixture consisting of 2.5-cm thick open-celled polyurethane foam layers, cut with an entrance hole for the projectile (Fig. 1).

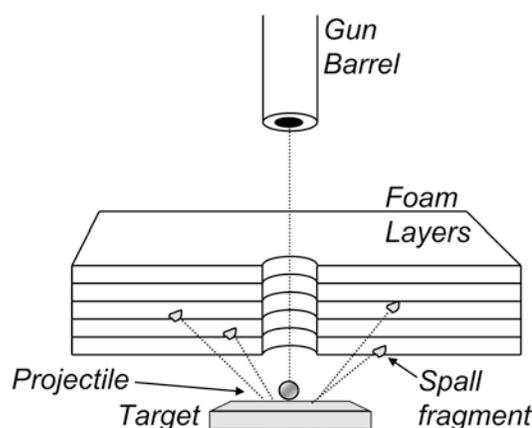


Fig. 1. Cut-away schematic of spallation experiment.

The projectiles were .25-caliber (6.3 mm) aluminum spheres fired perpendicularly into the target at 5.4 km/s. After disassembly of the recovery fixture, spall fragments were recovered aseptically and transported to the lab. Fragments were weighed, suspended in 0.1 ml PBS buffer and heat-shocked (80°C, 10 min) to select for spores. Aliquots of supernatants of each suspension were plated on nutrient medium, and incubated at 37°C for 2 days. To assure that colonies arose from authentic survivors and not contaminants, all isolates were checked for the prototrophic, Cm^R, Amy⁻ phenotype of WN511 and their molecular fingerprints were determined by rep-PCR as described previously [11].

To detect the shock damage in the tiny spall fragments, we concentrated on the characterization of the defect microstructure of quartz using

conventional transmission electron microscopy (TEM). Therefore, spall fragments were first suspended in ethanol and then loaded onto holey carbon grids for TEM analysis.

Results and Discussion: High-speed video (0.2 msec frames taken at 2 msec intervals) documented spall fragments leaving the target at a minimum of 0.25 km/sec (likely an underestimate as the leading edge of the spall cone had exited the video frame) (Fig. 2).

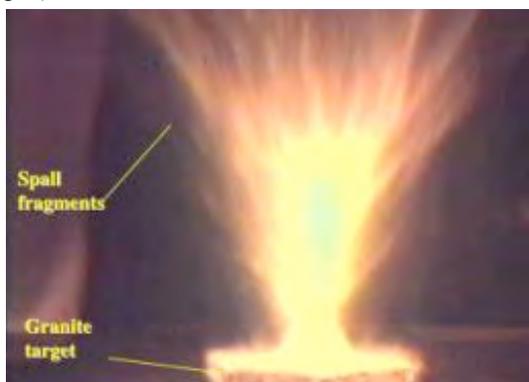


Fig. 2. High-speed video frame of impact.

Impacts created typical conical spall patterns with progressively fewer fragments penetrating upper foam layers (Fig. 3). Heating of particles was evident by partial melting and scorching of foam along the spall tracks (Fig. 3).

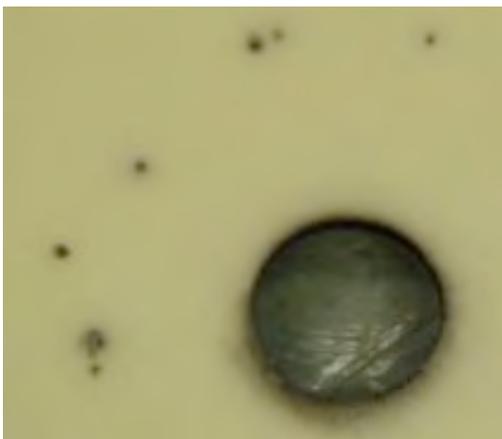


Fig. 3. Spall pattern from Shot #1, Foam Layer #7.

We were unable to determine the initial mass of spall fragments at launch, because the fragments disaggregated significantly during deceleration through the foam, leaving granite particles along the length of the spall tracks. Thus, spall fragments were recovered as small particles ranging from microscopic to ~1mm. Fragments were recovered

from the termini of 7 and 15 separate tracks from the sterile and spore-doped target, respectively, and subjected to analysis.

Significant numbers of spores (500 and 50 respectively) were recovered from 2 out of the 15 spall tracks from the spore-doped target, but none from the sterile target. These spores were confirmed to be WN511 by their prototrophic, Cm^R, Amy⁻ phenotypes and DNA fingerprints. Spore survival was calculated to be 10⁻⁴, in agreement with other non-spallation shock simulations [7, 9].

TEM observations revealed that most of the recovered quartz grains contained only few dislocations. However, in some of the quartz grains we observed sets of planar fractures that are parallel to the rhombohedral plane (10 $\bar{1}$ 1), suggesting slight shock damage. In addition, the absence of planar deformation features (PDFs) in quartz, i.e. amorphous shock lamellae, suggests that the shock pressures may have just exceeded the Hugoniot elastic limit (> 5-8 GPa).

In conclusion, bacterial spores were demonstrated to survive combined shock, heating, acceleration and jerk of ejection from a granite target surface resulting from a hypervelocity impact. These results provide quantitative support to the hypothesis that endolithic bacteria could survive ejection into space by spallation, a key component of lithopanspermia theory.

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