BALLISTIC IMPACT STUDIES OF A THERMOPHILIC BACTERIUM – THE IMPORTANCE OF GROWTH PHASE IN SURVIVAL. Carrine E. Blank[1] , Thomas J. Ahrens[2], Michael Long[2], L. Elizabeth Bertani[2], Mikhail Rashev[2], Sherry L. Cady[3], Richard C. Hugo[3], and Victoria Orphan[2], [1]Department of Earth & Planetary Sciences, Washington University, Saint Louis, MO, blank@wustl.edu; [2]Division of Geological & Planetary Sciences, California Institute of Technology, Pasadena, CA; [3]Department of Geology, Portland State University, Portland, OR.

Introduction: Our aim was to compare the relative survivability of mesophilic (organisms that grow optimally between 20-45˚C) and thermophilic microorganisms (those that grow optimally above 45˚C) to ballistic impact to test the hypothesis that thermophiles that branch deep in the tree of life are better able to survive impacts than mesophiles. Our chosen thermophile was *Thermus thermophilus*, an aerobe that grows rapidly at 70˚C. Cultures were concentrated and subjected to ballistic impact using a powder gun facility. Next, the cells were aseptically retrieved from the stainless steel capsule and plated to count the number of survivors. We then examined the cell morphologies using transmission electron and epifluorescence microscopy. Results were compared to that from *E. coli*, a typical mesophilic microorganism.

Here, we present an improved experimental design that allows for a greater recovery of shocked organisms and show that the growth phase of the organism is an important determinant in survivability. Actively growing cells (exponential growth phase) had a lower survival rate than starving cells (stationary growth phase). We also show differences in the cell wall integrity in the two growth phases after impact, suggesting that the cell wall is an important determinant to survival following impact events. Lastly, we show that *T. thermophilus* had a greater survival rate than the mesophile *E. coli.* While this may suggest that early branching thermophiles are more resistant to ballistic impact - and could be an explanation for why the last common ancestor of life is a hyperthermophile [1, 2] – we also plan to test for morphological, phylogenetic, genetic, and metabolic components to survivability.

Methods: Two control experiments were performed on cells loaded into stainless steel capsules, but not impacted. This was followed by two impact experiments, one with a stationary phase culture and one with an exponentially growing culture. *Thermus thermophilus* (strain HB27) was grown aerobically at 70˚C in Castenholz liquid medium (ATCC medium 461). Cell densities were determined using spectrophotometry and haemocytometry (total cells) and plate counts (colony forming units, or CFU). One mL (~10^9 cells) was transferred asceptically into an eppendorf tube, pelleted in a microcentrifuge, and resuspended in 18 µL Castenholz salt replacement (to buffer the pH and osmolarity, but disallow growth). A duplicate sample was held at room temperature to determine pre-impact plate counts and pre-impact morphologies.

The cells were loaded into a capsule and impacted in the 20 mm powder gun at the Lindhurst Laboratory of Experimental Geophysics at velocities of 0.67 km/sec and peak pressures of 2.4 GPa [3]. The cells were retrieved by flushing the capsule with medium in triplicate. The impacted and control cells were serially diluted and plated onto solid medium in duplicate. The plates were incubated at 65˚C for 48 hours and the number of colonies recorded to quantify the survival fraction. Ten µL from the 10^{-2} dilutions were stained with Live:Dead (Molecular Probes, Inc.) and visualized using epifluorescence microscopy to determine cell morphology and the proportion of live and dead cells (using a haemocytometer). The remaining cells were fixed with 3% glutaraldehyde and shipped to Portland State University for TEM analyses.

Results: During initial experiments, the cell suspension often disappeared during capsule opening, resulting in low sample recovery by pipetting [3]. Controls using the dye bromophenol blue showed that upon breaking the seal, the sample often dispersed along the capsule threads. Consequently, the new sample retrieval protocol involved washing the capsule with culture medium in triplicate followed by centrifugation, resulting in complete and repeatable cell recovery. To also prevent any potential leaks to the capsule and evaporation of the sample, the vacuum on the gun was pumped down for the minimum amount of time (~2 min.) to achieve a vacuum of <200 microns prior to the shot. These changes resulted in a higher rate of successful sample recovery than previously achieved.

Survivability. Cells in exponential phase exhibited a 0.27% survival fraction (ratio of pre-shock CFU to post-shock CFU) while cells in stationary phase had a survival fraction of at >43% (Table, below). Thus, survival following impact was strongly dependent upon the growth phase of the culture.

Organism	Optimal Growth Temperature	Growth Phase	Survival Fraction
E. coli	27˚C, Mesophile	Stationary Phase	>1% [3]
T. thermophilus	70˚C, Thermophile	Stationary Phase	>43% (this work)
T. thermophilus	70˚C, Thermophile	Exponential Phase	0.27% (this work)

There was also a significant difference between the survival fraction of stationary phase *E. coli* (~1%), compared to *T. thermophilus* (>43%). Although the *Thermus* had a markedly higher survival fraction, the impact experiments on *E. coli* will need to be repeated using the improved procedure to precisely quantify the survival difference of *Thermus* and *E. coli.*

Phase-contrast. T. thermophilus cells exhibit morphological variability. During exponential growth they form long rods that are often paired or in chains, while in stationary phase most form individual small diameter coccoids with a small number of filamentous cells (Live:Dead shows that many of these are dead).

Epifluorescence. In the shocked stationary phase culture, Live:Dead showed broken long filaments that mostly stained red (dead). The small coccoids, however, stained green (live). In the shocked exponential phase culture, the background fluorescence was high, presumably from massive lysis and cellular debris (substantiated below). Staining of the cell margins was altered, suggesting disruption of the cell wall or exopolysaccharide layer. Post-shocked morphologies showed a lower abundance of rods and a higher abundance of smaller coccoid cells that stained green. This suggests that rods and filaments may be preferentially ripped apart by the passing pressure waves.

TEM. T. thermophilus is a Gram Negative rod with a ruffled electron-dense outer cell wall that is well developed in both stationary and exponential phase [4]. The inner cell wall (peptidoglycan) is attached to the outer lipopolysaccharide layer, with a small periplasmic space between the peptidoglycan and the plasma membrane.

In stationary phase shocked cells (Fig. 1), nearly all cells show disruption in the cell wall layers, some with mild damage and others with significant damage. Damaged cells show a larger detached periplasmic space, and the cell wall layers are disrupted. Cells with the greatest amount of damage appear to have lysed, many having multiple membrane vesicles within the damaged cell wall. Cells with the least amount of cell wall damage appear to be intact, similar to unshocked cells.

Low magnification images show a significantly greater amount of cell debris and lysed cells in the shocked exponential phases culture. Most cells also show significantly more cell wall damage (Fig. 2). This damage was similar to that observed in the stationary phase sample and were consistent with the epifluorescence staining and plate counts (above).

Implications: Impacts likely had a significant influence in the origin and impact frustration of early life [2]. Hyperthermophiles likely evolved when the flux of impactors was much higher than today. Recent models predict that impacts on Mars could have led to long term hydrothermal circulation for hundreds of thousands of years, providing early habitats for hyperthermophiles [5]. Knowledge of what types of organisms were most likely to be spread between planetary bodies will be needed to direct future search strategies for present and past life in the solar system.

Figure 1:

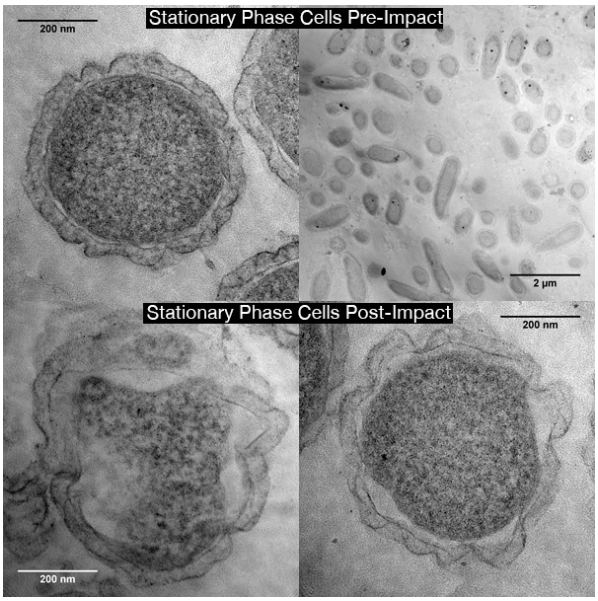
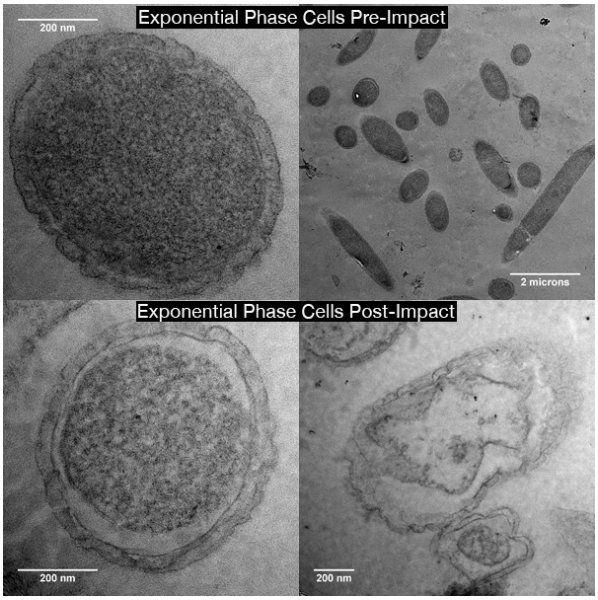


Figure 2:



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