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Shock Kill of Biological Agents

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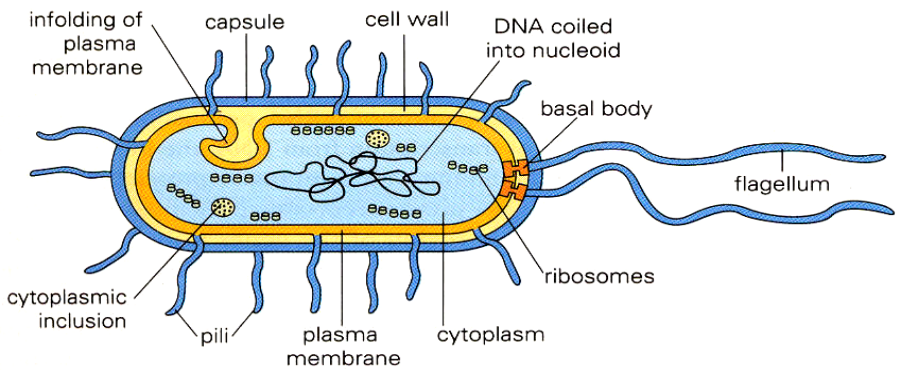
Shock Kill of Biological Agents



Shock Physics Team

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Biophysics Team

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- Combine Sandia's expertise in bio-physics and shock-physics
- Address pressing need for defense against bioagents by conducting the first systematic study of agent defeat via shock loading
- Provide data for scenarios involving kill of bioagents

Show 6 log of kill achievable via shock loading at μs time scales

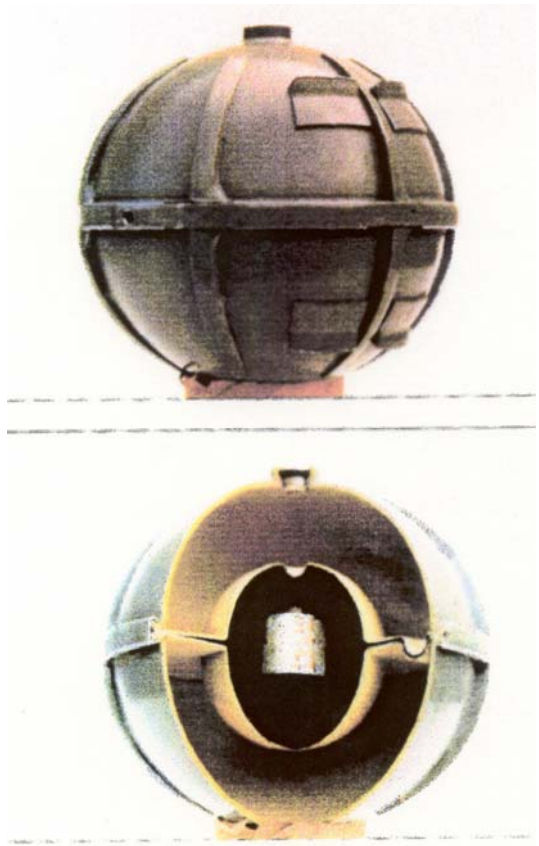
Related Lithopanspermia Work

- possibility of spread of life or building blocks of life from one body to another (e.g. from Mars to Earth) via meteorites or comets - originally suggested by Kelvin, Helmholtz, Arrhenius, etc.
- *S. oneidensis* and *E. coli* survived to 1.2-1.6 GPa in DAC [Sharma *et al.*, 2002]
- *R. erythropolis* survives at 10^{-7} in when loaded on projectiles impacting at 5 km/s [Burchell *et al.*, 2001]
- *B. subtilis* spores survive at 10^{-6} to 10^{-4} at 32 GPa [Horneck *et al.*, 2001]
- amino acids shocked to 10's of GPa form peptides [Blank, 2002]

data neither quantitative nor systematic

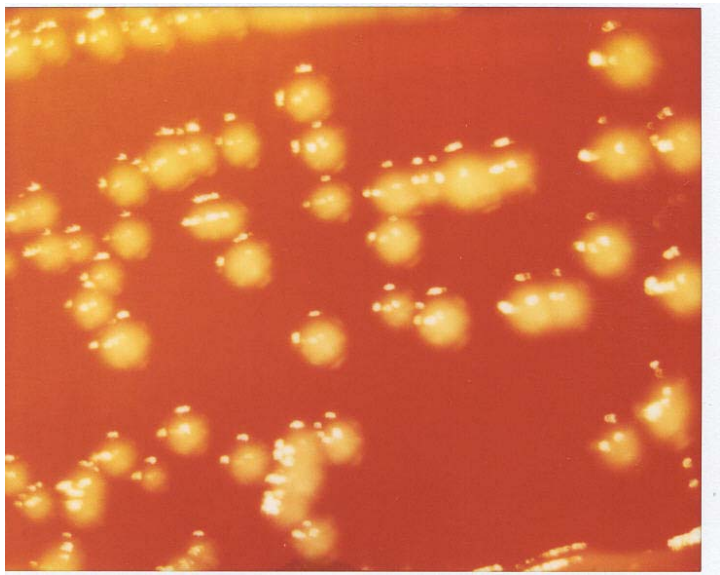
BW Dissemination

- Artillery
- MRLS
- Sprayer
- Missile
  - M139 Submunition
  - Explosive dispersal
  - Aerosurface spins generator
  - BW load outer shell



Agents Studied: *Yersinia Pestis* (Plague)

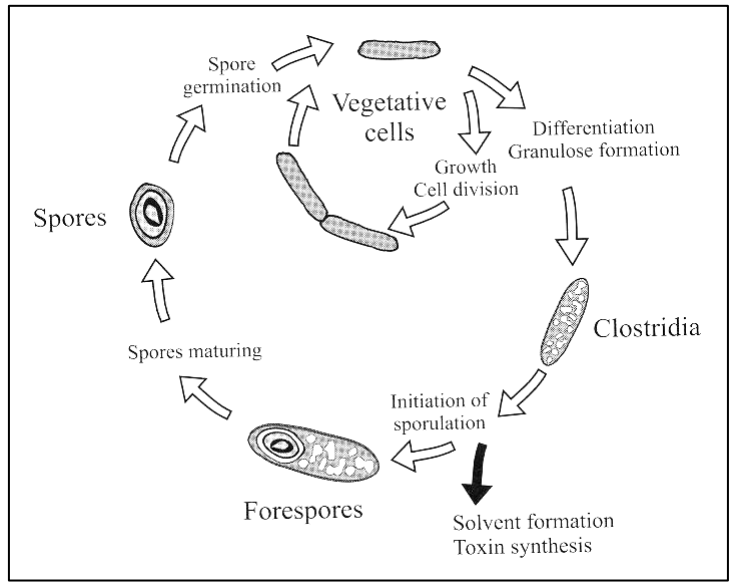
- highly infectious and high mortality
- spread person-to-person by aerosol
- significant epidemic potential
- study genetically modified form of *Y. pestis* with deleted Lcr- virulence gene
- Gram-negative bacteria



Agents Studied: *Bacillus Cereus* (close relative of *Bacillus Anthracis*)

- Gram-positive rod bacteria
- genetic analysis indicates it is in same clade as *B. anthracis* and *B. thuringiensis* [Priest et al., 2004] but not *B. subtilis*
- structurally essentially identical
- does not produce toxin crystallites of *B. thuringiensis*
- associated with food poisoning
- biosafety level 2
- initially study vegetative form

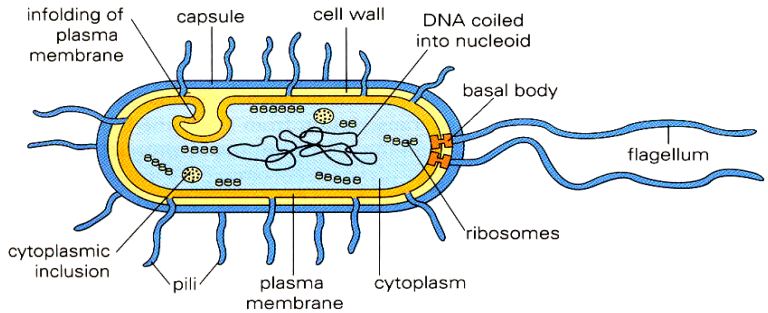
Koch & Pasteur: the cycle of sporulation and germination seen in *Bacillus* & *Clostidium* genera



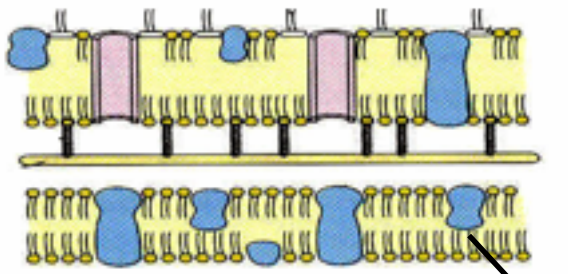
Source: Clostridia, Bahl and Durre, Eds

ultimately study sporulated B. cereus

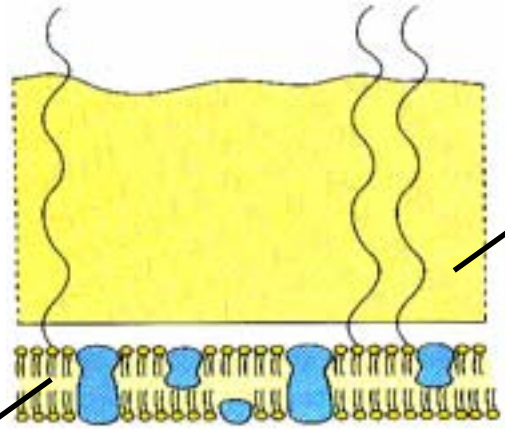
Bacterial Cell Membranes & Walls



Gram-Negative



Gram-Positive

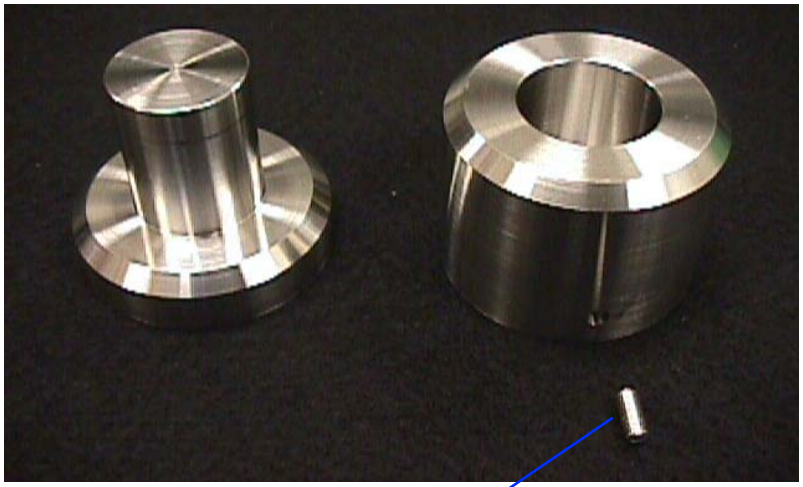


robust cell wall makes Gram-positive bacteria more resistant to static loading

Shock Physics Aspect of Project

- shock loading of wet agent simulants in capsule
- gas guns provide controllable velocities and stresses
- EOS models used to calculate pressures and temperatures
- three levels of bio-containment
  - primary containment: 304 SS capsule
  - secondary containment: steel urn or cup sealed by HDPE projectile
  - tertiary containment: evacuated debris vessel of gun

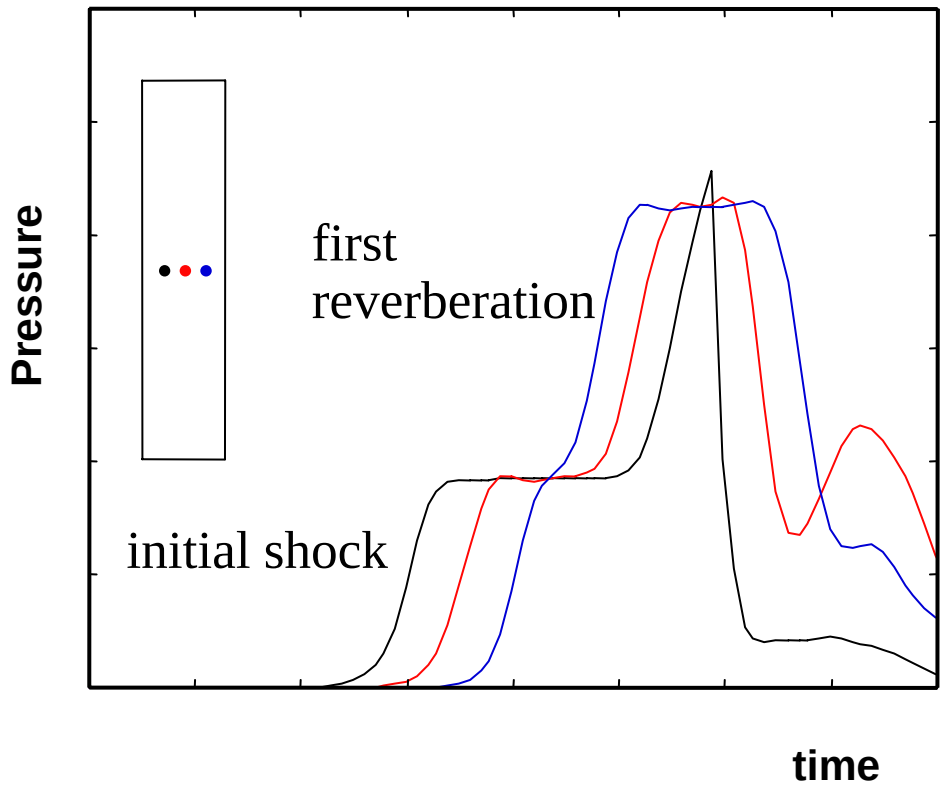
Sample Capsule



- stainless steel for biocompatibility
- small, robust design; holds ~1 mL
- welded shut and filled through hole sealed by gold BB and set screw
- interference fit with Macor ceramic sleeve for energy absorption and easy removal
- based on design used successfully at LLNL

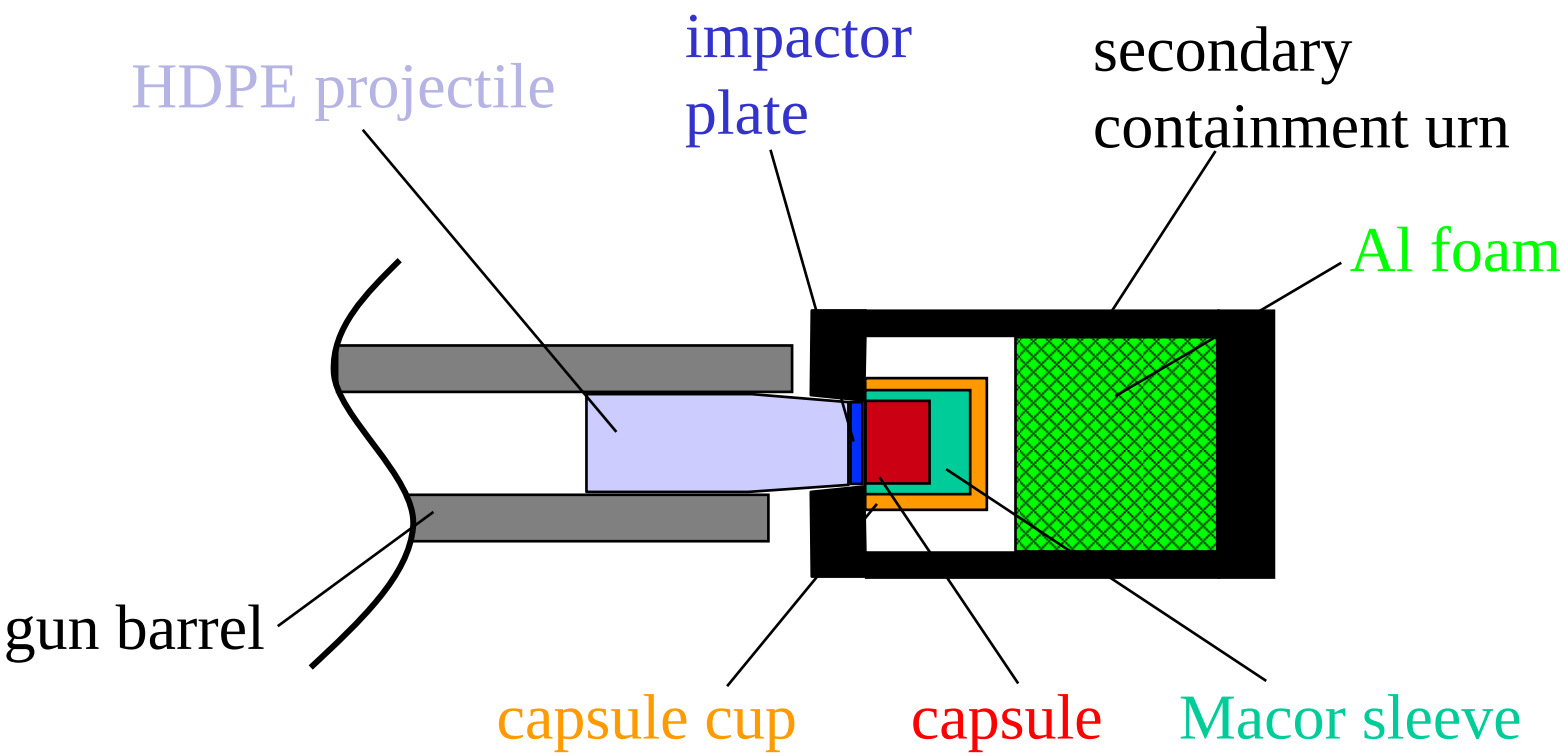
Sample Pressure & Temperatures

2-D CTH calculations



- pressure history consistent throughout most of sample
- pressure taken from first reverberation
- little if any negative pressure in sample
- temperature rises small
- late-time stresses no more severe than early stresses

Secondary Containment & Projectile

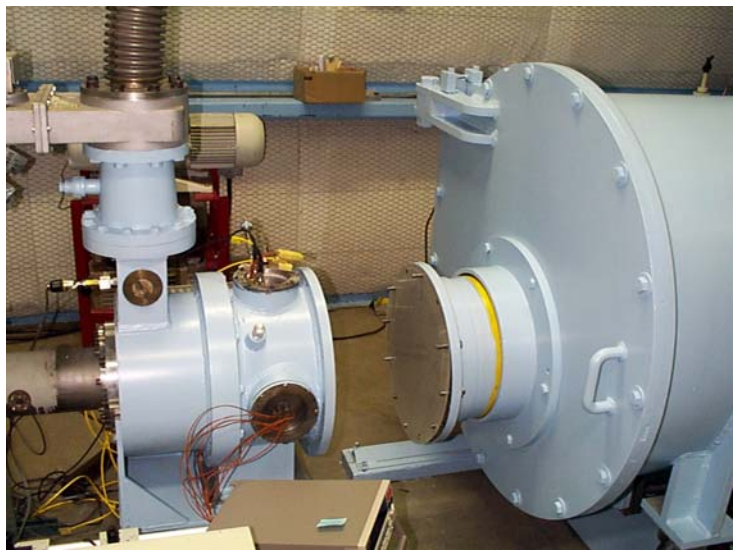


Gun & Tertiary Confinement

Single Stage Gun

~1 km/s
~300 kbar

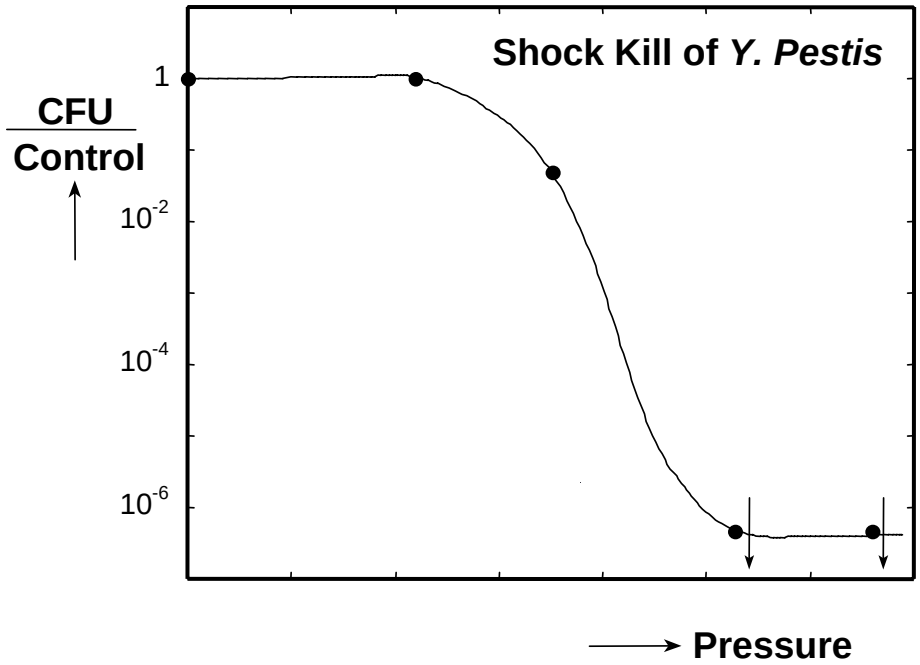
- catcher tank modified to hold vacuum
- bio-filters used in venting tank



Summary of Procedure

- bacteria grown
- bacteria diluted to known concentration and capsule and control filled
- vessels transported to gun facility; containment vessel loaded into gas gun chamber
- gun fired; vessel impacted; 2nd containment sealed
- recovery chamber vented through biological filters; tank opened and assessed for leakage
- capsule extracted and transported to biophysics lab
- shocked and control bacterial solution extracted
- survival assessed by growth of colony forming units

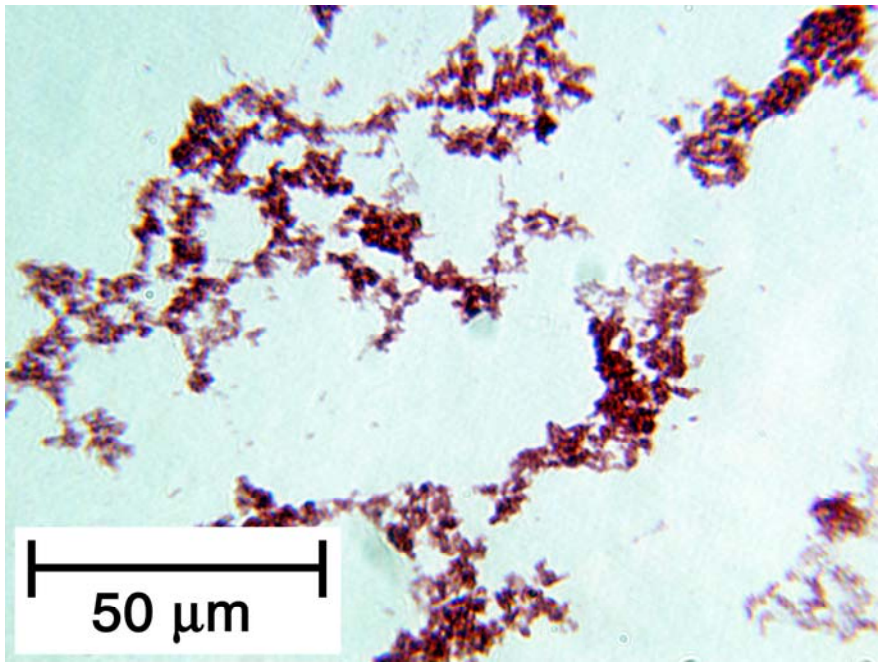
Kill Results for *Y. pestis*



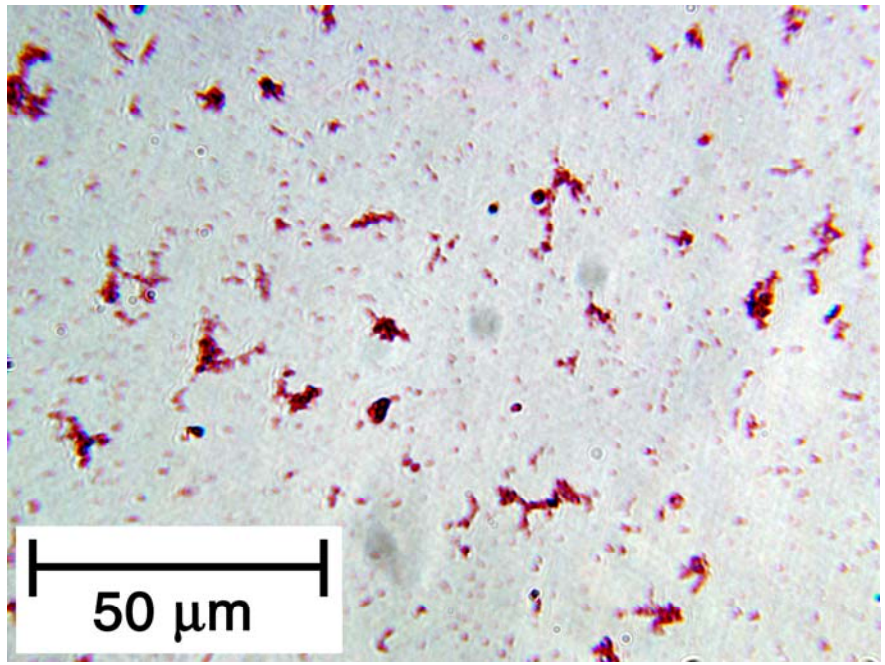
- steel-on-steel impact
- steep drop off in survival
- cells killed by rupture of cell membranes

Kill Results for *Y. pestis*

control

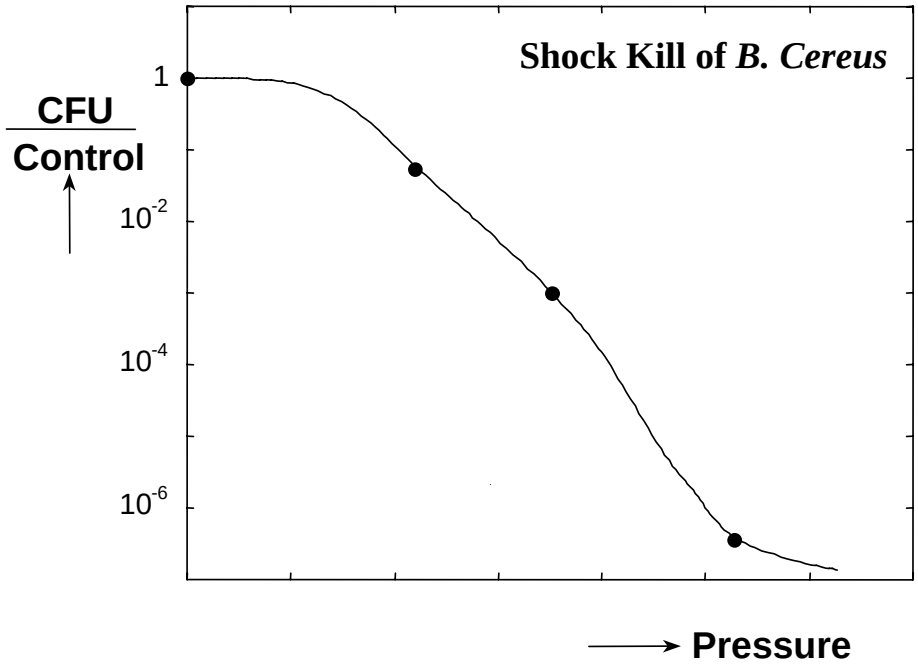


shocked to 3.3 GPa



- cells killed by rupture of cell membranes
- SEM images in preparation

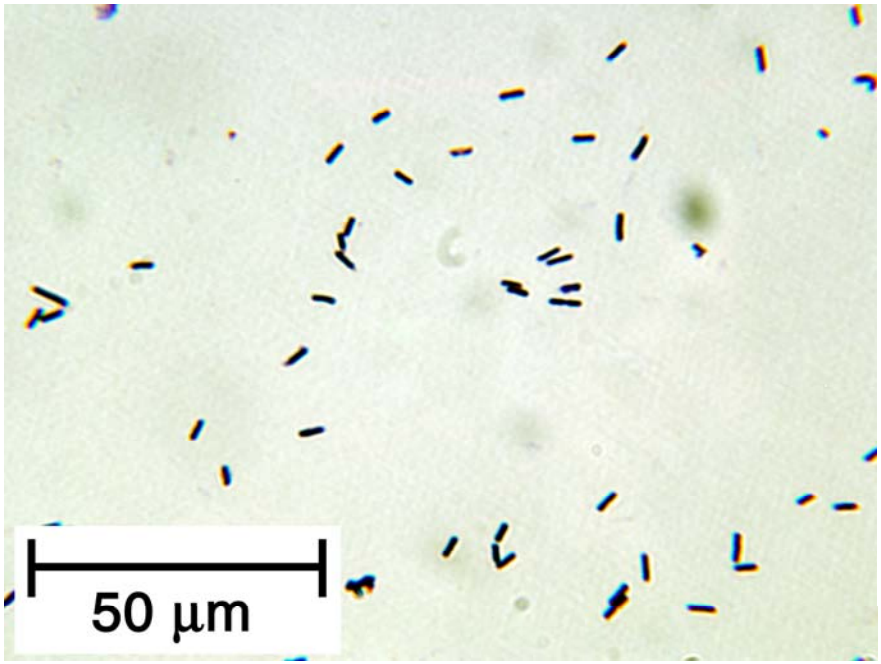
Kill Results for *B. cereus*



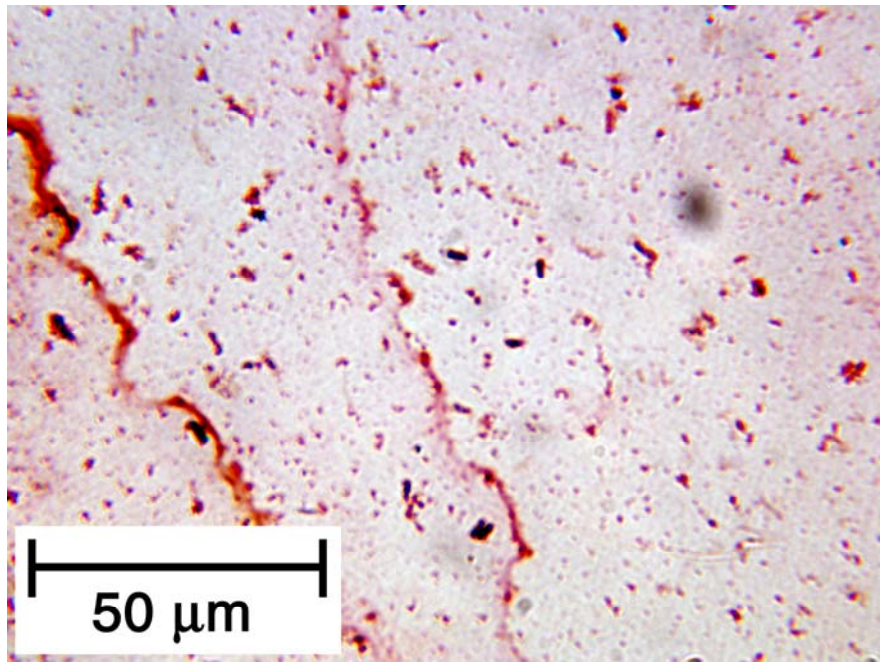
- gradual falloff in survival compared to Y. Pestis
- earlier kill onset despite robust cell wall
- gradual falloff may be associated with rod shape of *B. cereus*
- cells killed by rupture of cell membranes

Kill Results for *B. cereus*

control



shocked to 3.3 GPa



- cells killed by rupture of cell membranes
- some rods appear to remain intact

Conclusions

- first systematic study of bioagent defeat by shock loading
- high fidelity simulants for plague and anthrax studied
- *Y. pestis* shows steep drop in survival
- *B. cereus* displays gradual drop
- both high pressure and high loading rates appear to be critical to kill mechanism
- kill due to rupture of cell walls, though precise mechanism is unclear
- provides first parametric data for assessment of scenarios for bioagent kill via shock loading

Future Work

- examine realistic scenarios to determine if necessary pressures can be attained
- SEM of *Y. pestis* and *B. cereus* to study cell rupture mechanism
- determine shock kill of viruses (smallpox / vaccinia) and spores (anthrax)
- investigate potential clumping of spores by shock