The Interaction of Shock Waves with Bacterial Endospores

Principal Investigator: Professor Ronald K. Hanson

Research Associates: Dr. Jay Jeffries

Research Assistant: A. Daniel McCartt, Sean Gates

Motivation

We have developed a laboratory protocol to investigate the interaction of shock waves with aerosols of bacterial endospores. Main points of interest include laser-based real-time monitoring of shock wave-induced spore breakup and the assessment of DNA/RNA, protein and morphological damage. Understanding the shock generated mechanisms of spore deactivation both at the molecular and structural level remains an overarching goal of the project. Our methodology allows for the direct, laboratory-based simulation of blast wave - bioaerosol interactions in an atmospheric environment.

Overview

Aerosols of *Bacillus Atropheaus* (BA) are introduced into the test region of a gas-driven shock tube where they are subjected to shock waves of controlled strength. *In situ* laser diagnostics are used for time-resolved monitoring of the scattering from the aerosol, gas temperature, and the lysate produced by spore rupture. These time-resolved measurements are complemented by *ex situ* analysis of collected samples of control and shock-treated spores. Quanitative measurements of shock-treated spore viable fraction are assessed through the conjunction of standard agar plating techniques and flow cytometry. Post-shock spore morphology is observed by scanning electron microscopy.

Aerosol Shock Tube

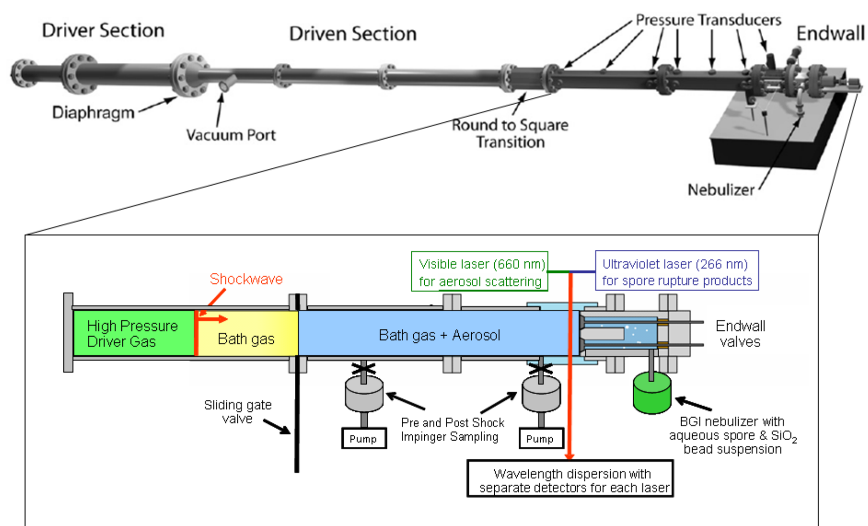

Figure 1: Stanford Aerosol Shock Tube with both the *in-situ* laser diagnostics and the *ex-situ* sampling used for quantitative measurements of spore response to shock heating

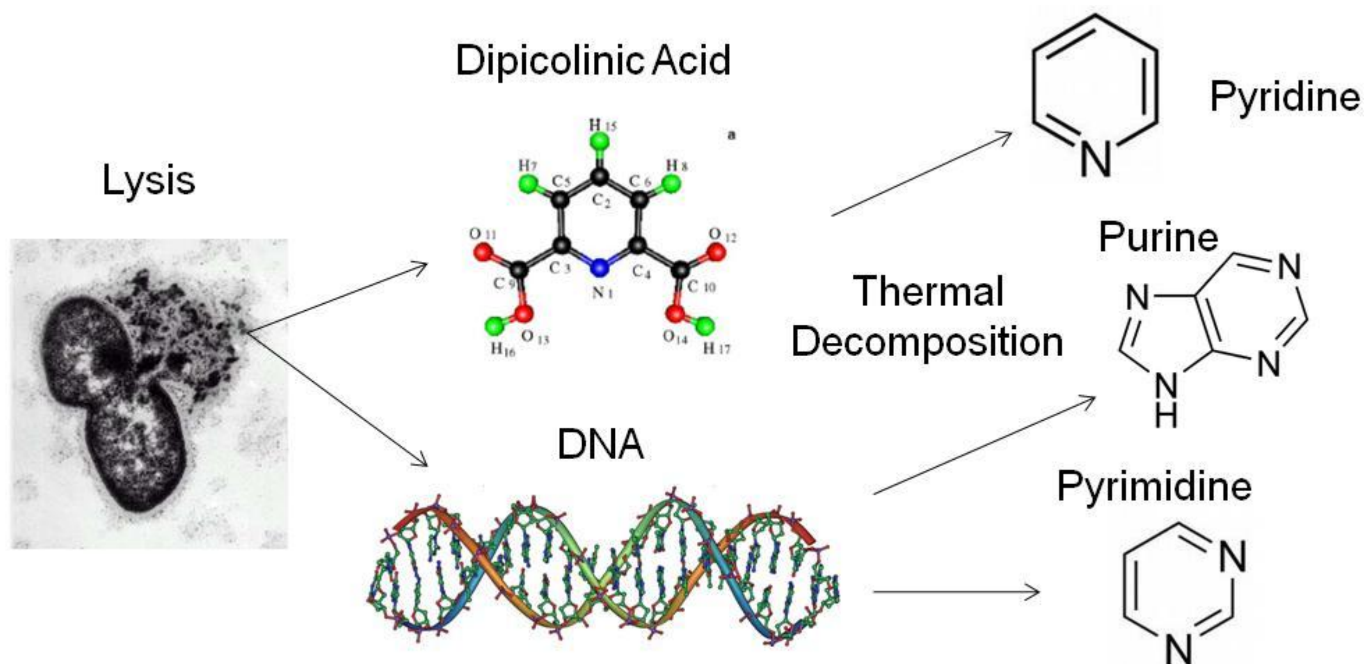

Figure 2: Shock treatment of endospores result in lysis and the release of UV active molecules

In-situ laser diagnostics provide time-resolved optical extinction measurements at 266 nm and 665 nm. In addition to scattering by the aerosol, the UV light at 266 nm is strongly absorbed by UV active endospore biochemicals. The visible light at 665 nm is only scattered by the bio-aerosol. Thus, the post-shock decay of 665 nm extinction provides a time history for the destruction of the spore morphology, while the UV light at 266 nm indicates the release of spore lysate. This method enables us to quantitatively measure the rate of spore destruction as a function of shock strength and post-shock temperature.

Flow cytometry measurements serve to enumerate the particles in the pre- and post-shock samples using forward and side scatter of laser light. Endospore resistance to shock heating is subsequently quantified by combining the results of the flow cytometer analysis with viability as determined by standard plating techniques. In addition, the inclusion of propidium iodide (PI) fluorescent dye in the spore samples allows for the interrogation of intact but morphologically damaged spores.

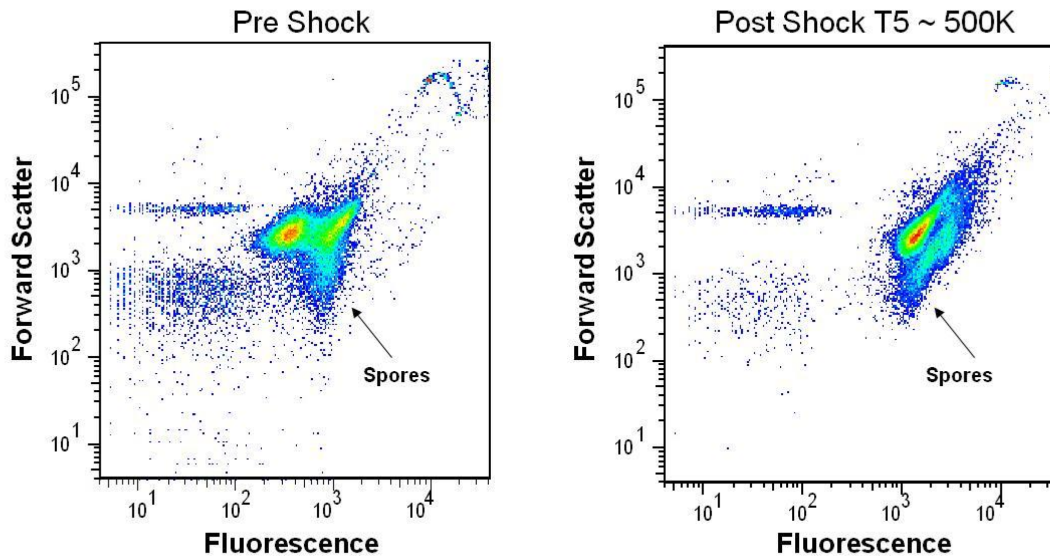

Figure 3: Flow cytometry data: PI, a nucleic acid binding dye, is added to the sample to indicate exosporium deterioration. The increased fluorescence signal in the post shock sample is indicative of PI infiltration and adhesion to the nucleic acid laden core.

Next Steps

Recent experiments have confirmed the presence of various temperature dependent modes of spore deactivation as well as a distinct elevated temperature demarcating a rapid loss in viability. To understand the deactivation mechanisms of critical components of the spore, mutants lacking select proteins and other biochemical agents will be investigated. Experimental tests will also be extended to strains with different structural properties. In addition, computational methods will be developed to model both the shock wave induced structural deterioration of the exosporium as well as biochemical deactivation due to heating of the core.

References

1. Gates, S.G., et al. "*Bacillus* Endospore Resistance to Gasdynamic Heating." Submitted to: Journal of Applied Microbiology, 2010