Decontamination of Anthrax Spores in Critical Infrastructure and Critical Assets

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Problem

Decontamination of anthrax spores in critical infrastructure (e.g., subway systems, major airports) and critical assets (e.g., the interior of aircraft) can be challenging because effective decontaminants can damage materials.

Current decontamination methods require the use of highly toxic and/or highly corrosive chemical solutions because bacterial spores are very difficult to kill.

- **Bacterial spores such as *Bacillus anthracis*, the infectious agent of anthrax, are one of the most resistant forms of life and are several orders of magnitude more difficult to kill than their associated vegetative cells.**
- **Remediation of facilities and other spaces (e.g., subways, airports, and the interior of aircraft) contaminated with anthrax spores currently requires highly toxic and corrosive chemicals such as chlorine dioxide gas, vapor-phase hydrogen peroxide, or high-strength bleach, typically requiring complex deployment methods.**

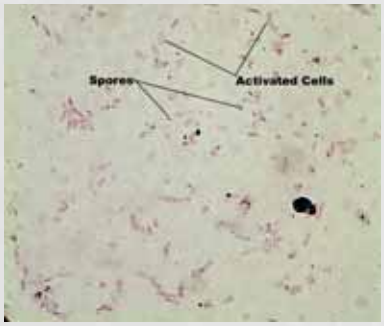
Corrosion of a metal lamp fixture following application of chlorine dioxide gas inside of an office building.

Complex deployment system for chlorine dioxide gas inside of a contaminated facility following the 2001 anthrax attacks.

Approach

We have developed a non-toxic, non-corrosive decontamination method to kill highly resistant bacterial spores in critical infrastructure and critical assets.

- **A chemical solution that triggers the germination process in bacterial spores and causes those spores to rapidly and completely change to much less-resistant vegetative cells that can be easily killed.**
- **Vegetative cells are then exposed to mild chemicals (e.g., low concentrations of hydrogen peroxide, quaternary ammonium compounds, alcohols, aldehydes, etc.) or natural elements (e.g., heat, humidity, ultraviolet light, etc.) for complete and rapid kill.**



Microscopic image of cells exposed to the germination solution — bacterial spores are stained blue while their associated vegetative (i.e., activated) cells are stained red.

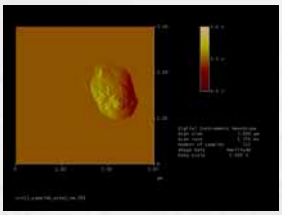
Results

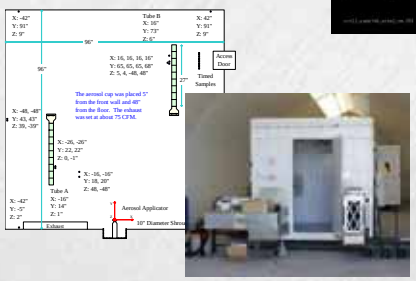
- **Our process employs a novel germination solution consisting of low-cost, non-toxic and non-corrosive chemicals.**
- **We are testing both direct surface application and aerosol delivery of the solutions.**

Germination Solution (GS)	Kill Solution (KS)	Time Exposed GS	Time Exposed KS	Log CFU's Remaining
PBS (Control)	None	-	-	6.27
None	3% H_2O_2	-	60 min	6.41
None	6% H_2O_2	-	60 min	6.42
5% of GS Mix in DI H_2O	3% H_2O_2	60 min	60 min	2.55
10% of GS Mix in DI H_2O	3% H_2O_2	60 min	60 min	2.22
15% of GS Mix in DI H_2O	3% H_2O_2	60 min	60 min	1.99
20% of GS Mix in DI H_2O	3% H_2O_2	60 min	60 min	1.59
25% of GS Mix in DI H_2O	3% H_2O_2	60 min	60 min	0
30% of GS Mix in DI H_2O	3% H_2O_2	60 min	60 min	0

Kill of *Bacillus cereus* spores (an anthrax surrogate) with and without the addition of a germination solution (CFU's = colony forming units).

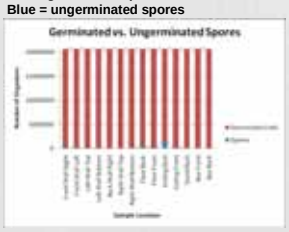
Atomic force microscopy image of a spore that has activated and is shelling its spore coat following exposure to the germination solution.





Aerosol dispersal testing of the germination and kill solutions is in progress. Initial tests have resulted in ~20M bacterial spores killed on coupons mounted in various locations in the test chamber (starting concentration is >10^7 spores)

Red = germinated spores
Blue = ungerminated spores



Green = spores that germinated and were killed
Red = spores that germinated and were not killed
Blue = ungerminated spores

Significance

A key Homeland Security need is to develop the capability to rapidly recover from an attack utilizing biological warfare agents. This project will provide the capability to rapidly and safely decontaminate critical facilities and assets to return them to normal operations as quickly as possible, sparing significant economic damage by re-opening critical facilities more rapidly and safely.

- **Facilities and assets contaminated with *Bacillus anthracis* (i.e., anthrax) spores can be decontaminated with mild chemicals as compared to the harsh chemicals currently needed.**
- **Both the "germination" solution and the "kill" solution are constructed of "off-the-shelf," inexpensive chemicals.**
- **The method can be utilized by directly spraying the solutions onto exposed surfaces or by application of the solutions as aerosols (i.e., small droplets), which can also reach hidden surfaces.**

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