SOUTHERN RESEARCH

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Aerosol Characterization of Three *Bacillus anthracis* Spore Preparations

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Abstract

Bacillus anthracis (*B. anthracis*) is the causative agent of cutaneous, gastrointestinal, and inhalational anthrax. In inhalational anthrax models, *B. anthracis* is induced to form spores that are used to challenge animals. Since many different *B. anthracis* spore preparation protocols exist that produce different aerosol particle size distributions, we characterized aerosol from different *B. anthracis* Sterne (*BAS*) purification processes to determine which process produces the best type of spore preparation. Our parameters for the ideal spore preparation were: 1) the most consistent and 2) the highest percentage of particles with the appropriate size (1 to 2 microns). We hypothesized that renografin purified spores would give us the most consistent results and analyzed three different purification processes: washed, pellet, and renografin purified. The mean aerosol concentration (cfu/L) and impinger concentrations (cfu/mL) were higher in the renografin preparations when compared to washed or pellet purified spores. However, only ~13% of the washed spores had a count median diameter between 1 to 2 microns. The impinger concentrations (cfu/mL) were higher in the renografin samples compared to washed or pellet purified. The renografin preparation demonstrated approximately 80% of the count median diameter between 1 to 2 microns. We conclude that renografin purification of *BAS* spores is necessary to obtain clean and consistent bioaerosols for use in inhalation anthrax models.

Methods

Preparations of bioaerosols: *BAS* was grown in Difco Sporulation Media for 48 hours and harvested by centrifugation. Then washed, pellet, and renografin purification were done. Following each purification all spore preparations were resuspended in USP water, plate counted in triplicate, and diluted to 1 x 10^9 CFU/mL. Spore bioaerosols were generated with a Collison 3-jet nebulizer and collected from a plenum with an all-glass impinger (AGI) containing USP water. Relative AGI concentrations of spores were determined by additional plate counting.

Wash purification: The spores were washed a total of three times with USP water.

Pellet purification: The spores underwent a process in which the outer layer of the spore pellet was removed by gently swirling and pipetting to remove the loosely pelleted material.

Renografin purification: The spores were passed though a 20:50% (v/v) renografin gradient.

Five different spore preparations were analyzed. Particle counts and size were taken for each preparation. Mean t-tests were performed to determine statistical significant for impinger and aerosol concentrations.

Methods

***BAS* pellet purification process**

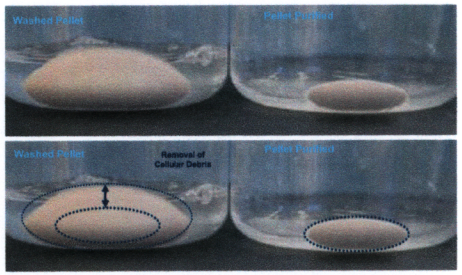

Figure 1. Representative sample of a spore pellet debris removed in the pellet purification process. A large amount of total cell volume is lost in the pellet purification process, which could lead to its variability between preparations.

***BAS* purification visual comparison**

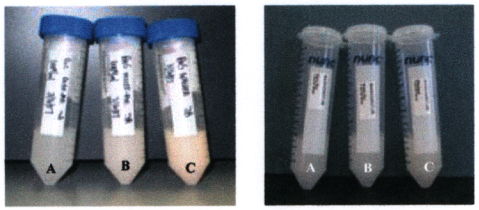

Figure 2. A representative sample of two separate preparations of spores. A) Renografin purified spores, B) Pellet purified spores, and C) Washed spores. A visual comparison of the purification process shows variability between preparations.

Rodent nose-only aerosol inhalation system

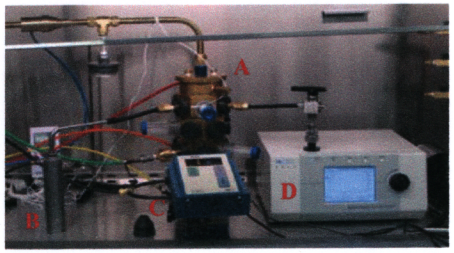

Figure 3. Rodent nose-only aerosol inhalation system. A) ITP 24-port chamber, B) Stainless steel impinger, C). DustTrak (particle counter), and D) Aerodynamic particle sizer.

Results

***BAS* impinger vs. aerosol concentration comparison**

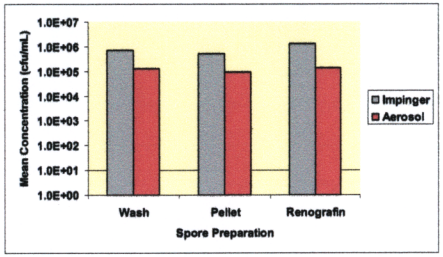

Figure 4. The mean concentration of the different spore preparations collected from the AGI and calculated aerosol concentrations. The mean aerosol concentration (cfu/L) and impinger concentration (cfu/mL) were higher in the renografin purified spore preparations compared to washed or pellet, but were not significant.

Particle number comparison of wash, pellet, and renografin purified spores

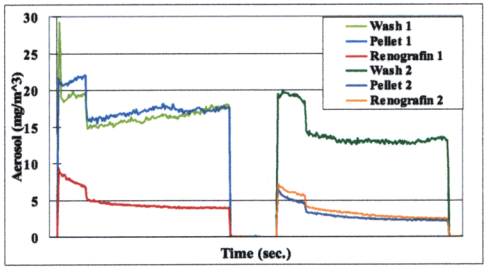

Figure 5. MicroDust comparison of two data sets. The aerosol particle concentration varies in both the wash and pellet purified samples; whereas, the renografin purified samples are reliably consistent.

***BAS* purification particle size comparison**

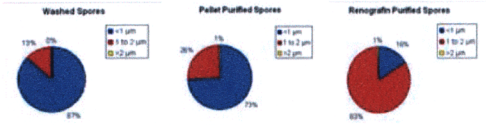

Figure 6. Size distribution of spores from each type of preparation. The mean count median values for washed, pellet, and renografin purified spores were 0.79, 1.03, and 1.26 respectively and statistically significant. The renografin purified spores had the most particulates between 1 micron and 2 microns compared to the washed and pellet purified spores.

Results

***BAS* purification mass comparison**

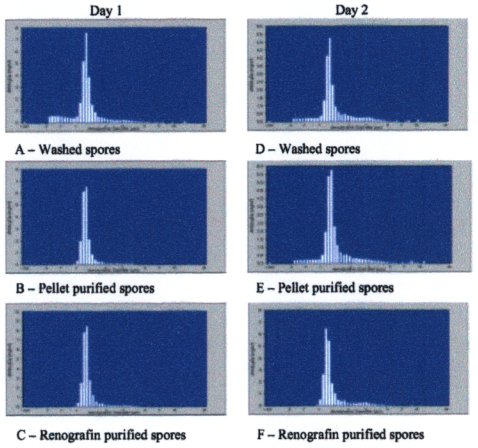

Figure 7. Representative aerosol particle size distributions of different spore preparations from two independent experiments. The aerodynamic diameter graph is weighted by particulate mass. The mean mass median for washed, pellet, and renografin purified spores were 1.36, 1.35, and 1.31 respectively but was not significantly different.

Conclusions

- Different spore preparations produce varying amounts of cellular debris.
- The wash purification retains the most particles of debris.
- The pellet purification was the most variable.
- Renografin purification generates more consistent spore preparations of particulates between 1 to 2 microns.

Acknowledgements

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