The Release of Calcium in *Bacillus anthracis* Pathogenicity

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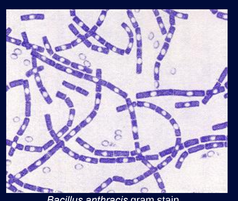

Bacillus anthracis gram stain

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Abstract

Anthrax infection starts with germination of *Bacillus anthracis* spores in macrophages. Some bacteria, including *B. anthracis*, can sporulate in response to environmental stress, such as starvation. During germination, a large concentration of calcium ions are released from the *B. anthracis* spore. Calcium ions are hydrophilic secondary messengers, and may therefore interfere with detection of the spore by confusing the cell signaling pathways. We investigated the effect of calcium release on infected macrophage viability by replacing the calcium stored in *B. anthracis* spores for other cations via demineralization/remineralization. It was discovered that calcium ions typically out-preformed other cations in germination of *B. anthracis*.

Introduction

Bacillus anthracis is a gram-positive, spore-forming, rod-shaped bacterium. *B. anthracis* spores are resilient, surviving extreme temperature, low-nutrient environments, and harsh chemical treatment (13). When spores are inhaled, ingested, or come into contact with a skin lesion on a host the spores may reactivate and multiply rapidly (6). The vegetative form of *B. anthracis* releases a lethal toxin (7). Macrophages are white blood cells within tissues that phagocytize cellular debris. When a macrophage engulfs a *B. anthracis* spore, the spore is not killed. The spore, instead, germinates inside the host macrophage. During the germination process, *B. anthracis* toxins are released (9). Calcium ions act as secondary messengers in macrophages, secondary messengers are involved in signal transduction. When the *B. anthracis* spore germinates, it releases up to 1M of calcium ions (13). It is possible that the sudden influx of calcium ions inside the macrophage, once the spore starts to germinate, can confuse the cell signaling system. This confusion may allow the newly germinated bacterium to go undetected; it then can release the toxin to kill the macrophage.

Figure 1: A living macrophage (opaque) and a dead macrophage (blue)

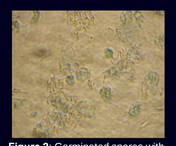

Figure 2: Germinated spores with killed macrophages

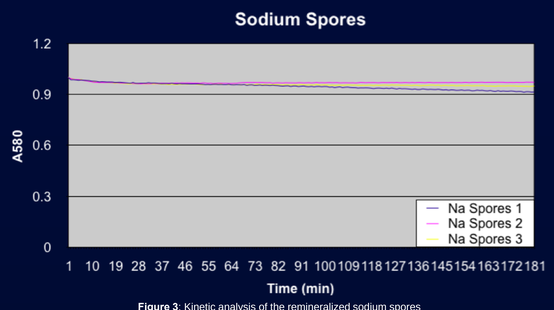

Figure 3: Kinetic analysis of the remineralized sodium spores

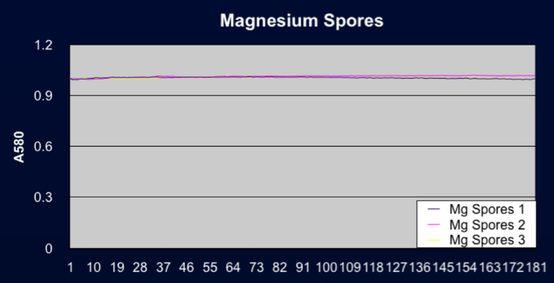

Figure 4: Kinetic analysis of the remineralized magnesium spores

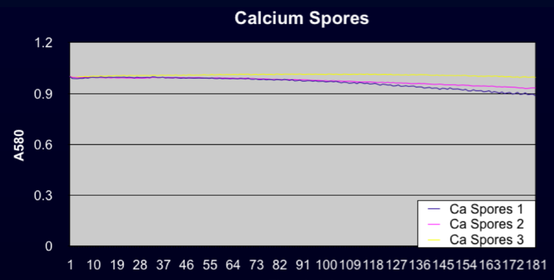

Figure 5: Kinetic analysis of the remineralized calcium spores

Hypothesis

The release of calcium ions during *Bacillus anthracis* spore germination impacts anthrax- mediated macrophage death.

Methods

- Spore Preparation
  - The remineralized spores were prepared using a modified method outlined in Igura et al
- Cell Culture
  - Murine macrophages were cultured in a HyClone HBS medium that included fetal bovine serum
- Infection
  - Remineralized spores were added to the macrophages and incubated
  - The macrophages were washed several times, a new medium containing an anitbiotic was added
- Microscopy
  - Macrophages were stained with 0.04% Trypan Blue
  - Macrophages were visualized with light microscopy
- Kinetics
  - Germination was measured as a decease in optical density (A_{580}) using the Tecan M200 and the coordinating iControl computer program

Results

The remineralized spores took over 18 hours to germinate and kill the macrophage. Of the three remineralized spores analyzed here, the remineralized calcium spores were most efficient at germination and macrophage killing. Through this and previous work (4), it can be inferred that calcium is important to spore germination and possibly to the eventual lysis of the macrophage.

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Acknowledgements

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