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## Is Heat Activation of *Bacillus anthracis* spores Reversible?

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**Background:** Bacteria of the *Bacillus* genera are capable of forming dormant and resilient cells called spores in response to starvation. These dormant spores can be reactivated in the presence of nutrients in a process called germination. Historically, spores are exposed to sublethal heat treatments to increase the extent and rate of germination. This process is known as heat activation (HA). HA reduces  $T_{lag}$ , the time between the addition of germinant to the rapid release of  $Ca^{2+}$  and dipicolinic acid (Ca-DPA) from the spore core, and increases the percentage of spore germination. This release of Ca-DPA initiates germination, and optical density loss follows. Previous studies on *Bacillus* species indicate that the effects of HA are reversible after 72 hours. After this time period, the spores must be reactivated. However, recent experiments by our lab suggest that this might not be the case for *Bacillus anthracis* spores.

**Methods:** *B. anthracis* spores were prepared by exhaustion in DSM and extensively water washed. Each sporulation was split into 3 samples: no heat treatment, heat activated on day one of the experiment (HA1) and activation on the particular day relative to day 1 (HAN). Spores were heated at 65°C for 30 minutes, cooled on ice for 15 minutes, and warmed to room temperature. Germination is initiated with 1mM Alanine and 1mM Inosine. Germination was measured by the loss of optical density at 580nm ( $OD_{580}$ ). The assays were performed on days 1, 3, 5, 7, and 14.

**Results:** As expected, heat activation had an impact on spore germination. The preliminary data shows that, over a period of seven days, heat activated spores had reduced  $T_{lag}$  and an increase in the final percent germination over non-activated spores. We are in the process of repeating this experiment to determine if the storage conditions of the spores impact these results.

**Conclusions:** Our data suggests that heat activation may impact spores longer than originally expected. This may have broader impacts on our understanding of heat activation.