Germination and sporulation of *Bacillus anthracis* in topsoil

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Background Information

Bacillus anthracis is a spore forming bacterium which causes fatal infections in mammals. Route of infections include ingestion or inhalation of the environmentally persistent spores which reside in the soil. Infection causes massive hemorrhaging in the host, which may contribute to contamination of the soil. The common belief is that spores germinate and proliferate in the host's body and then the vegetative forms sporulate in the carcass post mortem when they deplete the nutrients in their local environment. Whether *B. anthracis* spores are capable of germination, proliferation, and subsequent sporulation in soil is a topic of discussion and debate.

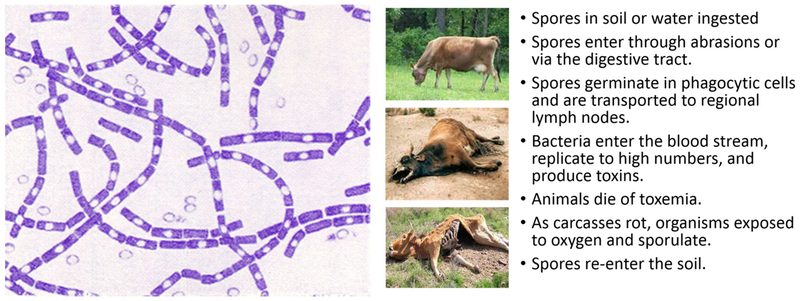

Figure 1: Gram stain of *Bacillus anthracis* vegetative forms (left) and the life cycle of *Bacillus anthracis* (right).

Methods

This study incorporates two experiments to A) determine if *B. anthracis* spores can germinate in sterile potting soil and B) determine if vegetative forms can sporulate in sterile potting soil and determine the effects of blood on the sporulation process. We hypothesized that spores are capable of germination and sporulation in topsoil environments and that blood has a positive effect on the sporulation of vegetative forms in topsoil. For experiment A spores where inoculated into soil samples, the bacteria were recovered from the soil using filters. Half of the recovered sample was heat treated to identify spores. Heat treated and non-heat-treated halves of the samples were diluted and plated to enumerate the number of spores and vegetative forms. For experiment B vegetative forms were inoculated into soil samples with varying amounts of blood. Samples were collected and enumerated as in experiment A.

Results

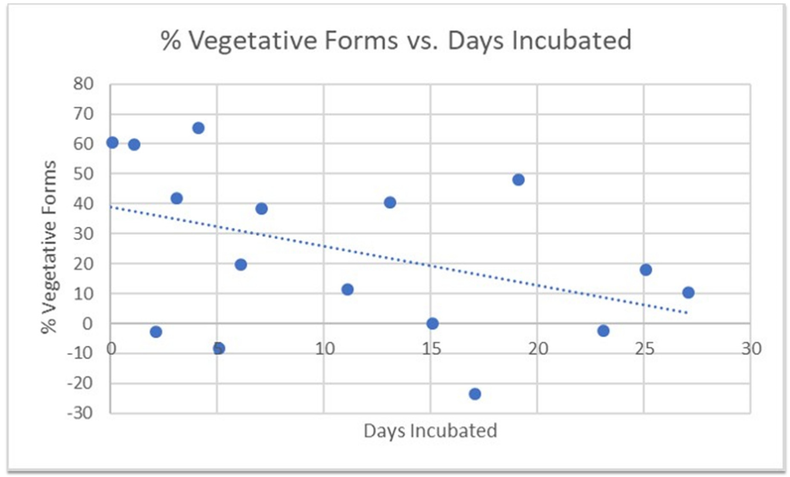

Figure 2: The results of experiment A. The percent of vegetative forms collected at each time point plotted against the number of days the soil sample incubated at 30˚C.

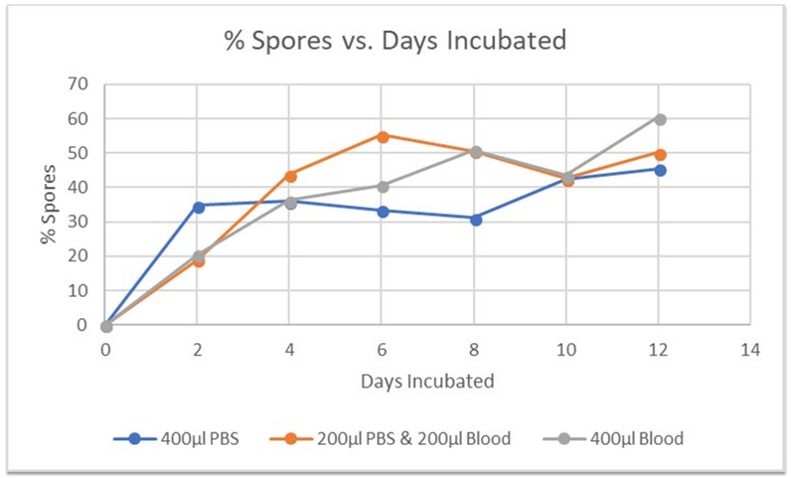

Figure 3: The results of experiment B. The percent of spores collected at each time point plotted against the number of day the soil sample incubated at 30˚C.

Conclusions

The results of experiment A were inconclusive. There is a slight downward trend but most date points do not fit on this line.

The results of experiment B showed that vegetative bacteria are capable of sporulation in soil and also show that the addition of blood to the soil seems to have a positive effect on the rate of sporulation.

Discussion

The results of experiment A were inconclusive. The presence of vegetative cells at time 0 is an unexpected result. It is possible that the soil was sufficiently nutrient dense to allow these cells to begin to germinate between the time of inoculation and the time of heat treatment. This would result in a loss of heat resistance in these cells.

The results from experiment B show that sporulation can occur outside the infected animal when bacteria are deposited into soil and that the presence of blood may have a modest positive effect on sporulation. This suggests that soil may become spore contaminated when viable bacterial cells are introduced and subsequently sporulate.

Acknowledgements

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