Effects of Immunoglobulins against Specific Exosporium Proteins on Spore Germination of *Bacillus anthracis in vitro*

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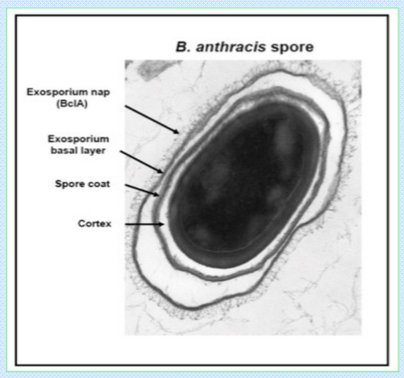
1 Background

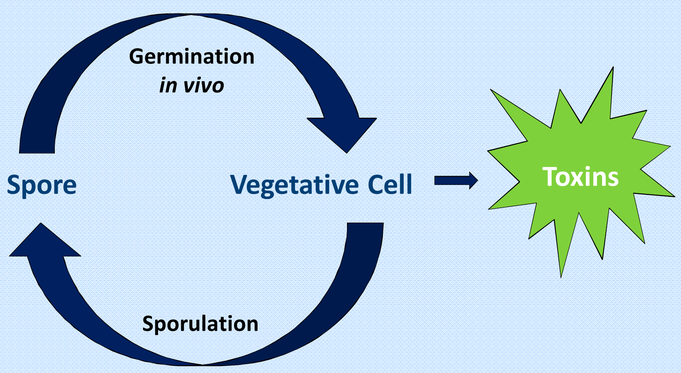
Bacillus anthracis is a rod-shaped, gram (+) spore-producing bacteria that is the causative agent of Anthrax – a deadly disease and potential bioterrorism threat. The infectious particles are spores which must germinate *in vivo* and produce toxins in order to cause illness. The spores are composed of a single bacterial cell surrounded by a multilayer protein shell – the outermost of which is the exosporium. The exosporium consists of the inner basal layer and an outer hair-like nap layer, that is comprised primarily by the immunodominant glycoprotein BclA.

The host's immune system produces antibodies against exosporium proteins upon vaccination with whole-spore preparations. Currently, the primary vaccine used in the United States results in antibody protection against a critical *B. anthracis* protein called Protective Antigen (PA). Brahmbhatt et al.[1] found that boosting with anti-BclA antibodies after a primary immunization against PA offered full protection from disease upon spore challenge in mice. Similarly, vaccination with PA with the BclA protein present provided better protection from infection than PA alone[2]. Together, these studies suggest that the addition of particular exosporium proteins may be beneficial to vaccination efficacy. It is not fully known which mechanisms are responsible for the additional protection. Studies have shown that anti-spore antibodies can delay or inhibit spore germination *in vitro*. However, how anti-sera raised against specific exosporium proteins will effect germination remains unclear.

The objective of this study is to assess the effects of polyclonal antibodies raised against specific exosporium proteins on spore germination of *Bacillus anthracis in vitro.* We hypothesize that, like anti-spore antibodies, anti-sera raised against specific exosporium proteins will delay germination.

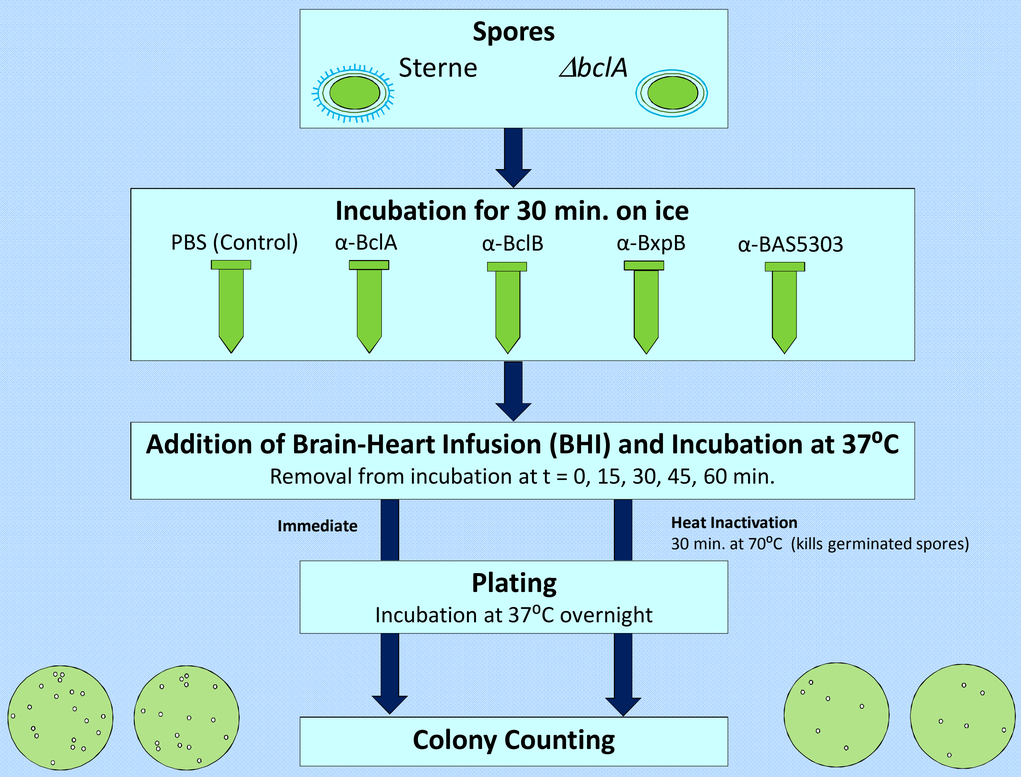


Spore EM courtesy of Dr. Brian Thompson.



2 Materials and Methods

Heat Inactivation Assay



3 Results

Western Blots Probed with antibodies against specific exosporium proteins

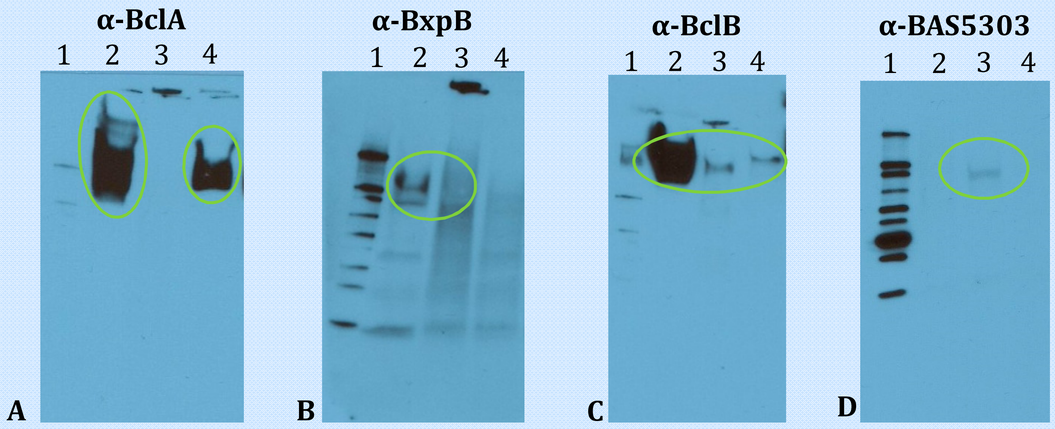


Figure 1 A-C Western Blots. Lanes 1: Protein Standard, 2: Sterne Strain, 3: *ΔbclA*, 4: *ΔbxpB*. Exosporium and some spore coat proteins were electrophoresed onto an SDS-PAGE, and subsequently transferred to a membrane. Western blot revealed antisera bound to their respective exosporium protein (circled), when present.

Heat Inactivation Assay

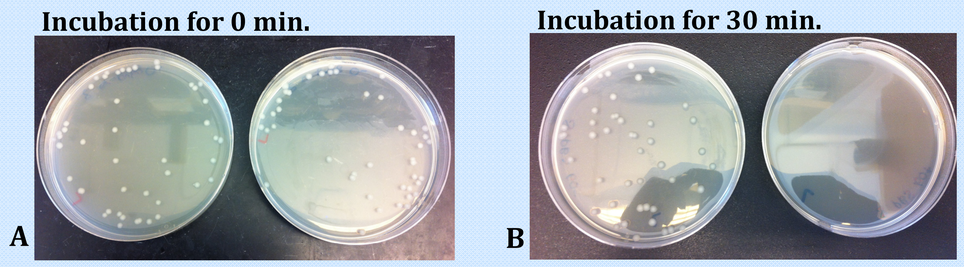


Figure 2 A-B Colony Growth on Plated Samples where left = immediate plating, right = heat inactivation. (**A**) Spores incubating in BHI for 0 min. were not inactivated by heat. In contrast, (**B**) shows that spores incubating in BHI for 30 min. showed no colony growth when inactivated by heat.

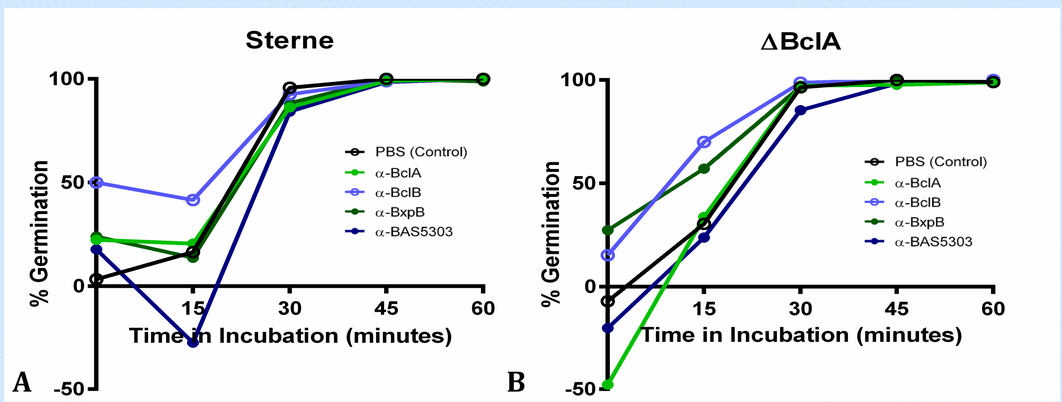


Figure 3 A-B Germination Curve. All treatments resulted in spores that were fully germinated (97% +) after 45 minutes of incubation in BHI. There were no clear differences in % germination through time between control and antibody treated groups.

4 Summary and Conclusions

- Previous studies showed that antibodies towards whole-spore preparations delayed spore germination *in vitro* for *Bacillus anthracis*. Therefore, immunization with particular exosporium proteins may be useful in improving vaccination success.
- In our experiments we observed that:
  - Western Blot: Antibodies against exosporium proteins (α-BclA, α-BclB, α-BxpB and α-BAS5303) bound to their respective proteins, when present.
  - Heat Inactivation: There were no obvious differences in % germination of spores (Sterne and *ΔbclA* strains) through time between control and treatment groups.
- **Antibodies to specific exosporium proteins were not found to effect germination of *Bacillus anthracis* spores *in vitro***

References

[1]Brahmbhatt, T. N., Darnell, S. C., Carvalho, H. M., Sanz, P., Kang, T. J., Bull, R. L., Rasmussen, S. B.,Cross, A. S., and O'Brien, A. D. Recombinant Exosporium Protein BclA of *Bacillus anthracis* Is Effective as a Booster for Mice Primed with Suboptimal Amount of Protective Antigen. Infec. Immun. 2007. 75:5240-5247.

[2]Hahn, U. K., Boehm, R., and Beyer, W. DNA vaccination against anthrax in mice – combination of anti-spore and anti-toxin components. 2006. Vaccine. 24:4569-4571.

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