# Fate of exosporium proteins BclA, BclB, and BxpB in germinating *Bacillus anthracis* spores.

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## BACKGROUND

*Bacillis anthracis* is a spore-forming, Gram-positive rod that is widely distributed throughout the world and responsible for zoonotic disease in mammals, especially ruminants. When the environment lacks sufficient resources, the bacteria undergo sporulation and enter a metabolically inert state, becoming surrounded by a hardy spore coat. *B. anthracis* spores also possess an additional outer layer, the exosporium, consisting of a basal layer and a hairlike glycoprotein nap layer. Exosporium proteins include BclA (in the nap layer), BclB (thought to reside beneath the nap layer), and BxpB (in the basal layer). The exosporium contains a “cap” region (accounting for about 25% of the exosporium) and “non-cap” region (the remaining 75%). When external conditions are again suitable to sustain growth, the cell will undergo germination and emerge from the spore via the opening of the cap (shown by Steichen and colleagues in 2007). Precisely how the cap opens to allow exit of the bacterium from the spore is not understood. BclB is found exclusively in the non-cap region. It has been demonstrated that mutants lacking BclB form spores with a defective cap, suggesting an essential role of BclB in cap formation. We hypothesized that BclB may be a target of proteases, serving to open the cap after activation and outgrowth of the bacterium. Finding smaller fragments of BclB complexes in germinating spores than those of non-germinating spores would support this theory.

## WESTERN BLOTTING RESULTS

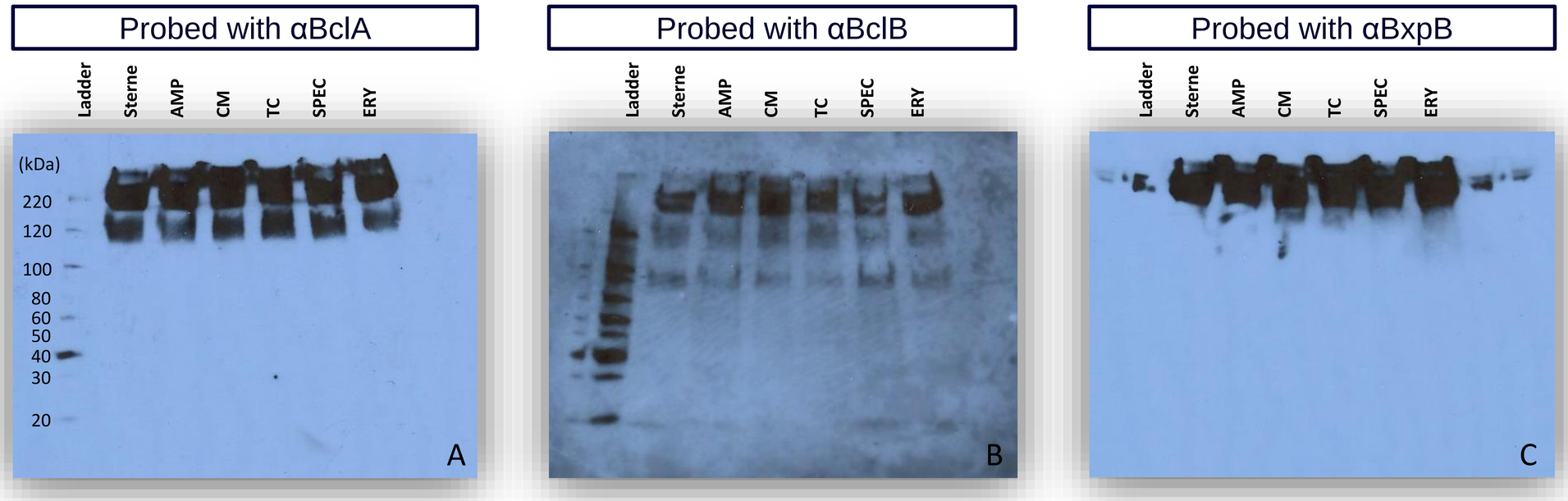


**FIGURE 2:** Western blotting after size-fractionating via SDS-PAGE. In each image, the lanes contain the following samples, from left to right: Protein size standard ladder, non-germinating Sterne vaccine strain *Bacillus anthracis* spores, spores germinating in the presence of ampicillin, spores germinating in the presence of chloramphenicol, spores germinating in the presence of tetracycline, spores germinating in the presence of spectinomycin, and spores germinating in the presence of erythromycin. **(A**) The blot probed with αBclA antibody (1:20,000 dilution), showing consistent bands at around 220 kDa and 120 kDa. 1 minute exposure time. **(B)** The blot probed with αBclB antibody (1:6,666 dilution), showing consistent bands at three sizes (>220 kDa, ~220 kDa, and ~110 kDa). 15 minutes exposure time. **(C)** The blot probed with αBxpB antibody (1:20,000 dilution), showing consistent bands at around 220 kDa. 1 minute exposure time.

## THE EXOSPORIUM

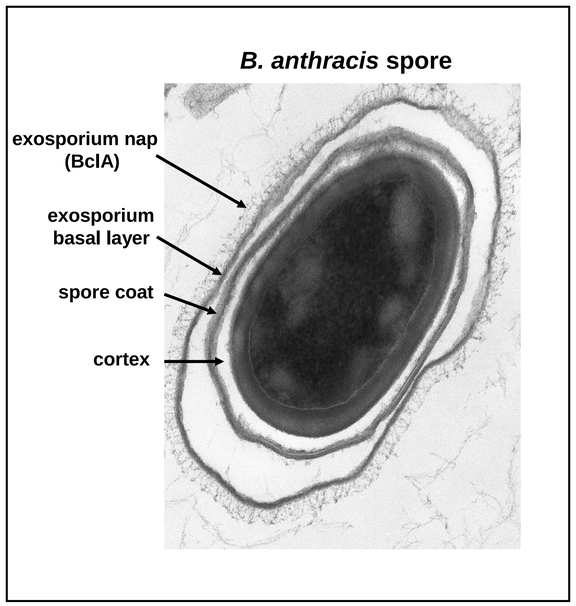


**FIGURE 1**: Scanning electron micrograph of the *B. anthracis spore*, showing the outer nap layer of the exosporium (containing BclA) and the exosporium basal layer (where BxpB is found).

## METHODS

**1: Preparation of the *Bacillus anthracis* spores.** Nutrient agar plates were inoculated with the Sterne vaccine strain of *Bacillus anthracis*. The Sterne strain lacks one of the two virulence plasmids possessed by wild-type *B. anthracis*. The missing PXO2 plasmid is that which produces the polyglutamic acid coat that protects the bacterium from host immune defense. The remaining plasmid is used to produce the toxins that are immunogenic. This is the only respect in which the Sterne strain differs from the wild-type bacterium.

**2: Prepare test groups – dormant and germinating spores.** As available nutrients in the dish medium decrease over time, the bacteria sporulate. One test group was created by harvesting these dormant spores. Other spores harvested and later stimulated to germinate by being placed in nutrient-rich broth (Brain Heart Infusion broth) containing one of five antibiotics. The antibiotics were added to impede cell growth after germination. The other test groups were the spores that germinated in the presence of these antibiotics – and thus had opened cap regions of their exosporia. Verification of germination was completed by preparing a control broth without antibiotic and observing vegetative cells with few (if any) remaining spores under a phase contrast microscope after incubation.

**3: Extract the exosporium proteins.** The spore samples were boiled for ten minutes in a solution containing 2% SDS (detergent properties), beta-mercaptoethanol (to break disulfide bonds), and 8M urea. This rendered a sample with proteinaceous chunks that contained the exosporium proteins of interest.

**4: Size-fractionate the extracts.** The boiled spore samples from each experimental group were electrophoresed on a gradient polyacrylamide gel and transferred to a PVDF membrane.

**5: Western blotting.** The Stewart Laboratory has rabbit polyclonal antibodies against exosporium proteins BclA, BclB, and BxpB. Blots probing for each of the three proteins were performed twice each to reveal any potential differences in the protein sizes between germinating and non-germinating samples.

## CONCLUSION

Western blotting revealed that there appears to be no significant difference between the size of BclB protein complex fragments in non-germinating *B. anthracis* spores and those in germinating *B. anthracis* spores. These results then reject the initial hypothesis that the removal of the cap region during germination involved the cleavage of BclB as a protease target.

## REFERENCES

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