# Role of the *gerQ* determinant in exosporium structure in *Bacillus anthracis*

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## Background

*Bacillus anthracis* is a gram-positive, spore forming bacterium that is the etiologic agent of anthrax. This zoonotic pathogen is associated with high levels of mortality in infected ruminants and humans with the pulmonary form of the disease. When nutrients are sparse, such as in the soil, the bacteria will undergo the process of sporulation, to produce the dormant, specialized structure, the spore. The spore is important as it is the infectious form of the bacterium. The outermost layer of the spore is the exosporium, which consists of a basal layer and a hairlike glycoprotein nap layer. The exosporium is the structure which directly interacts with the host innate immune system during the initial stages of the infection. Some of these proteins have been shown to have an effect on exosporium development, such as BclA (the prominent nap layer glycoprotein), BclB (found just below the nap layer), and BxpB (a basal layer protein)[a].

## Objective and Hypothesis

Experiments done with *B. cereus* that studied *gerQ* knockout spores showed a weakened exosporium, suggesting that this protein was integral in the formation and attachment of the exosporium[b]. Since this has not been proven in *B. anthracis*, we intend to create a *gerQ* knockout mutant and determine if it will show a similarly weakened exosporium.

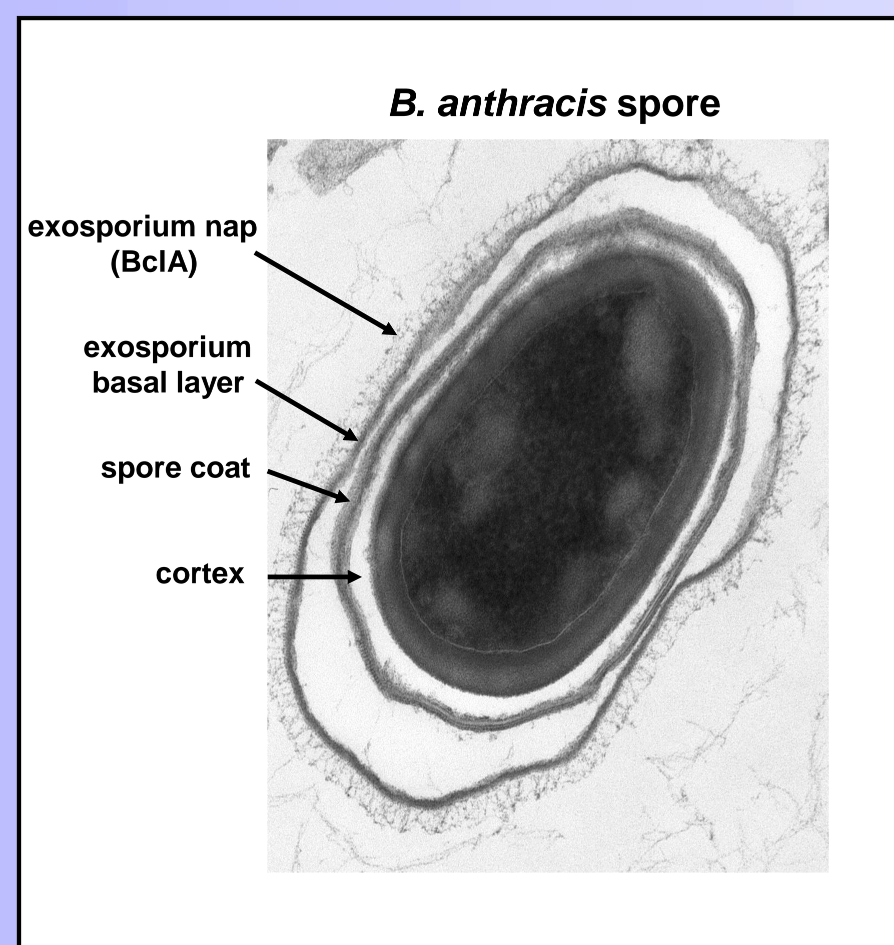


Figure 1
Transmission electron micrograph detailing spore structures

## Methods and Materials

We utilized a method known as allele replacement mutagenesis. We engineered a temperature-sensitive plasmid with erythromycin resistance and the target gene sequence, consisting of 1 kb of sequences upstream and downstream of *gerQ* and with the GerQ coding sequence replaced with a spectinomycin resistance cassette. We then electroporated this plasmid into the Sterne strain of *B. anthracis*. We then plated these electrotransformed cells onto spectinomycin containing plates, incubated them at 30°C. Resultant colonies were then toothpick inoculated onto spectinomycin plates and erythromycin plates to confirm both resistances. Cells were then inoculated into spectinomycin-containing broth and incubated at 42°C, a temperature at which the plasmid is defective in replication. An overnight broth culture was plated on spectinomycin containing plates. Resulting colonies were toothpick inoculated on spectinomycin-containing and erythromycin-containing plates. This was repeated until spectinomycin resistant, erythromycin-sensitive clones were obtained (herein numbered 18 and 19). DNA was isolated from these cells and PCR was carried out on these samples to determine if the *gerQ* determinant was missing and replaced with the spectinomycin resistance cassette. We knew that the wild type strain PCR product would be about 2.5 kb, and with the GerQ knockout gene we anticipated the size to be about 3.2 kb, and we achieved these results. Once we were confident that the strains were indeed *gerQ* deletion mutants, spores were prepared and we performed Western Blots on detergent extracts of spores to detect three known exosporium proteins: BclA, BclB, and BxpB. To determine if the exosporium layer in the mutant spores was more fragile than that of the wild-type strain, western blots were also conducted on spores that had been vortex-agitated for 5 minutes and spores subjected to bead-beating for ten one-minute intervals followed by 30 seconds rest at 4°C.

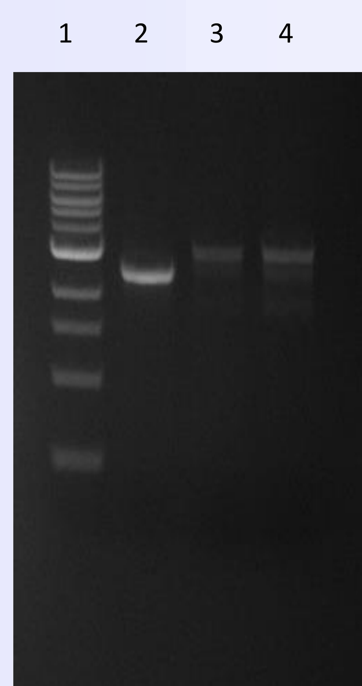


Figure 2: Gel Electrophoresis after PCR amplification.
Lane 1, DNA ladder.
Lane 2, Sterne wild-type.
Lane 3, mutant 18.
Lane 4, mutant 19.
The Sterne wild-type amplification was about 2.5 kb, while the two mutant strains- PCR products are about 3.2 kb.

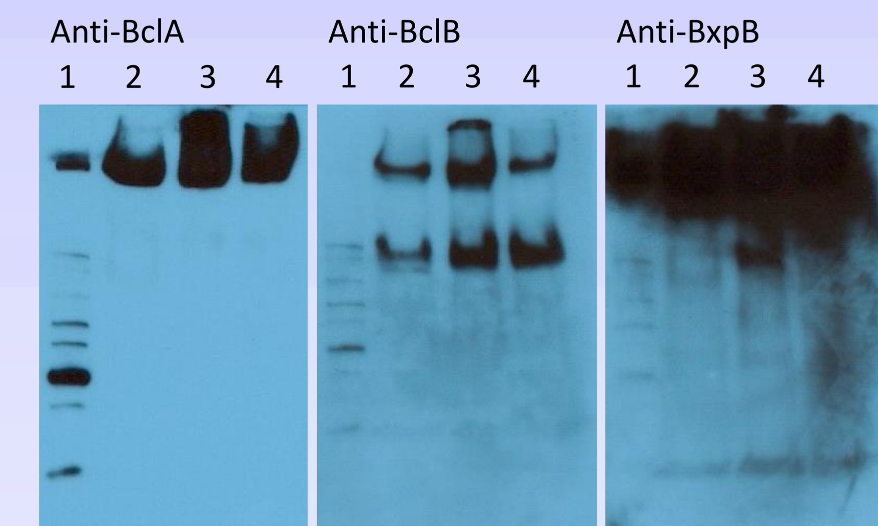


Figure 3: Western Blot with rabbit polyclonal sera. Proteins were extracted from spores by boiling in urea and SDS. Lane 1, protein marker. Lane 2, Sterne wild-type spore extract. Lane 3, GerQ-negative strain 18. Lane 4, GerQ negative strain 19. Blots were developed for 5 minute exposure times.

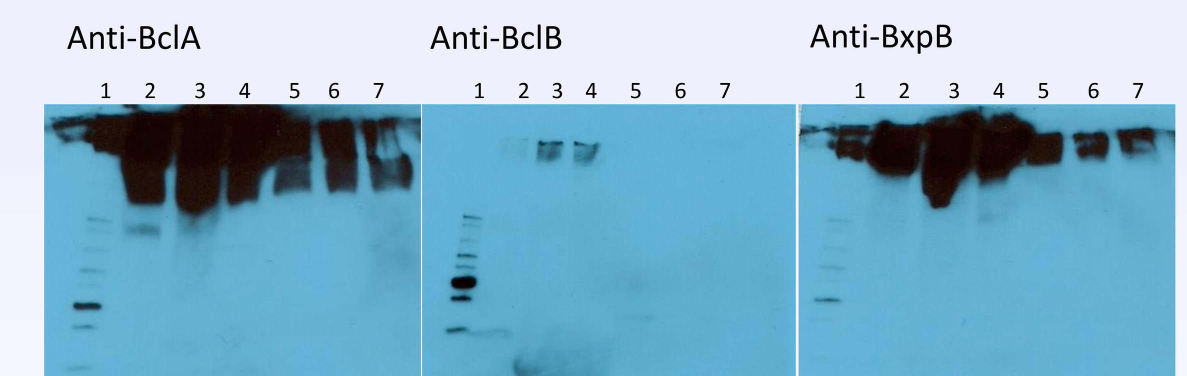


Figure 4: Western Blot with rabbit polyclonal antisera. Proteins were extracted from spores using two methods. Method A used vortex-agitation for 5 minutes. Method B utilized the bead-beating with 0.1 mm glass beads. Lane 1, protein marker. Lane 2, Vortexed Sterne wild-type spore extract. Lane 3, Vortexed mutant strain 18. Lane 4, Vortexed mutant strain 19. Lane 5, Bead-Beat Sterne wild-type. Lane 6, Bead-Beat mutant strain 18. Lane 7, Bead-beat mutant strain 19. 5 minute exposure times

## Summary and Conclusions

1. We were able to successfully create *gerQ* deletion mutant strains of *B. anthracis.*
2. In contrast to the observations with *B. cereus,* eliminating the GerQ protein of *B. anthracis* does not appear to have as great an effect on the integrity of the exosporium.
3. In the GerQ knockout mutants, we were able to extract more of the exosporium protein BclB than from the Sterne wild-type, suggesting a less stable attachment of BclB in GerQ-deficient spores.

## References


[a]Stewart GC. 28 October 2015. The exosporium layer of bacterial spores: a connection to the environment and the infected host. Microbiol Mol Biol Rev doi:10.1128/MMBR.00050-15

[b]Terry C, Shepherd A, Radford DS, Moir A, Bullough PA (2011) YwdL in Bacillus cereus: Its Role in Germination and Exosporium Structure. PLoS ONE 6(8): e23801. doi:10.1371/journal.pone.0023801


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