

Structure and Assembly of the *Bacillus anthracis* Exosporium

Cynthia M Rodenburg, Sylvia A McPherson, Charles L. Turnbough Jr., Terje Dokland

Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL, USA

Bacillus anthracis, the causative agent of anthrax, is a highly pathogenic Gram-positive soil bacterium that is also considered a serious threat as a potential bioterrorism agent. Like other bacilli, *B. anthracis* forms protective spores upon starvation. The spores are the main pathogenic agents, easily spread and highly resistant to environmental stress. When the spores enter a mammalian host, they germinate and undergo vegetative growth, followed by production of anthrax toxin, which leads to systemic bacteremia and toxemia that is often fatal.

The spore is a complex, multilayered structure, consisting of a nucleoid surrounded by a peptidoglycan cortex and a proteinaceous coat [1]. Spores of *B. anthracis* and other pathogenic bacilli are surrounded by an *exosporium*, a balloon-like layer that acts as the outer permeability barrier of the spore and is thought to contribute to spore survival in the environment, uptake by potential hosts, and as a virulence determinant. The exosporium consists of a hair-like nap and a paracrystalline basal layer [1]. The filaments of the nap are comprised of trimers of the collagen-like glycoprotein BclA, while the basal layer contains at least 20 different proteins. One of these proteins, BxpB, forms tight complexes with BclA and is required for attachment of essentially all BclA filaments to the basal layer [2]. Another basal layer protein, ExsB, is required for the stable attachment of the exosporium to the spore [3]. ExsY is required to complete exosporium assembly during sporulation [4]. The exosporium also harbors several enzymes, including alanine racemase (Alr), which functions as an anti-germinant, presumably functioning to prevent premature germination.

We have used a combination of cryo-electron microscopy (cryo-EM), cryo-sectioning (Tokuyasu method) and crystallographic analysis of negatively stained exosporium fragments to compare wildtype spores and spores with deletions of specific exosporium genes, including *bclA*, *bxpB*, *exsB* and *exsY*, to study the structure and assembly of the *B. anthracis* exosporium [5].

Cryo-EM reveals the intact spore in its native form and shows clearly the bilayered basal layer and the nap filaments with their distal knobs (Fig. 1A, B). The filaments appear to connect to protrusions in the basal layer, more clearly seen in negatively stained cryo-sections (Fig. 1C). $\Delta bclA$ spores lack the nap, while $\Delta bxpB$ spores lack both the nap and the protrusions, but retain the bilayered basal layer (Fig. 1D, E). The exosporium of $\Delta exsB$ spores is more fragile and is easily sloughed upon grown in liquid media, but looks otherwise completely normal and contains a normal complement of BxpB protrusions and BclA filaments (Fig. 1F). Taken together with crystallographic analysis of isolated exosporium fragments, these data suggest that the protrusions are made of trimers of BxpB (Fig. 1G, H). The protrusions interact with a crystalline layer of hexagonal subunits formed by other, still undetermined basal layer proteins (Fig. 1G). Although $\Delta bxpB$ spores retain the hexagonal subunits, the basal layer is not organized with crystalline order and lacks basal layer protrusions and most BclA filaments, indicating a central role for BxpB in exosporium organization.

References:

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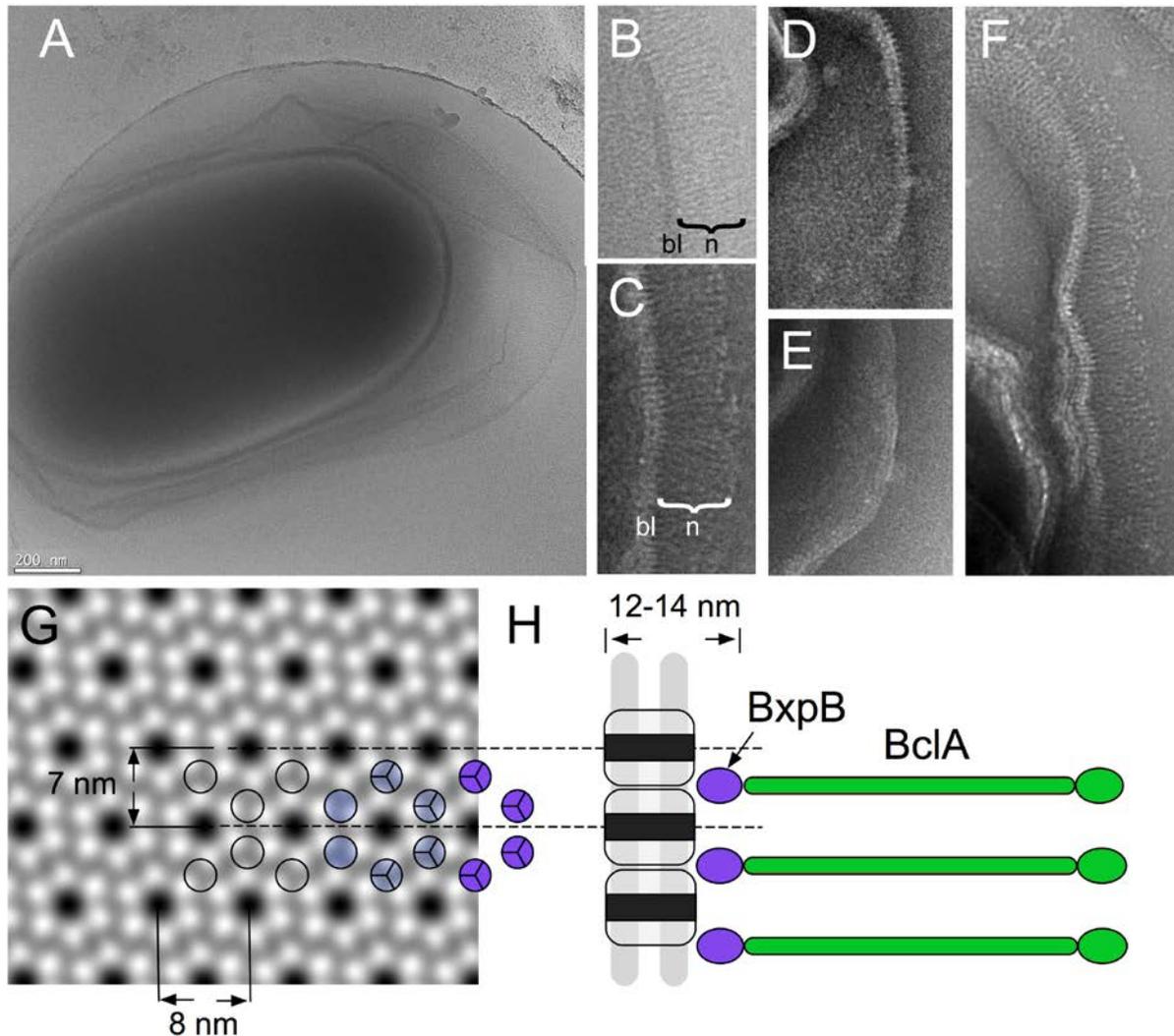


Figure 1. (A) Cryo-electron micrograph of the wildtype *B. anthracis* spore. (B) Cryo-EM (high-pass filtered) and (C) negatively stained cryo-section of the wildtype exosporium, showing the nap (n) and basal layer (bl). (D–F) Cryo-sections of the $\Delta bclA$ (D), $\Delta bxbB$ (E) and $\Delta exsB$ (F) exosporium. (G) 2D crystal average of the negatively stained, wildtype exosporium, showing the hexagonal subunits. BxbB trimers are indicated in purple. (H) Model for the arrangement of BxbB (purple) and BclA (green).