Playing Tag with Spores II: Incorporation of an Amino Acid Barcode into the BcIA Exosporium Protein of Bacillus thuringiensis

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Abstract

Tagging and tracking biological agents and surrogates in the environment requires markers or signatures that are specific to the organism of interest. Here, we describe an approach to tag Bacillus thuringiensis concomitant with expression of HA epitope tag by coexpressing BclA tagged with the TAG52 (α-HA) epitope tag. The tagged variants coexpress BclA in Bacillus thuringiensis exosporium via homologous recombination. The tagged variants are effective at detecting Bacillus thuringiensis and is recognized specifically by anti HA antibodies. Our vision is to create a suite of immunoassay test tickets to serve as unique biological tracers for environmental release studies and/or as training aids for first responders to bioterrorism scenarios.

Background

The exosporium of B. cereus group strains (B. thuringiensis and B. anthracis) forms a protective barrier around the spore and renders the spore considerably more hydrophobic than B. subtilis group strains including B. atrophaeus var globigii (BG), the historical surrogate for B. anthracis [1]. Therefore, B. thuringiensis strains including B. thuringiensis have been investigated as improved surrogates for B. anthracis [2]. The exosporium is a multicomponent structure, a dominant feature of which is the collagen-like BclA glycoprotein, which is an immunodominant antigen of B. anthracis [3] that is required for efficient entry into mammalian cells [4]. The crystal structure of BclA is known [5], and shows a trilayered homotrimeric structure and several potential surface-exposed loops. BclA proteins vary dramatically across B. cereus group species, mostly in the length of the highly repetitive collagen-like stalk domain, while the head domain is relatively conserved (Figure 1A, B). Previously, we had engineered BclA strains containing specific genetic barcodes integrated into a neutral genomic region [6] to facilitate detection in an “on-rail Anthony” [7]. It was hoped to augment our barcoding efforts by developing an immunologic marker for detection. In this study, we introduce a small matrix HA epitope tag into the BclA tag and demonstrate its exposure on the spore surface of various Bacillus thuringiensis strains. Our goal is to develop a genotypically tractable reporter gene that can be used to track infection and may allow translational studies.

Identification of Epitope Insertion Points

Figure 1: Design and construction of an epitope-tagged BclA protein for spore surface barcoding. A) Left panel: structural analysis of B. anthracis BclA protein adapted from (1) showing three potential surface-exposed regions; (B) Right panel: alignment of B. anthracis and B. thuringiensis BclA showing identified epitopes, designated motifs A-A and BclA.

BcIA-HA is Surface-Localized

Figure 2: Expression of epitope-tagged BclA in Btk anthracis strain BA-1028. BclA variants were probed with anti-HA antibodies. Only tagged variants of BA-1028 were positively stained with anti-HA antibodies. These data indicate that the tagged variants express additional epitope on the spore surface.

Allelic Replacement of bcia with bciaAM

Allelic Exchange Scheme

Diagram of BclA-Flag DNA Structure

Figure 3: Immunofluorescence analysis of epitope-tagged BclA constructs. Btk strain expressing BclAM (BA-1028) was freeze dried and stained with anti-HA or anti-Flag antibodies. FITC (green) and secondary antibodies were used to detect the primary antibody by fluorescence microscopy.

Future Work

• ELISA of whole spores and spore extracts to confirm tag accessibility on whole spores.
• Development of specific hand-held lateral flow immunoassay test kits to detect the tagged variants.
• Ability of nucleic acid tagged with tagged gene
• Substitution of BA-HA with alternative epitope tags

Conclusions

• Identified surface-exposed loops of BclAM by homology modeling of BclA of Bacillus cereus crystal structure
• Expression of HA-tagged BclA in Bacillus thuringiensis resulted in redirects detectable HA-Antibodies
• Migration of bands was consistent with authentic BclA and BclA variants
• HA-reactive material was extractable from the spore surface by denaturing polyacrylamide gel electrophoresis
• HA-reactive material colocalizes with BclA on the spore surface by immunofluorescence microscopy

References

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