Comparison of *Bacillus* spore display systems

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Abstract

Tethering of enzymes to a solid support has been shown to enhance their activity and stability in a variety of industrial and biological applications. Recently, bacterial endospores have been used as particle display systems for covalent attachment of proteins in an environmentally accessible format. Spores are dormant forms of certain soil bacteria, which have the ability to resist environmental degradation for long periods of time due to their unique protein coat. Genetic engineering of the *B. subtilis* bacterium has shown fusion of foreign proteins to certain spore coat proteins results in surface display of the foreign protein, and enzyme activity and immunogenicity of the attached proteins were also demonstrated. The spore coat proteins used in these studies were CotB, CotC, CotG, and CotX. However, incorporation of the fusion protein into the spore coat is thought to be relatively inefficient, thus limiting the amount of foreign protein that can be displayed on the spore surface. Conversely, *B. thuringiensis* has an additional structural layer of its spore coat called the exosporium. The architecture of the exosporium includes a surface hairlike nap layer consisting of the BclA glycoprotein. Our laboratory has identified the protein sequence that targets BclA to the spore surface allowing us to express high levels of foreign proteins on the *B. thuringiensis* exosporium surface. This study was initiated to determine the relative expression levels of foreign proteins on the surface of *B. thuringiensis* versus *B. subtilis* spores. Enhanced green fluorescent protein was incorporated into spores of *B. thuringiensis* using the BclA system or spores of *B. subtilis* using the CotB, CotC, or CotG systems. Furthermore, fluorescence levels relative to spore titer will be determined.

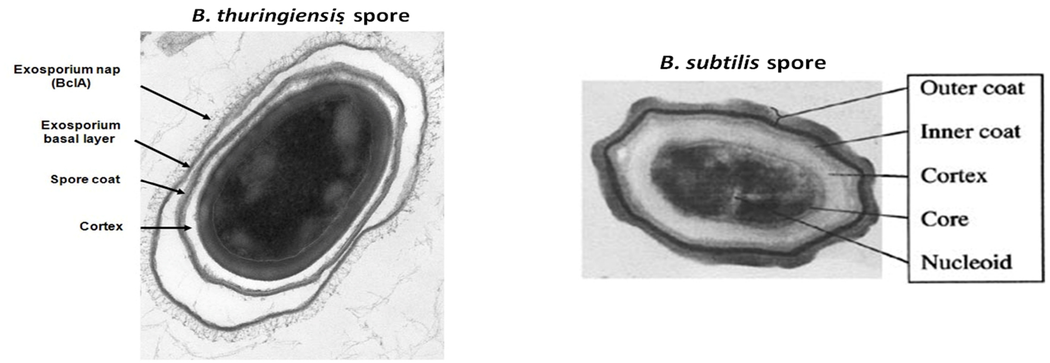

Figure 1: Left: The structure of *Bacillus thuringiensis* spore; Right: *Bacillus subtilis* spore

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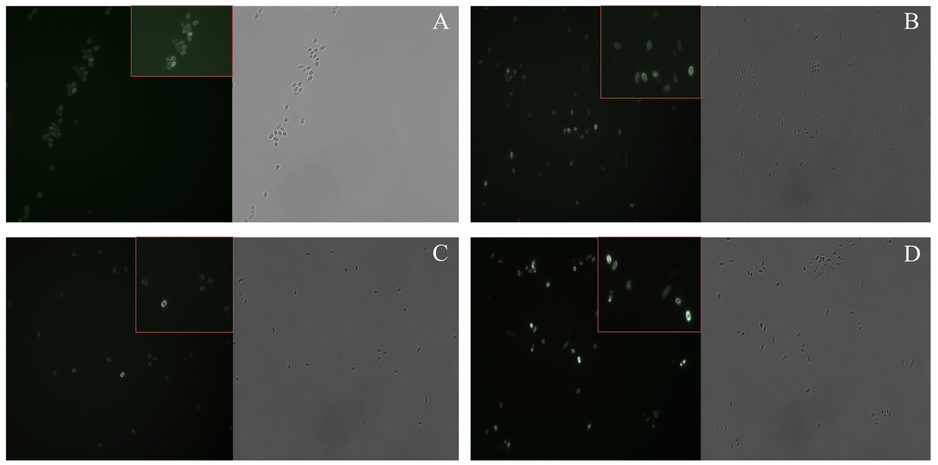

Figure 2: A nutrient agar plate containing selective antibiotics was swabbed with each overnight culture of *Bacillus* carrying target gene fusion construct and incubated at 30^{o}C incubator until sporulation complete. Spores were harvested from the plates, washed with PBS and fixed. The fixed spores were observed with fluorescent microscopy and photographed.
A. *B. thuringiensis* spores expressing BclA-eGFP; **B**. *B. subtilis* spores expressing CotB-eGFP; **C**. *B. subtilis* spores expressing CotC-eGFP; **D**. *B. subtilis* spores expressing CotG-eGFP. The inset at the corner of panels is the spore at a higher magnification for the observation of the expression pattern.

Summary and Future Directions

1. eGFP proteins were successfully expressed on spores in CotB, CotC or CotG system of *B. subtilis* or BclA system of *B. thuringiensis* with our fusion constructs.
1. The eGFP protein expression patterns were different among the four systems.
1. Quantifying the fluorescent expression level will be the next step for this project.

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