

Quantitative Measurements of Cellulase Display in the Model Gram+ Microbe *Bacillus subtilis* Define Determinants Required for Enzyme Display and Activity

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Project Goals:

Lignocellulose is a promising feedstock from which to sustainably produce biocommodities, but its recalcitrance to hydrolysis limits its use. One strategy to overcome this problem is to use consolidated bioprocessing (CBP) microbes to directly convert biomass into biochemicals [1]. We seek to develop a robust heterologous saccharolytic enzyme display system to give Gram+ bacteria cellulolytic activity and enable their use in CBP [2]. Here we discuss our efforts to define the determinants that affect heterologous surface protein display in *B. subtilis*, an industrially used model Gram+ microbe. We report quantitative measurements of cellulase display via the noncovalent LysM module. Utilizing five parent strains, we show that genetic elimination of eight proteases improves display levels. However, protein display is inefficient with only 28% of secreted cellulases bound to the cell surface, at much lower numbers than when cellulases are added *ex vivo*. Also, *B. subtilis* appears to shed cell wall enzymes as it enters stationary phase, perhaps caused by cell wall turnover. These quantitative measurements form the foundation for genetic engineering of *B. subtilis* for increased stability of displayed protein systems.

References:

1. Lynd, L.R., et al., *Consolidated bioprocessing of cellulosic biomass: an update*. Curr Opin Biotechnol, 2005. **16**(5): p. 577-83.
2. Huang, G.L. and R.T. Clubb, *Progress Towards Engineering Microbial Surfaces to Degrade Biomass*, in *Biomass*, J. Tumuluru, Editor. 2016, InTech. In press.

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