

132. Recombinant *Bacillus subtilis* that grows on untreated plant biomass

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Project Goals: The UCLA-DOE Institute for Genomics and Proteomics carries out research in bioenergy, structural biology, genomics and proteomics, consistent with the research mission of the United States Department of Energy. Lignocellulosic biomass is a promising feedstock to produce biofuels and other valuable biocommodities. A major obstacle to its commercialization is the high cost of degrading biomass into fermentable sugars. Here we explore the use of microbes to break down biomass. *Bacillus subtilis* was engineered to display a multi-cellulase containing minicellulosome. The complex contains a miniscaffoldin protein that is covalently attached to the cell wall and three non-covalently associated cellulase enzymes derived from *Clostridium cellulolyticum* (Cel48F, Cel9E, and Cel5A). A unique feature of the minicellulosome is that it spontaneously assembles, thus increasing the practicality of the cells. The recombinant bacteria are highly cellulolytic and grow in minimal media containing industrially relevant forms of biomass as the primary nutrient source (corn stover, hatched straw, and switchgrass). Notably, growth did not require dilute acid pretreatment of the biomass and the cells achieved densities approaching those of cells cultured with glucose. An analysis of the sugars released from acid pretreated corn stover indicates that the cells have stable cellulolytic activity that enables them to break down $62.3 \pm 2.6\%$ of the biomass. When supplemented with beta-glucosidase, the cells liberated 21% and 33% of the total available glucose and xylose in the biomass, respectively. The cells produce one third as much reducing sugars upon exposure to acid treated corn stover as compared to a multi-cellulase containing cocktail. As the cells display only three types of enzymes, increasing the number of displayed enzymes should lead to more potent cellulolytic microbes. This work has important implications for the efficient conversion of lignocellulose to value-added biocommodities.

References

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2. Anderson TD, Miller JI, Fierobe H-P and Clubb RT. Recombinant *Bacillus subtilis* that grows on untreated plant biomass. *Applied and Environmental Microbiology* 79 2013; 867-76.

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