Title: Population level analysis of mutations underlying improvements in biofuel production by *Clostridium phytofermentans*

Supratim Mukherjee[1], Stephen Godin[1], Jeffrey L. Blanchard[1]

[1]Department of Microbiology, University of Massachusetts, Amherst, MA 01003

http://www.bio.umass.edu/micro/blanchard/

Project Goals: Although cellulosic plant biomass is plentiful and cheap, the cost of degrading the cellulosic and hemicellulosic components of the plant cell wall currently limits the use of plant biomass as a competitive renewable alternative to gasoline and is a key challenge in developing a U.S. biomass-based industry for manufacturing biofuels from agricultural and forestry wastes. The goal of this project is to determine the mechanistic basis of improvements in *C. phytofermentans* strains evolved to produce biofuels more rapidly. Understanding the mechanistic details of individual mutations will be of particular interest to the larger scientific community for improving rates of biomass degradation and biofuel production.

Clostridium phytofermentans is a genetically tractable, anaerobic bacterium isolated from forest soil near the Quabbin Reservoir and the Harvard Forest Long Term Ecological Research site in Massachusetts, U.S.A. *C. phytofermentans* (1) can saccharify all major carbohydrate components of plant biomass; (2) has acquired many biofuel-related enzymes through horizontal gene transfer; and (3) produces ethanol as the primary product of plant carbohydrate fermentation. The combination of its broad nutritional versatility coupled with its high levels of ethanol production distinguishes *C. phytofermentans* from all other cultured microbes characterized to date. Using long-term experimental evolution we have selected for strains of *C. phytofermentans* that grow and produce ethanol faster on plant carbohydrates. Full genome re-sequencing through the DOE Community Sequencing Program has allowed us to identify mutations present at varying allele frequencies in cultures with increased rates of biomass degradation. The results reveal new strategies for evolving and engineering microorganisms for the production of biofuels from plant feedstocks.

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