

Gene Expression Differences between *Clostridium thermocellum* Biofilm and Planktonic Cells Lead to Specialized Activities and Growth

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Project Goals: The BioEnergy Science Center (BESC) focuses on fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves (1) improved plant cell walls for rapid deconstruction and (2) multi-talented microbes for converting plant biomass into biofuels in a single step [consolidated bioprocessing (CBP)]. Biomass research works with two potential bioenergy crops (switchgrass and *Populus*) to develop improved varieties and to understand cell wall biosynthesis pathways. We test large numbers of natural variants and generate specific modified plants samples. BESC's research in deconstruction and conversion targets CBP manipulating thermophilic anaerobes and their cellulolytic enzymes for improved conversion, yields, and titer. Enabling technologies in biomass characterization, 'omics, and modeling are used to understand chemical and structural changes within biomass and to provide insights into mechanisms.

Concerns around carbon and energy security have encouraged efforts to develop alternative fuel sources. Our goal was to present a fundamental and novel study into adherent biofilms and the non-adherent planktonic cells of the anaerobic thermophile *Clostridium thermocellum*, which is a model biofuel bacterium for direct conversion of lignocellulosic plant biomass. Unlike canonical biofilms that feed from the flux-limited diffusion of soluble nutrients and where non-adherent cells have prime access to labile carbon, *C. thermocellum* utilizes cellulose as the solid attachment surface which provides the only carbon source for energy and growth, thus placing planktonic cells at an inherent disadvantage. Expression studies that compare and contrast the biofilm lifestyle for *C. thermocellum* cells have not been reported, that we are aware of, and this growth mode represents the primary cell type for industrially relevant applications.

This model biofuel bacterium forms biofilms adherent to lignocellulosic feedstocks in a continuous cell-monolayer in order to efficiently break down and uptake cellulose hydrolysates. We developed a novel bioreactor design to generate separate sessile and planktonic cell populations for 'omics studies. Sessile cells had significantly greater expression of genes involved in the catabolism of carbohydrates through glycolysis, pyruvate fermentation and ATP generation by proton gradient; the anabolism of proteins and lipids and the cellular functions critical for cell division consistent with substrate replete conditions. Planktonic cells had notably higher gene expression for flagellar motility and chemotaxis, cellulosomal cellulases and anchoring scaffoldins, and a range of stress-

induced homeostasis mechanisms such as oxidative stress protection by antioxidants and flavoprotein co-factors, methionine repair, Fe-S cluster assembly and repair in redox proteins, cell growth control through tRNA thiolation, recovery of damaged DNA by nucleotide excision repair and removal of terminal proteins by proteases.

Using well-controlled and novel bioreactors, the current study demonstrates that microbial attachment and access to the solid carbon source produced widespread gene expression changes in the bacterium. This study provides insights into population heterogeneity within a constrained system which is of broad interest. Most notably, 59% of the protein coding genome recorded a minimum two-fold change in gene expression between biofilm and planktonic cell populations. The more productive sessile cells focus on both conversion and growth while the planktonic cells are more stressed – and are present in higher numbers at the end of the fermentation. We provided succinct summaries for key physiological insights and detailed information for researchers interested in the finest details. RNA-Seq data were verified by RT-qPCR and supporting proteomics data.

Insight into these cellular adaptations stands to benefit future genetic studies and strain engineering of industrial-ready phenotypes and contributes to our fundamental knowledge about adherent cellulolytic microbes.

The BioEnergy Science Center is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.