436g Genome-Wide Screening for Solvent Tolerance Genes in *Clostridium Acetobutylicum*

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The history of *Clostridium acetobutylicum* includes its productive exploitation to produce the solvents acetone, butanol, and ethanol (ABE). Renewable technology is a key remedy to exhaustion of petroleum reserves, therefore greater emphasis is being placed on overcoming the limitations imposed by solvent toxicity in the ABE fermentation. Single genes conferring solvent tolerance are being characterized, with verification of each genetic element on a time scale of years and not all investigations proving fruitful. In contrast, directed evolution techniques such as Parallel Gene Trait Mapping (PGTM) can efficiently screen the entire *C. acetobutylicum* genome for a desirable, solvent tolerant phenotype.

PGTM is being applied to *C. acetobutylicum* (*C. ac.*) and solvent tolerance genes identified on DNA microarrays. Plasmid libraries were constructed from *C. ac.* genomic DNA, sheared by sonication, and fragments inserted into a characterized shuttle vector. 12,000 fragment-bearing *E. coli* transformants have been gathered to generate a library constituting 99% coverage. This plasmid library will be transformed by electroporation back into *C. ac.* using current protocols, and an external butanol stress applied after initial outgrowth. Selective pressure (butanol stress) will provide preferential growth for transformants harboring solvent-tolerant gene fragments. Plasmid samples, isolated from cultures from the mid exponential through the stationary growth stage, will be sonicated and fluorescently labeled for microarray application.

Protocols for DNA-microarray hybridization using genomic (gDNA) and plasmid (pDNA) DNA will be discussed. The protocols for generation and hybridization of single-stranded cDNA from *C. ac.* mRNA for transcriptional profiling have been adopted, validated, and optimized for the hybridization of gDNA and pDNA. Labeled pDNA from each timepoint of the PGTM culture will be hybridized against a common reference of sonicated, fluorescently labeled genomic DNA. Gene signal intensitiesin the pDNA channel, normalized against the common gDNA reference, will be used to estimate the change in plasmid (and gene fragment) composition over time, with solvent tolerant genes becoming preferentially brighter over time.