Energy Biosciences Institute

THERMOPHILIC MICROBES AND ENZYMES FOR BIOFUEL PRODUCTION PROGRAM

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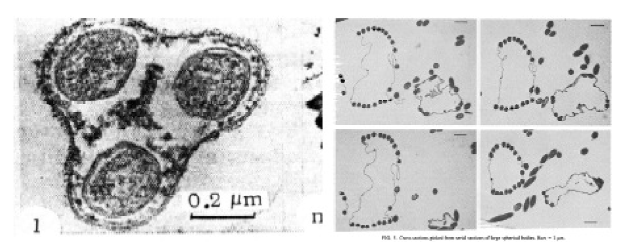
Overview

Our program addresses several bottlenecks impeding the practical production of biofuels from cellulosic feedstocks. Major goals include:
• discovery and application of new thermophilic organisms as enzyme sources and/or for biofuel production
• protein engineering and kinetic modeling of improved cellulases
• cellular engineering for improved solvent tolerance
• bioprocess engineering to optimize for optimal fermentation.

Bioprospecting for High-Temperature Conversion of Lignocellulose to Ethanol (with F.T. Robb)

Advantages of elevated operating temperatures for cellulose processing:
• lower risk of contamination
• reduced cooling costs
• higher reaction rates
• easier and more economical product recovery.



Left: Unusual "bundle" morphology of *Dictyoglomus turgidum* (*T_{opt} = 74-77ºC), which produces a thermostable amylase and cellulase complex* Right: Disruption of the cell bundles of *D. turgidum.*

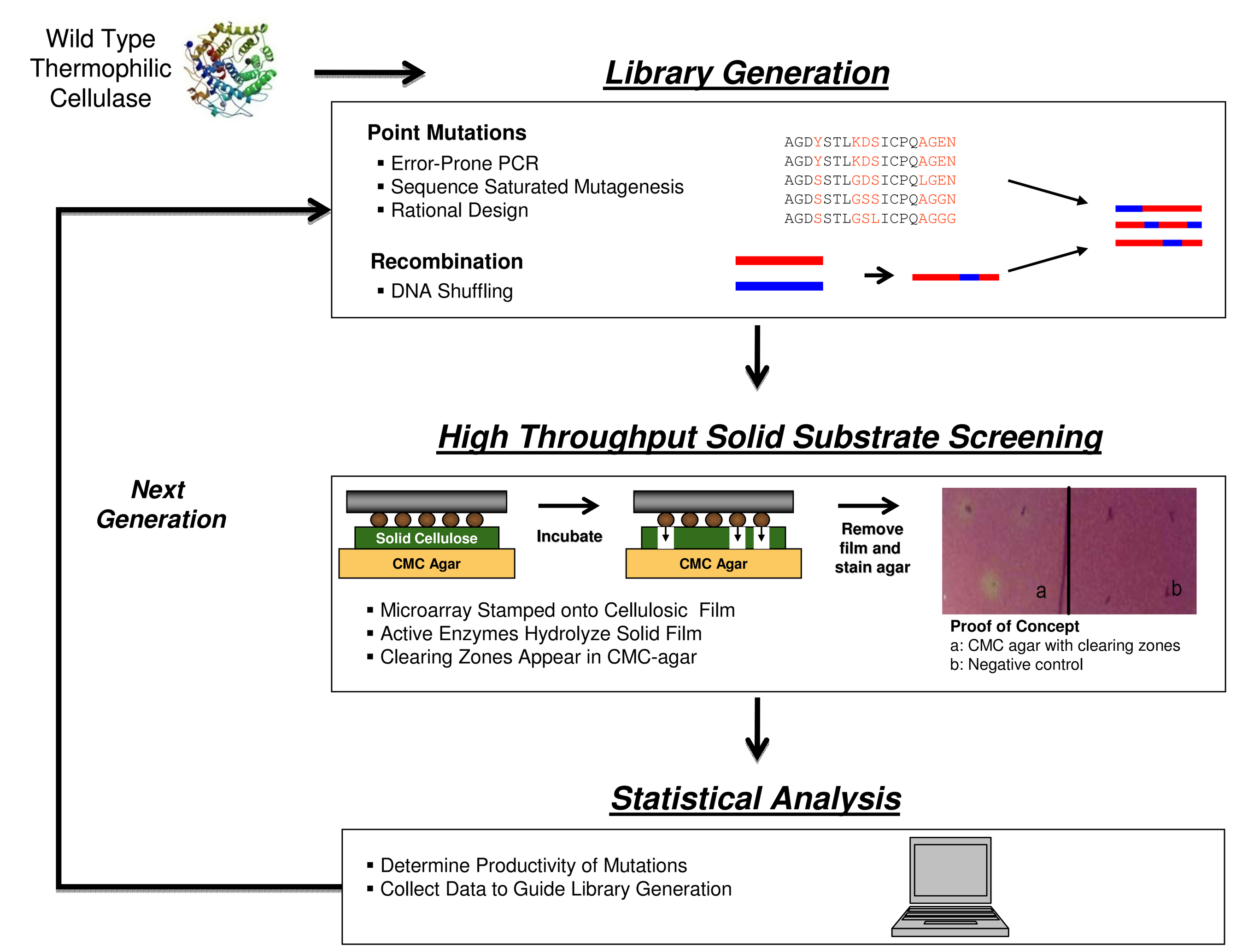
To translate of these advantages into practice, we propose to:
• isolate new ethanol/butanol-producing thermophiles from hot-spring enrichments
• isolate, characterize, and produce glycolytic enzymes from thermophiles adapted for cellulose/hemicellulose degradation
• develop a simultaneous saccharification/fermentation process for operation near the boiling point of ethanol.

Bioprospecting sample sites: (A) Eruption of Karymsky volcano Kamchataka Russia. No samples from this location yet. (B) Graduate student Stephen Techtmann collecting samples from a hot spring in Surprise Valley CA. A cellobiose-utilizing enrichment was obtained from this sample. (C) Sandy's Spring West in Gerlach NV. A cellobiose-utilizing enrichment was obtained from this spring. (D) A mud volcano in a thermal field adjacent to Mutnovsky geothermal power station, Kamchatka Russia. No samples from this location yet.

High-Throughput Solid-Substrate Cellulolytic Screens and Directed Evolution of Improved Cellulases

High-throughput screening and selection of new cellulases against *solid* cellulosic substrates remains an unmet challenge. To overcome this challenge and develop more cost-effective cellulases, we propose to:

• develop high-throughput solid substrate assays for cellulase activity
• generate new thermophilic cellulases by directed evolution.

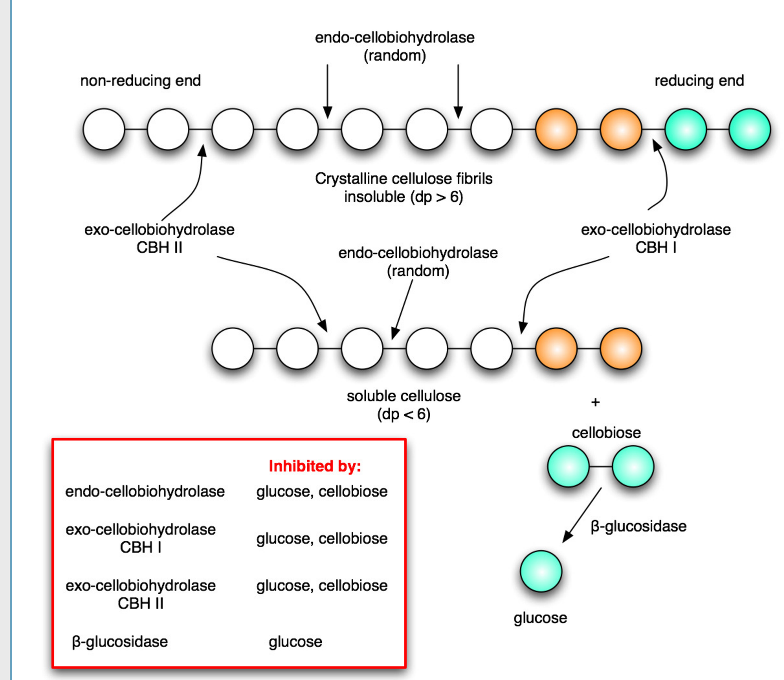


Starting points for directed evolution:
• β-endoglucanase, CBHI (Cel7A), CBHII, and β-glucosidase from *Trichoderma* sp.
• β-glucosidase from *Aspergillus* sp. and *P. furiosus* (a hyperthermophile)
• Thermophilic b-endoglucanase from *P. horikoshii* (a hyperthermophile)
• Thermophilic exoglucanase cellulosome subunit (CelS) from *C. thermocellum*
• Thermophilic reducing-end (Cel48A) and nonreducing-end (Cel6A) exoglucanases from *Thermobifida fusca*

Mechanistic Kinetic Modeling for Optimal Cellulase Design and Cellulose Hydrolysis

Accurate kinetic models of cellulose hydrolysis by cellulases are critical for designing/optimizing processes for cellulose conversion to biofuels. We propose to develop a comprehensive model of cellulose hydrolysis that can be used to:

• predict cellulase performance
• guide cellulase design and preparation of enzyme mixtures
• optimize the hydrolysis of various cellulosic substrates.

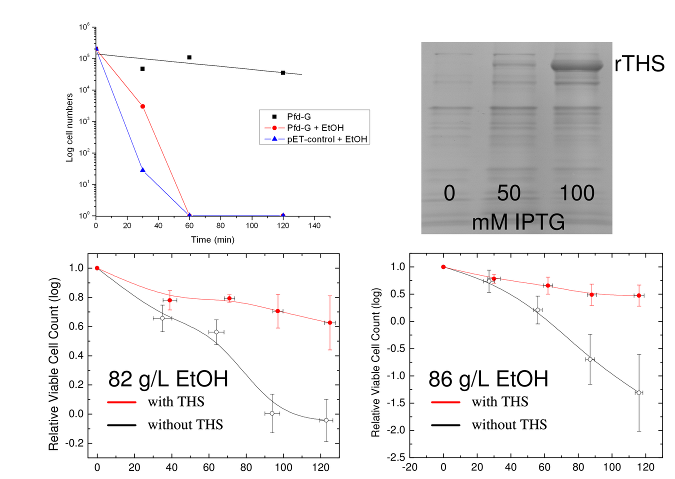


The model will include:

• reversible and irreversible adsorption of cellulase components
• cellulase reaction kinetics (including product inhibition)
• the nature of the substrates considered (e.g., crystalline versus amorphous)
• Reaction rate data will be collected in batch reactors for and analyzed using the model to determine hydrolysis kinetics and the corresponding rate law parameters.

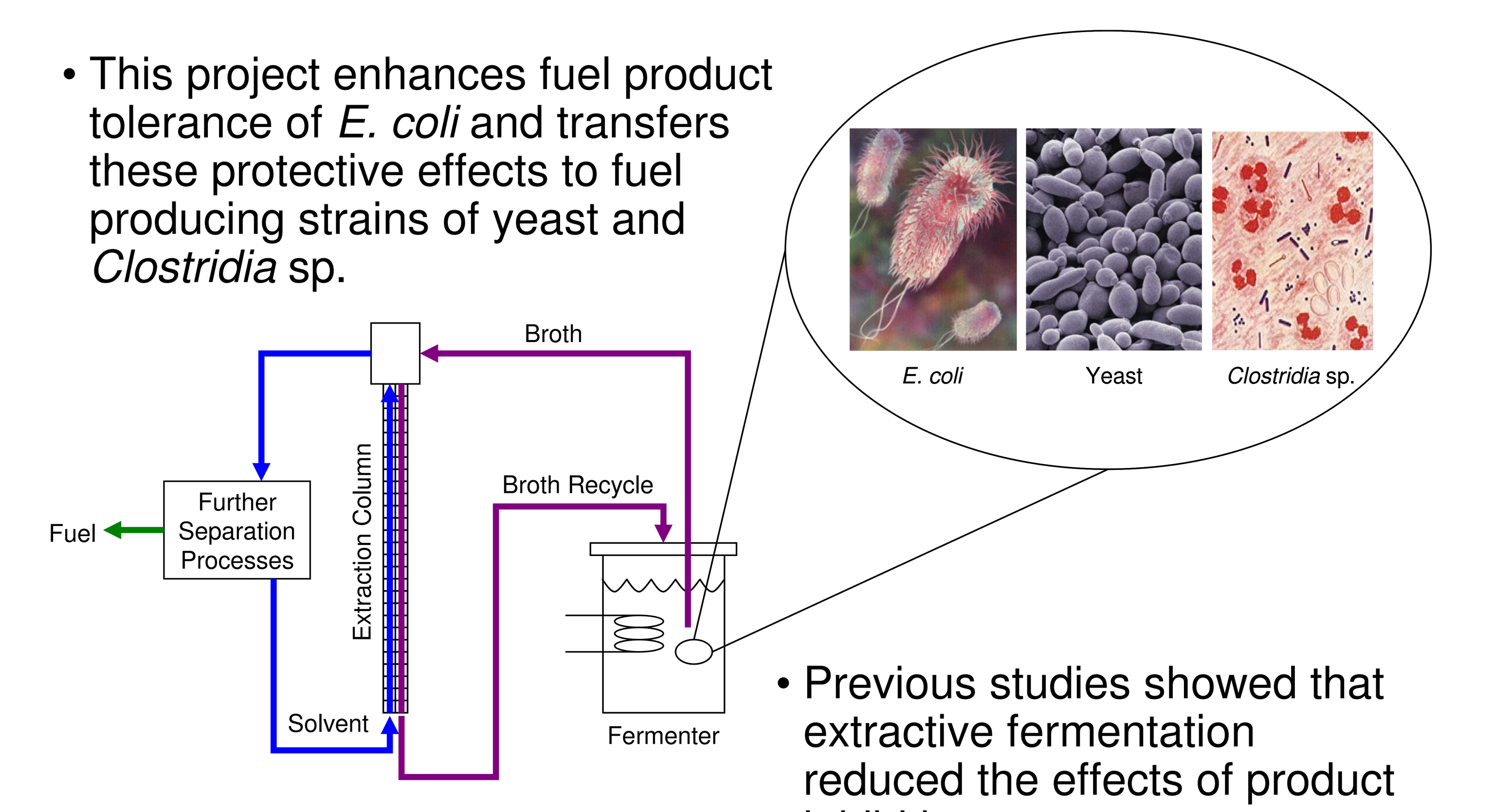
Alleviating Product Toxicity in Biofuel Production

Developing new microbes with greater tolerance toward biofuels could greatly improve the cost effectiveness of producing biofuels from cellulosic biomass. We recently discovered that expression of extremophilic chaperones confers significant protection of *E. coli* to ethanol at 50°C and 37°C. We propose to engineer enhanced solvent tolerance into several microorganisms.



Top: (Left) Survival of *E. coli* at 50°C with and without expression of the γ-PFD (Pfd-G in figure label). (Right) Expression of soluble rTHS in *E. coli* TUNER (labeled band) as a function of IPTG. Bottom: (Left) Survival of *E. coli* at 37°C with (red) and without (black) expression of *M. jannaschii* thermosome (rTHS) in 82 g/L ethanol and (right) 82 g/L ethanol.

• This project enhances fuel product tolerance of *E. coli* and transfers these protective effects to fuel producing strains of yeast and *Clostridia* sp.



• Previous studies showed that extractive fermentation reduced the effects of product inhibition.

http://www.uni-heidelberg.de/zentral/ztl/grafiken_bilder/bilder/e-coli.jpg
http://io.uwinnipeg.ca/~simmons/16cm05/1116/31-15-BuddingYeast.jpg
http://kepler.uag.mx/uagwbt/microbiologia/ciencias/images/clostridium.jpg

An extractive fermentation system will also be set up and used to optimize *in situ* product removal in fed-batch fermentations. developed via chaperone expression. Higher intrinsic solvent tolerance combined with extractive fermentation should result in extremely high production rates and volumetric productivities of biofuels such as biobutanol.