Synthetic Biology of Novel Thermophilic Bacteria for Enhanced Production of Ethanol from 5-Carbon Sugars

LDRD
LABORATORY DIRECTED RESEARCH & DEVELOPMENT

Sandia National Laboratories

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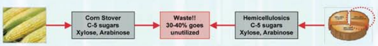
The Problem: Energy Independence Part of The Energy Solution … Plant Biomass

- **Plant Biomass-** inexhaustible and renewable resource
- **Economic Benefits:** Rural development and employment
- **Environmental Benefits:** Reduction in Green House Gases
- **Lowest cost feedstock:** Farm waste, woods, grasses
- **Feedstock to fuel ⟶ Bioethanol**

Ref. for Graphic: DOE/EERE

Bioethanol And Bottlenecks with State of the Art

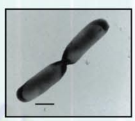


- Optimal temperature for enzymatic saccharification is 50-60° C, optimal temperature for most ethanol fermentations is 30° C
  - *–Solution: Simultaneous Saccharification and Fermentation (SSF)*
- Conventional fermentation organisms (Yeast) cannot process five-carbon sugars, reducing efficiency and increasing chance of process contamination.
  - *–Solution: Organisms that utilize wide range of sugars for ethanol production*

>> Need fermentative organism(s) that can use C5 and C6 sugars to produce ethanol at high temperatures in SSF process

The Problem: Energy Independence Part of The Energy Solution … Plant Biomass

- Geobacillus thermoglucosidasius M10EXG grows optimally at ~60 °C, is tolerant to 10% ethanol (v/v) and can grow in aerobic and anaerobic conditions
  - All other known thermophilic bacteria are tolerant to <4% (v/v) ethanol
- Produces ethanol, acetate, lactate, from five and six-carbon sugars
- Excellent candidate for SSF if engineered to overproduce ethanol

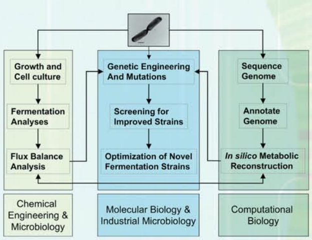


Scientific Advantages of Our Proposed Research

- G. thermoglucosidasius (Gth) M10EXG ferments wide range of sugars C5 sugars (xylose)
  - Fermentation enzyme already present in Gth M10EXG
- Metabolic engineering of bacterial pathways is currently employed for production of preferred molecules (Keasling, Nature 2006)
  - Gth M10EXG has the necessary prerequisites for metabolic engineering
- Engineering thermotolerance in a whole organism is difficult (~impossible)
  - –Gth M10EXG grown optimally at 60 °C
- Ethanol tolerance is dependent on multiple parameters- nature of membranes, membrane pumps for ethanol, enzymes and membrane proteins.
  - –Gth M10EXG has the highest tolerance for ethanol among all known thermophilic bacteria

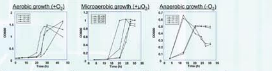
⟶ Our approach logically targets engineering for results over a short period of time

Synthetic Biology: Interdisciplinary Approach Leveraging Existing and Emerging Sandia Core Technologies



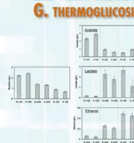
Geobacillus thermoglucosidasius M10EXG Metabolic Analysis: Glucose and Xylose Utilization with Varying O_2 Concentrations

	Glucose (C6)	Xylose (C5)	Glu + Xyl (C5+C6)
O_2 Aerobic	+	+	+
O_2 Microaerobic	+	+	+
O_2 Anaerobic	+	+	+



- Highest biomass yield when cells are grown aerobically, irrespective of the carbon source
- Biomass yield slightly higher on xylose than glucose in aerobic media
- Biomass yield decreases with decreasing oxygen concentration in the growth media

End-point Metabolite Analysis for G. thermoglucosidasius M10EXG

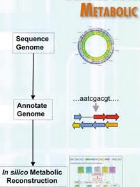


Metabolic ending products of G. thermoglucosidasius M10EXG under:
- aerobic (+O2),
- micro-aerobic (uO2) and
- anaerobic (-O2) conditions.

G: glucose and X: xylose.

- Acetate in the major carbon end product when cells are grown aerobically
- Major electron sink when cells are grown under oxygen limitation conditions is lactate
- Only L-lactate is produced; no D-lactate is produced (imp from industrial perspective)
- Ethanol yield increases under oxygen limitation conditions

Genome Sequencing and in silico Metabolic Reconstruction

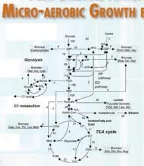


Genomic DNA has been sequenced
- Hybrid Approach to Genome Sequencing
  - Pyrosequencing + Sanger Sequencing
- Advantage:
  - Pyrosequencing: Speed (2-3 week turnaround time)
  - Sanger Sequencing: High quality

Genome annotation currently in progress
- Raw sequence data is converted to ORFs (genes)
- Genes are given putative function

Metabolic Modeling
- Metabolic reconstruction allows deduction of metabolism from the genome
- Advantage: Application of known protein functions and functional pathways to G. thermoglucosidasius M10EXG will allow us to integrate physiological and genomic data

Flux Balance Analysis of Glucose Metabolism Under Micro-aerobic Growth by G. thermoglucosidasius M10EXG.



The amino acids used for isotopomer models are in parenthesis. Numbers denote the arbitrary flux indices used in modeling the pathways.

Abbreviations:
ACoA, acetyl-coenzyme A;
CIT, citrate;
E4P, erythrose-4-phosphate;
C1, 5,10-Me-THF;
F6P, fructose-6-phosphate;
G6P, glucose-6-phosphate;
6PG, 6-phosphogluconate;
ICT, isocitrate;
MAL, malate;
OAA, oxaloacetate; OXO, 2-oxoglutarate;
PEP, phosphoenolpyruvate;
PGA, 3-phosphoglycerate;
C5P, ribose-5-phosphate (or ribulose-5-phosphate or xylulose-5-phosphate);
S7P, sedoheptulose-7-phosphate; SUC, succinate;
T3P, triose-3-phosphate;
PYR, pyruvate.

Flux Balance Analysis of Glucose Metabolism Under Micro-aerobic Growth by G. thermoglucosidasius M10EXG.

- Development of bacterial fermentation at high temperatures. Yeast cannot ferment C-5 sugars while bacteria can utilize C5 and C6 sugars.
- High temperature fermentations are compatible with and ideal for Simultaneous Saccharification and Fermentation (SSF) and Consolidated Bioprocessing (CBP) for fuel production & recovery from biomass.
- Improvement in the conversion and recovery of lignocellulose biomass to ethanol.
- Engineered thermophilic platform can be used for development of second generation hydrocarbon biofuels
- Project goals and approach fits well with the DOE mission and program for 5/10/15 year goals:
  - SSF in thermophilic bacteria for C5 and C6 sugars.'
- Development of cutting edge methods and protocols
  - 13C isotopomer analysis for FBA is a state-of-the-art HTP method of flux pathways.
  - Pathway engineering in thermophilic microorganisms- new genetic tools.
  - Metabolic and Kinetic Modeling to rationally engineer flux towards ethanol production

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