

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



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¹ The process of converting biomass to bioethanol is energy intensive. ² The process of converting biomass to bioethanol is energy intensive. ³ The process of converting biomass to bioethanol is energy intensive. ⁴ The process of converting biomass to bioethanol is energy intensive. ⁵ The process of converting biomass to bioethanol is energy intensive.

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

Plant biomass is a potential renewable energy source. Biomass is a potential renewable energy source. Biomass is a potential renewable energy source. Biomass is a potential renewable energy source.



Bioethanol And Bottlenecks with State of the Art



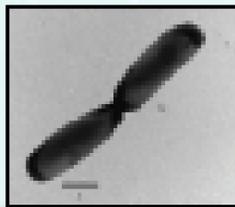
Optimal temperature for enzymatic saccharification is 50-60°C, optimal temperature for most ethanol fermentations is 30°C. **Solution:** Saccharify in Colder Climate and Ferment in Hot

Commercial fermenters have significant heat/cooling process flow-related issues, reducing efficiency and increasing chance of process upsets. **Solution:** Organisms that utilize a wide range of sugars for ethanol production.

• **Need for metabolic engineering** that can use C3 and C5 sugars to produce ethanol at high temperatures in SSF process.

Our Proposed Solution: Engineer Thermophilic Bacteria for Ethanol Production

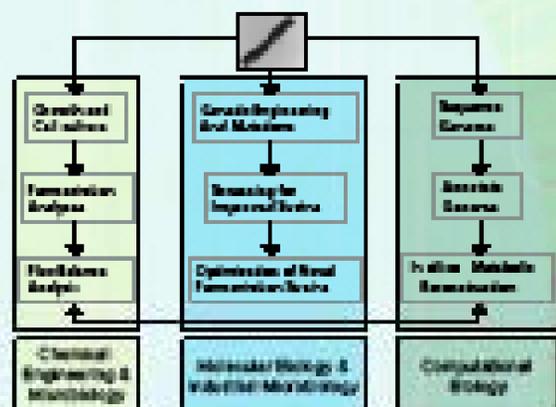
- Gemmatimonas thermophilus* M1000 grows optimally at ~60 °C, is tolerant to 10% ethanol (w/v), and can grow anaerobically and aerobically.
- all other known thermophilic bacteria are tolerant to 0-10% ethanol.
- Produce ethanol, acetate, but not 1,3-propanediol or xylitol.
- Genetically amenable for SSF engineering to anaerobically ferment.



Scientific Advantages of Our Proposed Research

- G. thermophilus* M1000 has a wide range of sugars (C3 sugars (xylose) - fermentation capacity already present in *G. thermophilus* M1000).
- Metabolic engineering of bacterial pathways is well-developed for production of potential inhibitors (Xanthine, Ribose, 3HG).
- G. thermophilus* has the necessary prerequisites for metabolic engineering.
- Converting thermotolerance to a whole organism trait that is inheritable.
- G. thermophilus* grows optimally at 60 °C.
- Ethanol tolerance is dependent on multiple parameters: nature of medium, medium prep for ethanol, oxygen and ammonia profiles.
- G. thermophilus* 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



Genetically thermotolerant M1000G Metabolic Analysis: Glucose and Xylose Utilization with Varying O₂ Concentration

Strain	Glucose (g/L)	Xylose (g/L)	Glucose (g/L)
M1000G	10	10	10
M1000G	10	10	10
M1000G	10	10	10



- Highly tolerant: yield also cells are grown anaerobically, irrespective of the carbon source.
- Glucose yield: slightly higher up to 10% than glucose yields.
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End-point Metabolite Analysis for *G. thermophilus* M1000G



Metabolic analysis products of a thermotolerant M1000G strain:
- aerobic (+O₂)
- anaerobic (-O₂), and
- anaerobic (-O₂) conditions.
O: glucose and D: xylose.

- tolerance to the water uptake and product release will not grow anaerobically.
- Major ethanol yield from cells are grown under oxygen but in hot conditions in batch.
- High 1,3-propanediol production (1,3-propanediol production) (high from industrial perspective).
- Glucose yield increases under oxygen limitation conditions.

Genome Sequencing and in silico Metabolic Reconstruction



Genome DNA has been sequenced
- High-throughput genome sequencing
- Sequencing - High-throughput
- Sequencing - High-throughput
- Sequencing - High-throughput

Genome annotation and gene prediction
- Gene prediction (supported by RNA-seq)
- Gene prediction (supported by RNA-seq)

Metabolic Modeling
- Metabolic modeling (flow balance) of metabolism from the genome
- Metabolic modeling (flow balance) of metabolism from the genome

Flux Balance Analysis of Glucose Metabolism Under Micro-aerobic Growth by *G. thermophilus* M1000G



Glucose metabolism under micro-aerobic growth by *G. thermophilus* M1000G.
- Glucose metabolism under micro-aerobic growth by *G. thermophilus* M1000G.
- Glucose metabolism under micro-aerobic growth by *G. thermophilus* M1000G.

- The pathway information generated will help in better design synthetic biology.

Significance of The Project

- Development of bacterial fermentation of high temperatures. Next round fermenter C-5 sugar-tolerant bacteria can utilize C3 and C5 sugars.
- High temperature fermentation are compatible with and ideal for Saccharomyces cerevisiae top and Fermentation (SSF) and Commercial Fermentation (CFP) for ethanol production & recovery from biomass.
- Improvement in the conversion and recovery of lignocellulosic biomass is achieved.
- Engineered thermophilic jet from can be used for development of second generation biofuels.
- Project goals and approach fit well with the DOE initiative and program for 2010/15 year goals.
- SSF for thermophilic bacteria for C3 and C5 sugars.
- Development of utility value analysis and protocols.
- C-5 sugar-tolerant bacteria for C5 is state-of-the-art. BT metabolic of the pathway.
- Pathway engineering in thermophilic microorganisms - pure genetic tools.
- Metabolic and flux-balance analysis to identify design for ethanol ethanol production.