More for Less:
Metabolic Engineering of Yeast for Ethanol Production

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Overview

1) The Ethanol Industry

2) Yeast and Yeast Metabolism

3) Metabolic Engineering of Yeast
Economics of Ethanol: Industry Statistics

- 13.3 Billion Gallons produced in 2013*
  - 86,000 Jobs
  - $44 Billion Contributed to the GDP
  - $8 Billion in Taxes Paid
  - $36 Billion Spent on Raw Materials

* Renewable Fuels Association 2014 Industry Outlook
Economics of Ethanol: Government Involvement

- Foreign Oil and Energy Independence
  - “Promote the development and use… of domestic replacement fuels in lieu of petroleum motor fuels”
- Energy Policy Act of 2005
  - The RFS
    - 7.5 Billion gallons of blended fuels
  - 36 Billion Gallons by 2022
Economics of Ethanol: Agriculture

• Corn
  • $36 Billion spent in 2013
  • The Midwest
  • 1997-2006 Corn was grown at a loss
  • Corn payments in 2012 were 82% lower than in 2006
Economics of Ethanol: Related Industries

- Animal Feed
- Fermentation Chemicals
  - $100 Billion Industry*
  - $400 Billion by 2030*

Ethanol as a Biofuel

- E10, E15, E85
- Engine Benefits
- Carbon Footprint and “Green Energy”
Takeaway:
Ethanol is a big, profitable industry.
Industrial Production of Ethanol

- Feedstocks
  - “Sugar” (It’s corn...)
  - Cellulose
- Dry Milling
- Wet Milling
- The Role of Yeast
  - Biological catalyst

http://www.theblaze.com/wp-content/uploads/2012/02/EthanolPlant.jpg
Production Paradigms

Dry Milling

Milling

Mashing

Fermentation (Yeast!)

Purification

Wet Milling

Soaking

Milling

Separation

Fermentation (Yeast!)

Purification
Takeaway: You want ethanol, you’re going to need yeast
Yeast

• Single-celled fungi
• "Brewers yeast"
• Well understood
  • Genetics
  • Proteomics
  • Lots of tools

http://3.bp.blogspot.com/-muYzxHc0lo/TWUeRJ5j0I/AAAAAAAACbl/WaEAVcjhWgU/s1600/yeast+proteome.GIF
Yeast Metabolism

Is there oxygen?

Aerobic Respiration

AKA “The mitochondria is the powerhouse of the cell!”
Alcoholic Fermentation

(b) Alcohol fermentation occurs in yeast.

2 ADP → 2 ATP

Glucose → 2 NADH

2 NAD+ → 2 Pyruvate

2 Acetylaldehyde → 2 CO₂

2 Ethanol
The Rho-Zero State

- **Ethanol Concentration**: The graph shows a comparison of ethanol concentration between Rho+ and Rho0 strains. Rho0 has a higher ethanol concentration.

- **Average Doubling Time**: The graph illustrates the average doubling time for Rho+ and Rho0 strains. Rho0 has a shorter doubling time compared to Rho+.
The Rho-Zero State

Ethanol Concentration After 52 Hour Fermentation

KCN Growth Curve
Rho-Zero Desiccation Tolerance

ERR vs ERY Desiccation Survival

<table>
<thead>
<tr>
<th></th>
<th>Desiccation</th>
<th>Control</th>
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<tbody>
<tr>
<td>3 Days Growth</td>
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<td>0.325</td>
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<td>4 Days Growth</td>
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<td>5 Days Growth</td>
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ERR GFP-2

ERY DIP
Takeaways:

1. Yeast can live with or without oxygen

2. Yeast living without oxygen produce ethanol

3. Yeast in the rho-zero state produce more ethanol, but grow slowly and don’t survive desiccation.
Metabolic Engineering of Yeast

http://blog.addgene.org/plasmids-101-what-is-a-plasmid
Inducing the Rho-Zero State

“...dominant inducible interfering transgene that will render yeast respiratory deficient during the production phase of commercial fermentations.”

“...using a gene to mess with mitochondria once yeast start fermenting”
The Transgene
Takeaways:

1. A plasmid containing a “toxic gene” will be introduced.

2. Lack of oxygen will lead to expression of the toxic gene.

3. The toxic gene will alter the gene expression in the mitochondria.

4. Altered gene expression will lead to the rho-zero state, increasing ethanol production.
Conclusions and Implications

The system proposed here, if successful, will be broadly applicable to current and future ethanol production models. Implementation of this system will save ethanol producers money and improve production efficiency.
Acknowledgements

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