**Advanced Yeast Biocatalysts for 2nd Generation Ethanol Production**

BIO Pacific Rim; December 8, 2014

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Value of Advanced Yeasts

Potential value created by bioengineered Yeast:

Assumptions:

• $1.75/gal EtOH selling price
• $12/Kg enzyme selling price

• 2 products being used at commercial scale
• >3 Billion gallons of EtOH made with Mascoma strains to date

Mill Size (MGY): 40 80 20

$MM/yr

Cane Sugar Corn Cellulosic

0 5 10 15 20 25

Enzymes Yield Increase

Native pathways

New pathways

Complex Sugars Simple Sugars

Enzymes

glucose

glycerol

ethanol

xylose

arabinose

acetate

Cane Sugar Corn Cellulosic

$MM/yr

Assumptions:

• $1.75/gal EtOH selling price
• $12/Kg enzyme selling price
Application in Grain-Based Fuel Ethanol Production: Transferm

**Concept**
- **Alpha amylase enzymes**
- **Reduced Glucoamylase (GA) Enzymes**
- **Fermentation**
- **Distillation**
- **FUEL ETHANOL**

**Scale-Up**
- **Slurry**
- **Liquefaction**
- **Jet cooker**

**Lab Scale Demonstration**
- 45 Hr Fermentation Results
- % of Reference GA dose
- MGT 1.0 vs Conventional

**Rapid Commercial Adoption**
- **Liquid Yeast dosing & Storage**
- **Market Penetration (%) vs Months In Market Place**
TransFerm® Yield+, Increased Yields and GA for Corn Ethanol

**TransFerm Yield+**

- Balances the redox during anaerobic growth with a new metabolic pathway
- Makes sufficient glycerol to balance osmotic stress

**Pilot Scale Data:**

- 4.5% Yield increase
- Same Kinetics

Note: pilot data based on n=3 for conventional yeast; n=5 for TransFerm Yield+
TransFerm® Yield+ Commercial Scale Results

<table>
<thead>
<tr>
<th>Customer</th>
<th>Plant Scale (MGPY)</th>
<th>Design Type</th>
<th>N=</th>
<th>Ethanol Yield Increase Achieved</th>
<th>Glycerol Reduction</th>
<th>Enzyme Reduction</th>
<th>Estimated Total Value per Year (MPY)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>110</td>
<td>ICM</td>
<td>13</td>
<td>2.9%</td>
<td>21%</td>
<td>45%</td>
<td>$6.3</td>
</tr>
<tr>
<td>2</td>
<td>125</td>
<td>ICM</td>
<td>10</td>
<td>1.6%</td>
<td>40%</td>
<td>30%</td>
<td>$3.9</td>
</tr>
<tr>
<td>3</td>
<td>115</td>
<td>ICM</td>
<td>29</td>
<td>2.0%</td>
<td>37%</td>
<td>25%</td>
<td>$3.6</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>ICM</td>
<td>53</td>
<td>2.0%</td>
<td>30%</td>
<td>40%</td>
<td>$2.1</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>Delta T</td>
<td>88</td>
<td>1.7%</td>
<td>26%</td>
<td>28%</td>
<td>$0.9</td>
</tr>
</tbody>
</table>

- Robust commercial scale results, kinetics
- Average value of $0.04/gal delivered
- >$500M value creation for the U.S. ethanol industry from this product
### Gen2 Yeast: Drop-in Value

#### Technology for Bagasse Sugar Fermentation

<table>
<thead>
<tr>
<th>Process</th>
<th>Yield +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylose Fermentation</td>
<td>33%</td>
</tr>
<tr>
<td>Glycerol Reduction</td>
<td>5%</td>
</tr>
<tr>
<td>Acetate Conversion to Ethanol</td>
<td>5%</td>
</tr>
<tr>
<td>Arabinose Utilization</td>
<td>5%</td>
</tr>
<tr>
<td>Cellulase and Hemicellulase Secretion</td>
<td>&gt;10%</td>
</tr>
</tbody>
</table>
Xylose Fermentation

A. Isolation of unique xylose isomerase
B. Optimization of core genes
C. Identification of new mutations

- Finishes fermentation in ~30 hours from small inoculum
- 0.45 g/g ethanol yield
- Directly Engineered, no adaptation

Commercial corn stover hydrolyzate fermentation

Graph showing:
- Glucose, Xylose, Ethanol, CO2 (L) concentrations over fermentation time
- Data points indicating the production of ethanol and CO2 over time

MASCOMA
Arabinose Fermentation

- 93% arabinose utilized
- 0.45 g/g yield on added arabinose
- Directly engineered, no adaptation

**Diagram: L-arabinose metabolic pathway**

- L-arabinose → AraT → L-arabinitol
- L-arabinose → AraA → L-ribulose → AraB → L-ribulose-5-P → AraD → D-xylulose-5-P
- Pentose phosphate pathway: TKL1, TAL1, RKI1, RPE1
- Glycolysis: ethanol

**Graph:**

- 5 g/L glucose + 30 g/L arabinose
- 0.45 g/g yield on added arabinose
- Directly engineered, no adaptation
Acetate Fermentation

Novel Pathway for Acetic Acid Fermentation

- Acetate $\rightarrow_{adhE} Ethanol$
- NADH $\rightarrow_{adhE} NAD^+$
- Biomass $\rightarrow_{adhE} Glucose$
- CO$_2$ $\rightarrow_{adhE} Glucose$

\[ \sim 10\% \text{ via Glycerol reduction} \]

\[ \sim 90\% \text{ via new pathway} \]

- 120 g/L glucose + 10 g/L acetate
- Wild Type
- Engineered
- 9 g/L acetic acid consumed
- 9.3% yield increase
- Yield on glucose is 0.513 g/g
Yeast Secreted Hemicellulase Enzymes

Direct Fermentation of Hardwood Xylo-oligomers from 2-stage Pretreatment with no Enzyme:

Unique Enzyme Combinations:

Screened activities
- xylanase
- xylosidase
- acetyl xylan esterase
- glucuronyl esterase
- acetyl esterase
- endoglucanases
- α-galactosidase
- β-glucosidase
- α-glucuronidase
- β-mannanase
- β-mannosidase
- others

Applied in Pilot Scale Fermentations:

Need for Enzyme Eliminated

Conventional Xylose Yeast

CBP Yeast
Cellulase Secretion

Enzymatic Activities Expressed in Yeast

<table>
<thead>
<tr>
<th>Activity</th>
<th>Enzyme/Chimera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellbiohydrolase I</td>
<td>CBH I chimeras*</td>
</tr>
<tr>
<td>Endoglucanases</td>
<td>Arabinofuranosidase</td>
</tr>
<tr>
<td>Swollenin</td>
<td>Alpha-expansin</td>
</tr>
<tr>
<td></td>
<td>Beta-expansin</td>
</tr>
<tr>
<td></td>
<td>Feruloyl esterase</td>
</tr>
</tbody>
</table>


Impact of secreting cellulases:

Hydrolysis of cellulose

<table>
<thead>
<tr>
<th>Enzyme Loading (mg/g TS)</th>
<th>Concentration at 96 Hours (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
</tr>
<tr>
<td>8</td>
<td>45</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
</tr>
</tbody>
</table>

Xylose Utilizing Background Strain

+CBH1, 2, and 6 accessory enzymes

(~$0.16/gal savings @ $0.50/gal enzyme cost)

Highest Reported CBH1 and CBH2 Expression by 10X

Source: NREL.

Hydrolysis of cellulose

MASCOMA
Hemicellulase Secretion in Corn Stover HHF Process

10% Yield improvement by hemicellulase enzyme expression

@20MM gal scale, Value = +2 MM gal x $3/gal = $6M/yr = $0.30/gal
Co-Fermentation to Extend 1G Capital

**Corn Mash and Xylose:**

- Ethanol Titer (g/L)
- Xylose Concentration (g/L)

**Molasses and Xylose:**

- Ethanol
- Fructose
- Xylose
- Glucose

Dash = wild type

Solid = C5 strain
Summary

- Proven ability to deploy GM Yeasts for commercial fuel production
- Suite of technologies developed for 2G ethanol production (xylose, glycerol, acetic acid, enzyme secretion)
- Yield increases of 25% and cost savings of ≥$0.30/gal above xylose utilizing strains
- Novel process configurations to extend 1G fermentation capital are now possible