Comparison of Laboratory and Industrial *Saccharomyces cerevisiae* Strains for Their Inhibitor Resistance and Xylose Utilization

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Overview

• Lignocellulosic ethanol process
• Biocatalysts development at Ngee Ann Polytechnic
• Inhibition and stresses studies
  – S. cerevisiae strains
  – Inhibitor resistance
  – Stress tolerance
  – Xylose utilization
Lignocellulosic Biomass Composition

- **Cellulose**
  - Very high molecular weight
  - Highly crystalline
  - Uniform polymer of glucose
- **Hemicellulose**
  - Non-homogeneous
  - Non-crystalline
  - Short branches
  - Polymers of C5, C6 sugars
- **Lignin**
  - Aromatic
  - Complex structure
- **Extractives**
  - Low molecular weight
  - Mostly lipophilic
Crops and Lignocellulose

1. Crops

A. Sucrose
F \rightarrow \text{G}

B. Starch
G \rightarrow G \rightarrow G \rightarrow G

2. Lignocellulose

C. Cellulose

D. Hemicellulose

E. Lignin
G \rightarrow G \rightarrow G \rightarrow G
G \rightarrow Gal \rightarrow Man \rightarrow X \rightarrow Ara \rightarrow Other \rightarrow Lignins (coniferyl, sinapyl, vanillyl)
Biomass to Fuel Ethanol

Crops → Starches Sugars → Amylase → Glucose → Yeast → Ethanol

Biomass → Cellulose Hemicellulose Lignin → Cellulase hemicellulase → Hexose (C6) Glucose Galactose Mannose → Yeasts Bacteria → Ethanol

Pretreatment → Hydrolysis → Fermentation

BIOCATALYSTS

NGEE ANN POLYTECHNIC
Cellulosic Ethanol Biocatalyst Development at Ngee Ann Poly

Consolidated Bioprocessing

High-Strength Enzyme Complex

Robust Ethanologens

Consolidated processing, i.e. hydrolysis and fermentation in one step, for fuel ethanol production from biomass could make this process more economically feasible
Our Focuses

High-strength enzyme cocktails
- Celluase
- Hemicellulase
- Pectinase
- Peroxidases etc.

Robust ethanologens
- Cofermentation of glucose and xylose
- Inhibitor resistant
- Temperature tolerant
- Ethanol tolerant
Our Approaches

- Strain Selection
- Strain Improvement
- Medium Engineering
- Biocatalysts
- Induction Depression
- Proteomics
- Genomics
Our People
Our People
Objectives

• Comparison of lab and industrial *Saccharomyces Cerevisiae* strains on
  – Inhibitor resistance
  – Stress tolerance
  – Xylose utilization
Biomass Hydrolysate Fermentation

**Sugar mixture**
- Hexose
  - Glucose
  - Galactose
  - Mannose
- Pentose
  - Xylose
  - arabinose

**Stresses**
- pH
- Ethanol
- Xylose
- Temperature

**Inhibitors**
- Furans
- Weak acids
- Phenolics
Robust Ethanologens

- Inhibitor resistance
- Stress tolerance
- Sugar mixture utilization

Yeast *Saccharomyces cerevisiae*
- More resistant to inhibitors
- More tolerant to stresses such as ethanol, low pH and high temperature
Saccharomyces Cerevisiae strains

- **Laboratory strains**
  - ATCC 44771 (haploid)
  - CBS 8066 (diploid) – xylulose-utilizing
- **Industrial strains**
  - ATCC 24860 (diploid) – xylulose-utilizing
  - ATCC 96581 (polyploid)
  - ATCC 4126 (polyploid)
  - TJU (polyploid)
Inhibitor Cocktail

The 100% (v/v) inhibitor stock cocktail

- 75 mM formic acid (Sigma–Aldrich),
- 75 mM acetic acid (Merck)
- 30 mM furfural (Sigma–Aldrich)
- 30 mM 5-hydroxymethyl-2-furaldehyde (HMF) (Sigma–Aldrich).
Stresses

- pH
- Ethanol concentration
- Xylose
- Temperature
Inhibitor Resistance

- ATCC 24860 and ATCC 96581 demonstrated the highest resistance
- ATCC 44771 and CBS 8066 demonstrated the lowest resistance
- TJU and ATCC 4126 growth were sensitive to inhibitor concentration
pH Tolerance

- pH below 4, all strains showed less growth and the optimal pH is 5
- ATCC 44771 showed the least tolerance to the lower pH followed by CBS 8066
- Strains TJU and ATCC 24860 demonstrated the highest tolerance to the lower pH
Ethanol Tolerance

- Strains TJU and ATCC 24860 demonstrated the highest tolerance to ethanol
- ATCC 44771 showed the lowest ethanol tolerance followed by CBS 8066
- ATCC 4126 and ATCC 96581 demonstrated similar moderate ethanol tolerance
Xylose Tolerance

- When xylose concentration is greater than 20g/L, all strains showed significant drop in cell density.
- ATCC 96581 demonstrated the highest xylose tolerance.
- ATCC 4126 showed the lowest xylose tolerance followed by strain TJU.
Temperature Tolerance

- All strains died off at 50°C.
- ATCC 24860 and ATCC 96581 demonstrated moderate tolerance to temperature increase.
- ATCC 4126 and Strain TJU were quite sensitive to temperature change.

![Temperature Tolerance Chart]

**OD600**

- 0
- 5
- 10
- 15
- 20
- 25

**Temperature**

- 30 ºC
- 40 ºC
- 50 ºC
## Summary

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<th>Inhibitor</th>
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Xylose Utilization

- Random mutagenesis by UV irradiation and ethyl methanesulfonate (EMS) and directed evolution
- Except ATCC 44771, the rest strains can all grow on xylose aerobically
- No growth was observed under anaerobic conditions
Conclusion

- ATCC 24860 and ATCC 96581 are the best candidate strains for further improvement
  - Sugar mixture utilization
  - Inhibitor resistance and stress tolerance
  - Biomass hydrolysis
Thank You