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**

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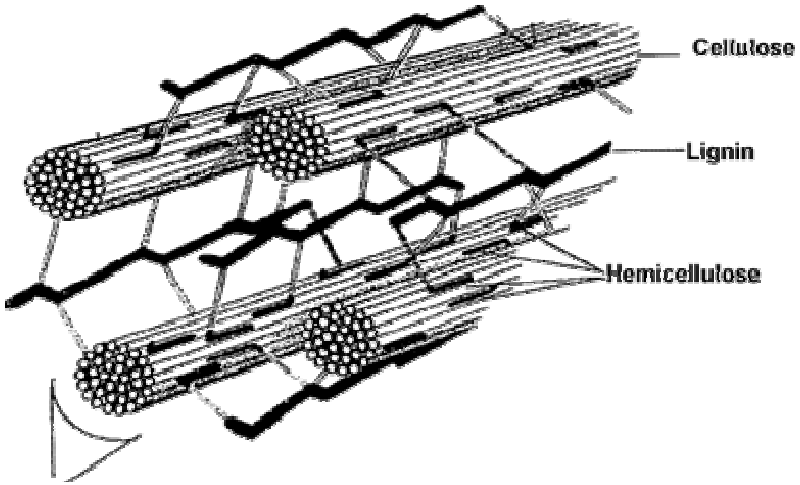
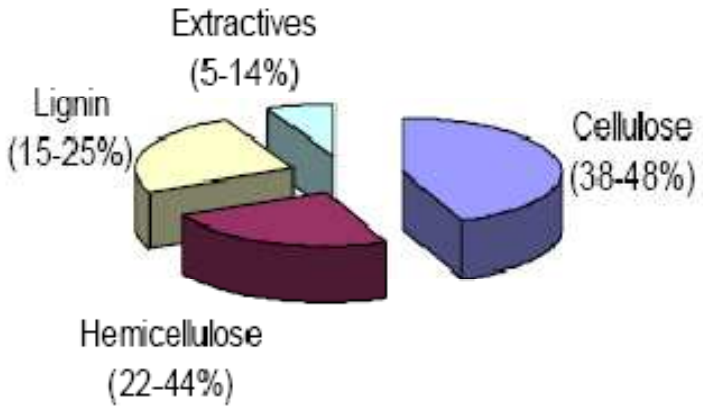
Lignocellulosic Biomass Composition

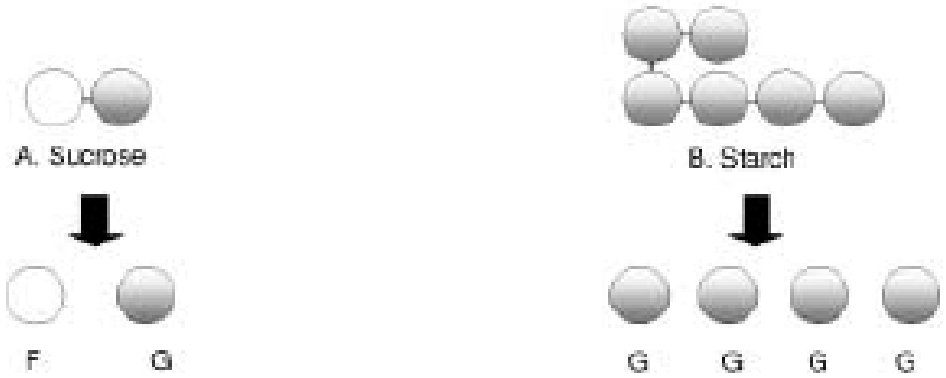
- **Cellulose**
  - Very high molecular weight
  - Highly crystalline
  - Uniform polymer of glucose
- **Hemicellulose**
  - Non-homogeneous
  - Non-crystalline
  - Short branches
  - Polymers of C5, C6 sugars
- **Lignin**
  - Aromatic
  - Complex structure
- **Extractives**
  - Low molecular weight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



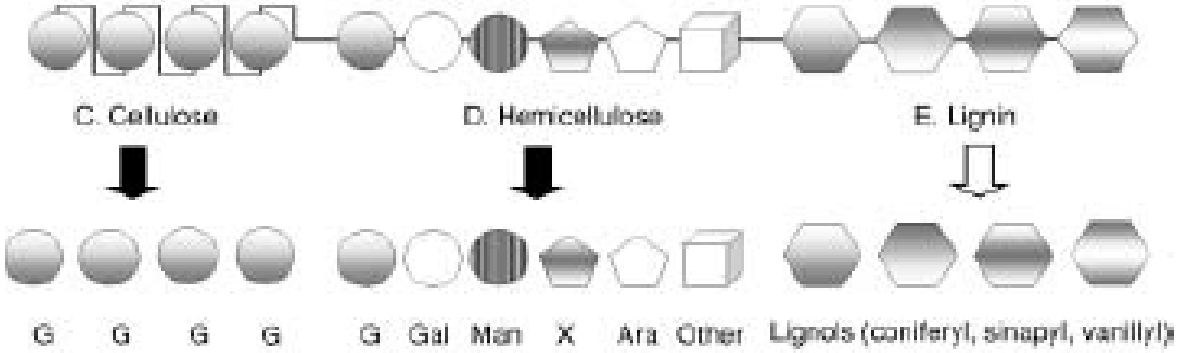


Crops and Lignocellulose

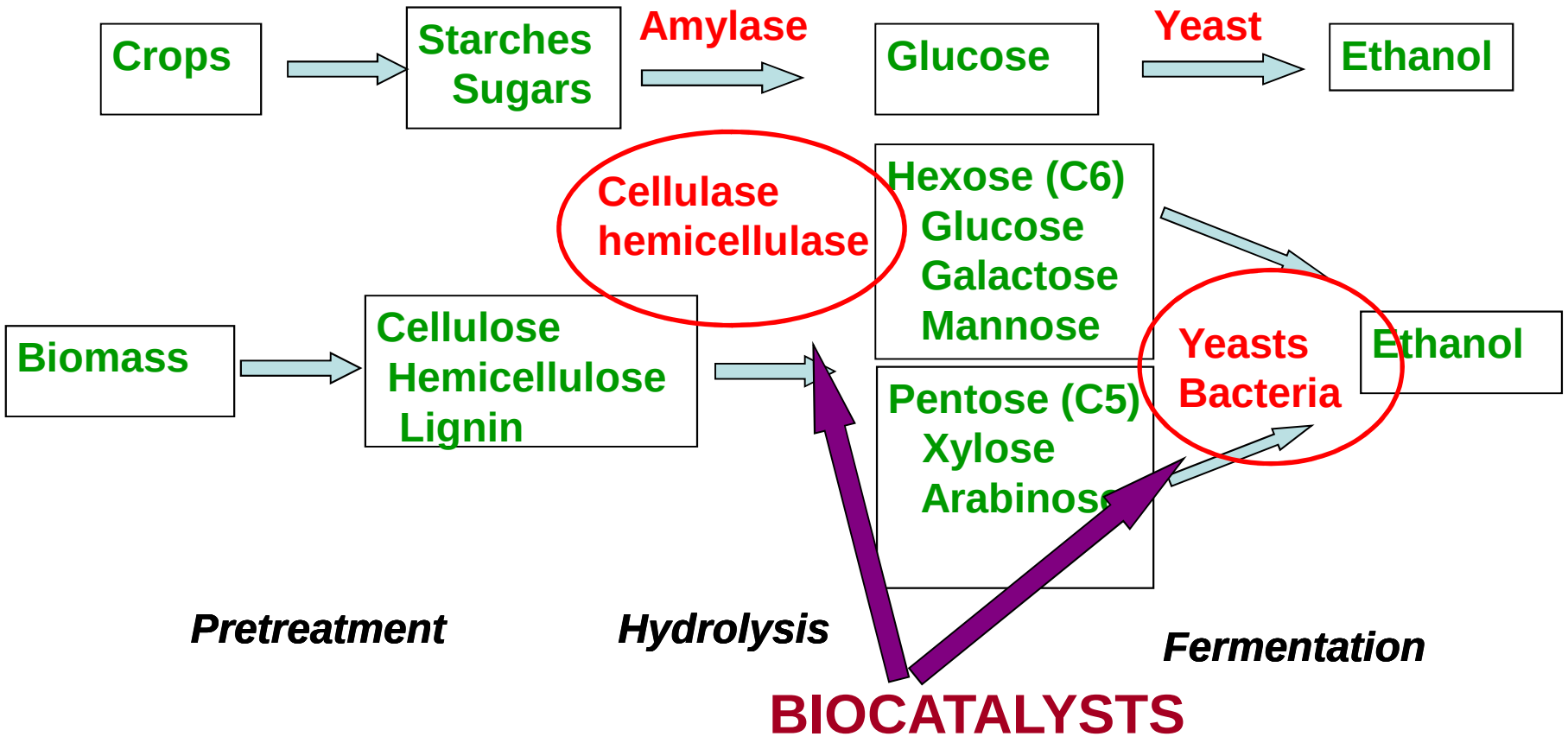
1. Crops



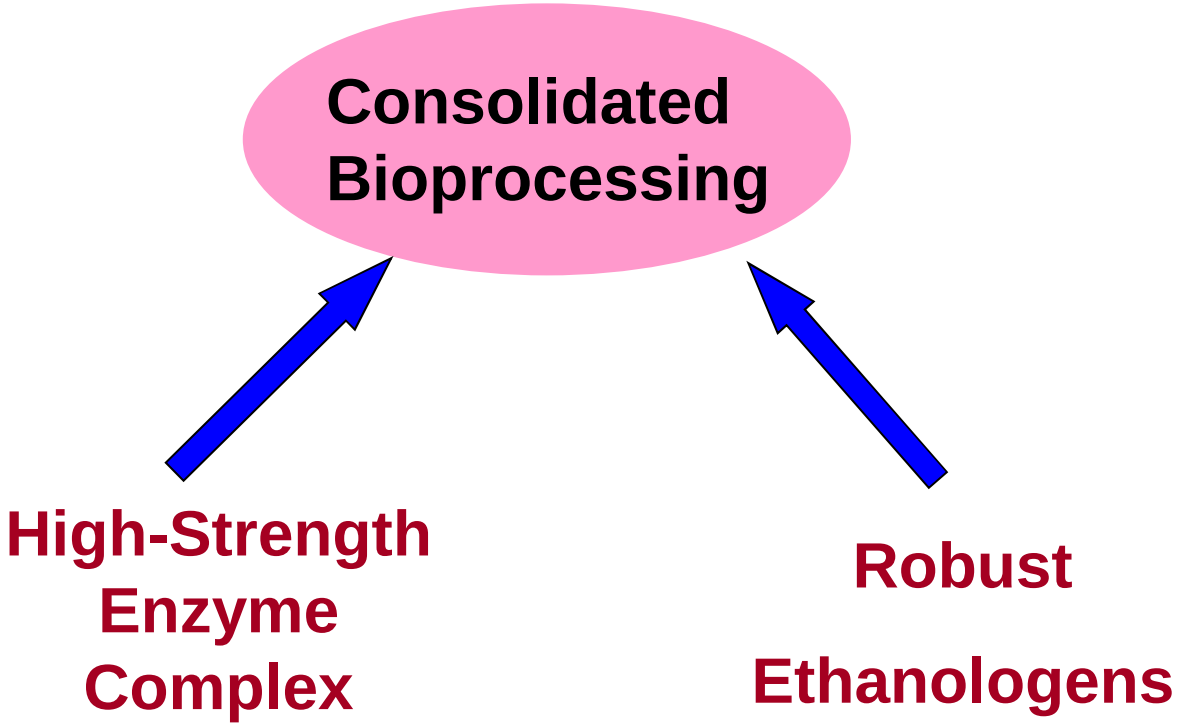
2. Lignocellulose



Biomass to Fuel Ethanol



Cellulosic Ethanol Biocatalyst Development at Ngee Ann Poly



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

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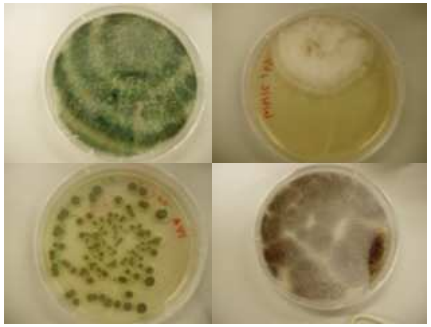
Our Focuses

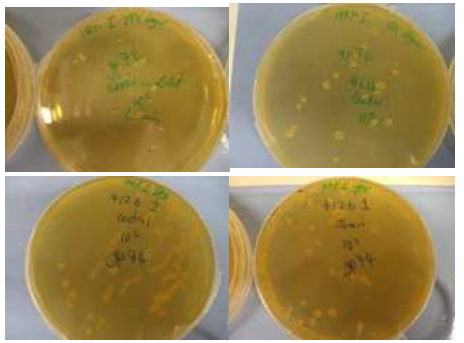
High-strength enzyme cocktails

- Celluase
- Hemicellulase
- Pectinase
- Peroxidases etc.



• Robust ethanologens

- Cofermentation of glucose and xylose
- Inhibitor resistant
- Temperature tolerant
- Ethanol tolerant



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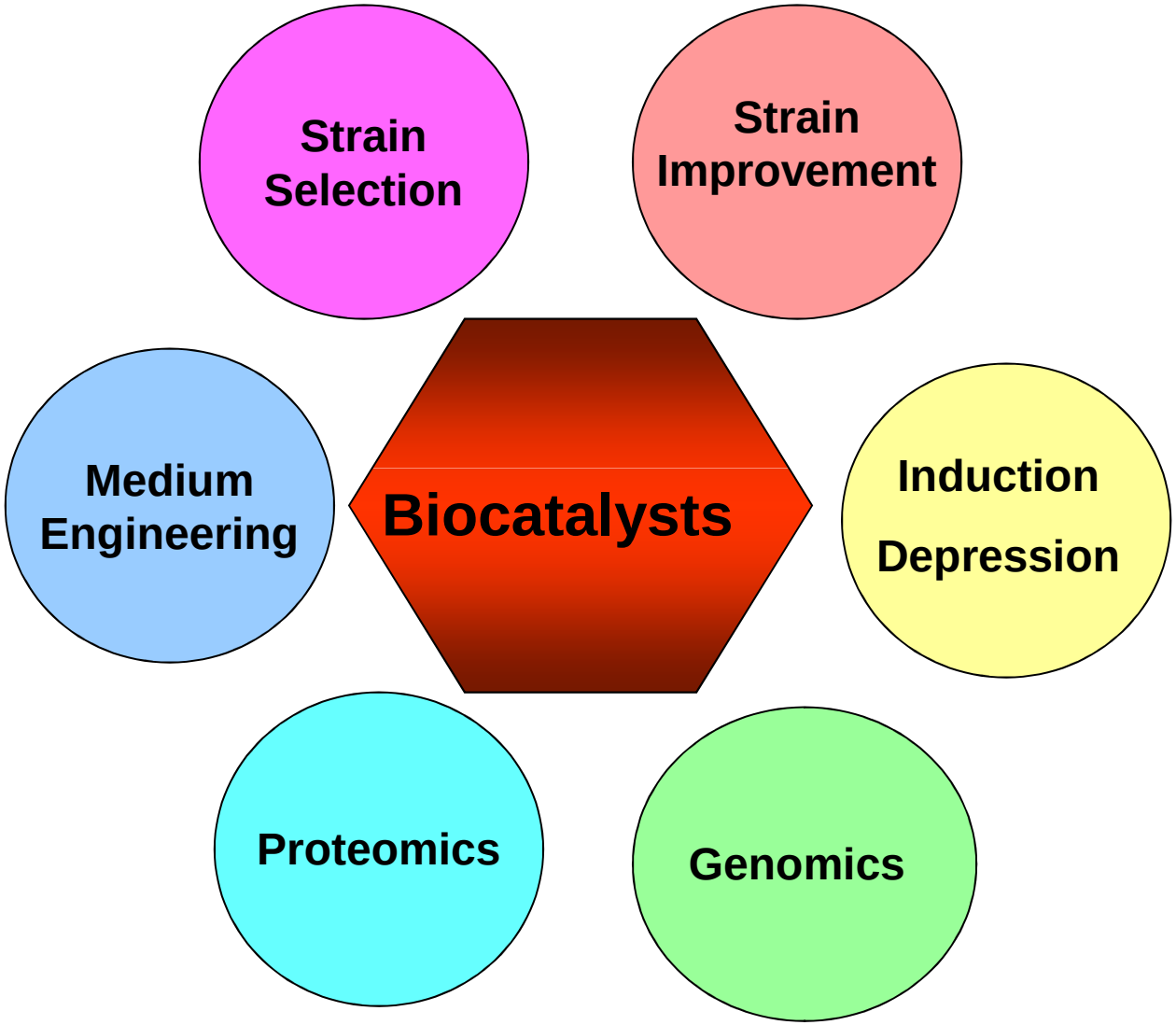
Our Approaches



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Our People




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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

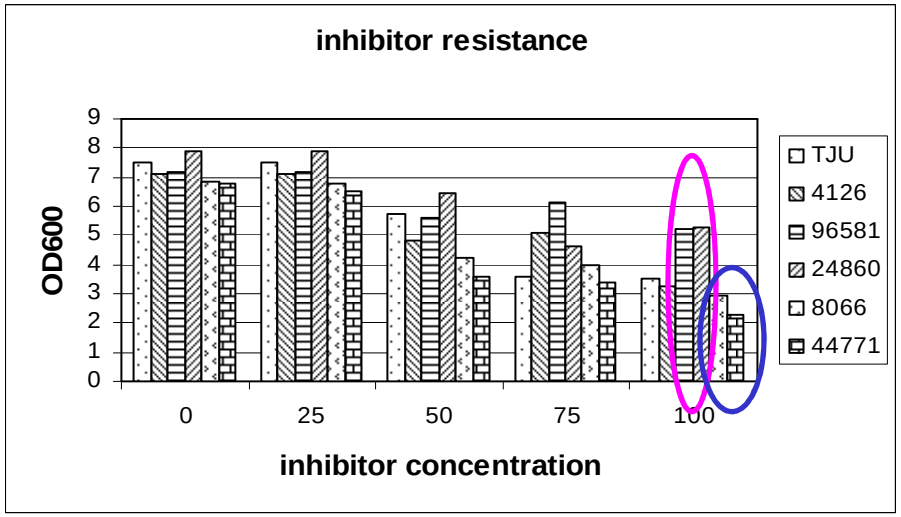
The 100% (v/v) inhibitor stock cocktail

- 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**

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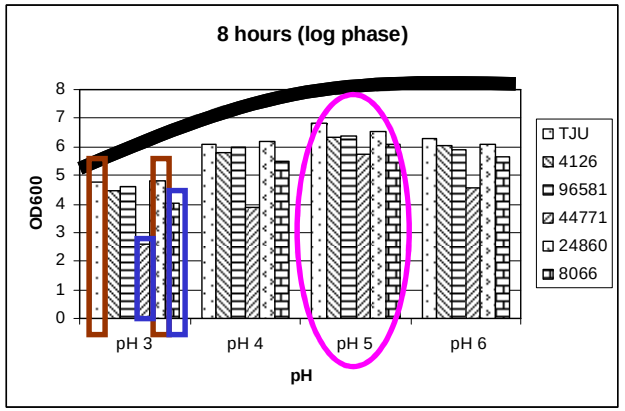
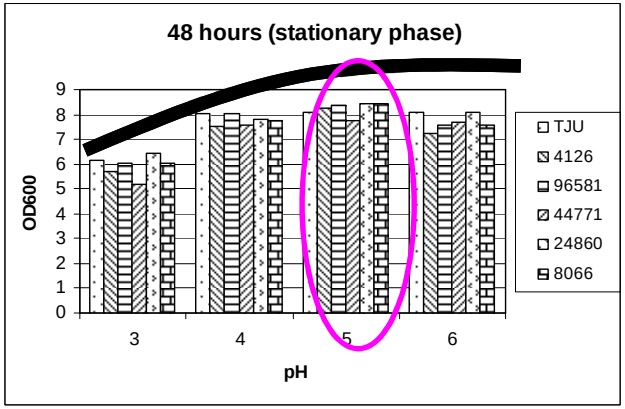
pH Tolerance

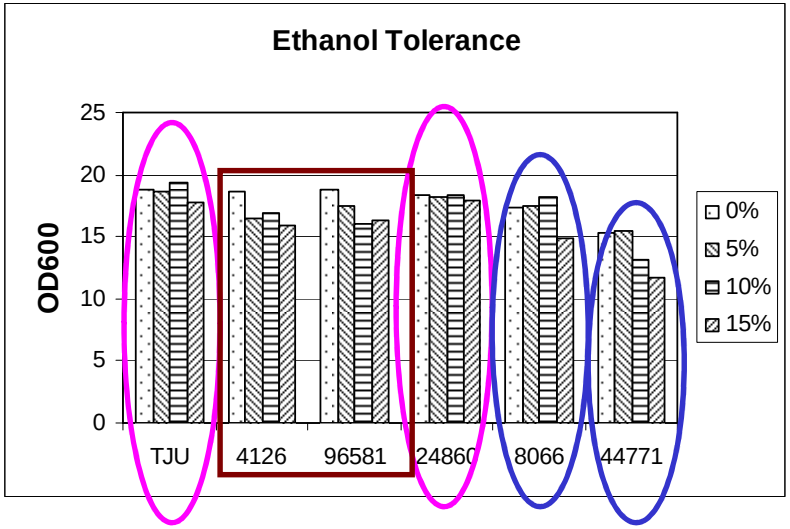
- pH below 4, all strains showed less growth and the optimal pH is 5
- ATCC 44771 showed the least tolerance to the lower pH followed by CBS 8066
- Strains TJU and ATCC 24860 demonstrated the highest tolerance to the lower pH



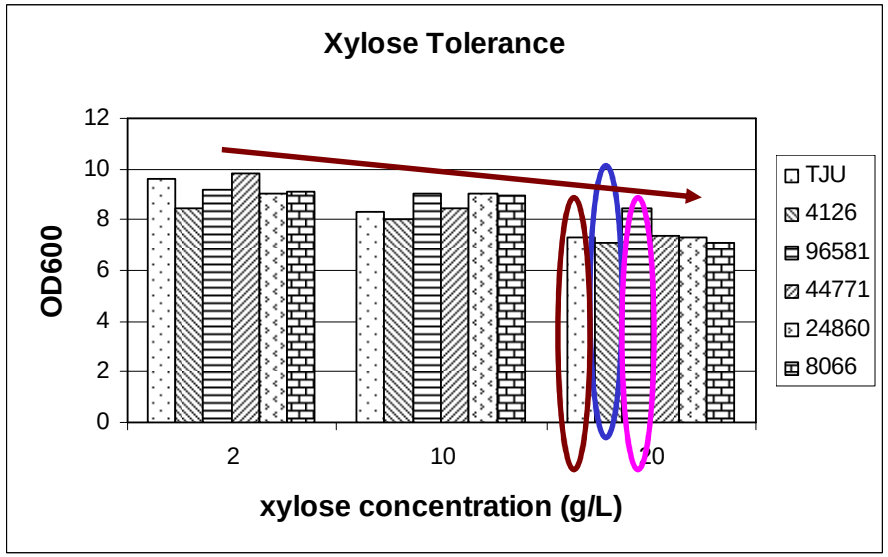


Ethanol Tolerance

- **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

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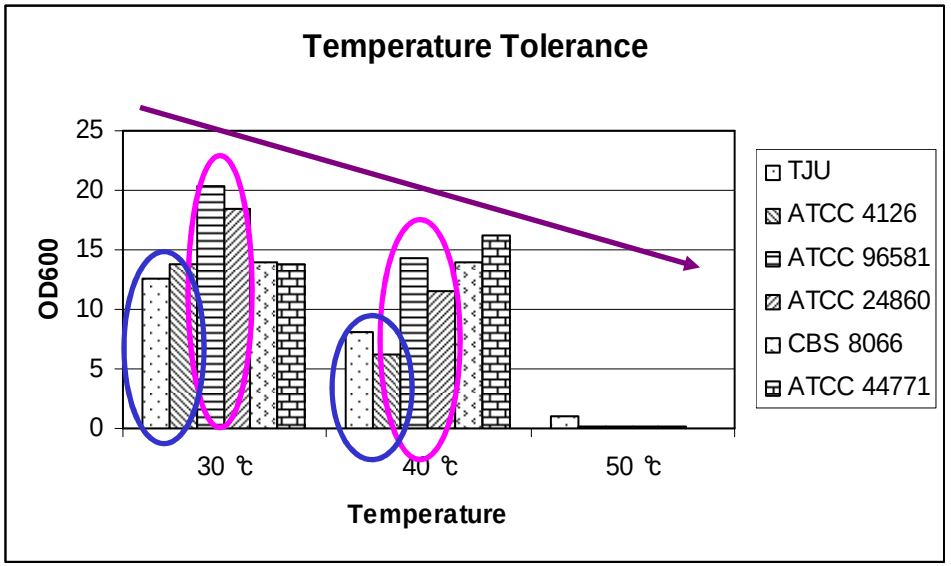
Temperature Tolerance

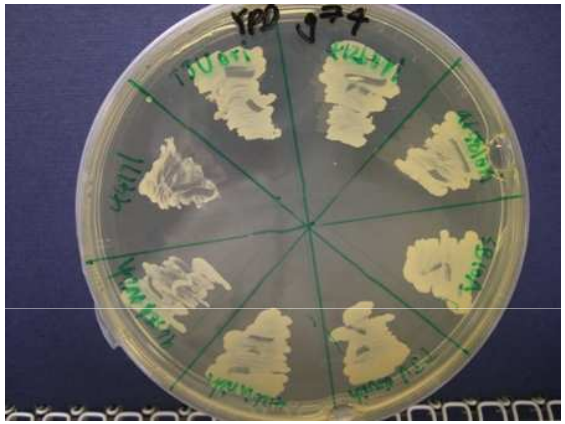
- All strains died off at 50ºC.
- ATCC 24860 and ATCC 96581 demonstrated moderate tolerance to temperature increase
- ATCC 4126 and Strain TJU were quite sensitive to temperature change

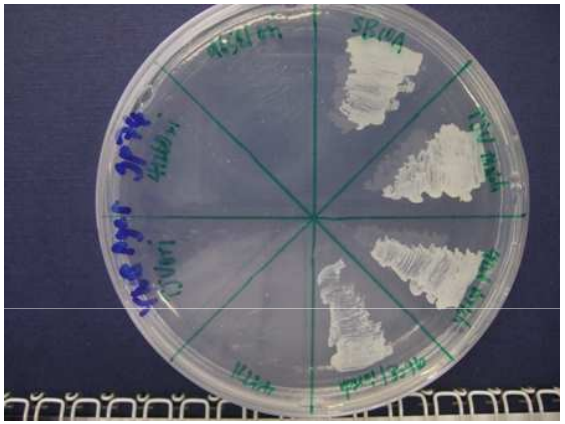


Summary

	Inhibitor	pH	Ethanol	Xylose	Temperature
TJU	+	++	++	-	-
ATCC 4126	+	+	+	--	-
ATCC 24860	++	++	++	+	+
ATCC 96581	++	+	+	++	+
CBS 8066	-	-	-	-	+
ATCC 44771	--	--	--	-	++

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**

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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

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