Metabolic Engineering of a Strain of *Saccharomyces cerevisiae* Capable of Utilizing Xylose for Growth and Ethanol Production

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**Bioethanol** as a fuel supply

**Bioethanol**: Ethanol produced from the fermentation of sugars from the breakdown of plant matter, typically as a transportation fuel.
Starch Bioethanol
Cellulosic Bioethanol
Cellulosic Bioethanol: Why?

- Produced from waste products of agriculture and industry.
- Very abundant feedstock.
- Economical.
- Environmentally friendly.
- Minimal changes in agricultural harvesting processes or land use.
Xylose In Cellulosic Biomass

Xylose ("wood sugar"):  
- The second most common sugar found in cellulosic biomass.
- The glucose:xylose ratio in cellulosic biomass is between 2:1 and 3:1.

PROBLEM:

*Saccharomyces cerevisiae* are incapable of fermenting pentose sugars, such as xylose, to produce ethanol. Until efficient co-fermentation of glucose and xylose to produce ethanol is attained, cellulosic bioethanol is not economically viable.
Saccharomyces cerevisiae: Baker’s Yeast

For use in producing cellulosic ethanol:

**Pros:**
- Very high ethanol yield.
- Traditionally used for ethanol production (baking, brewing).
- Very resilient in industrial fermentation conditions.
- Easily can adapt existing industrial systems to enable fermentation by *S. cerevisiae*.

**Cons:**
- Can not ferment pentose sugars, like xylose.
Xylose Metabolism to Ethanol

a) The yeast pathway:

PROBLEMS:

- Limited by cofactor imbalances.
- Many studies have shown this cofactor imbalance greatly limits ethanol production.

b) The bacterial pathway:

PROBLEMS:

- Bacterial xylose isomerase expression in yeast has very low activity.
- Interest has faded after many failed attempts.

Obtained From: Chandrakant and Bisaria, 1998
Piromyces sp E2 Xylose Isomerase

Piromyces sp E2:
- An anaerobic fungus found in Indian elephant dung.
- Capable of xylose fermentation via xylose isomerase pathway.
- This xylose isomerase has high activity in yeast.
In order for the yeast to ferment xylose, it must be able to transport xylose into the cell.

*S. cerevisiae* does naturally transport xylose with its hexose transporter, but at a very low rate.

The expression of the Glucose/Xylose Facilitator (GXF1) from the yeast *Candida intermedia* in the modified yeast should increase the rate of xylose uptake.
Xylulokinase

D-Xylose → NAD(P)H

NAD(P)+ → Xylitol (Xylitol Dehydrogenase)

Xylose isomerase

D-Xylose → ATP + NADH (Xylosokinase)

D-Xylulose - 5-P

Pentose phosphate pathway

Glyceraldehyde - 3 - P

Glycolytic pathway

Pyruvate

Pyruvate decarboxylase

Acetaldehyde

Alcohol dehydrogenase

Ethanol
Research Goal

To produce a novel industrial strain of *S. cerevisiae* capable of the simultaneous fermentation of glucose and xylose to produce bioethanol by overexpressing the XI, XK, and/or GXF1 genes.

Producing this novel xylose-fermenting yeast should increase fermentation efficiency and ethanol yield from cellulosic biomass.

2. Construct three separate yeast expression vectors carrying XI, XK, or GXF1 with three dominant negative selectable markers.

3. Produce and characterize the genetically engineered yeast strains through:

   (i) XI, XK, and GXF1 assays.

   (ii) Fermentation experiments.
pXKB: Xylulokinase Yeast Expression Vector

- TDH3 promoter
- Xylulokinase
- Amp<sup>R</sup>
- pUC ori
- Bsd<sup>R</sup>
- CYC1 Terminator
- 2µ ori
- pXKB (8.6kb)
pGXFZ: Glucose/Xylose Facilitator Yeast Expression Vector

pGXFZ (8.6kb)

TDH3 promoter

Amp<sup>R</sup>

Glucose/xylose facilitator 1

Zeo<sup>R</sup>

CYC1 Terminator

pUC ori

2μ ori
Yeast Transformations

- Single-transformants:
  - XIG-expressing
  - XKB-expressing
  - GXFZ-expressing

- Double-transformants
  - XIG/XKB-expressing
  - XIG/GXFZ-expressing
  - XKB/GXFZ-expressing

- Triple-transformant
  - XIG/XKB/GXFZ-expressing
Batch Fermentation Experiments

- Currently, batch fermentation experiments have been performed on wild-type, XIG-expressing, and XIG/XKB-expressing strains.
- Yeast grown in variants of YPD media containing glucose and/or xylose.
  - Biomass measured spectrophotometrically.
  - Sugar depletion measured using HPLC.
  - Ethanol production measured using GC.
Fermentation Experiments (Biomass)
Fermentation Experiments (Glucose)
Fermentation Experiments (Xylose)
Ethanol Production

WT Fermentation (Ethanol)

XIG Fermentation (Ethanol)

XIG/XKB Fermentation (Ethanol)
Fermentation Results Summary

- The expression of XI allows slow metabolism of xylose for the production of biomass, but not fermentation.

- The expression of both XI and XK allows for even faster xylose metabolism and yeast cell growth on xylose.
  - Still too slow for ethanol production to occur.
Future Work

For remaining recombinant strains:

a) Determine the enzyme activities of xylose isomerase and xylulokinase (through coupled assays).

b) Determine the relative levels of glucose/xylose facilitator expression (real-time RT-PCR).

c) Perform further fermentation experiments and analyze yeast growth, ethanol production, and glucose and xylose depletion.

d) Perform larger scale fermentations on any “interesting” strains.

e) Compare double- and triple-transformant xylose metabolism and ethanol production.
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Questions?