Pichia stipitis: Rapid ethanol from biomass

A. What is this technology?

Culture conditions supporting inhibitor tolerance and rapid production of ethanol from biomass sugars by the natural xylose-fermenting yeast *Pichia stipitis* NRRL Y-7124

B. What problem does it address?

- New process strategies are needed to expand the biomass to fuel ethanol industry.
- Predominant sugars produced from biomass include glucose (C6) and xylose (C5). Unlike traditional *Saccharomyces* yeast strains, *Pichia stipitis* NRRL Y-7124 is yeast naturally able to ferment C5 in addition to C6 sugars, but its fermentation efficiency and inhibitor tolerance need improvement.
- Culture conditions must foster production of yeast populations able to survive and ferment C6 and C5 sugars while exposed to inhibitors (ethanol, furfural, and hydroxymethylfurfural, or HMF). Furfural and HMF are key hydrolytic byproducts of dilute-acid pretreatment, currently the most economical pretreatment of biomass that allows efficient enzymatic release of fermentable sugars.

C. What is the significance of this solution?

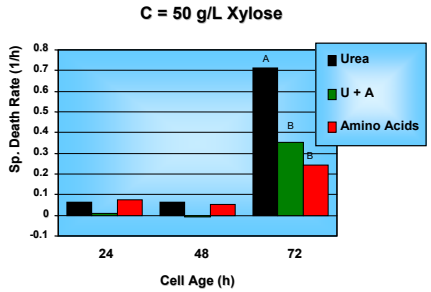
- **Culture nutrition optimized for ethanol production on xylose and inhibitor tolerance**
  Optimal ethanol yield (up to 70 g/L) and productivity of *P. stipitis* on xylose were obtained when nitrogen and carbon were supplied at a C:N ratio of 37:1 and when 80% of the nitrogen was supplied by urea and 20% by amino acids. Supplying N as either urea or amino acids alone led to > 50% reduction of ethanol yield. Amino acid enrichment of the growth medium significantly enhanced the ability of cells to resist furfural, HMF and ethanol exposure (Fig.1). Addition of an optimized mineral supplement of Mg, Fe, Mn, Ca, Zn and others improved ethanol yield by more than two-fold.
- **Priming of *P. stipitis* on xylose reduced lag and fostered rapid utilization of sugar mixtures**
  Producing large inocula on xylose (rather than glucose) induced faster fermentation rates in ethanol production fermentors and eliminated diauxic lag during mixed sugar conversion by *P. stipitis* (Fig.2)

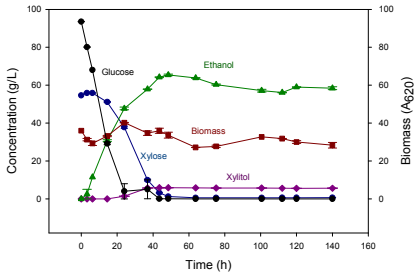
D. Who could use this technology?

Use of these process strategies in industrial plant designs by businessmen and engineers has the potential to lower the cost of ethanol from biomass.

E. How is this technology unique?

Using both nutrition and culture priming technologies, *P. stipitis* Y-7124 yields an economically recoverable 66 g/L ethanol in 48 h at a conversion efficiency of 88% (0.44 g ethanol/g sugar) from 95 g/L of glucose and 55 g/L xylose. This is a vast improvement over "stuck" fermentations commonly occurring during the glucose to xylose switch, which may lower the cost of ethanol from biomass.





Stage of Development

This research has been published:

- Slininger PJ, Dien BS, Gorsich SW, Liu ZL (2006) Nitrogen source and mineral optimization enhance D-xylose conversion to ethanol by the yeast Pichia stipitis NRRL Y-7124. Appl Microbiol Biotechnol 72:1285-1296
- Slininger PJ, Gorsich SW, Liu ZL (2008) Culture nutrition and physiology impact the inhibitor tolerance of the yeast *Pichia stipitis* NRRL Y-7124. Biotechnol. Bioeng. (submitted)
- Slininger PJ, Liu ZL (2008) Impact of inoculum production conditions on stress tolerance and fermentation efficiency of natural xylose-fermenting yeasts presented xylose and glucose. 30th Symposium on Biotechnology for Fuels and Chemicals, May 4-7, 2008, New Orleans, LA, Abstract 2-11

Moving Forward

Partnering is needed to examine use of these strategies to boost efficiency of a biomass to ethanol plant.

PARTNERSHIP OPPORTUNITY

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