Use of Genetically Modified *Saccharomyces cerevisiae* to Convert Soluble Starch Directly to Bioethanol

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What is *Bioethanol*?

- Ethanol produced by fermentation process
- *Saccharomyces cerevisiae*
Metabolic pathways in yeast

- **Aerobic catabolism**
  - Glucose + 36Pi +36 ADP + 6O$_2$ -> 6 CO$_2$
  - + 6 H$_2$O + 36 ATP

- **Anaerobic catabolism (fermentation)**
  - Glucose -> 2Ethanol + 2CO$_2$ + 2ATP
Industrial fermentation process

Raw Starch → Grinding → Cooking → liquefaction → Saccharification → Fermentation

Enzymes

Yeast
Breaking down the starch molecules

- $\alpha$-amylase
- Glucoamylase
- Starch particles
Industrial fermentation process (continued)

- Energy costs
- Starch hydrolyzing enzymes
- Expensive equipment
Possible Solution

Starch Molecules

DNA Recombinant Technique

DNA Recombinant Yeast Expressing Barley $\alpha$-amylase

Yeast

Barley $\alpha$-amylase
Possible Solution (continued)

- Grinding
- Cooking
- Liquefaction
- Saccharification
- Fermentation

Grinding

Fermentation
Research plan

1. Construction of a novel plasmid;
2. Yeast transformation & suitable transformants selection;
3. Detection of cell wall anchored barley $\alpha$-amylase activity;
4. Fermentation on soluble starch.
Novel Plasmid Design

Barley α-amylase gene

3’ α-agglutinin gene

Secretion signal sequence

ADH1

CYC1

2μ Origin

Bsd+

Amp+

pAMY
DNA recombinant yeast
Selection of plasmid containing yeast - NRRL Y-132

Blasticidin (µg/ml)

0  20  60  100

pAMY

pSac

W.T.

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Iodine Vapor

YPD + Soluble Starch plate

Starch - Amylose

\[ \text{Iodine slides into starch coil to give a blue-black color} \]
Starch plates stained with iodine vapor

NRRL Y-132 transformed with pAMY

Munton’s yeast transformed with pAMY
Iodine assay

0                2               4                 6 hours
DNS assay

0  2  4  6 hours
Superior Yeast?

Growth curve of NRRL Y-132/pAMY and NRRL Y-132 on 2% soluble starch

Cell Number (X 10^10)

Time (hours)
Starch hydrolysis
The active site…

SS \(\alpha\)-amylase \(\alpha\)-agglutinin

Cell Membrane

Cell Wall

N-terminal C-terminal
The active site…
Not the end of the story yet…

- Other hydrolysing enzymes
- Different anchoring genes
- Broad application
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Questions?

NRRL Y-132

NRRL Y-132

Munton’s yeast

Solar eclipse

W.T.

pAMY

pAMY

in 1999