

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

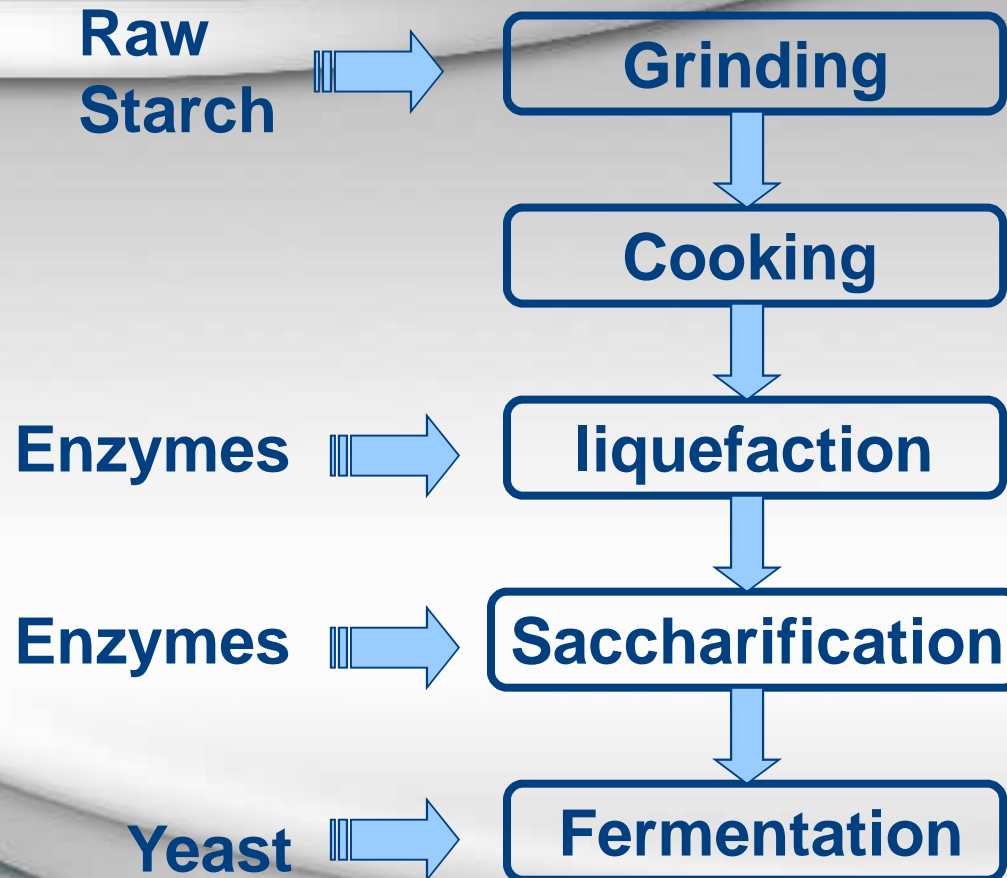
- **Aerobic catabolism**



- **Anaerobic catabolism (fermentation)**

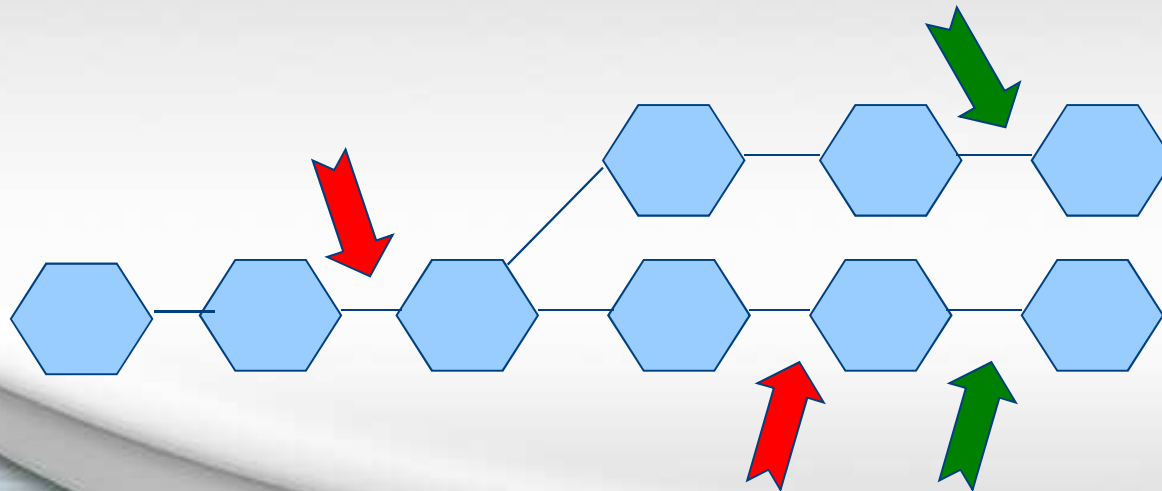


Industrial fermentation process



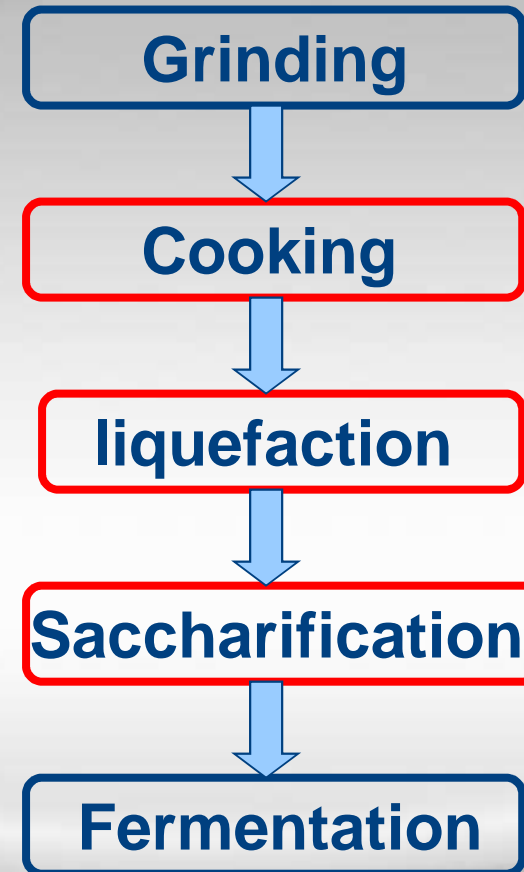
Breaking down the starch molecules

- α -amylase
- Glucoamylase
- Starch particles

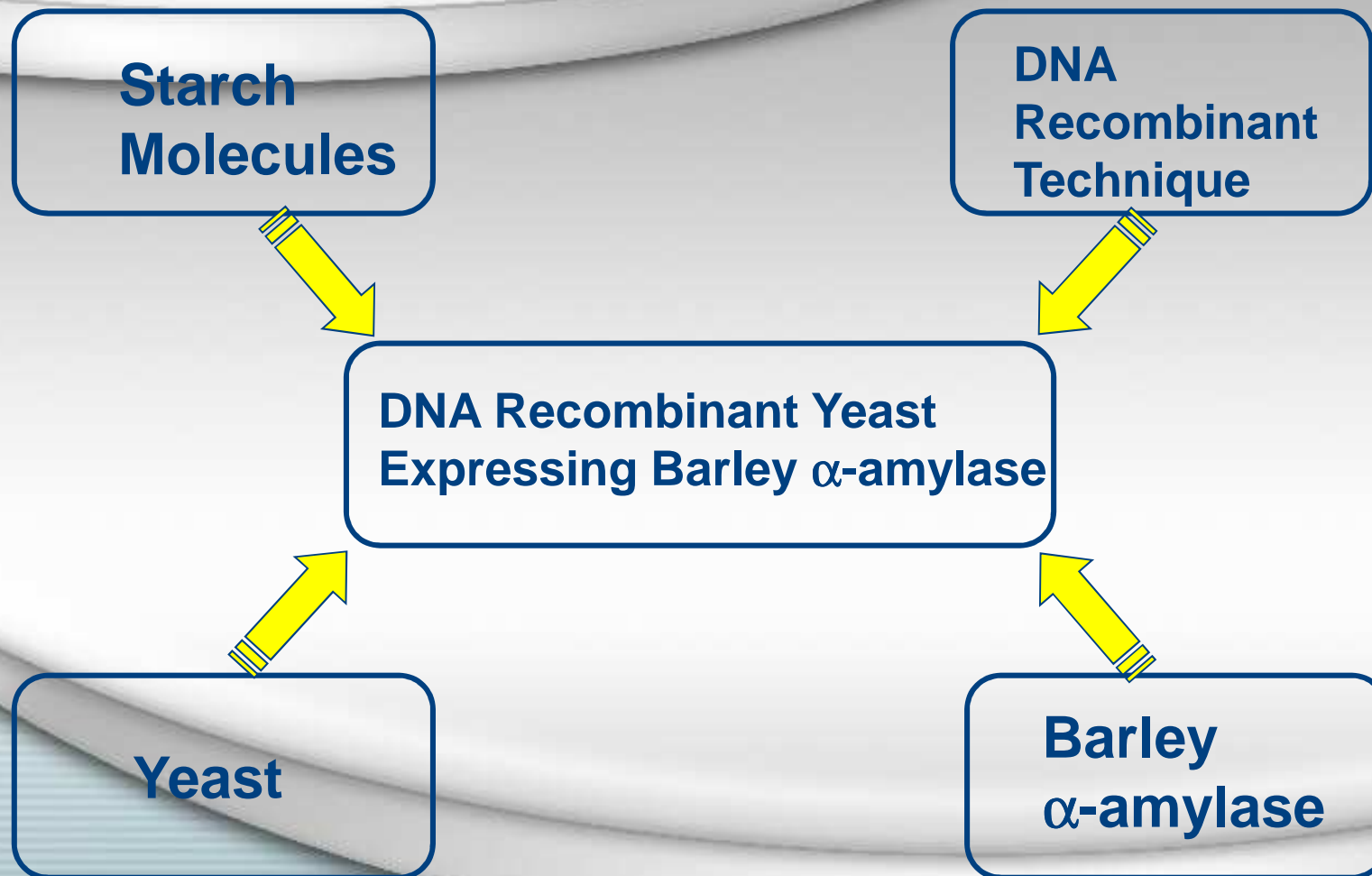


Industrial fermentation process (continued)

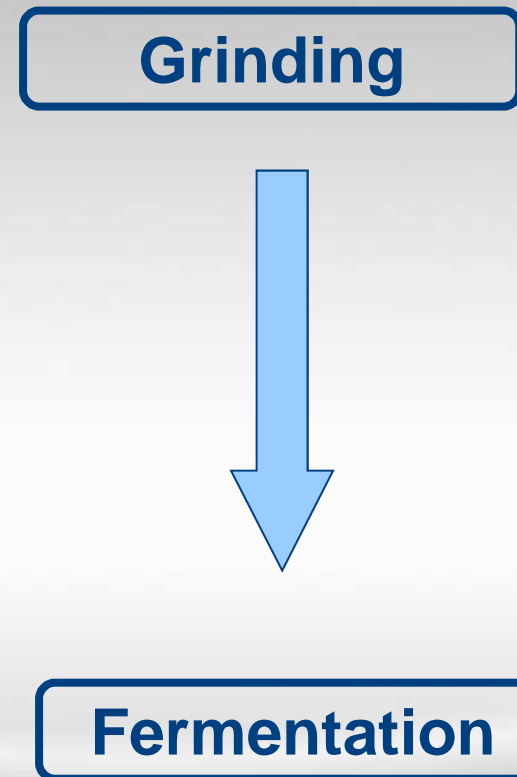
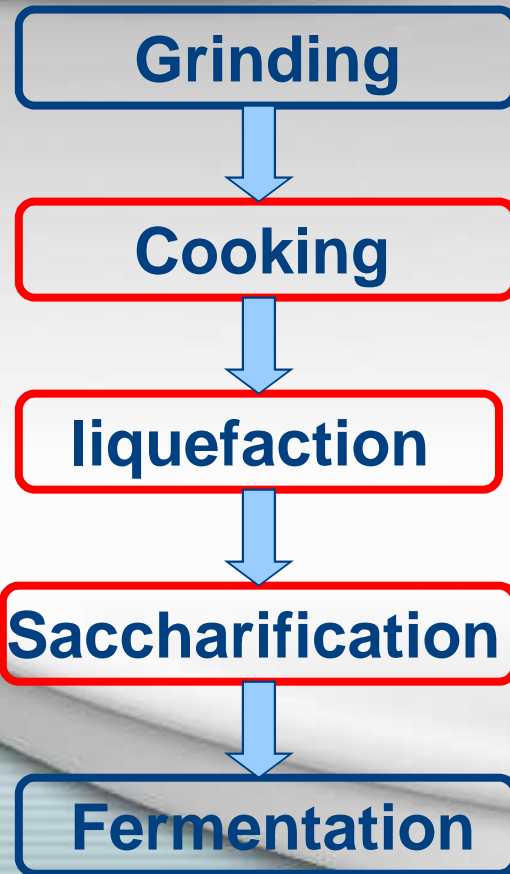
- Energy costs
- Starch hydrolyzing enzymes
- Expensive equipment



Possible Solution



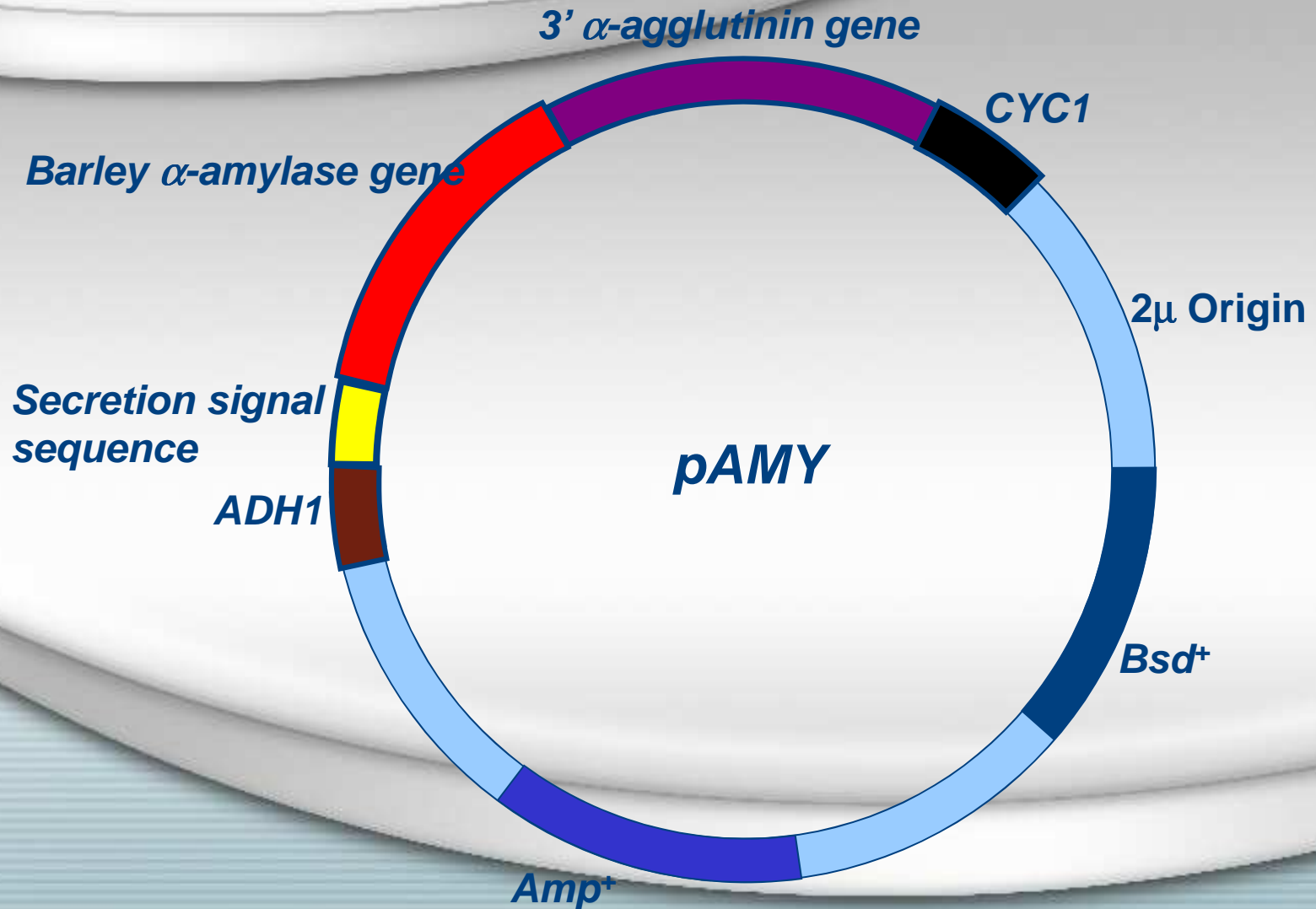
Possible Solution (continued)



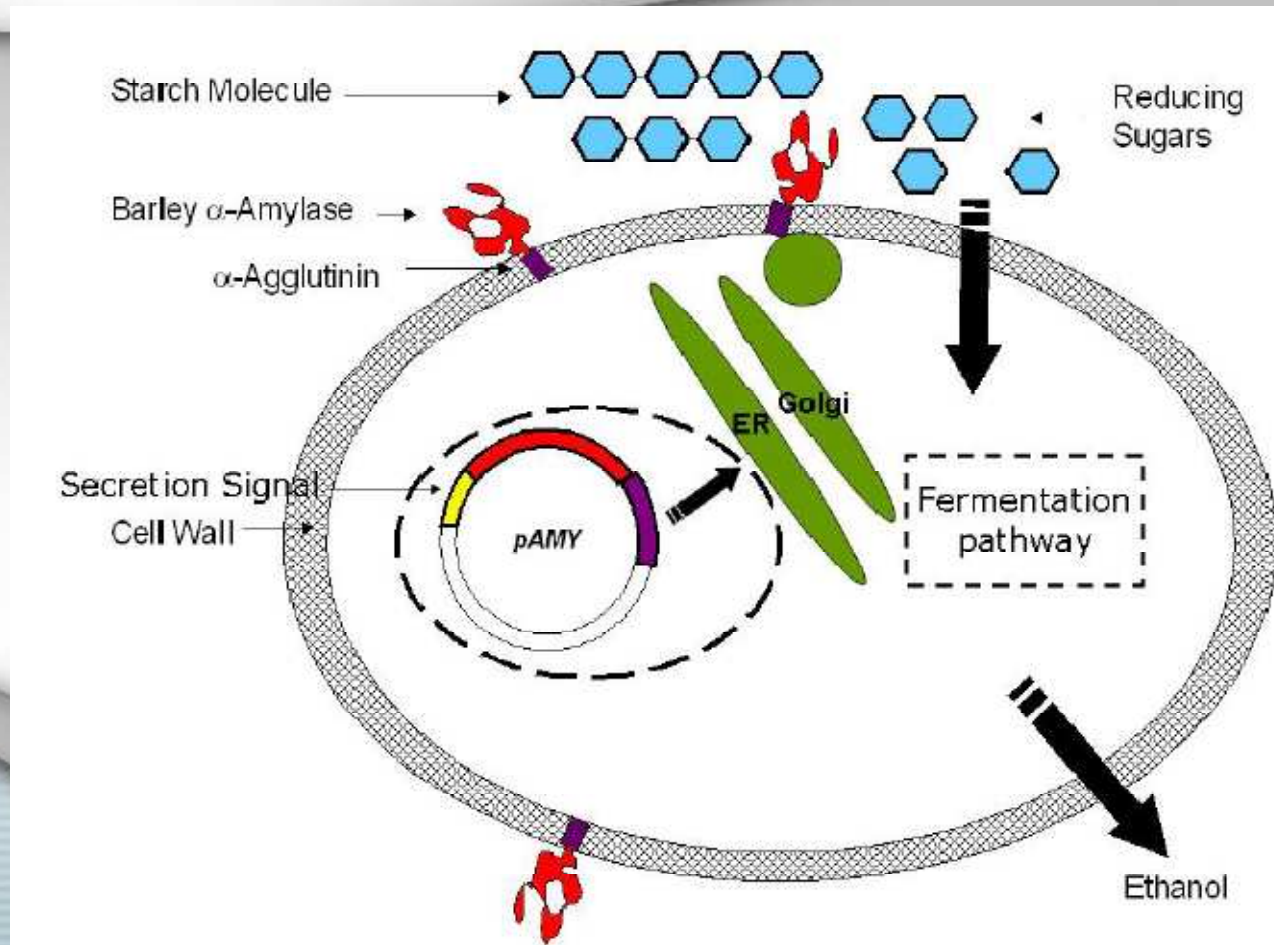
Research plan

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

Novel Plasmid Design



DNA recombinant yeast



Selection of plasmid containing yeast -NRRL Y-132

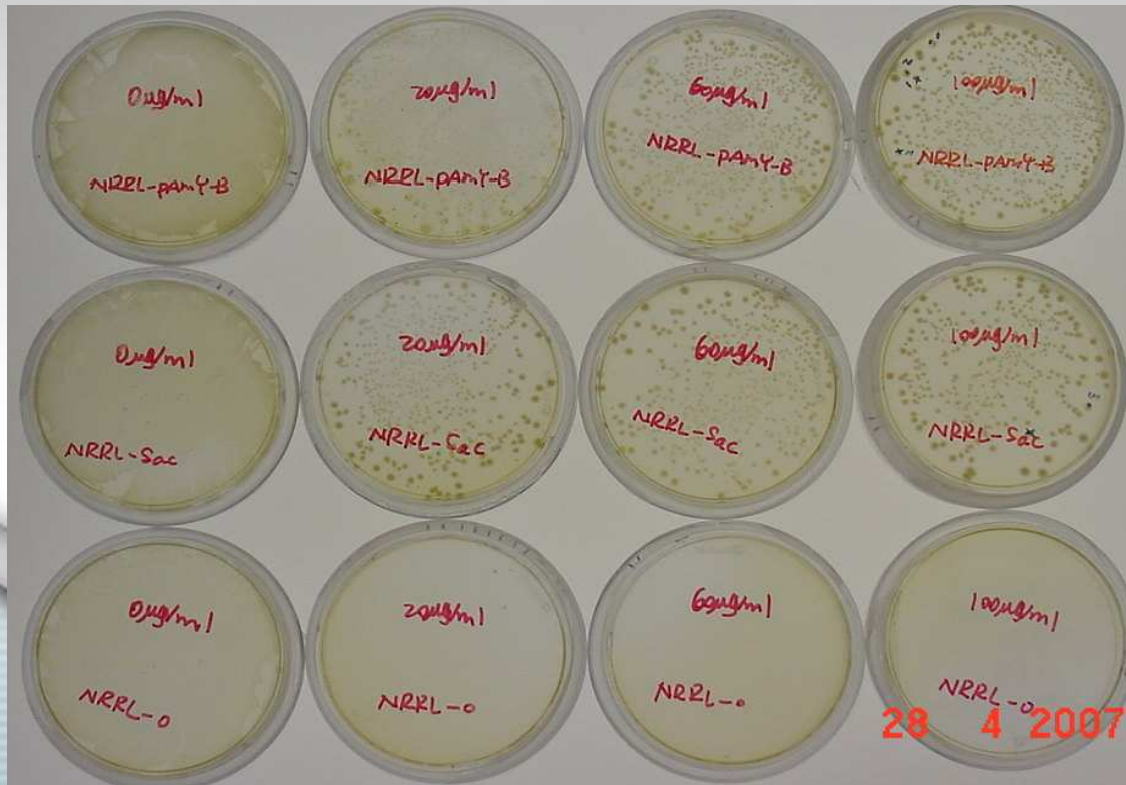
0

20

60

100

Blasticidin($\mu\text{g/ml}$)

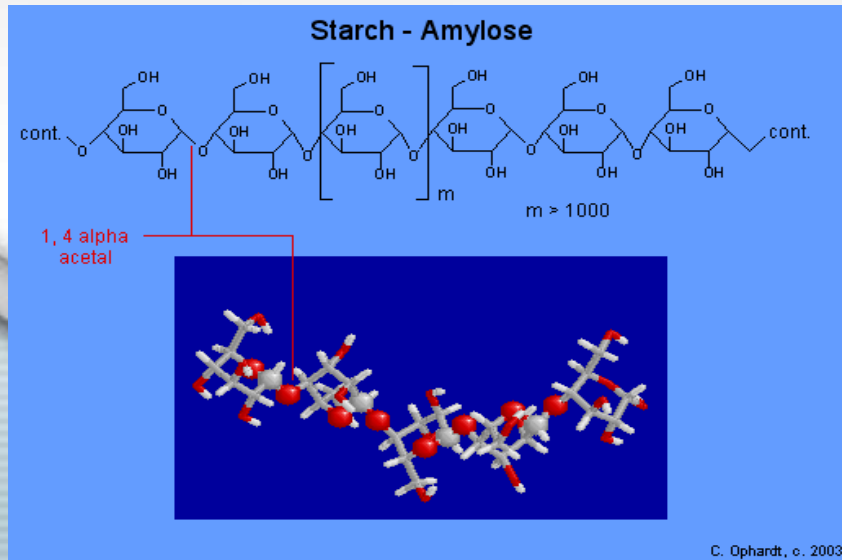
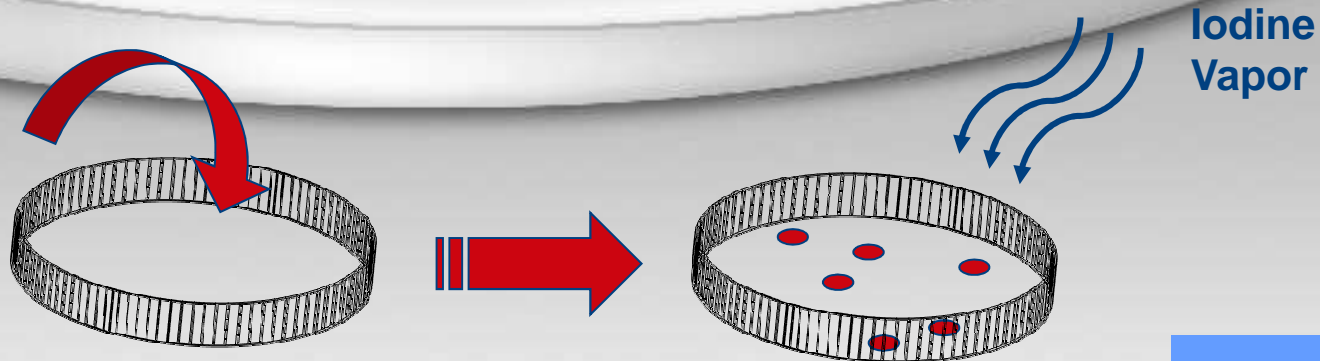


pAMY

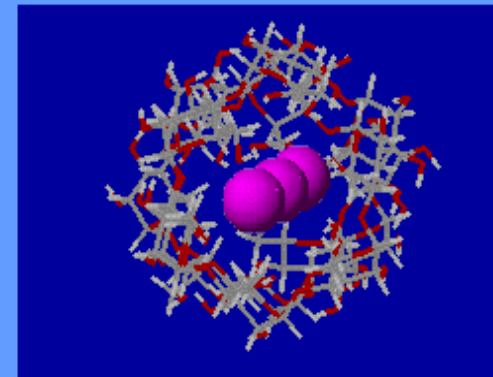
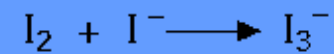
pSac

W.T.

YPD + Soluble Starch plate



Starch - Iodine Complex



Iodine slides into starch coil to give a blue-black color

C. Ophardt, c. 2003

Starch plates stained with iodine vapor



NRRL Y-132 transformed with pAMY



Munton's yeast transformed with pAMY

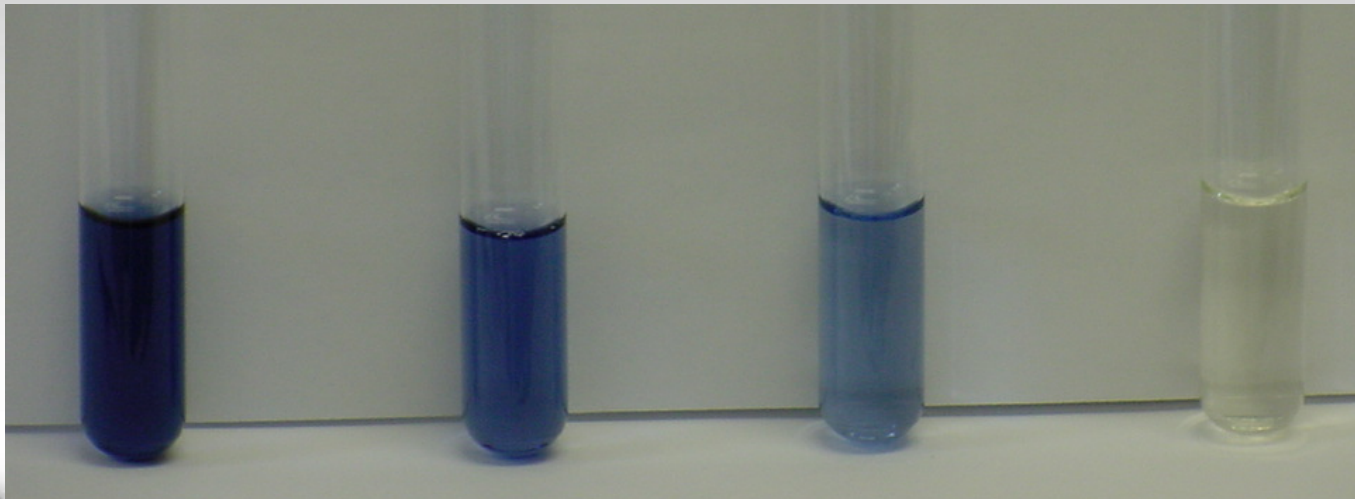
Iodine assay

0

2

4

6 hours



DNS assay

0

2

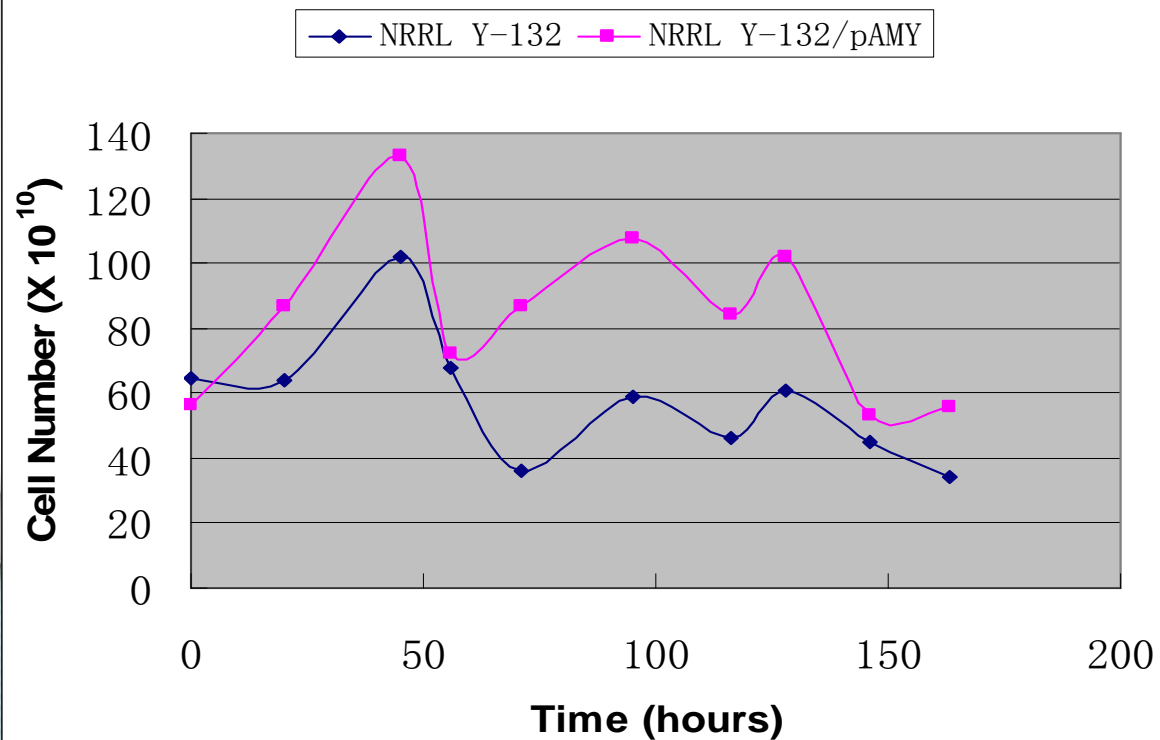
4

6 hours

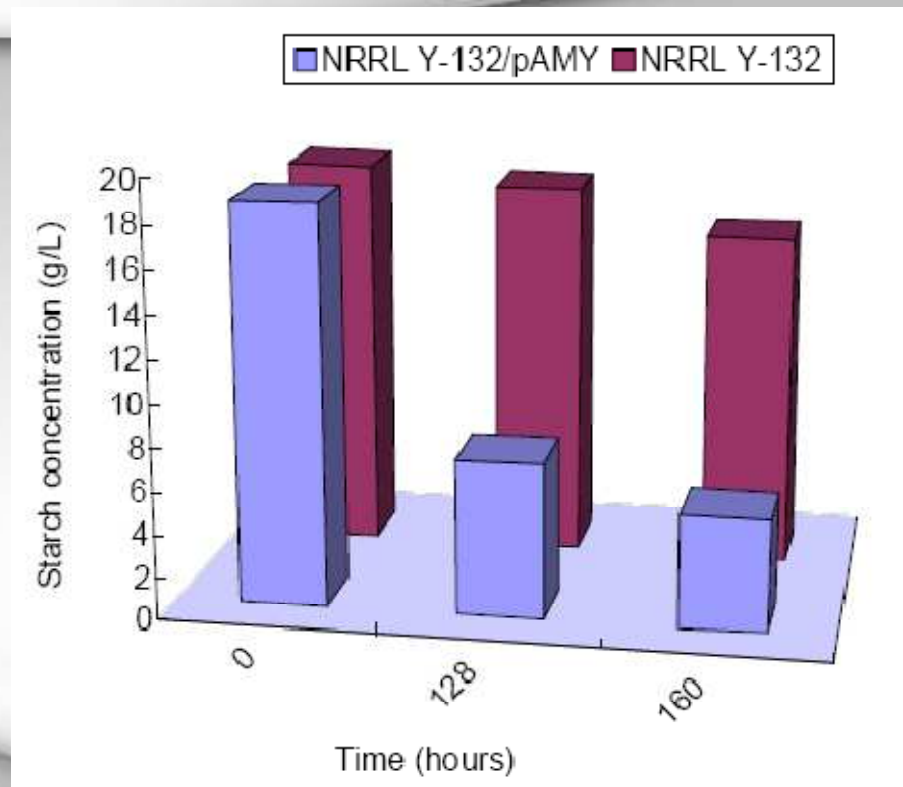


Superior Yeast?

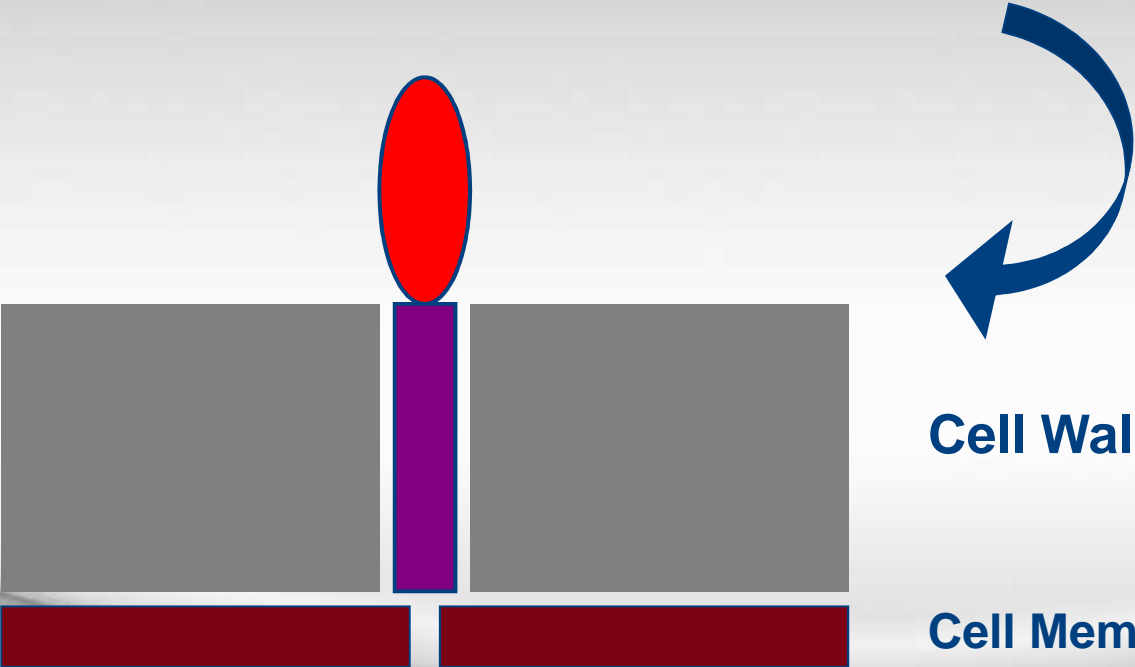
Growth curve of NRRL Y-132/pAMY and NRRL Y-132 on 2% soluble starch



Starch hydrolysis



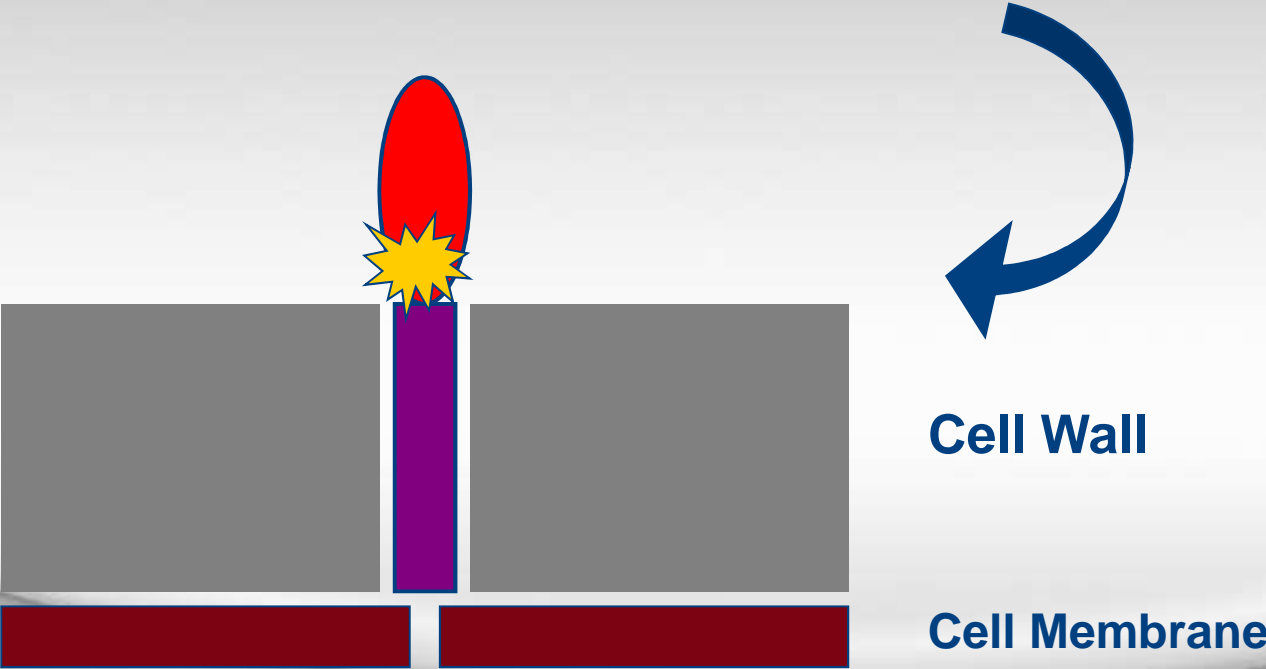
The active site...



Cell Wall

Cell Membrane

The active site...



Not the end of the story yet...

- Other hydrolysing enzymes
- Different anchoring genes
- Broad application

Acknowledgement

- Dr. William Roesler
- Dr. Gordon Hill

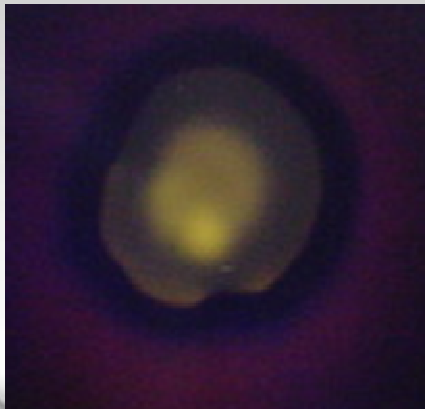


Agriculture Development
Fund (ADF)

Agriculture Canada's
ABIP Fund

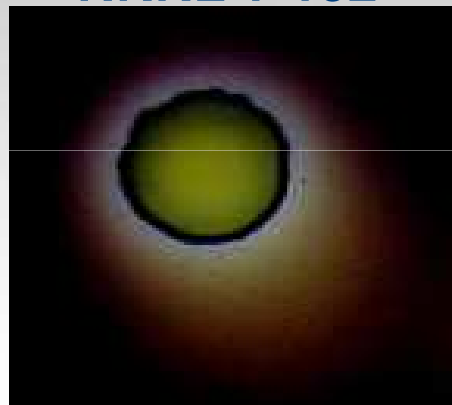
Questions?

NRRL Y-132



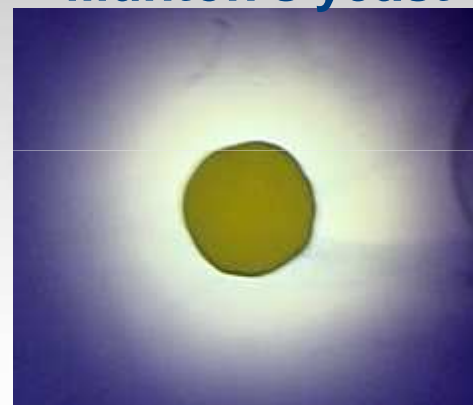
W.T.

NRRL Y-132



pAMY

Munton's yeast



pAMY

Solar eclipse



in 1999