Development of Pentose Fermenting Yeast Strains for Ethanol Production from Lignocellulosic Biomass

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Fermentation of Lignocellulosic Hydrolysate

PRETREATMENT

HYDROLYSIS

LIGNOCELLULOSIC BIOMASS

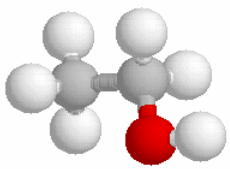
Cellulose
Hemicellulose
Lignin
Extractives

Hexoses
Pentoses
Extractives

Pretreatment by-products

SSF

ETHANOL



ETHANOLIC FERMENTATION

DISTILLATION

OTHER PRODUCTS

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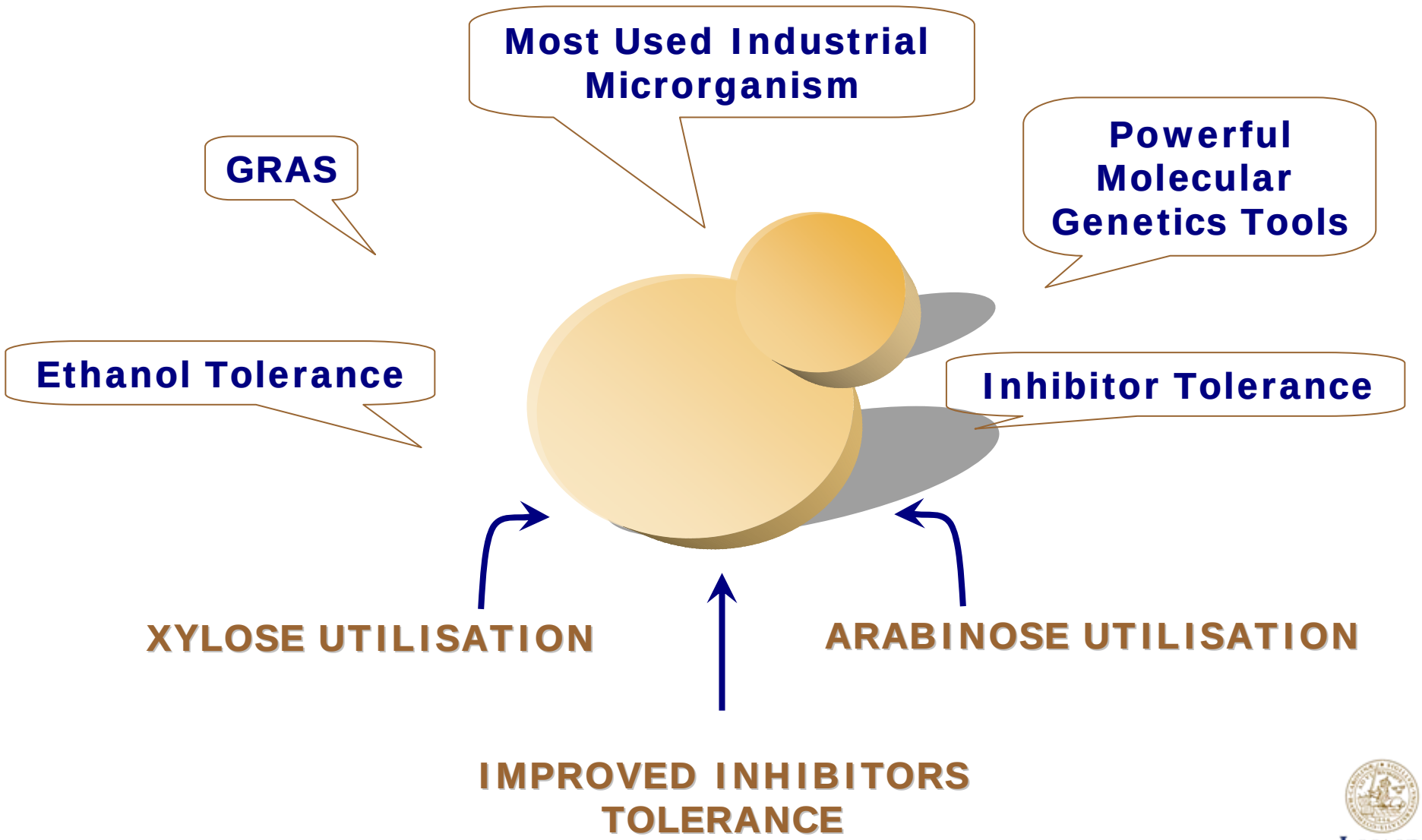
Saccharomyces cerevisiae Is a Good Choice



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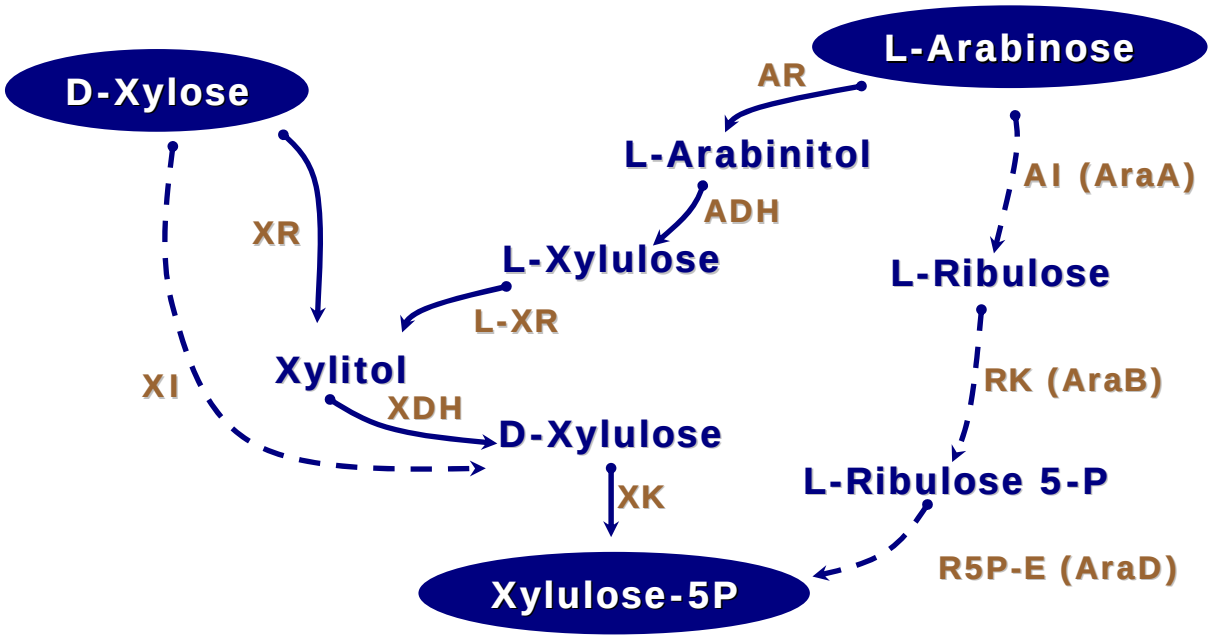
New Pentose Fermenting Strains

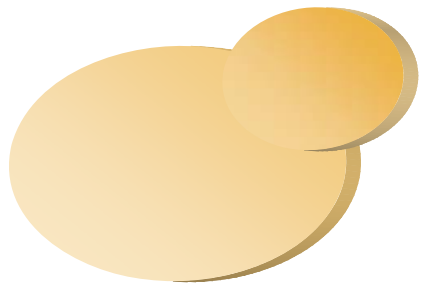


Available pathways

BACTERIAL

FUNGAL

Genetic engineering



New strain with the required pathways

Pentose fermenting strain?

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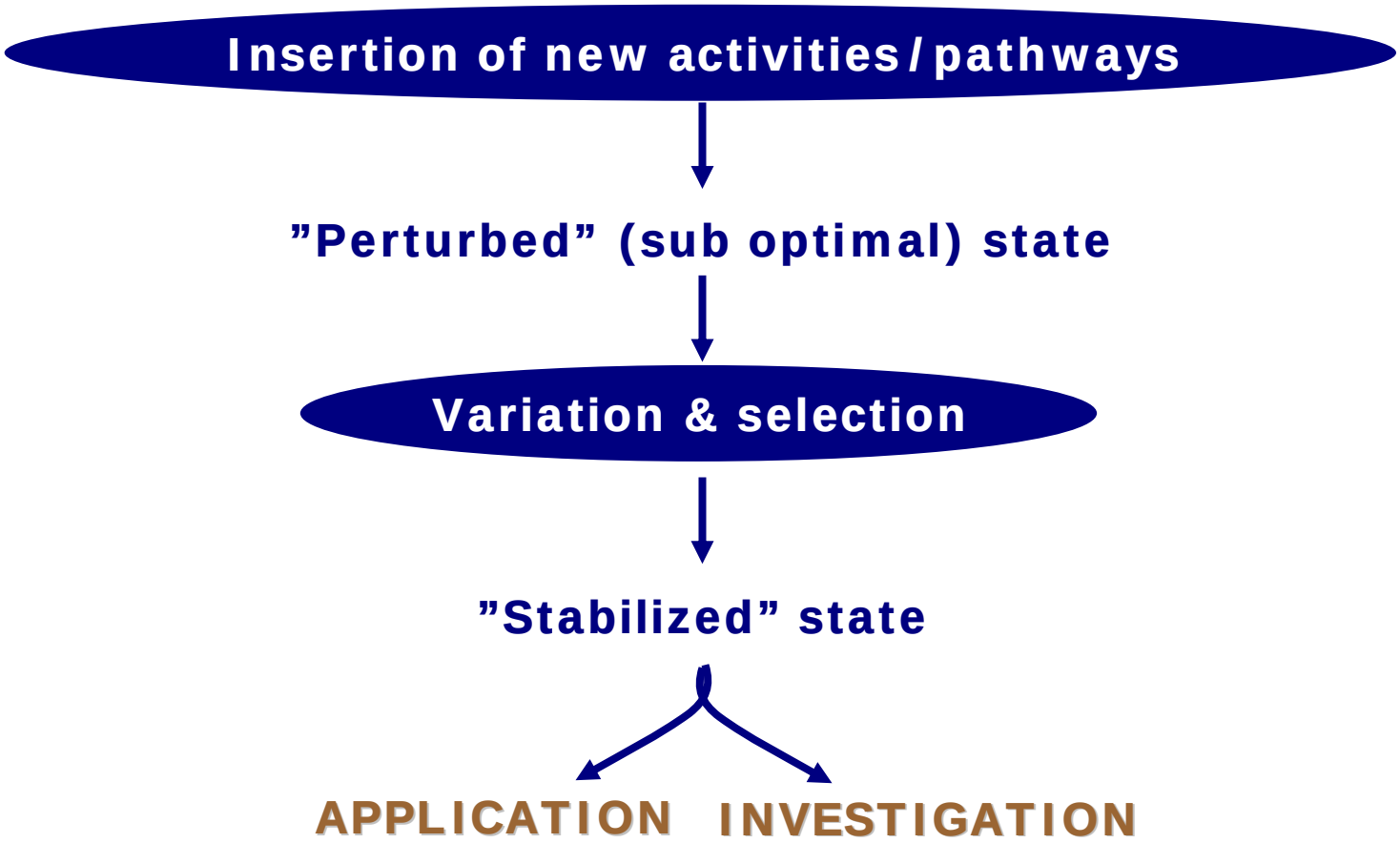
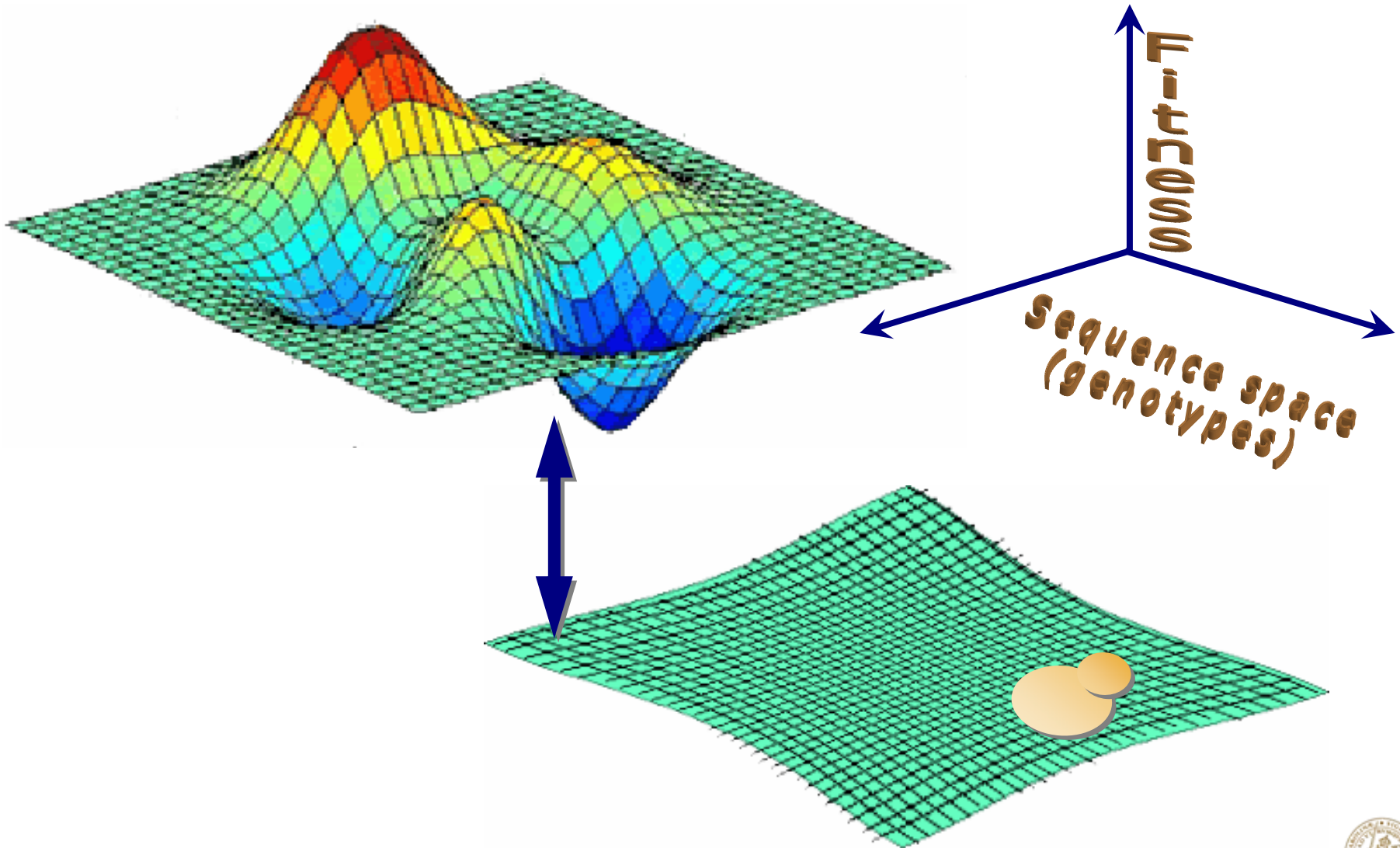
A Combined Strategy to Reach the Goal

Metabolic systems work in a highly coordinated status

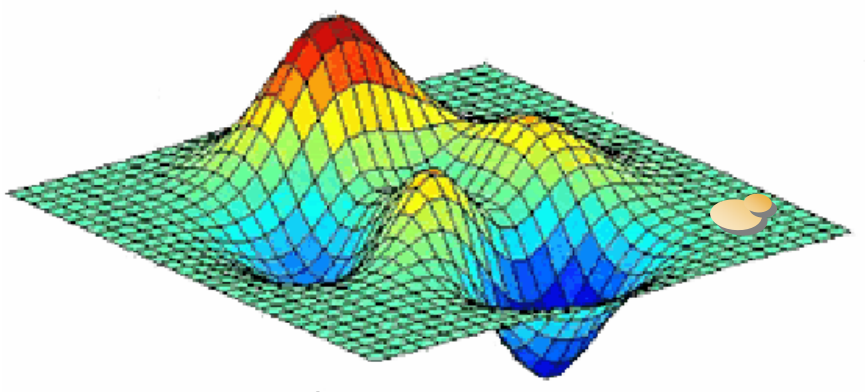


The Fitness Landscape



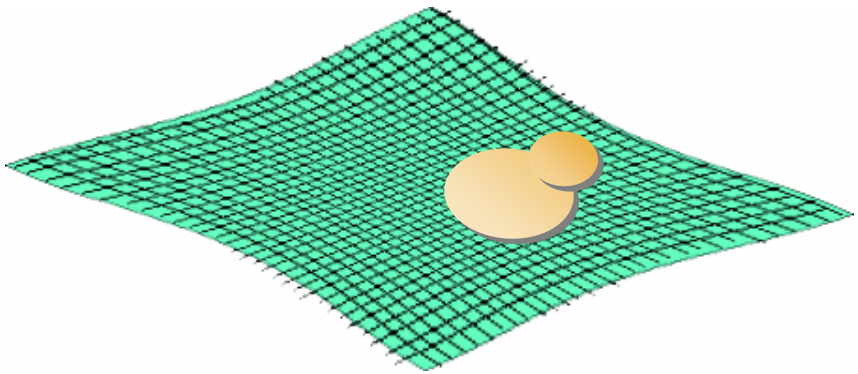
- Wright (1982) Evolution 36:427.
- Kauffman (1993) The origins of order. Oxford University press.

Variation Scans the Fitness Landscape



Evolutionary engineering / mutagenesis (Scan the fitness landscape)

Increased mutational rate: Increased driving force through the fitness landscape



Genetic engineering (Jump)
Metabolic system in ”suboptimal” state

Point 0: No pentose genes

•Sauer (2001) Adv Biochem Eng Biotechnol 73:130-169

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Xylose Fermenting Strain

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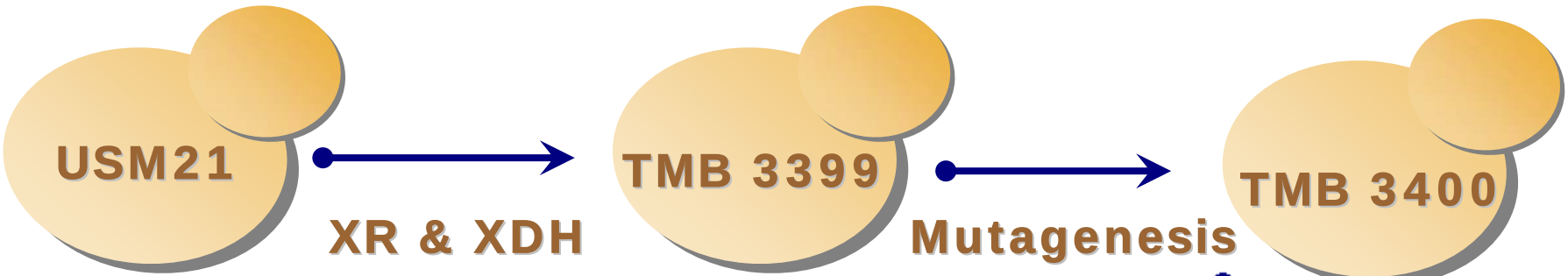
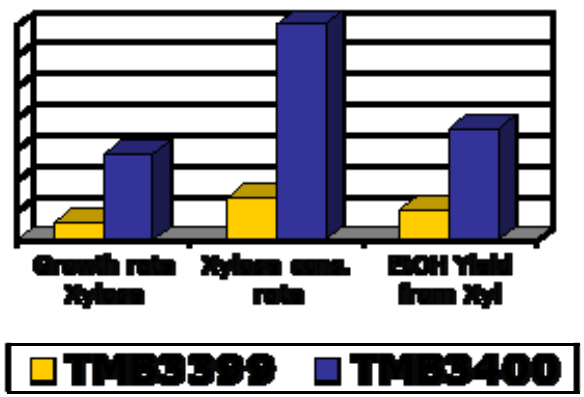
jou kennisvennoot·your knowledge partner



Better performing strain
(Application)



What's happened?
(Investigation & Knowledge)

↑PPP
↑XR/XDH

Transcriptome

(Proteome under study)
Karhumaa, submitted

- Wahlbom et al (2003) FEMS Yeast Res 3:319-326
- Wahlbom et al (2003) Appl Environ Microbiol 69:740-746

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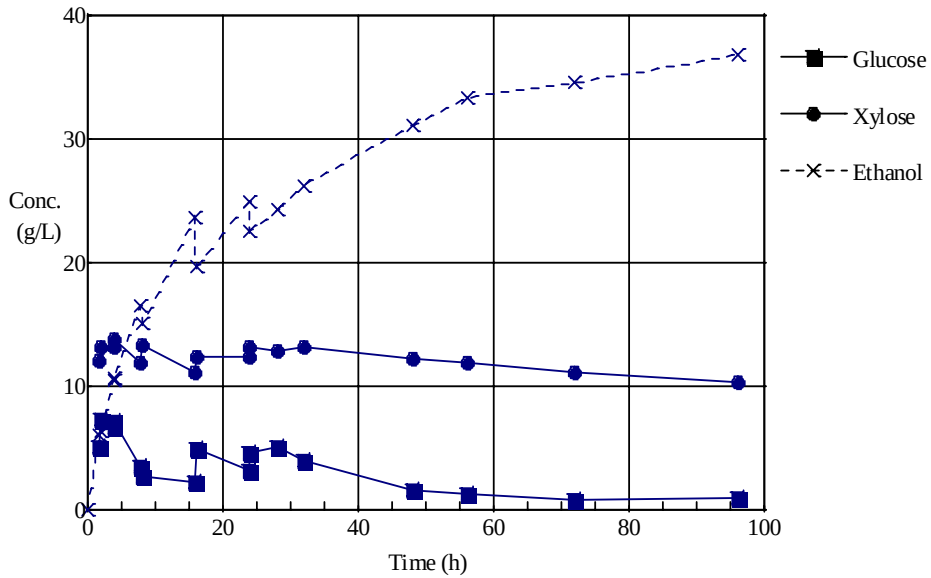
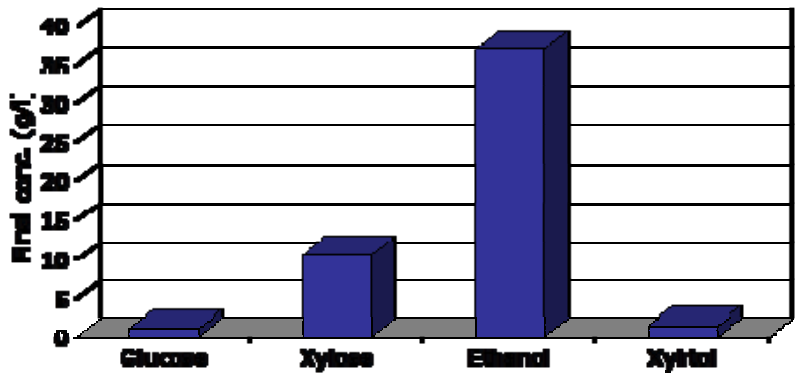
Performances in Operational Conditions

SSF, Fed Batch, Corn Stover

Xylose is co-consumed with glucose



By product (Xylitol) production is low



•Öhgren et al (2006) J Biotechnol, in press (doi:10.1016/j.jbiotec.2006.05.001)

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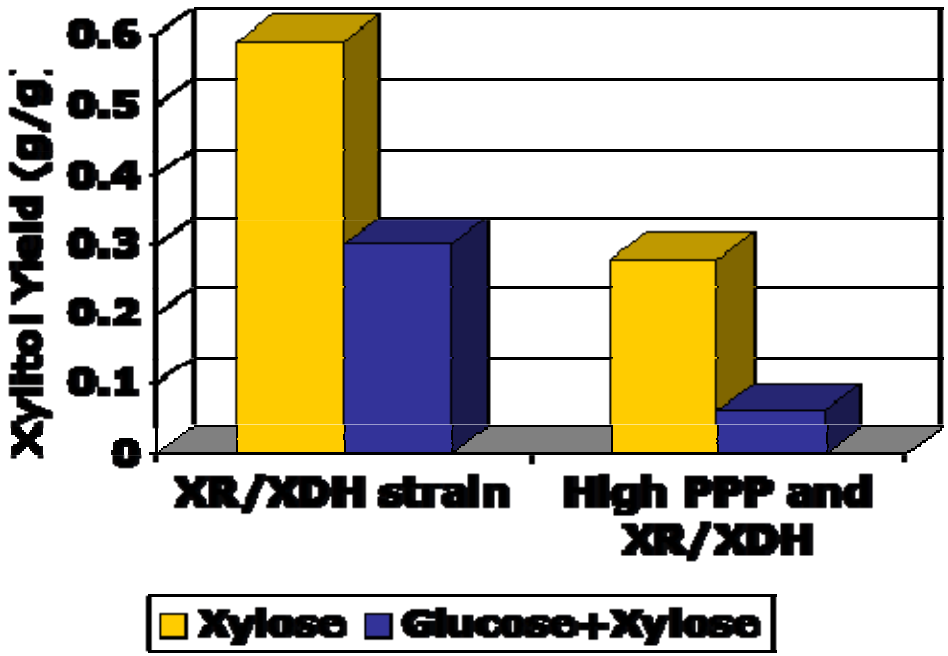
Lower Xylitol Production May Result from:

Increased Overall Metabolic Flux by Presence of Glucose



- Karhumaa et al (2006) Appl Microbiol Biotechnol. In press.

- Jeffries et al (1984) Biotechnol Bioeng 27:171
- Jin et al (2004) Appl Env Microbiol 70:6816-25

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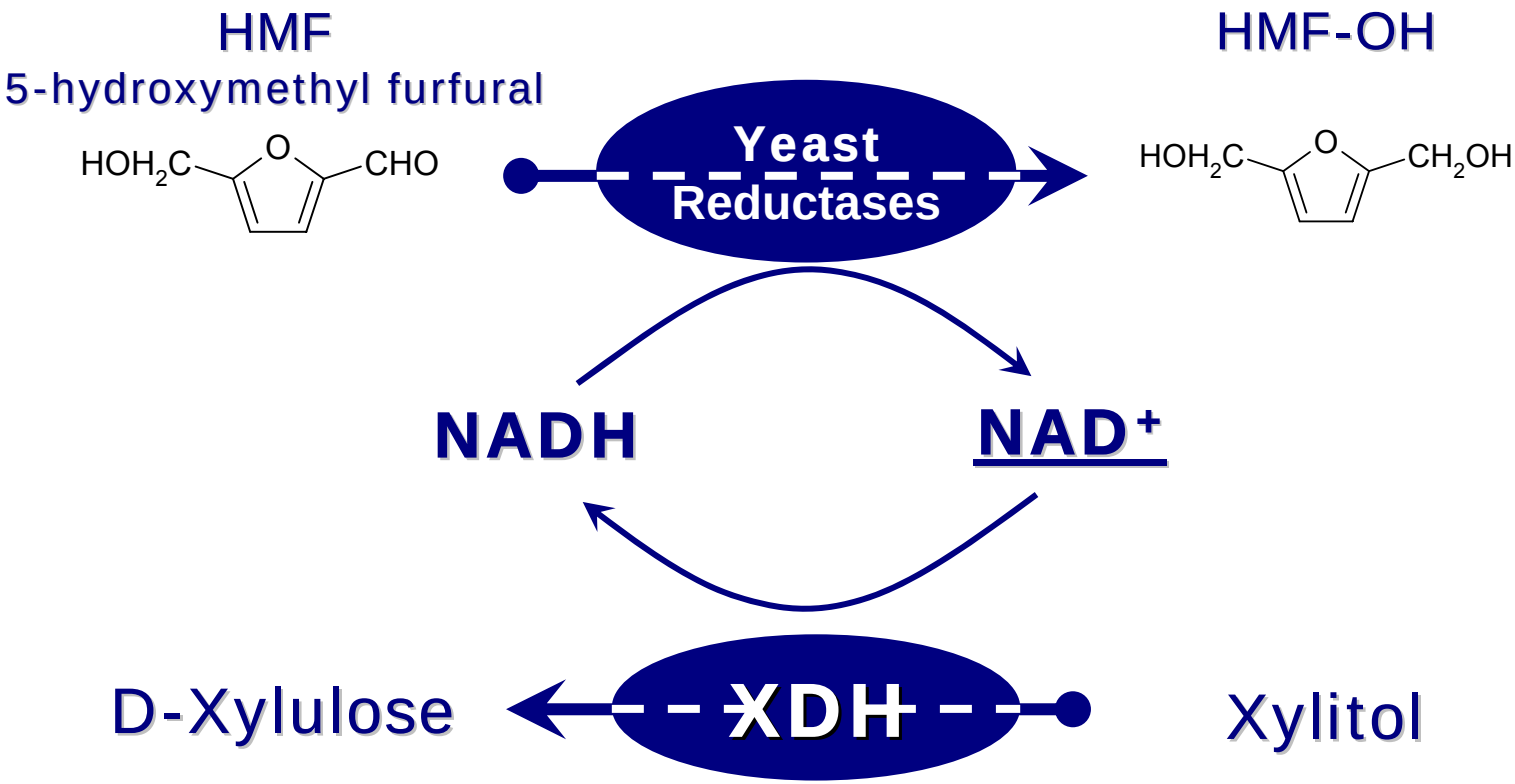
Lower Xylitol Production May Result from:

Presence of electron acceptors

(Pretreatment by-products)



- Wahlbom and Hahn-Hägerdal (2002) Biotechnol Bioeng 78:172-178.

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Arabinose Fermenting Strain / 1

JOHANN WOLFGANG GOETHE
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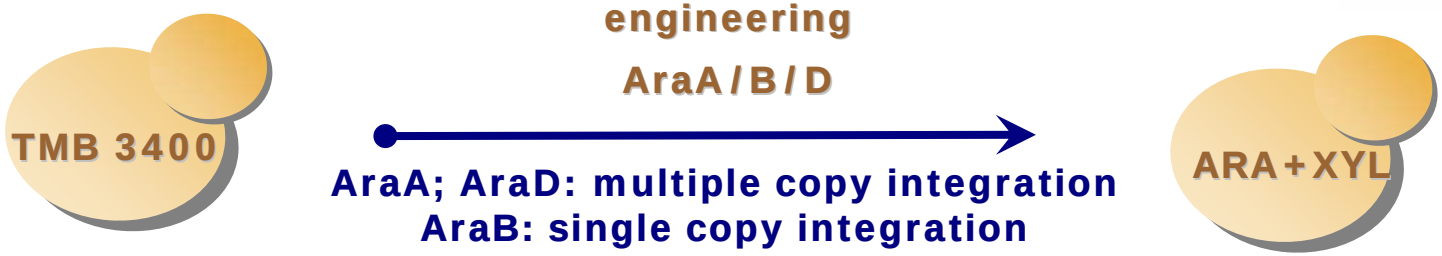
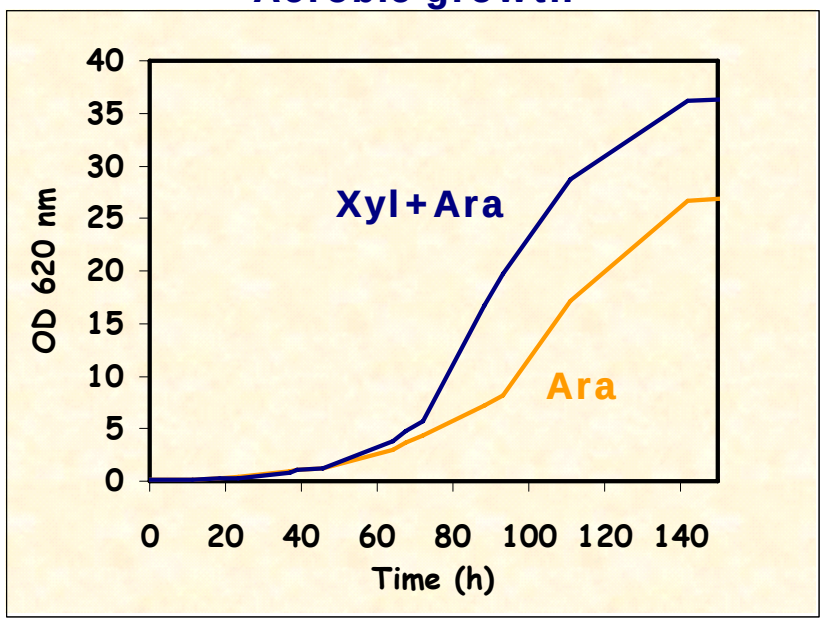
FRANKFURT AM MAIN



AraA; AraD: multiple copy integration
AraB: single copy integration

Aerobic growth



•Karhumaa et al. (2006) Microbial Cell Factories 5:18.

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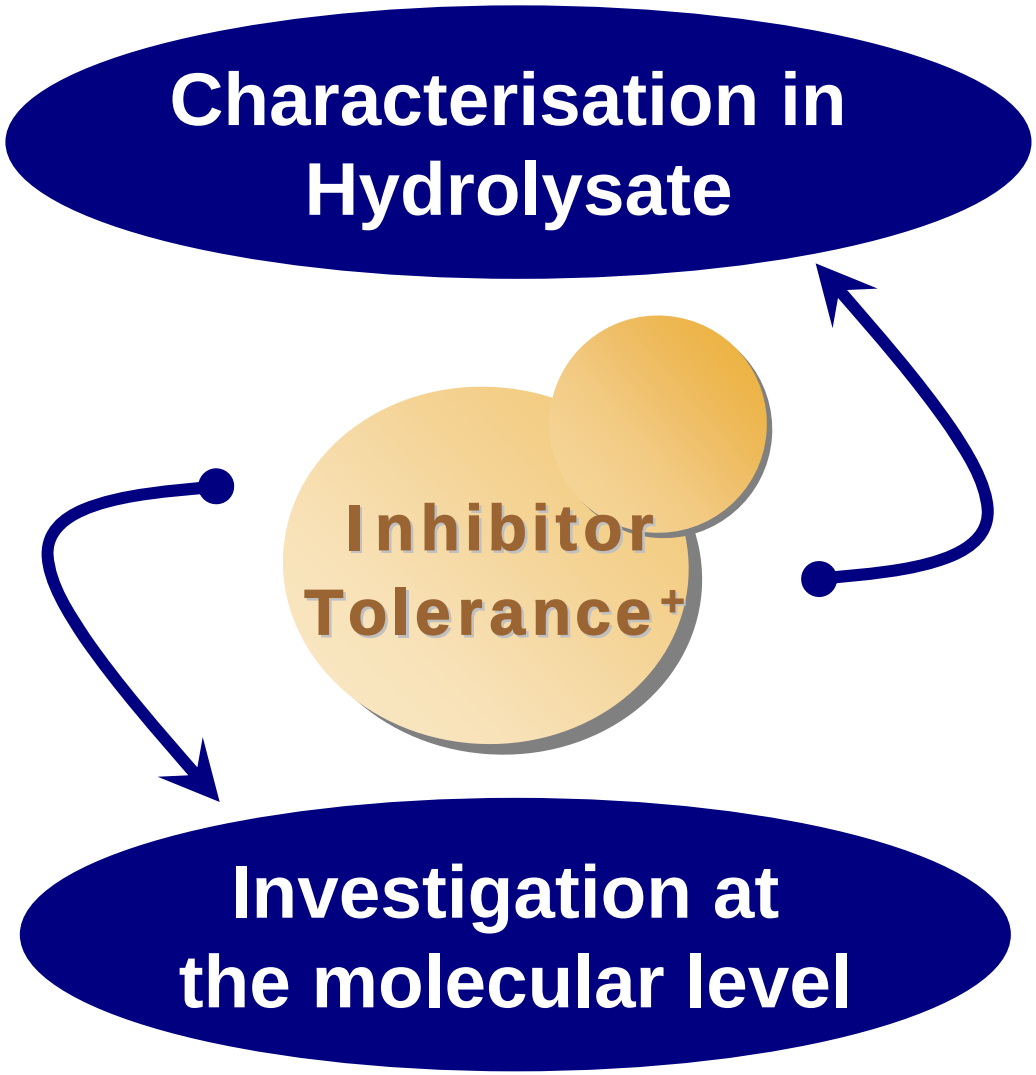
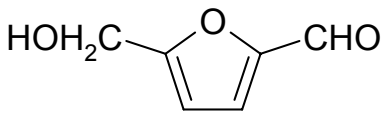
Proof of Principle



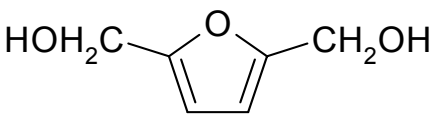
- Nilsson et al (2005) Appl Env Microb. 71:7866-71
- Petersson et al (2006) Yeast 23:455-64

Reduction Of Furans Improves Tolerance

HMF
5-hydroxymethyl furfural



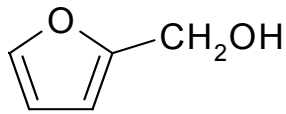
HMF-OH



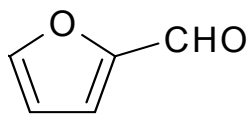
NAD(P)H

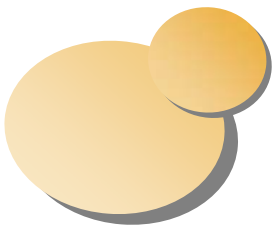
NAD(P)+

furfural

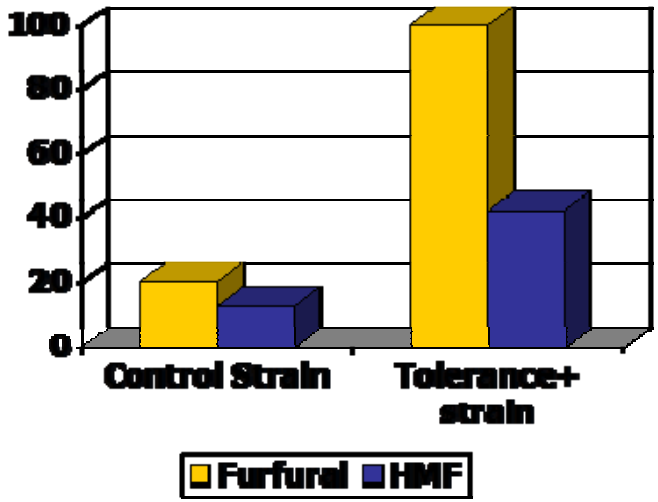


F-OH





Tolerance+ strain



- Nilsson et al (2005) Appl Env Microb. 71:7866-71
- Wahlbom and Hahn-Hägerdal (2002) Biotechnol Bioeng 78:172-78.

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Identification of HMF-reducing Enzymes / 1

NAD(P)H-dependent activity

1

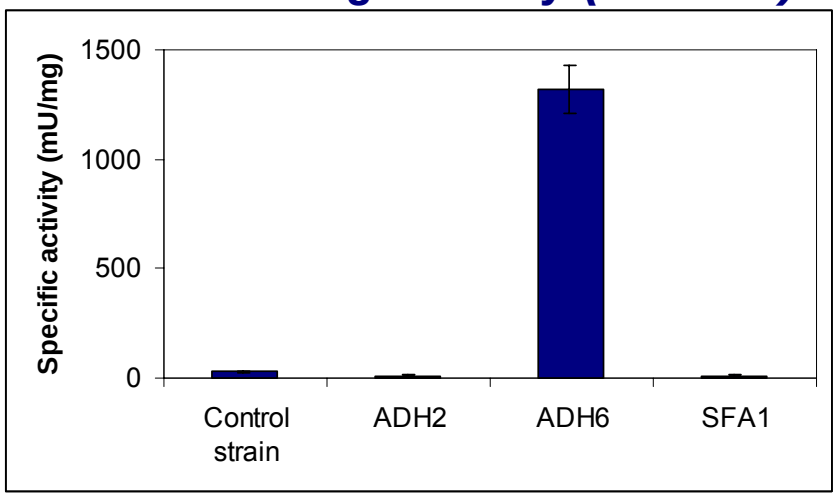
Microarray analysis

Non-Tolerant Vs Tolerant strain
Genes Induced?

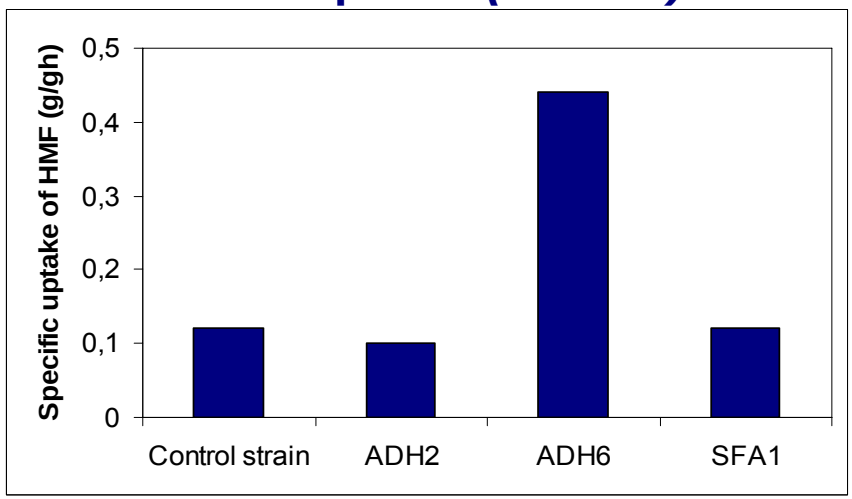
2

Over expression of best candidates

HMF-reducing activity *(in vitro)*



HMF Uptake *(in vivo)*



ADH6

•Petersson et al (2006) Yeast 23:455-64

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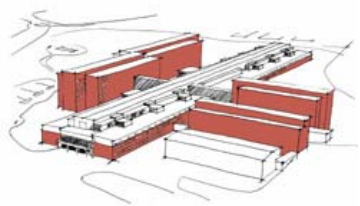

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