Evaluation of *S. cerevisiae* promoters during growth on xylose

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Motivation

- Identify a promoter that is strongly induced during growth on xylose
- Increase production of recombinant enzymes needed during the degradation of lignocelluloses
- Recombinant enzymes such as xylanases, beta-glucosidase, cellulases etc
Introduction

- *S. cerevisiae* has been used for years in the production of recombinant proteins
- *S. cerevisiae* is a widely used organism
  - fast cell growth
  - tolerate high ethanol concentration
  - tolerate wide spectrum of inhibitors
  - well-characterised physiology & genetics
  - GRAS status
Introduction

- Glucose is the most abundant sugar in nature (cellulose)
- S. cerevisiae is a Crabtree positive yeast
- It produces ethanol on glucose under aerobic conditions (little biomass)

- High expression level of recombinant protein is linked to the amount of biomass obtained during fermentation (Ferndahl et al., 2010)
Introduction

- Xylose is the second most abundant sugar in nature and considered a waste in most industries

- S. cerevisiae cannot utilise xylose as a carbon source

- S. cerevisiae engineered to grow on xylose has been reported to produce more biomass on xylose

- Xylose is the more suitable carbon source in recombinant protein production
Introduction

- **Promoter**
  - ENO1, ENO2, YG100, PGK1, ADH2, GPD3
- **Reporter gene**
  - Xylanase gene
- ** Terminator**
  - ENO1, ENO2, YG100, PGK1, ADH2, GPD3
- **Episomal selectable marker**
  - URA3
Engineering *S. cerevisiae*(Y294)

**Bacteria**

- D-xylose
  - Xylose isomerase
- D-xylulose
  - Xylulokinase
  - ATP
  - D-xylulose-5-P

**Fungi**

- D-xylose
  - Xylose reductase
  - NAD(P)H
  - NAD(P)+
- D-xylitol
  - Xylitol dehydrogenase
  - NAD+
  - NADH
- D-xylulose
  - Xylulokinase
  - ATP
  - D-xylulose-5-P
- D-xylulose-5-P

**Gene and Strain Information**

- sXI gene (*B. thetaiotaomicron*)
- *XYL3 (P. stipitis)*
- GRE3
Figure 1. pPGK1 used as base for the construction of all episomal plasmids.
Results and Discussion

Figure 2. Engineered *S. cerevisiae* on xylose and SC –URA3 with glucose.
Results and Discussion

Figure 3. Growth curve of the wild type and engineered yeast on glucose and on xylose
Results and Discussion

- Transformants spotted on RBB-xylan plates with different carbon source

Figure 4. The RBB-xylan plates used to confirm xylanase activity
Results and Discussion

- DNS ASSAY was done to determine the xylanase activity following the method by (Bailey et al, 1992)

Figure 5. Determination of the amount of xylanase enzyme produced using DNS assay
Results and Discussion

Figure 6. Determination of the amount of xylanase enzyme produced using DNS assay
Construction of *fur1::LEU2* strains

De novo synthesis

Glutamine

Orotidine 5'-P

Uridine 5'-P

*URA3*

Cytidine 5'-PPP

DNA

Kern *et al.*, 1990
Construction of \textit{fur1::LEU2} strains

Yeast chr 8R

\textbf{pUC plasmid}

\textit{FUR1} gene disruption

\textbf{pDF1}

\textit{fur1'} \hspace{1cm} \textit{LEU2} \hspace{1cm} 'fur1'

\textbf{FUR1 gene replacement}

\textbf{Yeast chr 8R}

\textbf{FUR1}

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1.33 \\
3.27
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FUTURE WORK

- Evaluation of episomal promoters during growth on xylose
- Determine metabolic burden during growth on xylose in the bioreactor
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Fur1 Disruption

- *fur1::LEU2* allele was isolated as 3.27kb from pDF1 plasmid
- NcoI-NsiI restriction enzymes
- Disrupted using the gene replacement method
Introduction

(Aristidou and Pentila, 2000)
Transformation of integrated plasmids

- Transformation in *S. cerevisiae* S228c strain
- Transformants selected on geneticin (G418)
Integrating expression cassettes

- *PGK1p* and *PGK1t* isolated from plasmid by restriction digest
- G418 isolated from plasmid by PCR
- 40bp *URA3* overhangs added to expression cassette (target cassette to *URA3*-locus)
- Expression cassette cloned into pUC19
Rapid genomic DNA isolation, “Bust and Grab” method
Construction of the strain that grows on xylose

- S. cerevisiae Y294 (Matα leu 2-3, 112 ura 3-52, his 3, trp1-289)

- Transformation with pMJM121 with Synthetic codon optimised xylose isomerase
  - B. thetaiotaomicron XI
  - Selective marker (zeocin)

- Disruption cassette (gre3::Xyl3Hygromycin) was used to knock out GRE3 gene
Figure 2: (A) Agarose gel electrophoresis of the PCR with XYN2 primers (B) Gel electrophoresis of PCR with promoter specific primer and XYN2 right primer.