Development of yeast strains for production of second-generation bioethanol and bio-based chemicals

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12 March 2019

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2G bioethanol and bio-chemicals production

Waste streams

Forest residues

Recycled paper

Rice straw

Empty fruit bunches

Bagasse

Corn cob & stover

Wheat straw

+ many other potential feedstocks

Bioenergy crops
1G and 2G bioethanol production

1G YEAST
- 1G Bioethanol

Sugar

2G YEAST
- 2G Bioethanol

Biomass

Main challenges
- Efficient xylose fermentation
- High inhibitor tolerance
Engineering of *S. cerevisiae* for xylose fermentation

**Two pathways**

- Xylose reductase (fungal pathway): redox problem → xylitol accumulation
- Xylose isomerase (bacterial pathway) → difficult expression in yeast

**Xylose isomerase expression in yeast**

*Eubacterium species*: VIB/GlobalYeast (IP)

- Proprietary xylose isomerase
- Many gene copies → high xylose isomerase activity
- Few other xylose isomerases are active in yeast → very tight IP space → small number of commercial proprietary 2G bioethanol strains
- Proprietary 2G yeast: bottleneck → 2G bioethanol + 2G bio-based chemicals
Engineering of *S. cerevisiae* for high inhibitor tolerance

Cheaper pretreatment methodologies → higher inhibitor levels → more robust yeast → cheaper process

- Acetic acid
- Furfural and hydroxymethylfurfural

High toxicity

- Reductases
- Export pumps

Corresponding alcohols
Low toxicity

Medium

Improvement by targeted and random engineering:
*(site-directed) mutagenesis, evolutionary adaptation, genome shuffling, whole-genome transformation*
Polygenic analysis platform for complex traits: pooled-segregant whole-genome sequence analysis

2 parent strains → Pooled → Extraction of genomic DNA → Whole-genome sequence analysis (Illumina) → QTL mapping (Quantitative Trait Locus)

Screen of *S. cerevisiae* strain collection

Diploid strain with superior trait

Industrial diploid strain with inferior trait

F1 superior haploids min. ± 30 segr.

F1 haploid segregants

superior haploid  x  inferior haploid

diploid

sporulation
Acetic acid tolerance of fermentation

**Known:**
Haa1: transcription factor involved in acetic acid tolerance

**New:**
Cup2: homolog of Haa1
Dot5
Glo1
Vma7

**F1 segregants**

**F7 segregants**

HAA1*: unique mutation in acetic acid tolerant strain
Insertion of G → A mutation in *HAA1* (2 alleles) of T18

- T18
- **T18 HAA1***

Predictable improvement of stress tolerance
Industrial strains with high xylose fermentation capacity

Further improvement by evolutionary adaptation, genome shuffling, targeted genetic engineering with superior alleles, whole-genome transformation → steady improvement of performance

Goal for commercial E2G production
> 80% of sugar in 48 h with 1 g DW yeast/L and > 5% (v/v) ethanol titer
2G bioethanol and bio-chemicals production

Other major challenges

• Pretreatment of the biomass
  → robustness of machinery

• Enzymatic hydrolysis
  → ±20% of the cost of the ethanol

E2G yeast with secreted enzymes
  - reduce enzyme requirement
  - Holy grail: ‘Consolidated bioprocessing’
    yeast: enzymatic hydrolysis + fermentation
Types of enzymes required

Cellulolytic enzymes:
- β-glucosidase (BGL)
- Endoglucanase (EG)
- Cellobiohydrolase I (CBH I)
- Cellobiohydrolase II (MCBH II)

Hemicellulolytic enzymes:
- β-xylosidase (β-XYL)
- Xylanase (XYN)
Secreted β-glucosidase (SfBGL1) supports cellobiose fermentation

HPLC analysis of components during fermentation by MD4 and AC1 at 35°C
YP + 5% Glucose + 4% Xylose + 1% Cellobiose

→ No negative effect of β-glucosidase expression on glucose or xylose fermentation

Expression of β-glucosidase results in efficient cellobiose utilization
AC2 strain: secreted β-xylosidase (AnXlnD) and xylanase (TrXyn2) support xylan fermentation

4% Xylan + yeast extract/peptone

AC2: Xylan is consumed

Parent strains do not degrade xylan

MD4: parent
AC1: MD4-TrBGL1
AC2: MD4-TrBGL1-AnXlnD-TrXyn2

50 mL YP + Xn4%
35°C
120rpm
Production of muconic acid with glucose + xylose mixture

Muconic Acid pathway

Sugars $\rightarrow$ PCA $\rightarrow$ Catechol $\rightarrow$ Muconic acid

Glucose (2.2%) + Xylose (2%)

20 mL CSM-FWY + Glucose (2.2%) + Xylose (2%) + EtOH (1%) + citrate buffer (0.1M, pH 5.5) in shaking flasks (300 ml), 250 RPM at 30°C. Start OD$_{660}$: 1
Conclusions

2G bioethanol and bio-chemicals production

- Efficient industrial yeast strains for second-generation bioethanol production available: xylose utilization + high inhibitor tolerance
- Can still be improved further for better performance in undetoxified lignocellulose hydrolysates → cheaper pretreatment technologies
- Strong platform for secreted enzyme expression
  Reduction of enzyme load/cost - Consolidated BioProcessing
- Strong platform for cell factory strains to produce bio-based chemicals with lignocellulosic biomass
Thank you for your attention