Background

Towards a fossil-free transport sector

Saccharomyces cerevisiae as a bioethanol production host

Engineered S. cerevisiae is able to ferment glucose and xylose for ethanol production.

- Lignocellulosics hydrolysates contains a broad range of inhibitors for yeast.
- Inhibitor composition depends on the type of raw material and pretreatment.

An efficient genome editing strategy for engineering industrial yeast strains is needed.

PHASE 1 – Genetic construction

Expression downregulation driven by:

Expression upregulation driven by:

Vectors expressing dCas9+repressor/activator constructs have been constructed using the Moclo Yeast Toolkit.

CRISPR will be used to establish permanently gene modifications successfully tested with CRISPRi.

In order to analyse a large number of strains a rapid, high-throughput system to characterise the performance of new strains in lignocellulosic hydrolysates is essential.

Various culture conditions and parameters have been tested in the Biorector® microbioreactor platform:

- Lignocellulose hydrolysate concentration (v/v)
- Size of inoculum
- Addition of salt to the medium
- Preculture adaptation
- Aerobic / anaerobic conditions

PHASE 2 – Establishment of a high-throughput screening platform

In order to develop inhibitor tolerant strains, the expression of genes involved in tolerance will be systematically altered using CRISPR interference (CRISPRi). This methodology uses a catalytically inactive, dead Cas9 (dCas9).

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MATERIALS AND METHODS

- • Culture volume: 1 mL
- • Polypeptide industrial strain
- • Media: Wheat straw hydrolysate with YPD supplementation (Final sugar concentration: 60 g/L glucose + 30 g/L xylose), pH 5.5
- • Adaptation in anaerobic conditions was performed by addition of different lignocellulosic hydrolysate concentration (0%, 14% and 40%) in the YPD preculture media, with an overnight incubation

Conclusions

- An efficient platform for high-throughput screening of yeast directly in lignocellulosic hydrolysates was established
- Preliminary results showed a reliable correlation in terms of growth with shake flask cultures

References