**Background**

Towards a fossil-free transport sector

Saccharomyces cerevisiae as a bioethanol production host

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).

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

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

**PHASE 1 – Genetic construction**

Expression downregulation driven by:

- Targeting dCas9 to a promoter can lead to a repression in transcription by directly blocking RNA polymerase binding.

Expression upregulation driven by:

- Co-expression of dCas9 + VP64 (mammalian transcriptional repressor) has been shown to improve CRISPRi based transcriptional repression.

**PHASE 2 – Establishment of a high-throughput screening platform**

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

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

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

**MATERIALS AND METHODS**

- Culture volume: 3 ml
- Polyploid 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 (5%, 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**