**Background**

Towards a fossil-free transport sector

Saccharomyces cerevisiae as a bioethanol production host

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

**OBJECTIVE**

To develop an efficient design-build-test-learn cycle for creating robust production strains using CRISPRi based systems as a tool for studying the impact of genetic alterations.

**PHASE 1 – Genetic construction**

Expression downregulation driven by:

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

Expression upregulation driven by:

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

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

1) AEROBIC CONDITIONS + SALT ADDITION

Salt addition improve growth in 70% and 80% hydrolysate cultures. 90% cultures were not able to grow in any condition tested.

2) AEROBIC CONDITIONS + ADAPTATION

Pre-culture adaptation in cultures with higher degree of hydrolysate and lower initial OD shortened lag phase (from 80% to 20%).

3) ANAEROBIC CONDITIONS + ADAPTATION LEVELS

Under anaerobic conditions, preculture adaptation at high hydrolysate concentration had a high impact in growth performance. Furthermore, HPC measurements showed a drastic increase in ethanol production. Concrete, about a 10-fold increase in ethanol production was measured for the strains adapted in 40% hydrolysates compared with non-adapted strains.

**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**