YEAST IMPROVEMENT BY METABOLIC AND EVOLUTIONARY ENGINEERING

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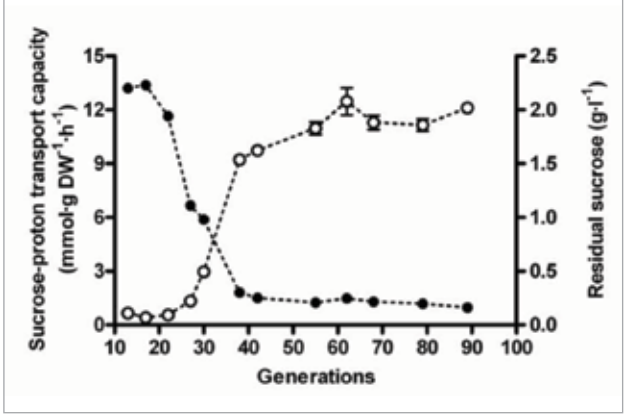

Figure 1. Ethanol yield improvement of Saccharomyces cerevisiae *by evolutionary engineering. Here the improvement of sucrose active transport capacity is depicted. For further details, see the article published in* Metabolic Engineering. *(DOI 10.1016/j.ymben.2011.09.005)*

Using evolutionary and metabolic engineering, either individually or in combination, our global aim is to improve yeast for its use in biorefineries. First-generation bioethanol production in Brazil, in which sucrose from sugarcane is converted into ethanol by *Saccharomyces cerevisiae* with high yields, was chosen as a first case study. We started with a yeast strain which had already been metabolically engineered to hydrolyze sucrose exclusively in the intracellular environment (Prof. Boris Stambuk, Federal University of Santa Catarina, Brazil). Without the capacity of hydrolysing sucrose extracellularly, this strain is obliged to transport this sugar actively into the cells via symport, which causes ATP expenditure to extrude protons from the cells back to the culture medium, in order to avoid acidification of the citoplasm. This energy drain forces the cells to produce more ATP, which, under anaerobiosis, is basically coupled to ethanol formation. As a first aim, we will characterize this strain quantitatively, in order to demonstrate that it converts sucrose into ethanol with a higher yield, when compared to strains with normal invertase activity. Subsequently, this strain will be subjected to evolutionary engineering, in order to increase the ethanol yield on sucrose even further. Future studies will focus on the metabolic and evolutionary engineering of industrial yeast strains, with the aim of improving tolerance towards the most relavant stressors present in the industrial bioethanol production, such as high ethanol concentration, high temperature, high osmolarity, and acid environment. The improvement of second-generation biofuels will also be tackled, by investigating tolerance of yeast towards common inhibitors released during hydrolysis of lignocellulosic materials, such as acetate, furfural, and hydroxymethylfurfural.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

- Yeast invertase was relocated to the cytosol by removal of N-terminal signal peptide
- Improved sucrose uptake kinetics obtained by evolutionary engineering in chemostats
- Strain evolved for intracellular sucrose metabolism shows deregulated MAL genes
- AGT1-encoded proton symporter was involved in sucrose uptake by evolved yeast strain
- The engineered yeast strain shows an 11% increase of the ethanol yield on sucrose

MAIN PUBLICATIONS

Basso TO, Kok S, Dario M, do Espirito-Santo JCA, Müller G, Schlölg PS, Silva CP, Tonso A, Daran JM, Gombert AK, van Maris AJA, Pronk JT, Stambuk BU. 2011. Engineering topology and kinetics of sucrose metabolism in *Saccharomyces cerevisiae* for improved ethanol yield. *Metabolic Engineering*. DOI:10.1016/j.ymben.2011.09.005.

Basso TO, Dario MG, Tonso A, Stambuk BU, Gombert AK. 2010. Insufficient uracil supply in fully aerobic chemostat cultures of *Saccharomyces cerevisiae* leads to respiro-fermentative metabolism and double nutrient-limitation. *Biotechnology Letters*. **32(7)**: 973-977.

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