YEAST IMPROVEMENT BY METABOLIC AND EVOLUTIONARY ENGINEERING

Aldo TONSO
Polytechnic School / University of São Paulo (USP)

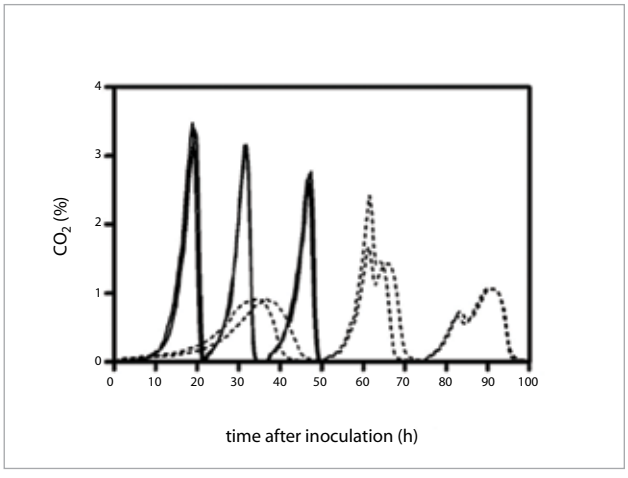

Figure 1. CO_2 profiles during evolution of Saccharomyces cerevisiae *in Sequential Batch Reactors*

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

The *Saccharomyces cerevisiae* strain that transports sucrose actively into the cells and hydrolyses it intracellularly was quantitatively characterized using a combination of chemostat cultivations and defined culture media. We were able to show that it delivers a 5% higher ethanol yield on sucrose, when compared to the reference strain (with normal extracellular invertase activity). Having achieved this first milestone, this strain is now being subjected to evolutionary engineering, both using chemostat cultivations and sequential batch reactors, aiming at improving the affinity of the active sucrose transport system. This, at least in theory, will increase the ethanol yield even further.

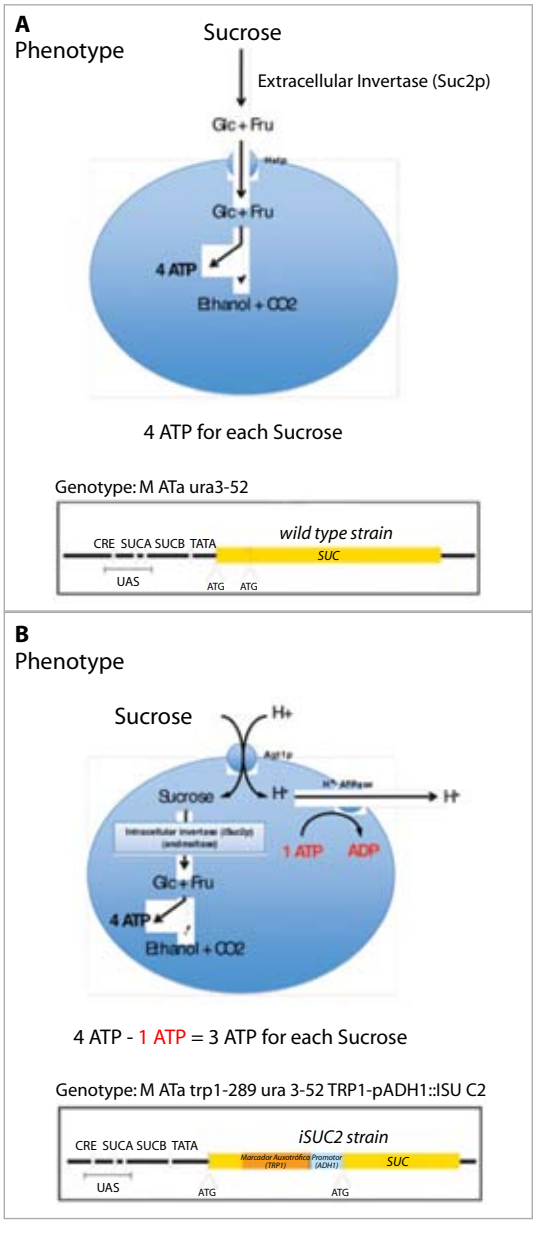

Figure 2. Sucrose uptake and metabolism, as well as the respective genotypes, in the reference strain (A) and in the modified strain (iSUC2) (B)

MAIN PUBLICATIONS

Basso TO, Dário 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*. (*accepted for publication*)

Basso TO, Dário MG, Tonso A, Stambuk BU, Gombert AK. 2009. Estudo da limitação por uracila em cultivos contínuos de *Saccharomyces cerevisiae*. Presented orally in XVII Simpósio Nacional de Bioprocessos (SINAFERM), August 2-5, 2009, Natal–RN, Brazil. Published in the annals of the symposium.

Basso TO, Dário MG, Espírito-Santo JC, Schlogl P, Silva CP, Tonso A, Gombert AK, Stambuk BU. 2009. Cellular relocalization of disaccharide metabolism in yeast for improved production of biofuels. Presented as poster in 27th International Specialized Symposium on Yeasts (ISSY), Yeasts for Health and Biotechnologies, August 26-29, 2009, Paris, France. Published in the Abstracts Book of the conference.

Aldo Tonso

Escola Politécnica
Universidade de São Paulo (USP)
Departamento de Engenharia Química
Av. Prof. Luciano Gualberto, travessa 3, nº 380
CEP 05508-970 – São Paulo, SP – Brasil

+55-11-3091-2283
atonso@usp.br