

# Transcriptome analysis of xylose and glucose co-fermentation by industrial engineered yeast for second generation bioethanol

Sheila Tiemi Nagamatsu<sup>1</sup>, Luige Armando Llerena Calderon<sup>2</sup>, Lucas Salera Parreiras<sup>3</sup>, Bruna Tatsue Grichowski Nakagawa<sup>2</sup>, Angelica Martins Gomes<sup>4</sup>, Gonçalo Amarante Guimarães Pereira<sup>5</sup>, Marcelo Falsarella Carazzolle<sup>6</sup>

*1 BRAZILIAN BIOETHANOL SCIENCE AND TECHNOLOGY LABORATORY),  
BRAZILIAN CENTER FOR RESEARCH IN ENERGY AND MATERIALS),  
BIOLOGY INSTITUTE - UNICAMP*

*2 BIOLOGY INSTITUTE - UNICAMP*

*3 BRAZILIAN BIOETHANOL SCIENCE AND TECHNOLOGY LABORATORY,  
BRAZILIAN CENTER FOR RESEARCH IN ENERGY AND MATERIALS, BIOLOGY  
INSTITUTE - UNICAMP*

*4 BRAZILIAN BIOETHANOL SCIENCE AND TECHNOLOGY LABORATORY,  
BRAZILIAN CENTER FOR RESEARCH IN ENERGY AND MATERIALS*

*5 BRAZILIAN BIOETHANOL SCIENCE AND TECHNOLOGY LABORATORY,  
BRAZILIAN CENTER FOR RESEARCH IN ENERGY AND MATERIALS, BIOLOGY  
INSTITUTE - UNICAMP*

*6 BIOLOGY INSTITUTE - UNICAMP, NATIONAL CENTER FOR HIGH  
PERFORMANCE COMPUTING/UNICAMP*

## Abstract

Second-generation (2G) ethanol is a promising technology which can increase production and reduce costs related to first-generation (1G) ethanol. Both process differ basically in the raw material for fermentative step, while 1G is based on fermentable sugars (glucose, fructose and sucrose) from sugarcane, 2G is based on deconstruction of biomass releasing fermentable sugars. This process generates non-fermentable sugars (mainly xylose) and inhibitors of yeast growth (acetic acid, furfural and HMF). To overcome these problems and increase yeast productivity in 2G ethanol production is essential select robustness microorganisms and perform genetic modifications to allow xylose consumption through insertion of endogenous xylose pathway genes, as xylose isomerase. Furthermore, approaches as evolutionary engineering can be used to improve some characteristics. Our previous work performed a comparative genomic analysis in genetically modified yeast followed by evolutionary adaptation for xylose consumption showing several point mutations and an increase of xylose isomerase genes during the evolution process. In this work we are showing a transcriptomic analysis from one parental (A) and two evolved strains studied before in xylose and glucose co-fermentation: one haploid strain (C) from intermediate round of evolution and the other, a diploid strain (E), from the final round of evolution. It was sequenced in biological duplicate and three different fermentation points for each strain, the first one with high glucose concentration and inhibition of xylose

consumption by catabolic repression (Glu), the second, low glucose concentration and high xylose concentration with consumption of both carbon sources (Glu-Xyl), and the last one, only xylose consumption (Xyl). The analysis of differentially expressed genes (DEG) was performed by comparison Parental versus C and E strains in each fermentation time independently and considering time-series correlation. For the Parental versus C, a total of 1.951, 2118, and 3.945 DEG were identified in Glu, Glu-Xyl and Xyl, respectively. For the Parental versus E, a total of 1.706, 937, and 3.893 DEG were identified in Glu, Glu-Xyl and Xyl, respectively. While, in a time-series analysis was detected 3.844 and 3.876 DEG for Parental versus C and Parental versus E comparisons, respectively. These results and its correlation with previous genome analysis can contribute to a better understanding of metabolic bottleneck of xylose consumption in industrial yeast, as it can show how genome variations are related to the expression profile.

Funding: FAPESP, CAPES