XXIII SINAFERM
XIV SHEB
XIV ENZITEC
Búzios, Rio de Janeiro, Brasil
28 a 31 de agosto de 2022
XXIII Simpósio Nacional de Bioprocessos
XIV Simpósio de Hidrólise Enzimática de Biomassas
XIV Seminário Brasileiro de Tecnologia Enzimática

METABOLIC ENGINEERING OF *SACHAROMYCES CEREVISIAE* FOR SECOND-GENERATION ETHANOL PRODUCTION FROM XYLO-OLYGOSACCHARIDES

Dielle P. Procópio[1], Jaewon Lee[2], Jonghyeok Shin[2], Rosana Goldbeck[3], Yong-Su Jin[2] and Thiago O. Basso [2]

[1] *Universidade de São Paulo, Departamento de Engenharia Química*

[2] *Universidade de Illinois, Departamento de Ciência dos Alimentos e Nutrição Humana*

[3] *Universidade Estadual de Campinas, Departamento de Engenharia de Alimentos*

E-mail: diellepierotti@gmail.com, thiagobasso@gmail.com

1. INTRODUCTION

Xylo-oligosaccharides (XOS)-consuming *Saccharomyces cerevisiae* strain can represent an essential step to reach a more cost-effective second-generation ethanol production. Xylan is one of the most abundant polysaccharide chains present in lignocellulosic residues (Stovicek et al., 2015). Engineered *S. cerevisiae* expressing NADPH-linked xylose reductase (XR) and NAD^+-linked xylitol dehydrogenase (XDH) for xylose assimilation, as well NADH-linked acetylating acetaldehyde dehydrogenase (AADH) and acetyl-CoA synthetase (ACS) for an NADH-dependent acetate reduction pathway (Zhang et al., 2016) was used as the host for expressing two β-xylosidases, GH43-2 and GH43-7, and one transporter, CDT-2, from *Neurospora crassa,* yielding the engineered strain, SR8A6S3-CDT_2-$GH43_{2/7}$. The engineered strain was able to produce ethanol through simultaneous co-utilization of XOS, xylose, and acetate. When a hemicellulosic hydrolysate was used, the yielded yeast strain produced 60% more ethanol and 12% less xylitol than the control strain without the XOS consumption pathway.

2. RESULTS

Rational metabolic engineering strategies were performed to introduce XOS metabolism in the *S. cerevisiae* SR8A6S3 strain. A high expression CDT-2 cassette was introduced into the *SOR1* locus, and a high expression cassette carrying both two enzymes, GH43-2, and GH43-7, was introduced into the *GRE3* locus through the locus-specific integration tool, CAS-9-based system (Stovicek et al., 2015). To assess the impact of the additional genetic modifications, we cultivated both SR8A6S3-CDT_2-$GH43_{2/7}$ and SR8A6S3 strains in YPXAH (YP medium containing hemicellulosic hydrolysate, xylose, and acetate) with an initial OD_{600} of 1 (Fig. 1).

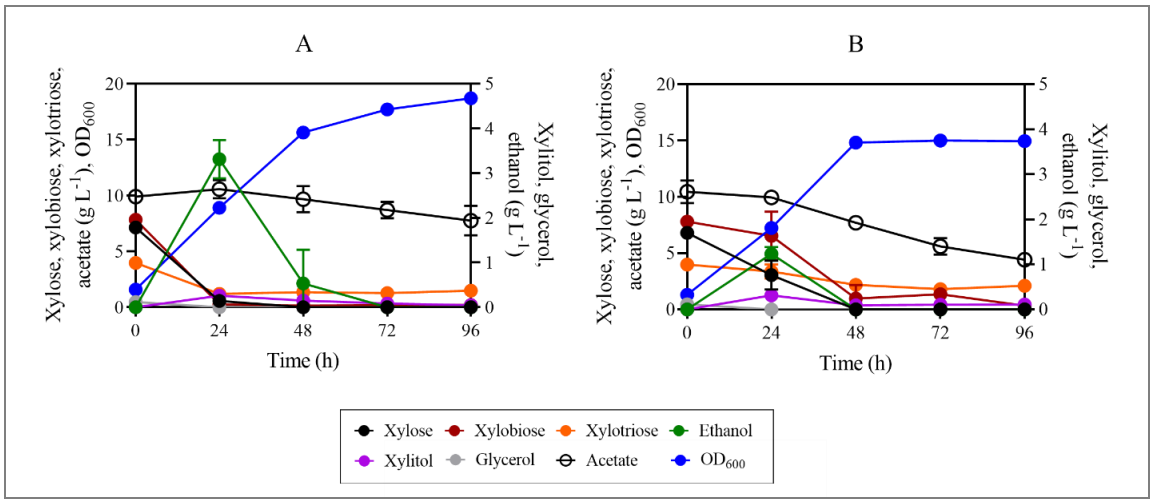

Fig. 1. Fermentation profiles of SR8A6S3-CDT$_2$-GH43$_{2/7}$ (A) and SR8A6S3 (B) during batch cultivation in YPXAH (YP medium containing xylose, acetate, and hydrolyzed hemicellulose). Cultivations were performed at 30 °C and 100 rpm with an initial OD$_{600}$ of 1. Data are presented as mean values and standard deviation of two independent biological replicates.

High fermentative capacity was observed for SR8A6S3-CDT$_2$-GH43$_{2/7}$, which produced more ethanol and achieved higher cell density than the control cultivation. The ethanol yield of the SR8A6S3-CDT$_2$-GH43$_{2/7}$ strain increased substantially as compared to the SR8A6S3 strain, from 0.33 ± 0.08 to 0.50 ± 0.03. XOS combined with xylose could improve bioethanol production from hydrolyzed hemicellulose since they represent a large source of renewable material which is available at low cost (Gírio et al., 2010).

3. CONCLUSION

The dataset presents indicate important physiological changes taken from the integration of the XOS-consumption pathway into two xylitol-production-related genes.

4. REFERENCES

Gírio, F. M., Fonseca, C., Carvalheiro, F., Duarte, L. C., Marques, S., and Bogel-Łukasik, R. (2010). Hemicelluloses for fuel ethanol: A review. *Bioresour. Technol.* 101, 4775–4800. doi:10.1016/j.biortech.2010.01.088.

Stovicek, V., Borodina, I., and Forster, J. (2015). CRISPR–Cas system enables fast and simple genome editing of industrial Saccharomyces cerevisiae strains. *Metab. Eng. Commun.* 2, 13–22. doi:10.1016/J.METENO.2015.03.001.

Zhang, G. C., Kong, I. I., Wei, N., Peng, D., Turner, T. L., Sung, B. H., et al. (2016). Optimization of an acetate reduction pathway for producing cellulosic ethanol by engineered yeast. *Biotechnol. Bioeng.* 113, 2587–2596. doi:10.1002/bit.26021.

5. ACKNOWLEDGMENTS

Acknowledgment for FAPESP (2018/01759-4 2019/18075-3) for the financial support.