

Analysis of *Komagataella phaffii* metabolites using mass spectrometry

Miria de Almeida Souza^{1,2}, Clara Vida Galvão Corrêa Carneiro¹, João Ricardo Moreira de Almeida¹ & Patrícia Verardi Abdelnur^{1,2}

¹Brazilian Agricultural Research Corporation

²Federal University of Goiás.

miria17a.souza@gmail.com & patricia.abdelnur@embrapa.br

Keywords: metabolomics, mass spectrometry, building blocks, xylose, fermentation

Introduction: The use of fermentation of lignocellulosic biomass by *Komagataella phaffii* produces biomolecules through the conversion of xylose, as ethylene glycol building block for the production of molecules in the chemical and pharmaceutical industry^[1, 2]. The synthetic way of producing ethylene glycol from xylose, being identified and characterized by metabolomics-based mass spectrometry^[2,3].

Methods: After the growth of *K. phaffii*, aliquots of the supernatant were centrifuged and stored at -20°C until analysis. Metabolites in the culture supernatant were identified using ultrahigh performance liquid chromatography–electrospray ionization tandem mass spectrometry (UHPLC-ESI-qTOF-MS/MS), using a BEH Amide column. Of fermentation supernatants were diluted before injection into ultrafiltered water (Milli Q).

Results: Five ions were identified by UHPLC-ESI-qTOF-MS/MS: m/z 165, 0298; 147,0211; and 151,0526 (ESI(-)-MS) and m/z 189.0369 (ESI(+)-MS). The ions m/z 165, 0298; 147,0211; 151,0526 and 189.0369 were identified as Xylonic acid (C₅H₁₀O₆), 2-keto-3- deoxy-D-xylonic acid (C H O), 3,4-dihydroxybutyric acid (C H O), Xylitol (C H O) and Xylonic acid (C₅H₁₀O₆), respectively. These compounds are intermediaries of the d-xylose oxidative pathway (XOP) and can produce several valuable compounds, such as ethylene glycol from xylose, present in lignocellulosic biomass^[2].

Conclusions: It was possible to identify intermediate compounds in the synthesis of the d- xylose (XOP) oxidative pathway in *K. phaffii*. These compounds can favor the production of ethylene glycol, adding value to the production chain of biofuels, mainly pharmacochemicals. Studies are being carried out in order to quantify these compounds in various cultivation changes.

Acknowledgments: We thank CNPq, Embrapa and UFG for financial support.

References:

1. Francois, J. M., et al. (2020). *Biotechnol Biofuels*. DOI: 10.1186/s13068-020-01744-6.
2. Valdehuesa, K. N. G. et al. (2018). *Applied Microbiology and Biotechnology*. DOI: 10.1007/s00253-018-9186-z.
3. Campos, C. G., et al. (2019). *Methods Mol. Biol.* doi: 10.1007/978-1-4939-8757-3_8.