Exploring the *Cyberlindnera jadinii* transportome for the identification of novel carboxylate transporters

Maria Sousa-Silva[1,2], Daniel Vieira[1,2], David Ribas[1,2], Pedro Soares[1,2], Margarida Casal[1,2] & Isabel Soares-Silva[1,2]

[1] Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Portugal
[2] Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Considering the global problems of resource scarcity and environmental damage, new technologies based on renewable biological sources are needed as current model of natural resource exploitation is unsustainable. Novel strategies to boost bio-based production of organic acids are based on the expression of carboxylate transporters in microbial cell factories. In this work we have focused on the identification and characterization of novel carboxylate transporters in the *Cyberlindnera jadinii* yeast. The transportome of *C. jadinii* was analysed by two approaches. First, the *C. jadinii* homologs of the carboxylate transporters Jen1p (Major Facilitator Superfamily) and Ady2p (AceTr Family) were identified and expressed in *Saccharomyces cerevisiae*. The *S. cerevisiae* strain W303-1A *jen1*Δ *ady2*Δ, lacking carboxylate uptake capacity, was used for the heterologous expression. Genes were identified through sequence alignment and homology prediction. In a parallel bioinformatic approach, the proteome from *C. jadinii* NRRL-1542 was downloaded from NCBI database and explored using a pipeline developed together with the CBMA bioinformatic team. This tool was designed to retrieve data from a specific database: a) that contains a single representative genome/proteome on the species level; b) where multiple matches within a species directly reflect homologs within the same genome, and c) e-values from BLAST searches that are statistically more reliable. A set of genes were selected using this tool and expressed in the IMX1000 strain, which is deleted in 25 genes related to carboxylic acid transport [1]. GFP-fusions versions were used to determine protein expression and localization. Transport activity was determined through growth on different carbon sources and measurement of the uptake of several radiolabelled CAs. The full characterization of the Ady2 and Jen1 homologs as well as others candidate CAs transporters from *C. jadinii* is ongoing.

References

1. Mans, R., Hassing, E. J., Wijsman, M., Giezekamp, A., Pronk, J. T., Daran, J. M., and van Maris, A. J. (2017). *FEMS Yeast Research*, 17:8.

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