Characterization of *Cyberlindnera jadinii* carboxylate transporters by heterologous expression in *S. cerevisiae*

Sousa-Silva, M.[1,2], Ribas, D.[1,2], Casal, M.[1,2], Soares-Silva, I.[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

Concerning the global problems of resource scarcity and environmental damage, new technologies based on renewable biological sources are needed as the current model of natural resource exploitation is unsustainable. Cell factories with specific genetic and physiological traits, namely suitable protein transporters, may be key players in the bio-based production of organic acids, as an alternative approach to the production of these chemical building-blocks from petrochemical derivatives.

The present work focused on the identification and characterization of novel organic acid transporters from the *Cyberlindnera jadinii* yeast. *C. jadinii* homologues of the monocarboxylate proton symporter Jen1p (Major Facilitator Superfamily) and the acetate permease Ady2p (AceTr Family) were identified and expressed in *S. cerevisiae*. The *S. cerevisiae* strain W303-1A *jen1*Δ *ady2*Δ, lacking carboxylate uptake capacity, was used as an expression host. Genes were identified through sequence alignment and homology prediction and cloned in the p416GPD vector, under the control of a GPD constitutive promoter. 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 CAs, namely [1-^{14}C] acetic acid, D,L-[U-^{14}C] lactic acid and [2,3-^{14}C] succinic acid. Molecular docking of these transporters was performed to unveil the amino acids that play a major role in the substrate binding of CAs tested.

In this study, 4 CjADY2 and 6 CjJEN1 homologs were identified and revealed to be functional carboxylate transporters in *S. cerevisiae*. Further studies are underway to fully characterize these ten new plasma membrane transporters.