

The *Cyberlindnera jadinii* carboxylate transporters Ady2 and Jen1 homologs are functional in *Saccharomyces cerevisiae*

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

¹CBMA – Centro de Biologia Molecular e Ambiental, Universidade do Minho, 4710-057 Braga, Portugal

In *Saccharomyces cerevisiae*, two permeases are responsible for the uptake of carboxylates (CA) at the plasma membrane, Jen1p a monocarboxylate proton symporter (Major Facilitator Superfamily) and Ady2p an acetate permease (AceTr Family). In *Cyberlindnera jadinii*, different uptake systems for CAs were functionally characterized however until now the genes encoding these transporters remain unidentified. In this work, CA transporter homolog genes from *C. jadinii* were identified and expressed in *S. cerevisiae*. The *S. cerevisiae* strain W303-1A *jen1Δ ady2Δ*, lacking carboxylate uptake capacity, was used to express *C. jadinii* ScJEN1 and ScADY2 homologs. 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 labelled CAs, namely D,L-[U-¹⁴C] lactic acid, [2,3-¹⁴C] succinic acid and [1-¹⁴C] acetic acid.

In *C. jadinii*, 6 genes homolog to ScJEN1 (Cjj23088, Cjj21966, Cjj22358, Cjj21989, Cjj21602, Cjj25129) and 4 genes homolog to ScADY2 (Cja24587, Cja20823, Cja20690, Cja20822) were identified. All proteins are being expressed to uncover their subcellular localization and the characterization of transporter specificity is currently underway.

In this work, we identified 6 CjJEN1 and 4 CjADY2 homologs that are functional carboxylate transporters in *S. cerevisiae*. All the CjJEN1 homologs are lactate transporters and CjADY2 homologs present different specificities. Further studies are underway to fully characterize these ten new plasma membrane transporters from *C. jadinii*.

References

Soares-Silva, I., et al. (2007). Mol Membr Biol. 24(5-6), 464-474.

Acknowledgments

Work supported by the strategic programme UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569) and PTDC/BIAMIC/5184/2014 funded by national funds through the FCT-IP and by the ERDF through the COMPETE 2020-POCI; the project EcoAgriFood (operação NORTE-01-0145-FEDER-000009), supported by NORTE-2020, under the PORTUGAL 2020 Partnership Agreement, through the ERDF and the TransAcids project with FCT reference PTDC/BIAMIC/5184/2014. MSS acknowledge the Norte2020 for the UMINHO/BD/25/2016 grant with the reference NORTE-08-5369-FSE-000060. DR acknowledges FCT for the SFRH/BD/96166/2013 PhD Grant.