Characterization of *Cyberlindnera jadinii* carboxylate permeases by heterologous expression in *Saccharomyces cerevisiae*

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Background: The wide applicability of organic acids for direct use as commodity chemicals and as polymer building blocks has evidenced their importance in diverse types of industries. 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).

Objectives: In *Cyberlindnera jadinii*, different uptake systems for CAs were functionally characterized however until now the genes encoding these transporters remain unidentified. In this study, CA transporter homolog genes from *C. jadinii* were identified and expressed in *S. cerevisiae*.

Methods: The *S. cerevisiae* strain W303-1A *jen1*Δ *ady2*Δ, lacking carboxylate uptake capacity, was used to express *C. jadinii 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-^{14}C] lactic acid, [2,3-^{14}C] succinic acid and [1-^{14}C] acetic acid.

Results: In *C. jadinii*, 4 genes homolog to *ScADY2* were identified. These are functional carboxylate transporters in *S. cerevisiae,* localized at the plasma membrane*,* presenting different specificities for the mono- and di-carboxylates. Further studies are underway to fully characterize these four new plasma membrane transporters, including molecular docking of these transporters to unveil the amino acids that play a major role in the substrate binding of CAs tested.

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/BIA-MIC/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.