

# 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 *jen1Δ ady2Δ*, 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-<sup>14</sup>C] lactic acid, [2,3-<sup>14</sup>C] succinic acid and [1-<sup>14</sup>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.

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