Exploring genetic tools for the overexpression of the lactate permease Jen1p of *Saccharomyces cerevisiae*: constitutive expression in *S. cerevisiae* and heterologous expression in *Pichia pastoris*

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In *Saccharomyces cerevisiae* the active transport of monocarboxylic acids is dependent upon the expression of the permease Jen1p. Cells of *S. cerevisiae jen1* were transformed with the plasmids p416GPD (centromeric) and p426GPD (multicopy) containing *JEN1* gene under the control of the GPD constitutive promotor. Lactic acid uptake and *JEN1* expression was found in cells grown in glucose, acetic acid, or glucose and acetic acid. Constitutive expression of the permease was achieved in the p416GPD-JEN1 transformants; the highest V_{max} (0.84 nmol s^{-1} mg^{-1} d.w.) was obtained in acetic acid-grown cells. In a second approach, *Pichia pastoris* strains X-33 (Mut^+) and KM71H (Mut^S) were transformed with the vectors pPICZB-JEN1 (integrative) and pZPARS-JEN1 (episomal). Activity for the lactate permease was only obtained in *JEN1*-transformed cells, the highest level (V_{max} 0.86 nmol s^{-1} mg^{-1} d.w.) being detected in the KM71H strain containing genomic insertions of the vector pPICZB-JEN1, after 24 h of methanol induction. RT-PCR, Northern and Western-blotting analysis confirmed the expression of *JEN1* in *P. pastoris* and *S. cerevisiae*. Both strategies described for *JEN1* expression resulted in a two-fold increase for the activity of the lactate transport system when compared to the data previously found in lactic acid-grown cells of native *S. cerevisiae* strains. Project financially supported by the FCT and FSE (POCTI/1999/BME/36625); ISS received a fellowship SFRH/BD/4699/2001