FUNCTIONAL EXPRESSION OF THE LACTATE PERMEASE JEN1P OF *SACCHAROMYCES CEREVISIAE* IN *PICHIA PASTORIS*

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Pichia pastoris was transformed with an integrative plasmid containing the *Saccharomyces cerevisiae JEN1* gene. After 24 h of methanol induction, Northern and Western-blotting analyses indicated the expression of *JEN1* in the transformants. Lactate permease activity was obtained in *P. pastoris* cells with a V_{max} of 2.1 nmol s^{-1} mg^{-1} dry weight. Reconstitution of the lactate permease activity was achieved by fusing plasma membranes of *P. pastoris* methanol-induced cells with *Escherichia coli* liposomes containing cytochrome oxidase, as proton-motive force. These assays in reconstituted heterologous *P. pastoris* membrane vesicles demonstrate that *S. cerevisiae* Jen1p is a functional lactate transporter. Moreover a *S. cerevisiae* strain deleted in the *JEN1* gene was transformed with a centromeric plasmid containing *JEN1* under the control of the glyceraldehyde 3-phosphate dehydrogenase constitutive promotor. Constitutive *JEN1* expression and lactic acid uptake were observed in cells grown either on glucose and/or acetic acid. The highest V_{max} (0.84 nmol s^{-1} mg^{-1} dry weight) was obtained in acetic acid-grown cells. Thus overexpression of the *S. cerevisiae JEN1* gene in both *S. cerevisiae* and *P. pastoris* cells resulted in increased activity of lactate transport when compared to the data previously reported in lactic acid-grown cells of native *S. cerevisiae* strains. *Jen1p* is the only *S. cerevisiae* secondary porter characterized so far by heterologous expression in *P. pastoris* at both the cell and membrane vesicle levels.

Functional expression of the lactate permease jen1p of Saccharomyces cerevisiae *in P*ichia pastoris