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 $V_{\text{max}}$ 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 $V_{\text{max}}$ (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 Pichia pastoris*