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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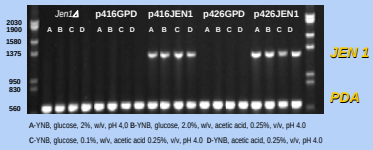
Objective

In *S. cerevisiae* the active transport of lactate and pyruvate is dependent on the expression of *JEN1* (1). *JEN1* is the only *S. cerevisiae* member of the Sialate-Proton Symporters subfamily (TC#2.A.1.12) belonging to the Major Facilitator Superfamily (2). However members of other phylogenic subfamilies can be expected to transport monocarboxylic acids such as the five MCP Monocarboxylate Porters, the FNT Acetate:H^+ Symporter YHL008c or even the *SSU1* Putative Transporter of Unknown Mechanism. To the data the possibilities whether Jen1p has regulatory (or sensor) or transport function haven't been discarded. The purpose of our work is to demonstrate non-ambiguously that Jen1p is a monocarboxylate transporter. Therefore the *ScJEN1 gene* was cloned in *Pichia pastoris* to produce significant amounts of active protein allowing heterologous reconstitution of lactate active transport in isolated membrane vesicles. The *JEN1* gene was also overexpressed in *S. cerevisiae* (at a lower efficiency however) to characterize the kinetic properties of Jen1p at the cell level.

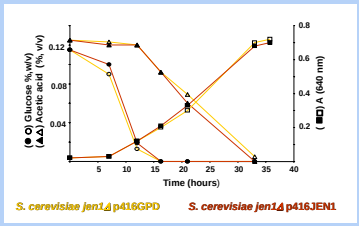
Constitutive Expression in *Saccharomyes cerevisiae*

RT-PCR



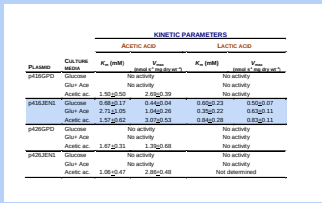
JEN1* was detected in the transformant containing *ScJEN1

Growth assays



The two strains presented a similar growth and consumption rate of glucose and acetic acid

Kinetic parameters for the transport of monocarboxylic acids



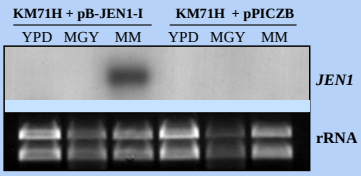
KINETIC PARAMETERS

Plasmid	Culture media	Acetic acid K_m (mM)	Acetic acid V_{max} (nmol L^{-1} mg dry wt $^{-1}$)	Lactic acid K_m (mM)	Lactic acid V_{max} (nmol L^{-1} mg dry wt $^{-1}$)
p416GPD	Glucose	No activity		No activity	
	Glyc+ Ace	No activity		No activity	
	Acetic ac.	1.50±0.50	2.66±0.39	No activity	
p416JEN1	Glucose	0.69±0.17	0.44±0.04	0.65±0.23	0.50±0.07
	Glyc+ Ace	2.71±1.05	1.94±0.26	0.35±0.22	0.63±0.11
	Acetic ac.	1.57±0.62	3.07±0.53	0.84±0.28	0.83±0.11
p426GPD	Glucose	No activity		No activity	
	Glyc+ Ace	No activity		No activity	
	Acetic ac.	1.67±0.31	1.35±0.68	No activity	
p426JEN1	Glucose	No activity		No activity	
	Glyc+ Ace	No activity		No activity	
	Acetic ac.	1.06±0.47	2.86±0.48	Not determined	

Constitutive expression of the Jen1p in *S. cerevisiae jen1Δ* transformed with p146JEN1

heterologous expression in *Pichia pastoris*

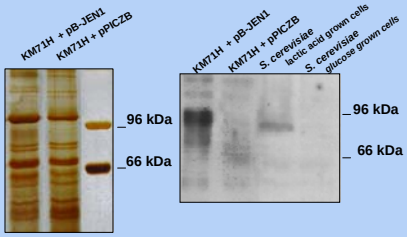
Northern-blot analysis



20 μg of total RNA extracted from transformant *P. pastoris* cells grown in YPD or MYG or incubated for 24 h in MM media were used.

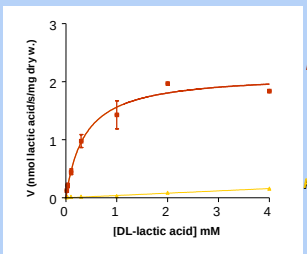
***JEN1* mRNA was only detected in the transformant containing a copy of *ScJEN1* after incubation in MM medium**

Western-blot analysis



Jen1p was detected in *P. pastoris* pB-JEN1

Kinetic parameters of lactic acid uptake in *P. pastoris* KM71H and *P. pastoris* KM71H pBJEN1



***P. pastoris* pB-JEN1**
V_{max} = 0.86 nmol/s/mg d.w.
K_m = 1,43 mM lactic acid

***P. pastoris* KM71H**
k_d = 0,040 μl /s/mg d.w.

The activity for the lactate permease is present in 24h methanol- induced cells

Final remarks

- **Constitutive expression in *S. cerevisiae* was achieved**
- **A 6-fold increase was obtained in Jen1p V_{max} in *P. pastoris* and only a 2-fold increase in *S. cerevisiae***
- **Jen1p was heterologous expressed in *P. pastoris***
- ***JEN1* is a fully functional lactate permease**

References

1 Casal, M., Paiva, S., Andrade, R. P., Gancedo, C., and Leão, C. (1999) The lactate-proton symport of *Saccharomyces cerevisiae* is encoded by *JEN1*. J Bacteriol 181:2620-2623

2 De Hertogh, B., Carvajal, E., Talla, E., Dujon, B., Baret, P., and Goffeau, A. (2002) *Funct. Integr. Genomics* 2, 154-170

This study was supported by the portuguese grant POCTI/1999/BME/36625 (Eixo 2, Medida 2.3, QCAIII - FEDER); Isabel Soares-Silva (SFRH/BD/4699/2001) received a fellowship