Molecular and physiological characterization of mono- and dicarboxylic acids permeases in the yeast *Kluyveromyces* lactis

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Monocarboxylic acids

Dicarboxylic acids

INTRODUCTION

The physiological and biochemical characterisation of permeases for carboxylic acids has been described in the literature for several yeast species. In the present work we focused on the genetic and molecular studies carried out in the mono- and dicarboxylate transport systems in *Kluyveromyces lactis*:

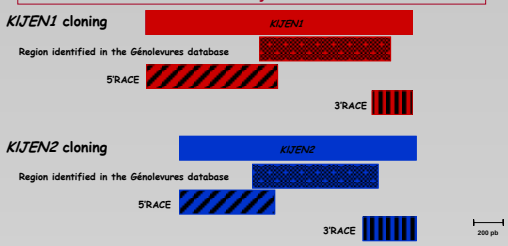
Previous studies developed in the species *S. cerevisiae* led to the identification of the lactate permease gene (*JEN1*/YKL217w) by our group. In Part I we describe the cloning and *in silico* analysis of 2 homologues of *JEN1* found in the non-conventional yeast *K. lactis* (*KlJEN1* and *KlJEN2*). Results concerning lactic acid transport by this species are presented as well as some results concerning expression of *KlJEN1* gene, associated with the carbon source used in the media.

K. lactis is able to use malic acid as the sole carbon and energy source. In the Part II of this work we describe the identification of a *K. lactis* gene, associated with the regulation of malate utilization (*MUP1*), which was detected by heterologous gene expression in *S. cerevisiae*. The medium YPmalate with the pH indicator bromocresol purple (YPMI medium) was used for the screening of the transformants. Southern analysis revealed the presence of a second copy of this gene in the *K. lactis* genome *(MUP2*) and functional analysis of *MUP1* and *MUP2* was carried out by the construction of a *K. lactisΔ mupΔ1mup2* deleted strain.

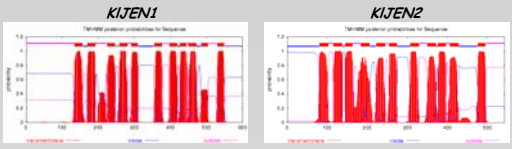
I – Cloning and *in silico* analysis of *KlJEN1/2*

Search in Génolevures Database → Identification of 2 *K. lactis* clones containing *JEN1* homologues partial sequences (*KlJEN1* and *KlJEN2*)

Identification of the whole sequences of *KlJEN1* and *KlJEN2* by RACE



Transmembrane domains in *KlJEN1* and *KlJEN2*



Probable localization of *Kljen1p* and *Kljen2p* in the plasma membrane (PSORT software analysis)

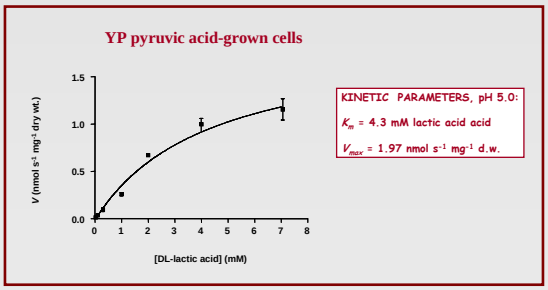
II – *JEN1/2* expression in different carbon sources

***JEN1/2* expression:**

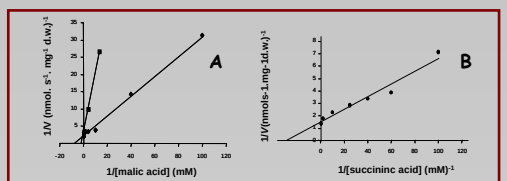
Strain		Carbon source						
		glucose	glycerol	ethanol	citric acid	malic acid	lactic acid	acetic acid
K. lactis	*JEN1*	+	+	+	++	+	++	+
	JEN2	+	+	+	+	+	++	+
S. cerevisiae W303 (*JEN1*)		-	-	++	ND	ND	++	-

Levels of *JEN1/2* transcript after 4 h incubation in YNB containing the indicated carbon source: (-) absent; (+) present; (ND) not determined

III – Lactic acid transport in *K. lactis*



IV – Dicarboxylic acid transport in *K. lactis*

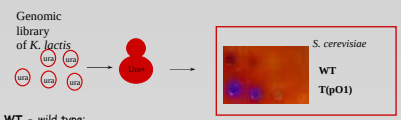


Initial uptake rates of labelled acid as a function of its concentration in *K. lactis* malic acid grown cells:
A) Uptake of malic acid ◆ in the absence of other dicarboxylic acids; ■ in the presence of succinic acid
B) Uptake of succinic acid

KINETIC PARAMETERS:
Malic acid: K_m = 0.12 mM malic acid and V_{max} = 0.47 nmol malic acid s^{-1} mg^{-1} d.w.
Succininc acid: K_m = 0.04 mM succinic acid and V_{max} = 0.75 nmol succinic acid s^{-1} mg^{-1} d.w.

V – Cloning of *MUP1*, a gene involved in malate utilisation

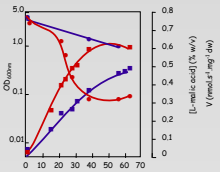
Identification



WT – wild type;
T(pO1)–pO1 transformant.

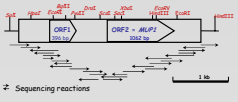
Recovery of the plasmid: pO1 containing *MUP1* gene

Physiological characterisation



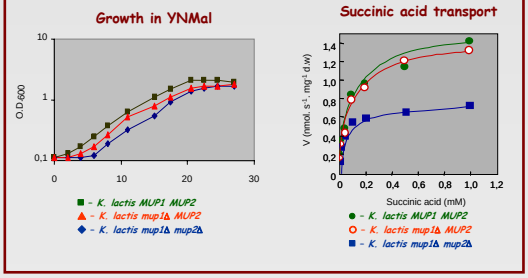
Growth in YPmalate medium and malic acid consumption:
WT: ■, OD_{600nm}; ●, L-malic acid concentration;
T(pO1): ■,OD_{600nm} ●,L-malic acid concentration.

Sequencing



Partial restriction map and DNA sequencing strategy of the insert contained in pO1.
MUP1 gene was found to be responsible for the phenotype observed and it is duplicated in the *K. lactis* genome

VI – Characterization of *mup1Δ mup2Δ K. lactis* double mutant



RESULTS AND DISCUSSION

K. lactis is able to use mono- and dicarboxylic acids as the sole carbon and energy source and presents mediated transport systems for these acids. *S. cerevisiae* although presents a carrier for monocarboxylates, is not able to transport dicarboxylic acids.

The gene encoding lactic acid permease has been cloned in *S. cerevisiae (JEN1* gene). Using the Génolevures database, two clones of *K. lactis* (named *KlJEN1* and *KlJEN2*) containing a partial sequence of two genes homologues to *JEN1* were identified. The RACE technique allowed the identification of the whole sequence of these genes. *KlJEN1* and *KlJEN2* expression has been assayed, and although in *S. cerevisiae JEN1* expression is glucose repressed, a transcript of both *JEN1/2* genes is present in glucose grown cells of *K. lactis*, indicating a different regulation in this species. Indeed, the analysis of the *KlJEN1/2* promoters sequenced so far did not reveal cis elements for repressors like mig1p, in opposition to what happens with *ScJEN1* promoter.

On the second part of this work, a *K. lactis* gene, associated with the regulation of malate utilization (*MUP1*) is presented. This gene was isolated by heterologous gene expression in *S. cerevisiae*. A second copy of this gene is present in the *K. lactis* genome *(MUP2*) and functional analysis of *MUP1* and *MUP2* was carried out by the construction of a *K. lactisΔ mupΔ1mup2* deleted strain. The growth in YNMal is very similar in the three strains; however, concerning dicarboxylic acid transport, the double mutant presented half of the activity for this transport system.