

Saccharomyces cerevisiae

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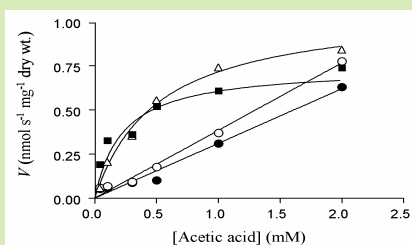
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Several yeast species, including *Saccharomyces cerevisiae*, are able to utilize acetic acid as a sole carbon and energy source under aerobic conditions (Barnett *et al.*, 1990), acetate being a normal and frequent end-product of fermentation (Flores *et al.*, 2000). When cells of *S. cerevisiae* are grown on respiratory substrates, activity for at least two monocarboxylate proton symporters are found, with differences in their mechanisms of regulation and specificity (Casal *et al.*, 1996). A lactate-pyruvate-acetate-propionate transporter, induced in lactic or pyruvic acid-grown cells, is encoded by the gene *JEN1* (Casal *et al.*, 1999). In addition to *JEN1*, another permease, which accepts acetate, propionate or formate, is present in cells grown in non-fermentable carbon sources (Casal *et al.*, 1996; Makuc *et al.*, 2001). To identify new genes involved in acetate uptake in *S. cerevisiae*, an analysis of the gene expression profiles of cells shifted from glucose to acetic acid was performed. This strategy allowed us to identify the membrane protein *Ady2p* as an essential component of the acetate active transport in these conditions and a valuable candidate as a new acetate transporter in yeast.

Acetate is transported by a mediated transport system

Glucose-grown cells → Shift to YNB-acetic acid medium, pH 6.0



After 4 h induction
○ W303-1A (*JEN1*)
● W303-1A (*jen1Δ*)

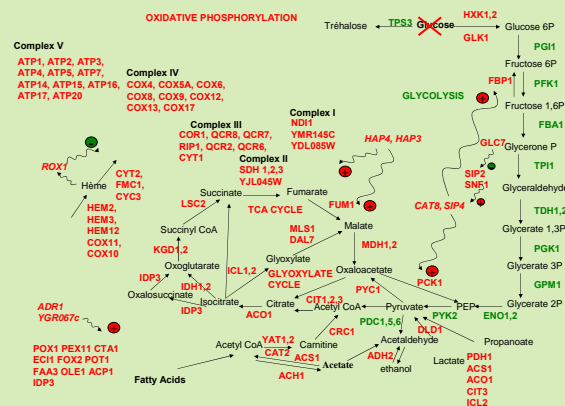
After 6 h induction
△ W303-1A (*JEN1*)
■ W303-1A (*jen1Δ*)

► The activity of the acetate permease was present only after 6 h of induction

► The deletion of *JEN1* did not affect the ability of the cells to transport acetic acid by a mediated mechanism

Setting up the best conditions for microarray analyses

Glucose-grown cells → Shift to acetic acid medium, 4 h



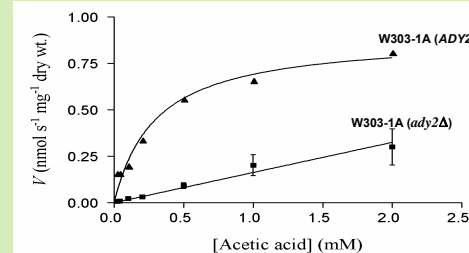
Expression changes in genes encoding known or putative transporters after 4h in acetic acid

Gene	Fold induction after 4 hours in acetate	Substrate
Mitochondrial transporters		
<i>PC1</i>	38	Bicarbonate/oxalate
<i>EFY1</i>	22	Bicarbonate/oxalate
<i>OCY1</i>	9.3	Oxalate/acetate
<i>OCY2</i>	-2.1	Oxalate/acetate
Oxalate/alpha-ketoglutarate products		
<i>AAO1</i> , <i>AAO3</i> , <i>PET1</i>	0.1 to 0.6	ATP/ADP export
<i>MEI1</i>	12	Isopropyl phosphate
Others		
<i>ACF1</i>	-3.0	Fatty acid precursors
<i>ACO3</i>	-3.0	Dicarboxylates
Plasma membrane transporters		
<i>CDY1</i>	42	Lactate, propionate, acetate
<i>MDJ1</i> , <i>MDJ2</i> , <i>MDJ3</i>	-2 to -3.7	Isotonic
Glucose and sorbitol		
<i>AGP1</i>	2.3	Arabinose, glucose, xylose, etc.
<i>TAT1</i>	6.0	Yeast hexose transport, etc.
<i>MDJ2</i>	3.4	Isotonic
<i>AGP2</i>	5.7	Isotonic
Hemolysins		
<i>HOT1</i> , <i>HOT2</i> , <i>HOT3</i> , <i>HOT4</i> , <i>HOT5</i> , <i>HOT6</i> , <i>HOT7</i> , <i>HOT8</i>	1 to 6.3	Hemolysins
Acetate ions		
<i>SHI2</i>	2.5	Magnessite
<i>CTR1</i>	4.2	Copper
<i>FEI1</i> , <i>FEI2</i>	2.0 to 3	Iron
<i>ERS1</i> , <i>ERS2</i> , <i>ERS3</i>	2.3	Sulfur, selenium
Unknown membrane proteins		
<i>ADY2</i>	37	Isotonic
<i>PUS14</i>	9.8	Isotonic
<i>ATO3</i>	2.6	Isotonic

► Genes *YCR10c/ADY2*, *YNR02c/FUN34* and *YDR384c/ATO3*, homologous to the *GPR1* gene of *Yarrowia lipolytica* were clearly activated

ADY2/YCR10c is an essential gene for the mediated transport of acetic acid in S. cerevisiae

Initial uptake rates of labelled acetic acid pH 5.0 by 6 h of acetic acid-derepressed cells

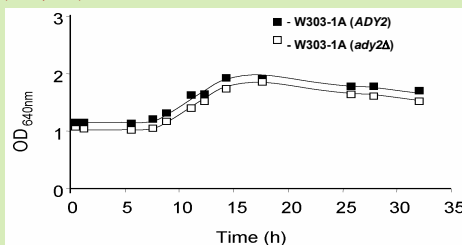


► *ADY2*-deleted strain (■) exhibited a dramatic decrease in the activity of the acetate permease when compared to the wt strain (▲)

► In the *ADY2*-deleted strain acetate crossed the plasma membrane by a simple diffusion mechanism

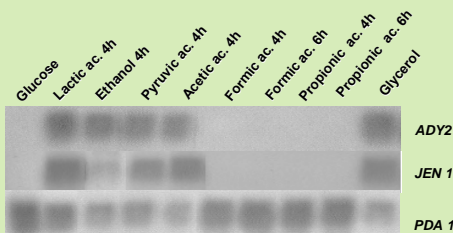
Adaptation of S. cerevisiae W303-1A and ady2Δ strains to acetic acid (0,5%, pH 6.0)

Cells were cultivated in glucose and transferred to a medium containing acetic acid (0,5%, pH 6.0)



► Cells of the *ady2Δ* have a normal growth in acetic acid when compared with the wild type strain

Expression of ADY2 in different carbon sources



Northern-blot analysis of *JEN1* and *ADY2* transcripts from W303.1A strain. Total cellular RNA was prepared from cells growing exponentially in YNB media containing different carbon sources

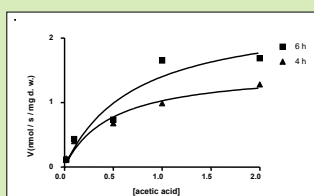
Comparison of gene expression profiles between W303-1A and ady2Δ strains



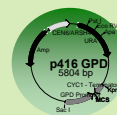
After 4 hours in acetate, the *ady2Δ* strain does not have a dramatic difference in the global transcriptional response compared to the wild type suggesting that *ADY2* is not a key regulator of the transcriptional response to acetic acid

Analysis of acetate uptake in S. cerevisiae ady2Δ containing the gene ADY2 in the plasmid p416GPD

YNBgluc → YNBace



Presence of a mediated transport system for acetic acid



	4h	6h
K_m (mM)	0.48	0.72
V_{max} (nmol s ⁻¹ mgdw ⁻¹)	1.5	2.4

The transcriptional adaptation of yeast cells to a shift from glucose to non-toxic conditions of acetic acid was characterized. These conditions of metabolic shift revealed to be very powerful for functional assignment of unknown proteins and for the study of mechanisms like translation regulation or mitochondria biogenesis.

► *ADY2* gene is an essential component of acetate transport system in *S. cerevisiae*