



D-Xylose Transport by *Candida succiphila* and *Kluyveromyces marxianus*

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Abstract

Fermentation of the pentose sugar D-xylose into ethanol is essential for an economically feasible production of fuel ethanol from plant biomass. Since the preferred organism in industrial ethanol fermentation processes is the yeast *Saccharomyces cerevisiae* (a microorganism unable to utilize xylose), several laboratories have attempted to engineer this yeast for efficient xylose fermentation. Although the results of such genetically engineered yeasts have been encouraging, it was recently shown that xylose uptake limits the xylose flux and metabolism by these recombinant strains. We have characterized the xylose transport system(s) in the yeast strains *C. succiphila* and *K. marxianus*. *C. succiphila* is one of the few yeasts capable of fermenting both D-xylose and L-arabinose under microaerobic conditions. This yeast strain showed a high affinity and active xylose transport activity when grown on xylose. When grown on glucose, xylose appeared to be transported by a facilitated diffusion mechanism with low affinity for the substrate. Sugar competition studies indicated that the high affinity system is probably mediated by a general monosaccharide transporter, while the low affinity system occurs through glucose transporters. A very different pattern was found with *K. marxianus* that utilizes pentoses without significant ethanol production.

Materials and Methods

Materials:

Strains: *Candida succiphila* (NRRL Y-11998) and *Kluveryomyces marxianus* (ATCC #52486, formerly *K. fragilis*)

¹⁴C D-xylose (ARC)

Cell Growth: Cells were grown in 2% (w/v) D-xylose in YP medium, either aerobically (50mL in a 250mL baffled Erlenmeyer flask at 220 rpm) or microaerobically (50mL in a 125mL unbaffled Erlenmeyer flask shaken at 100 rpm) at 30°C.

Transport Assay: Cells were harvested during mid-log growth phase, washed twice in ice-cold water, and resuspended in a final OD₆₀₀ density between 250-300. For the assay, cells were allowed to equilibrate to room temperature for 5 minutes in 50 mM succinate-Tris, pH5.0 buffer. ¹⁴C D-xylose mixed with varying concentrations of cold D-xylose (~.02-2 μCi/μmole) was added. Transport continued for 30 seconds, until the reaction was terminated by the addition of 5 mL ice-cold water and immediately filtered onto 0.8 micron 24 mm mixed cellulose ester filters from Millipore and washed with an additional 5 mL of water. Filters were immediately transferred to Liquid Scintillation vials and counted.

Table I. Kinetic parameters for xylose transport

Yeast and Growth conditions:	K_m (mM)	V_{max} (nmol/mg.min)
<i>C. succiphila</i>		
Xylose grown cells	3.8	15
Glucose grown cells	140	130
<i>K. marxianus</i> (xylose grown)		
Microaerobically grown cells	103	190
Aerobically grown cells	0.2 / 110	10 / 190

Table II. Effects of reagents upon xylose transport by *C. succiphila*.

Inhibitor (concentration in mM)	Mechanism of inhibition	Relative xylose transport (%)	
		Xylose grown cells	Glucose grown cells
None	-	100 ^A	100 ^B
NaN ₃ (10)	Protonophore	5	107
DNP (2.5)	"	2	86
CCCP (2.5)	"	2	62
DESB (5)	ATPase inhibitor	31	68
DCCD (5)	"	9	107
Glucose (250)	-	2	2
Galactose (500)	-	3	24
Arabinose (600)	-	11	56

Assay concentration of ¹⁴C-xylose was 10 mM

^ARate of xylose transport was 9.0 nmol/mg.min

^BRate of xylose transport was 8.5 nmol/mg.min

Table III. Effects of reagents upon xylose transport by *K. marxianus*.

Inhibitor (concentration in mM)	Mechanism of inhibition	Relative xylose transport (%)	
		Aerobic cells	Microaerobic cells
None	-	100 ^A	100 ^B
NaN ₃ (5)	Protonophore	16	77
DNP (1.25)	"	15	70
CCCP (1.25)	"	16	68
DESB (2.5)	ATPase inhibitor	34	80
DCCD (2.5)	"	39	nd
Glucose (250)	-	9	2
Galactose (500)	-	23	53
Arabinose (600)	-	12	89

Assay concentration of ¹⁴C-xylose was 10 mM

^ARate of xylose transport was 29.1 nmol/mg.min

^BRate of xylose transport was 21.2 nmol/mg.min

Conclusions

- While *C. succiphila* fermented xylose under microaerobic conditions (producing ethanol and xylitol), *K. marxianus* diverted a larger fraction of the metabolic energy from xylose degradation into cell growth.
- The slow rates of growth and substrate uptake observed with *C. succiphila* is probably a consequence of a low capacity of the active xylose transport system present in these cells. Under the same conditions *K. marxianus* exhibited a high-capacity facilitated diffusion transport system.
- *C. succiphila* transported xylose with higher capacity after growth on glucose, an uptake probably mediated by passive glucose transporters. When *K. marxianus* growth conditions were changed from microaerobic into fully aerobic conditions, the cells exhibited not only the low affinity transporter, but also an active high affinity xylose transport activity.
- Thus, our results indicate the presence in yeast of a variety of xylose transporters with not only different affinities and specificities for the substrate, but also with different patterns of expression regulated by both the sugar substrates and process conditions.

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