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D-Xylose Utilization by *Saccharomyces cerevisiae*

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Although it is generally accepted that *Saccharomyces cerevisiae* is unable to assimilate D-xylose, four strains were found to utilize xylose aerobically at different efficiencies in the presence of a mixture of substrates. The degree of D-xylose utilization by *S. cerevisiae* ATCC 26602 depended upon the presence of other substrates or yeast extract. The greatest amount of xylose (up to 69% over 7 d) was utilized when sugar substrates such as D-ribose were co-metabolized. Much lower degrees of utilization occurred with co-metabolism of organic acids, polyols or ethanol. A mixture of D-glucose, D-ribose, D-raffinose, glycerol and D-xylose resulted in greater xylose utilization than the presence of a single substrate and xylose. The absence of growth on a co-substrate alone did not prevent the utilization of xylose in its presence. Xylose was co-metabolized with ribose under anaerobic conditions but at a much slower rate than under aerobic conditions. When [^{14}C]xylose was utilized in the presence of ribose under anaerobic conditions, the radioactive label was detected mainly in xylitol and not in the small amounts of ethanol produced. Under aerobic conditions the radioactive label was distributed between xylitol (91·3 ± 0·8%), CO_2 (2·6 ± 2·3%) and biomass (1·7 ± 0·6%). No other metabolic products were detected. Whereas most xylose was dissimilated rather than assimilated by *S. cerevisiae*, the organism apparently possesses a pathway which completely oxidizes xylose in the presence of another substrate.

INTRODUCTION

According to the taxonomic literature, *Saccharomyces cerevisiae* lacks the ability to utilize D-xylose for growth (Kreger-van Rij, 1984). Consequently, *S. cerevisiae* has not been investigated extensively in the search for xylose-fermenting yeasts. *Pichia stipitis*, *Candida shehatae* and *Pachysolen tannophilus* are the most promising yeasts for ethanol production from hemicellulose hydrolysate (du Preez & Prior, 1985; Watson *et al.*, 1984*b*). However, the poor ethanol tolerance and slow fermentation rates of these yeasts in comparison with *S. cerevisiae* will probably limit their commercial application (du Preez *et al.*, 1987; Watson *et al.*, 1984*b*). The failure of *S. cerevisiae* to utilize D-xylose has been ascribed to its inability to convert xylose to xylulose (Gong *et al.*, 1981). According to other reports, however, *S. cerevisiae* produces all the enzymes necessary for xylose utilization. Xylose transport in *S. cerevisiae* occurs by facilitated diffusion using the glucose uptake system (Cirillo, 1968; Kleinzeller & Kotyk, 1967; Kotyk, 1967). Low activities of xylose reductase (XR) and xylitol dehydrogenase (XD) have also been detected in *S. cerevisiae*, indicating that it has the potential to convert xylose to xylulose via xylitol (Batt *et al.*, 1986). Xylulose is utilized by a large number of *S. cerevisiae* strains (Ueng *et al.*, 1981; Gong *et al.*, 1981).

Abbreviations: XD, xylitol dehydrogenase; XR, xylose reductase; YEP, yeast extract/peptone/salts; YNB, yeast nitrogen base.

Hsiao *et al.* (1982) suggested that *S. cerevisiae* can catabolize xylose in combination with other substrates, including glucose, xylulose and xylitol. Batt *et al.* (1986) have shown that xylose can be taken up and utilized in the presence of galactose.

The objective of this study was to examine the ability of *S. cerevisiae* strains to utilize D-xylose and to elucidate the properties of D-xylose utilization in strain ATCC 26602. The disappearance of xylose from the medium will subsequently be termed xylose utilization.

METHODS

Organisms. Saccharomyces cerevisiae strains were obtained from the American Type Culture Collection (ATCC cultures), Centraal Bureau voor Schimmelcultures (CBS cultures) Delft, The Netherlands and Professor J. P. van der Walt, Pretoria, South Africa (CSIR-Y cultures). The yeasts were routinely maintained on yeast-malt (YM) agar slants (Wickerham, 1951). The identity of the strains was confirmed by conventional classification methods (Barnett *et al.*, 1983) and long-chain fatty acid composition (Kock *et al.*, 1985).

Inoculum preparation. The inoculum was prepared by two different methods: (*a*) growth on fresh YM slants was suspended in sterile distilled water to an optical density equivalent to 200 Klett units (640 nm; Klett–Summerson colorimeter) and 1 ml was used to inoculate 30 ml of medium; (*b*) for evaluation of xylose utilization by *S. cerevisiae* ATCC 26602 a 48 h culture was transferred to medium containing 10 g glucose l^{-1} and 6·7 g yeast nitrogen base (YNB) l^{-1}. The culture was incubated aerobically at 30 °C until it reached an optical density of 1·0 (640 nm; LKB spectrophotometer) in the mid-exponential growth phase (100 ml medium yielded 0·05 g cells). The cells were harvested aseptically by centrifugation (2500 ***g*** for 5 min), washed twice and resuspended in sterile water to give an initial cell concentration of 2 to 3 g l^{-1} after inoculation. The high initial biomass concentration was used to ensure measurable utilization of xylose in the absence of growth.

Media. The culture medium was either YNB (6·7 g l^{-1}) medium or yeast extract/peptone/salts (YEP) medium containing yeast extract (10 g l^{-1}), peptone from pepsin-digested meat (5 g l^{-1}) and salts as follows (l^{-1}): 1·2 g $(NH_4)_2SO_4$; 0·5 g NaCl; 0·1 g $CaCl_2.2H_2O$; 0·64 g $MgCl_2.6H_2O$; 0·79 g KH_2PO_4; and 3·7 mg $FeCl_2.4H_2O$ (Quain & Boulton, 1987). Various carbon sources were added to a final concentration of either 5 or 10 g l^{-1} (pH 5·5). The pH of media containing organic acids was adjusted to pH 6 in order to minimize toxicity.

All chemicals were obtained from Merck except for yeast extract and YNB which were obtained from Biolab Chemicals (Pretoria, South Africa) and Difco, respectively. Concentrates of the yeast extract and peptone were heat-sterilized, whereas YNB, sugar and salt concentrates were filter-sterilized by ultrafiltration.

Yeast cultivation. The yeasts were cultivated aerobically in triplicate in either 100 ml or 1 litre Erlenmeyer flasks containing 30 or 100 ml of medium respectively. Some experiments were done in test-tubes containing 10 ml of medium. The flasks were incubated at 30 °C on an orbital shaker (160 r.p.m.; 27·5 mm throw) while the test-tubes were incubated in a slanting position at 30 °C in an incubator oscillating at 40 oscillations min^{-1}. Anaerobic cultivation was done in tightly sealed 25 ml serum vials containing 20 ml of medium. Prior to inoculation, scrubbed nitrogen gas was passed through the liquid for 15 min (Hungate, 1969). The vials were incubated without shaking at 30 °C.

Evaluation of growth on solid medium. YNB medium containing 5 g l^{-1} of the carbon source as indicated and 20 g agar l^{-1} in Petri dishes was point-inoculated from a fresh slant and incubated at 30 °C. The development of a colony at the point of inoculation was taken as evidence of the ability of the yeast to grow on a given carbon source.

Aerobic labelling experiments. Warburg flasks (14·5 ml total volume) containing 2 ml of YNB medium to which 5 g l^{-1} each of D-[U-^{14}C]xylose (specific activity 7·9 MBq $mmol^{-1}$) and D-ribose were added were inoculated to a final concentration of 2 g l^{-1} with either viable or autoclaved cells and incubated on an orbital shaker (160 r.p.m.) at 30 °C. CO_2 produced during incubation was absorbed by 0·3 ml of 1 M-KOH in the centre well of the flask. After 3 d the cells were removed from the medium by centrifugation and the supernatant was analysed by high-performance liquid chromatography (HPLC). Fractions (125 µl) were collected from the column eluant and the distribution of the radioactivity between the cells, CO_2 and column eluant was determined.

Anaerobic labelling experiments. YNB medium (1·8 ml) containing xylose and ribose was added to 5 ml vials and each vial was gassed with oxygen-free nitrogen (Hungate, 1969). To each vial 7·12 µl D-[U-^{14}C]xylose was added to give a specific activity of 0·79 MBq $mmol^{-1}$. The final concentration of both ribose and xylose was 5 g l^{-1}. The vials were capped and autoclaved, and then inoculated with 0·2 ml of a *S. cerevisiae* culture to a final cell concentration of 2 g l^{-1} and incubated at 30 °C. To trap CO_2 produced during fermentation the headspace of one vial was connected to another small vial containing 0·3 ml 1 M-KOH. After 6 weeks the cells were separated from the medium by centrifugation. The supernatant broth was analysed by HPLC and the distribution of the radioactivity was determined as described above.

Determination of radioactivity in biomass. The perchloric acid extraction method of D'Amore *et al.* (1988) was used with modifications as follows: cells were harvested by centrifugation, washed twice with water, held in 1 ml 0·58 M-perchloric acid for 12 h at 4 °C and separated from the broth by centrifugation at 15000 ***g*** for 5 min. The

hydrolysed cells were collected on a glass microfibre filter (Whatman GF/F), washed three times with 1 ml ice-cold 0·58 M-perchloric acid and three times with 5 ml ice-cold 70% (v/v) ethanol. The filter was air-dried and transferred to a scintillation vial for counting.

Analytical methods. Biomass was determined gravimetrically. Cells were collected on Whatman GF/F filters, washed and the filter containing the cells was dried to constant mass at 105 °C. Ethanol was determined by gas chromatography (Watson *et al.*, 1984*a*) whereas sugars and polyols were determined by HPLC (Waters). Medium components were separated on a Waters Sugarpak 1 column maintained at 85 °C using double-distilled deionized and degassed water as eluent at a flow rate of 0·5 ml min^{-1}. Radioactive samples were placed in a scintillation vial containing 8 ml scintillation fluid (Aquagel 1; Chemlab, South Africa) and radioactivity was counted in a liquid-scintillation counter (LKB RackBeta, model 1217).

RESULTS

Screening of yeasts

All the strains of *S. cerevisiae* tested utilized xylose to varying degrees in the presence of raffinose, glucose, glycerol and ribose but not in the absence of this substrate mixture (Table 1). The most extensive utilization of xylose (74%) was obtained with strain ATCC 26602. Xylose was converted nearly stoichiometrically to xylitol by all strains tested (Table 1). *S. cerevisiae* ATCC 26603 utilized 31% of the xylose in the substrate mixture, which is considerably greater than reported previously for this strain (Gong *et al.*, 1983).

Effect of medium composition and oxygen on D-*xylose utilization by S. cerevisiae ATCC 26602*

S. cerevisiae ATCC 26602 failed to utilize D-xylose as the sole carbon source in liquid YNB medium and did not grow on agar plates containing D-xylose and YNB (Table 2; Fig. 1). Utilization of xylose, however, occurred in the presence of sugars (Table 2), polyols (Table 2), organic acids (Table 3) or ethanol (Table 3). The rate of xylose utilization was very slow. The specific rate of xylose utilization in the presence of D-xylose, D-raffinose, D-glucose, glycerol and D-ribose was 0·007 h^{-1}. (Obtained by dividing the overall volumetric rate of xylose utilization by the final biomass concentration.) The biomass in the sugar mixture increased from an initial value of 0·06 g l^{-1} to 2·70 g l^{-1} within 7 d. In the presence of D-ribose 69% of the xylose was utilized within 7 d (Table 2). Furthermore, xylose and ribose were utilized simultaneously in shake-flasks (Fig. 1) with the concomitant production of xylitol. Under aerobic conditions no ethanol production was detected. Growth on the co-substrate was not a prerequisite for xylose utilization. Although *S. cerevisiae* is described in the taxonomic literature as unable to assimilate D-ribose (Barnett *et al.*, 1983), strain ATCC 26602 utilized 42% of the D-ribose as sole carbon source (Table 2) without growth. The absence or presence of growth on the sugar and polyol substrates was in accordance with the classical description of *S. cerevisiae* by Kreger-van Rij (1984) and Barnett *et al.* (1983).

Xylose was utilized in the absence of another substrate in YEP medium (Table 4). Yeast extract appeared to be the most important component stimulating xylose utilization since xylose was not utilized in a medium containing only peptone and salts. The addition of glutamic acid to

Table 1. *Utilization of* D-*xylose in a substrate mixture*

The *S. cerevisiae* strains listed were incubated in D-xylose (10 g l^{-1}) and YNB medium in the presence or absence of 10 g l^{-1} each of D-raffinose, D-glucose, glycerol and D-ribose in 100 ml shake-flasks for 6 d at 30 °C under aerobic conditions. Values are means of three replicates; standard coefficients of variation were less than 10% of the means. In the absence of the substrate mixture no xylose was utilized.

	Mixture present	
Strain	Xylose utilized (g l^{-1})	Xylitol formed (g l^{-1})
ATCC 26602	7·4	7·8
ATCC 26603	3·1	3·3
CBS 1907	5·7	6·6
CSIR-Y2	3·9	4·2

Table 2. *Substrate utilization and xylitol formation by S. cerevisiae ATCC 26602*

The yeast was incubated in YNB medium plus substrate(s) indicated in test-tubes for 7 d at 30 °C under aerobic conditions. Values in parentheses are SDs of three determinations. ND, Not determined; NA, not applicable.

Substrate combination (each 5 g l^{-1})	Ability to grow on co-substrate(s)*	Xylose utilized (%)	Ratio xylitol produced/xylose utilized	Co-substrate utilized (%)
D-Xylose, raffinose, D-Glucose, glycerol, D-Ribose	++	74 (1·8)	0·91	60†
D-Xylose, raffinose	++	32	0·83	ND‡
D-Xylose, D-glucose	++	37 (2·9)	0·95	100 (0)
D-Xylose, D-ribose	+	69 (0·6)	0·88	55 (0·8)
D-Xylose, D-galactose	++	31 (2·2)	1·25	ND§
D-Xylose, L-arabinose	−	2 (3·9)	1·00	0
D-Xylose, glycerol	+	34 (0·6)	1·00	46 (0·1)
D-Xylose, ribitol (adonitol)	+	7 (0·9)	0·75	0
D-Xylose	−	0	0	NA
Raffinose	++	NA	NA	ND‡
D-Glucose	++	NA	NA	100 (0)
D-Ribose	−	NA	NA	42 (0·4)
D-Galactose	++	NA	NA	ND
L-Arabinose	−	NA	NA	35 (1·3)
Glycerol	+	NA	NA	71 (3·6)
Ribitol (adonitol)	−	NA	NA	0

* Growth on agar plates: ++, growth after 1 d; +, growth after 7 d; −, no growth after 14 d.

† Raffinose was not determined and from the 60% of the other substrates utilized, 100% glucose, 34 ± 3% ribose and 46 ± 3% glycerol were utilized.

‡ Raffinose was converted to melibiose, but the amounts were not quantified.

§ Galactose could not be separated from xylose by HPLC and 100% utilization was assumed in order to calculate the amount of xylose utilized.

Table 3. *Xylose utilization and xylitol formation by S. cerevisiae ATCC 26602*

The yeast was incubated in YNB medium plus organic acids and ethanol as indicated in test-tubes for 7 d at 30 °C under aerobic conditions. Values in parentheses are SDs of three determinations. ND, Not determined; NA, not applicable.

Substrate combination (each 5 g l^{-1})	Ability to grow on combined substrates*	Xylose utilized (%)	Ratio xylitol produced/xylose utilized
D-Xylose, acetate	++	10	0·80
D-Xylose, citrate	+	10 (4·0)	0·40
D-Xylose, gluconate	+	0	NA
D-Xylose, glyoxylate	ND	0	NA
D-Xylose, pyruvate	++	10 (0·1)	0·80
D-Xylose, ethanol	++	21 (1·5)	1·27

* Growth on agar plates: ++, growth after 3 d; +, growth after 7 d; −, no growth after 14 d.

YNB containing xylose and ribose increased the amount of xylose utilized suggesting a role in promoting xylose utilization (Table 4).

D-Xylose was not fermented when supplied as the sole carbon source under anaerobic conditions. In the presence of D-ribose, however, 53% of the xylose was utilized and stoichiometrically converted to xylitol within 4 weeks, while 82% of the ribose was utilized and 1·2 g ethanol l^{-1} was produced (Table 5). Ribose was also utilized as sole carbon source under anaerobic conditions with the production of 0·9 g ethanol l^{-1}.

Table 4. *Effect of medium composition on xylose utilization by S. cerevisiae ATCC 26602 in the presence or absence of ribose*

The yeast was incubated in 5 ml of the stated medium in test-tubes for 7 d at 30 °C under aerobic conditions. Values in parentheses are SDs of three determinations. ND, Not determined; NA, not applicable.

	Xylose alone (5 g l^{-1})		Xylose + ribose (each 5 g l^{-1})		
Medium	Xylose utilized (%)	Ratio xylitol produced/xylose utilized	Xylose utilized (%)	Ribose utilized (%)	Ratio xylitol produced/xylose utilized
YNB	0	NA	43 (3·5)	9 (1·6)	1·20
YNB, glutamic acid (0·75 g l^{-1})	ND	ND	63 (2·1)	24 (2·8)	1·17
YEP	49 (2·5)	1·05	69	23	1·07
Half-strength YEP	29 (1·4)	1·17	55 (1·2)	19 (1·5)	1·12
Yeast extract, salts	49 (1·8)	1·00	71 (0·3)	30 (1·0)	1·07
Yeast extract, peptone	40 (4·6)	1·06	70 (2·7)	25 (3·1)	1·04
Yeast extract	41 (0·7)	1·24	ND	ND	ND
Peptone, salts	0	NA	35 (2·5)	10 (3·9)	1·20
Peptone	0	NA	36	9	1·06

Table 5. *Sugar utilization and product formation by S. cerevisiae ATCC 26602*

The yeast was incubated in YNB medium plus substrate(s) as indicated for 4 weeks at 30 °C under anaerobic conditions. Values in parentheses are SDs of three determinations. NA, Not applicable.

Substrate(s) (each 5 g l^{-1})	Xylose utilized (%)	Ratio xylitol produced/xylose utilized	Utilization of co-substrate (%)	Ethanol produced (g l^{-1})
D-Xylose	0	NA	NA	0
D-Ribose	NA	NA	72 (1·0)	0·9 (0·20)
D-Xylose, D-ribose	53 (3·3)	1·00	82 (1·0)	1·2 (0·08)
D-Xylose, D-glucose	14 (2·0)	1·00	100 (0)	1·5 (0·08)
D-Glucose	NA	NA	100 (0)	1·9 (0·12)

Table 6. *Distribution of radioactivity between metabolic products produced from* D-[^{14}C]*xylose by S. cerevisiae ATCC 26602*

The yeast was incubated in YNB medium containing D-ribose (5 g l^{-1}) and D-[^{14}C]xylose (5 g l^{-1}) for 3 d at 30 °C under aerobic conditions. Of the total D-xylose and D-ribose, 21·8 ± 3·3% and 6·0 ± 3·1% respectively, were utilized (mean of three experiments ± SD).

Metabolic product	Radioactivity (%)
Xylitol	91·3 ± 0·8
CO_2*	2·6 ± 2·3
Biomass†	1·7 ± 0·6
Other products	0
Total radioactivity recovered	95·6 ± 2·1

* Control experiments show contamination of the KOH accounted for not more than 16% of the radioactivity associated with CO_2.

† Control experiments with autoclaved cells showed that not more than 25% of the radioactivity could originate from non-specific binding of labelled xylose or xylitol to the biomass.

Fate of D-[^{14}C]*xylose under aerobic and anaerobic conditions*

HPLC analyses indicated that there was an approximate stoichiometric conversion of xylose to xylitol (Tables 2 to 5). This was confirmed by an aerobic labelled-xylose experiment in which 91% of the ^{14}C was converted to xylitol (Table 6). No other metabolic products were found in the medium (Fig. 2). Small amounts of radioactivity were detected in CO_2 and biomass (Table 6)

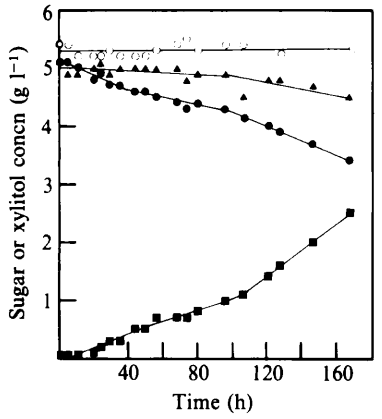

Fig. 1

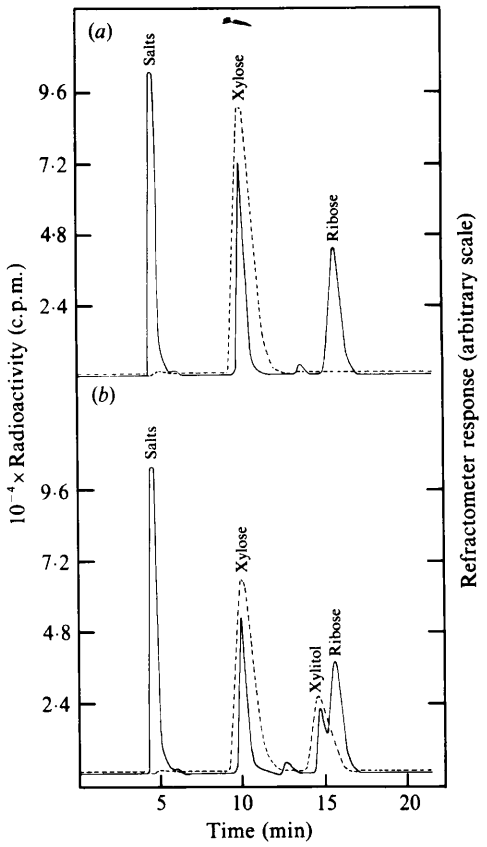

Fig. 2

Fig. 1. Aerobic utilization of 5 g D-xylose l^{-1} in shake-flasks in the presence (●) or absence (○) of 5 g D-ribose l^{-1} by *S. cerevisiae* ATCC 26602 at 30 °C. ▲, D-Ribose utilization; ■, xylitol production. Each data point represents the mean of triplicate experiments.

Fig. 2. HPLC of medium components immediately following inoculation with *S. cerevisiae* ATCC 26602 (*a*) and after incubation for 3 d (*b*) at 30 °C under aerobic conditions in YNB medium containing [^{14}C]xylose (5 g l^{-1}) and unlabelled D-ribose (5 g l^{-1}). ——, Refractometer response; ---, radioactivity.

which suggests that xylose may be metabolized beyond xylitol. Similarly, 92% of the labelled xylose (5 g l^{-1}) was converted to xylitol under anaerobic conditions in the presence of ribose (5 g l^{-1}) while only $0{\cdot}66 \pm 0{\cdot}17\%$ and $0{\cdot}12 \pm 0{\cdot}17\%$ were incorporated into biomass and CO_2 respectively. No significant levels of other ^{14}C-labelled metabolites such as ethanol were detected, indicating that *S. cerevisiae* failed to produce ethanol from xylose (results not shown).

DISCUSSION

The results presented here show that several strains of *S. cerevisiae* are unable to grow on xylose as sole carbon source but can convert most of the xylose to xylitol when another substrate is co-metabolized. Previously, xylose utilization has been reported in combination with other substrates including D-glucose and xylitol (Hsiao *et al.*, 1982) as well as D-galactose (Batt *et al.*, 1986). However, our studies show that a greater degree of xylose utilization could be achieved in combination with certain substrates such as D-ribose. The most extensive utilization of xylose occurred in combination with some sugars, ethanol or glycerol while xylose was utilized poorly or not at all in the presence of Krebs cycle intermediates, ribitol and L-arabinose.

Gong *et al.* (1983) found that *S. cerevisiae* ATCC 26603 utilized only 1·04% of 50 g xylose l^{-1} aerobically and 0·51% fermentatively when xylose was present as the sole substrate in a yeast extract/malt/peptone medium and that xylitol was the main metabolic product. However, the incubation time used was shorter (3 d) and the initial biomass concentration was not specified. The initiation of xylose utilization by yeast extract may be explained by the fact that yeast extract contains carbohydrates (Bridson & Brecker, 1970) and the detection of a peak compatible with ribose by HPLC in the batch of yeast extract used in this study (data not shown). Likewise, Quain & Boulton (1987) found that YEP medium but not YNB supported growth of *S. cerevisiae* on mannitol, another substrate on which growth of *S. cerevisiae* was reported either not to occur or to occur after a delay of 14 to 21 d (Barnett *et al.*, 1983).

The mechanism of stimulation of xylose utilization by the co-substrates is unknown. The uptake system is probably not the site of stimulation. Xylose is taken up by the general monosaccharide transport system in *S. cerevisiae* at a rate approximately tenfold faster than the rate of utilization observed in this study (Cirillo, 1968). The co-substrate is also unlikely to be involved in the induction of XR since xylose utilization starts immediately in mixtures of xylose and ribose. Morever, XR does not appear to be repressed by glucose since xylose utilization was observed without a delay when glucose-grown cells were transferred to medium containing xylose and ribose. Furthermore, xylose was utilized at similar rates in the presence of co-substrates ranging from sugars to ethanol. These results suggest that XR may be constitutive in our strain of *S. cerevisiae* in contrast to a report stating that it is inducible in another strain (Batt *et al.*, 1986). This enzyme has a broad substrate specificity in a number of organisms including yeasts (International Union of Biochemistry, 1979; Bolen & Detroy, 1985; Verduyn *et al.*, 1985) and is present when *Pachysolen tannophilus* is grown on substrates other than xylose (Bolen & Detroy, 1985). Non-specific reduction of xylose by an enzyme induced by another carbon source can therefore not be excluded as a possible mechanism for the conversion of xylose to xylitol by *S. cerevisiae*. For example, transketolase isolated from spinach acted on xylose although at a rate considerably slower than on the natural substrate (Villafranca & Alexrod, 1971).

The presence of XR and XD in *S. cerevisiae* (Batt *et al.*, 1986) would permit the conversion of intracellular xylose to xylulose. The conversion of [^{14}C]xylose into mainly xylitol with only minor amounts being incorporated into biomass and CO_2 under aerobic and anaerobic conditions suggests that although a pathway does exist to assimilate xylose, the rate-limiting step is the conversion of xylitol to xylulose. In yeasts such as *Candida utilis*, XR has a specific requirement for $NADPH_2$ whereas XD requires NAD^+ (Bruinenberg *et al.*, 1984). Under anaerobic conditions co-factor imbalance occurs as $NADH_2$ cannot be re-oxidized and fermentation does not take place. However, yeasts possessing an $NADH_2$-specific XR circumvent this co-factor imbalance and are able to ferment xylose anaerobically (Bruinenberg *et al.*, 1984). The co-factor requirements of these two initial steps in *S. cerevisiae* are poorly understood although Batt *et al.* (1986) reported that XR and XD use $NADPH_2$ and NAD^+ respectively. A similar imbalance may occur in *S. cerevisiae* as indicated by the accumulation of xylitol in this yeast. It is, however, unclear why $NADH_2$ could not be re-oxidized under aerobic conditions, if its accumulation is limiting the conversion of xylitol to xylulose.

Why ribose should promote the utilization of xylose to a greater degree than other substrates remains unclear. Ribose could possibly be transported into the cell via the general monosaccharide transport system (Maxwell & Spoerl, 1971) and be substantially metabolized as no metabolic intermediates such as ribitol were detected in the culture medium and ethanol accumulated under anaerobic conditions. This process could yield co-factors involved in xylose utilization. However, this requires further investigation.

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REFERENCES

BARNETT, J. A., PAYNE, R. W. & YARROW, D. (1983). *Yeasts: Characteristics and Identification.* Cambridge & New York: Cambridge University Press.

BATT, C. A., CARVALLO, S., EASSON, D. D., AKEDO, M. & SINSKEY, A. J. (1986). Direct evidence for a xylose metabolic pathway in *Saccharomyces cerevisiae*. *Biotechnology and Bioengineering* **28**, 549–553.

BOLEN, P. L. & DETROY, R. W. (1985). Induction of NADPH-linked D-xylose reductase and NAD-linked xylitol dehydrogenase activities in *Pachysolen tannophilus* by D-xylose, L-arabinose, or D-galactose. *Biotechnology and Bioengineering* **17**, 302–307.

BRIDSON, E. Y. & BRECKER, A. (1970). Design and formulation of microbial culture media. *Methods in Microbiology* **3B**, 229–295.

BRUINENBERG, P. M., DE BOT, P. H. M., VAN DIJKEN, J. P. & SCHEFFERS, W. A. (1984). NADH-linked aldose reductase: the key to anaerobic alcoholic fermentation of xylose by yeasts. *Applied Microbiology and Biotechnology* **19**, 256–260.

CIRILLO, V. P. (1968). Relationship between sugar structure and competition for the sugar transport system in baker's yeast. *Journal of Bacteriology* **95**, 603–611.

D'AMORE, T., PANCHAL, C. J. & STEWART, G. G. (1988). Intracellular ethanol accumulation in *Saccharomyces cerevisiae* during fermentation. *Applied and Environmental Microbiology* **54**, 110–114.

GONG, C.-S., CHEN, L.-F., FLICKINGER, M. C., CHIANG, L.-C. & TSAO, G. T. (1981). Production of ethanol from D-xylose by using D-xylose isomerase and yeasts. *Applied and Environmental Microbiology* **41**, 430–436.

GONG, C.-S., CLAYPOOL, T. A., MCCRACKEN, L. D., MAUN, C. M., UENG, P. P. & TSAO, G. T. (1983). Conversion of pentoses by yeasts. *Biotechnology and Bioengineering* **25**, 85–102.

HSIAO, H.-Y., CHIANG, L.-C., UENG, P. P. & TSAO, G. T. (1982). Sequential utilization of mixed monosaccharides by yeasts. *Applied and Environmental Microbiology* **43**, 840–845.

HUNGATE, R. E. (1969). A roll tube method for cultivation of strict anaerobes. *Methods in Microbiology* **3B**, 117–132.

INTERNATIONAL UNION OF BIOCHEMISTRY (1979). Nomenclature Committee Recommendations. In *Enzyme Nomenclature*, pp. 32–33. New York: Academic Press.

KLEINZELLER, A. & KOTYK, A. (1967). Transport of monosaccharides in yeast cells and its relationship to cell metabolism. In *Aspects of Yeast Metabolism*, pp. 33–45. Edited by A. K. Mills & H. Krebs. Philadelphia: F. A. Davis.

KOCK, J. F. L., LATEGAN, P. M., BOTES, P. J. & VILJOEN, B. C. (1985). Developing a rapid statistical identification process for different yeast species. *Journal of Microbiological Methods* **4**, 147–154.

KOTYK, A. (1967). Properties of the sugar carrier in baker's yeast. II. Specificity of transport. *Folia microbiologica* **12**, 121–131.

KREGER-VAN RIJ, N. J. W. (1984). *The Yeasts: a Taxonomic Study*, 3rd edn, pp. 45–104 & 384. Amsterdam: Elsevier Science Publishers.

MAXWELL, W. A. & SPOERL, E. (1971). Mannitol uptake by *Saccharomyces cerevisiae*. *Journal of Bacteriology* **105**, 753–758.

DU PREEZ, J. C. & PRIOR, B. A. (1985). A quantitative screening of some xylose-fermenting yeast isolates. *Biotechnology Letters* **7**, 241–246.

DU PREEZ, J. C., BOSCH, M. & PRIOR, B. A. (1987). Temperature profiles of growth and ethanol tolerance of the xylose-fermenting yeasts *Candida shehatae* and *Pichia stipitis*. *Applied Microbiology and Biotechnology* **25**, 521–525.

QUAIN, D. E. & BOULTON, C. A. (1987). Growth and metabolism of mannitol by strains of *Saccharomyces cerevisiae*. *Journal of General Microbiology* **133**, 1675–1684.

UENG, P. P., HUNTER, C. A., GONG, C.-S. & TSAO, G. T. (1981). D-Xylulose fermentation in yeasts. *Biotechnology Letters* **3**, 315–320.

VERDUYN, C., VAN KLEEF, R., FRANK, J., SCHREUDER, H., VAN DIJKEN, J. P. & SCHEFFERS, W. A. (1985). Properties of the NAD(P)H-dependent xylose reductase from the xylose-fermenting yeast *Pichia stipitis*. *Biochemical Journal* **226**, 669–677.

VILLAFRANCA, J. J. & AXELROD, B. (1971). Heptulose synthesis from nonphosphorylated aldoses and ketoses by spinach transketolase. *Journal of Biological Chemistry* **246**, 3126–3131.

WATSON, N. E., PRIOR, B. A., DU PREEZ, J. C. & LATEGAN, P. M. (1984*a*). Oxygen requirements for D-xylose fermentation to ethanol and polyols by *Pachysolen tannophilus*. *Enzyme and Microbial Technology* **6**, 447–450.

WATSON, N. E., PRIOR, B. A., LATEGAN, P. M. & LUSSI, M. (1984*b*). Factors in acid treated bagasse inhibiting ethanol production from D-xylose by *Pachysolen tannophilus*. *Enzyme and Microbial Technology* **6**, 451–456.

WICKERHAM, L. J. (1951). Taxonomy of Yeasts, Technical Bulletin no. 1029, United States Department of Agriculture.