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Endogenous Xylose Pathway in *Saccharomyces cerevisiae*

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The baker's yeast *Saccharomyces cerevisiae* is generally classified as a non-xylose-utilizing organism. We found that *S. cerevisiae* can grow on D-xylose when only the endogenous genes *GRE3* (*YHR104w*), coding for a nonspecific aldose reductase, and *XYL2* (*YLR070c*, *ScXYL2*), coding for a xylitol dehydrogenase (XDH), are overexpressed under endogenous promoters. In nontransformed *S. cerevisiae* strains, XDH activity was significantly higher in the presence of xylose, but xylose reductase (XR) activity was not affected by the choice of carbon source. The expression of *SOR1*, encoding a sorbitol dehydrogenase, was elevated in the presence of xylose as were the genes encoding transketolase and transaldolase. An *S. cerevisiae* strain carrying the XR and XDH enzymes from the xylose-utilizing yeast *Pichia stipitis* grew more quickly and accumulated less xylitol than did the strain overexpressing the endogenous enzymes. Overexpression of the *GRE3* and *ScXYL2* genes in the *S. cerevisiae* CEN.PK2 strain resulted in a growth rate of 0.01 g of cell dry mass liter^{-1} h^{-1} and a xylitol yield of 55% when xylose was the main carbon source.

The pentose sugar xylose is a major constituent of lignocellulose. *Saccharomyces cerevisiae* cannot use xylose, instead converting it primarily to xylitol with only a small fraction going into biomass or ethanol (44, 45). Recombinant xylose-metabolizing *S. cerevisiae* strains contain genes from the xylose-utilizing yeast *Pichia stipitis* coding enzymes for the first two steps in xylose conversion (23, 38, 46). However, the potential of *S. cerevisiae*'s own enzymes, if they are overexpressed, has not been evaluated.

In xylose-utilizing fungi, xylose reductase (XR) reduces xylose to xylitol, which is oxidized to xylulose by xylitol dehydrogenase (XDH). Xylulose is subsequently phosphorylated to xylulose 5-phosphate by xylulokinase and metabolized through the pentose phosphate pathway. *S. cerevisiae* cannot utilize xylose but can grow on xylulose (15, 43). Thus, the inability of *S. cerevisiae* to utilize xylose was attributed to its inability to convert xylose to xylulose (15), even though low XR and XDH activities are known in *S. cerevisiae* (4). A nonspecific aldose reductase, converting xylose to xylitol, was purified and characterized from *S. cerevisiae* (24); however, the genes coding for the putative XR and XDH enzymes remained unknown. The third enzyme in the xylose pathway, xylulokinase, is encoded by *XKS1*, a gene that has been cloned from, and probably is functional in, *S. cerevisiae* (19). Moderate increases in xylulokinase activity are beneficial in recombinant xylose-metabolizing *S. cerevisiae* strains (9, 18, 20, 21, 40).

Based on the *S. cerevisiae* genome sequence (14), the N-terminal amino acid sequence of the previously purified aldo-keto reductase corresponds to the open reading frame *YHR104w* (*GRE3*), which has 72% amino acid similarity to the XR enzyme of *P. stipitis*. This enzyme can reduce a wide variety of ketose substrates and requires a NADPH cofactor (24). The XR of *P. stipitis* can use either NADH or NADPH in the reduction reaction. *GRE3* is induced under various stress conditions and may act on the toxic intermediates generated (2, 11, 30, 31). Under some conditions, deletion of *GRE3* can decrease xylitol production (42). Two other aldose reductase gene homologs, *YPR1* and *YJR096w*, also encode enzymes requiring NADPH and can utilize xylose as a substrate, but they have much higher K_m values for xylose than does Gre3p (29, 41). Thus, several genes encoding aldose reductases that could utilize xylose are expressed in *S. cerevisiae*.

There are three genes in the *S. cerevisiae* genome that are similar to the gene encoding XDH, *XYL2* of *P. stipitis*. Open reading frame *YLR070c* encodes an enzyme with XDH activity (referred to here as *ScXYL2* and ScXDH, respectively) (32). The other two genes are *SOR1*, which encodes sorbitol dehydrogenase (SDH), and an open reading frame, *YDL246c*, that is almost identical to *SOR1*. The SDH enzyme also can use xylitol as a substrate (35).

The aim of this work was to study the endogenous xylose pathway in *S. cerevisiae*. The gene homologs needed for xylose metabolism exist in the *S. cerevisiae* genome, but it is not known how they are expressed or if the activities they encode form a functional pathway, collectively capable of metabolizing xylose. This study is the first attempt to use only endogenous genes for generating a xylose-metabolizing *S. cerevisiae* strain. The use of endogenous genes as alternatives in constructing recombinant xylose-utilizing strains is evaluated.

MATERIALS AND METHODS

Strains. The yeast strain W303-1B (MATα *leu2-3/112 his3-11/15 trp1-1 can1-100 ade2-1 ura31*) (39) was used for cloning of the *GRE3* and *ScXYL2* genes. The yeast strains S150-2B (H308) (MAT**a** *his3Δ-1 leu2-3/112 trp1-289 ura3-52 cir*$^+$ *gal*$^+$) (26) and CEN.PK2 (H1346) (*MAT***a** *leu2-3/112 ura3-52 trp1-289 his3Δ1 MAL2-8*^{c} *SUC2*) (6) were used as host strains for expressing the *XYL1* and *XYL2* genes of *P. stipitis* and the *GRE3* and *XYL2* genes of *S. cerevisiae*. The strains CEN.PK2 and ENY.WA-1A (*MAT*α *ura3-52 leu2-3/112 trp1-289 his3Δ1 MAL2-8*^{c} *MAL3 SUC3*) (7) were used for gene expression analysis. The previously described CEN.PK2-derived strains expressing the *XKS1* gene on a multicopy vector (strain H1695) and the corresponding control strain with an empty vector (strain H1697) were used as control strains in the *XKS1* expression analyses (33). The genomic DNAs of strains S288C (34) or CEN.PK2 were used as the template for PCR probes. The *Escherichia coli* DH5α strain was used for the bacterial cloning steps.

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TABLE 1. Primers used for gene cloning and probe generation

Gene	Primers
GRE3 (*YHR104w*)	fwd, AAATTGGATCCAGATGTCTTCACTGGTTA; rev, CATACGGATCCTGAGTATGGATTTTACTG
ScXYL2 (*YLR070c*) probe	fwd, GCCTTGAGGATAGAGAACACC; rev, GATTCATGTCCCAGCACCATT
SOR1 probe	fwd, ATGTCTCAAAATAGTAACCC; rev, TCATTCAGGACCAAAGATAATAGT
GRE3 (*YHR104w*) probe	fwd, AACCATCCAGGCAGTACCAC; rev, AGCATCGGAATGAGGGAAAT
TKL1 probe	fwd, TTTGAGTGTTGAAGCTGCTA; rev, CAAATCTGATGATCTACGATC
TAL1 probe	fwd, CGCTTAAGGAAGTATCTCGGA; rev, GCTTTGCTGCAAGGATTCAT
IPP1 probe	fwd, CCGATTGGAAAGTTATTGCC; rev, AGAACCGGAGATGAAGAACCA
ACT1 probe	fwd, AAGAAATGCAAACCGCTGCT; rev, TGGTGAACGATAGATGGACCA

Northern analysis. Total RNA was isolated with a Trizol reagent kit (Invitrogen, Carlsbad, Calif.). The samples taken at 70 h were treated with 10 mg of Zymolyase (Seikagaku Corporation, Tokyo, Japan) per ml for 10 min at room temperature (22 to 24°C) prior to the RNA isolation. Probes for the Northern analysis (Table 1) were prepared by PCR for all genes except for *XKS1* from the genomic DNA of strains S288C or CEN.PK2. To avoid cross-reactions with homologous genes, the probes for *ScXYL2*, *TKL1*, and *TAL1* were chosen partly from the 3′ or 5′ noncoding regions. The probe for *SOR1* also detects *YDL246c*. The PCR products were cloned into pCR2.1-TOPO (Invitrogen), excised from the vector by digestion with EcoRI, and verified by sequencing. *XKS1* was amplified by PCR as described previously (33). The fragments were purified from agarose gels, and labeled with a random primed DNA labeling kit (Roche, Basel, Switzerland) and [α-^{32}P]dCTP (Amersham Pharmacia Biotech, Uppsala, Sweden). Six identical gels were run and blotted to Hybond-N nylon filters (Amersham Pharmacia Biotech) and fixed with UV light (UV Stratalinker 2400; Stratagene, La Jolla, Calif.). Hybridized mRNAs were scanned with a Typhoon Phosphoimager and quantified with ImageQuant software (Amersham Pharmacia Biotech). The measured values were normalized by using the specified amount of pyrophosphate phosphohydrolase (*IPP1*) mRNA, coding for inorganic diphosphatase or actin (*ACT1*) mRNA, which codes for structural protein actin as a control for each sample.

Strain construction. *GRE3* (*YHR104w*) was amplified from the genomic DNA of strain W303-1B (Table 1). The resulting fragment was cloned into the BglII site between the *PGK* promoter and terminator of the pMA91 vector (27), resulting in plasmid B1165. The *ScXYL2* (*YLR070c*) gene was cloned into the pMA91 vector as described previously (32). The *ScXYL2* gene with the *PGK* promoter and terminator was released from pMA91 by digestion with HindIII and the fragment ligated into the HindIII site of the YEp24H (1) vector (plasmid B1180). The PCR fragment containing *ScXYL2* also was inserted into the BglII site of plasmid B1181, which contains the *PGK* promoter and terminator from the pMA91 vector as a HindIII fragment cloned into the corresponding site in the YEplac195 vector (13) (plasmid B1523). The *XYL1* gene of *P. stipitis* was previously amplified by PCR and cloned between the *PGK* promoter and terminator in the pMA91 vector (plasmid B383) (16). The *XYL2* gene of *P. stipitis* also was cloned into pMA91 (plasmid B731) (46). The *XYL2* expression cassette was ligated into the HindIII site of YEplac195 (plasmid B1530) or blunt ended and cloned into the PvuII site of YEp24 vector (17) (plasmid B733).

To give a control strain, the yeast strain H308 (S150-2B) was transformed with the empty pMA91 and YEp24 vectors. The strain H308 was also transformed with corresponding vectors containing the *P. stipitis XYL1* and *XYL2* genes (B383 and B733) to give strain H1356 and with vectors containing the *S. cerevisiae* genes *GRE3* and *ScXYL2* (B1165 and B1180) to give strain MTen. The CEN.PK2 strain was transformed with *GRE3*- and *ScXYL2*-containing vectors (B1165 and B1523) or with *GRE3*- and *P. stipitis XYL2* (B1165 and B1530)-expressing vectors, resulting in strains H2558 and H2560, respectively. Standard recombinant DNA methods were used (25). Yeast transformations were done as described by Gietz et al. (12).

Enzyme activities. XR and XDH activities were measured in cell extracts made by disrupting cells with glass beads either in 100 mM sodium phosphate buffer (pH 7.0) or in 50 mM HEPES buffer (pH 7.0), containing 1 mM $MgCl_2$, 0.1 mM EDTA, and 1 mM dithiothreitol. Both buffers were supplemented with the protease inhibitors phenylmethylsulfonyl fluoride and pepstatin A (final concentrations of 0.17 mg ml^{-1} and 0.01 mg ml^{-1}, respectively). XDH activity was measured as described earlier (32). XR activity was measured in 100 mM sodium phosphate buffer (pH 7.0) containing 1 M xylose and 0.2 mM NADPH as a decrease in A_{340}. Protein was measured with the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, Calif.). All enzymatic and protein analyses were carried out with the Cobas Mira automated analyzer (Roche).

Yeast cultures and metabolite analysis. The recombinant strains were cultured in shake flasks containing synthetic complete (YSC) medium (modified from that of Sherman et al. [37]) either with or without glucose and without either leucine or uracil for plasmid selection. The ratio of xylose to glucose was 19:1 or 20:1, with either 19 or 20 g of xylose liter^{-1} and 1 g of glucose liter^{-1}. Cultures were started from an initial optical density at 600 nm (OD_{600}) of 0.2. Alternatively, no glucose was added and the cultures were started with a high biomass (OD_{600} of approximately 2). Growth was measured as OD_{600}. Cells also were cultivated on 20 g of glucose liter^{-1} in YSC medium without leucine and uracil. For growth on xylulose, a mixture of xylose and xylulose (30 g of xylose liter^{-1} and 10 g of xylulose liter^{-1}) was prepared by isomerization of D-xylose (28). Cultures were made in 50 ml of media and carried out in 250-ml Erlenmeyer flasks at 30°C and 250 rpm on an orbital shaker. Each strain was cultured in duplicate.

Anaerobic growth experiments were performed in a 1.8-liter Chemap CMF bioreactor (Chemap AG, Volketswil, Switzerland) with an initial working volume of 1.2 liters. Mass flow controllers (Bronkhorst High-Tech BV, Ruurlo, The Netherlands) regulated the air and nitrogen flow rates. Nitrogen was passed through an Oxisorb oxygen-absorbing device (Messer Griesheim GmbH, Krefeld, Germany) to reduce the residual oxygen level to >50 ppb. The dissolved oxygen concentration was monitored by an Ingold polarographic probe (Mettler-Toledo, Columbus, Ohio). The temperature was maintained at 30°C, and the pH was controlled at 5.5 by the addition of 2 M NaOH. The medium was YSC without leucine and uracil supplemented with 47.5 g of xylose liter^{-1} and 2.5 g of glucose liter^{-1}. Cultures were started from an initial OD of 8 to 9. The total gas flow rate in this experiment was 0.5 standard liters per minute. Cell growth was measured as OD_{600} and cell dry mass (DM) as described previously (40).

Growth rates (Table 2) were calculated from time periods in which growth, xylose consumption, and xylitol production were linear, i.e., 63 to 121 h (Fig. 1) and 69 to 147 h (Fig. 2A). The growth rate was calculated as the change in DM per hour (gram liter^{-1} hour^{-1}). The specific production and consumption rates

TABLE 2. Growth rates and specific xylose consumption and xylitol production rates for the experiments of Fig. 1 and 2

Genotype[a]	Growth (g of DM liter^{-1} h^{-1})	Xylose consumption (C-mmol g of DM^{-1} h^{-1})	Xylitol production (C-mmol g of DM^{-1} h^{-1})
PsXYL1 PsXYL2[b]	0.014 ± 0.001	2.7 ± 0.1	0.44 ± 0.04
ScGRE3 ScXYL2[b]	0.008 ± 0.001	2.5 ± 0.2	0.89 ± 0.13
ScGRE3 ScXYL2[c]	0.010 ± 0.001	3.5 ± 0.1	2.0 ± 0.1
ScGRE3 PsXYL2[c]	0.011 ± 0.000	3.4 ± 0.0	1.9 ± 0.2

[a] *Ps*, gene originating from *P. stipitis; Sc*, gene originating from *S. cerevisiae*.
[b] Parental *S. cerevisiae* strain S150-2B. The rates were calculated between time points 63 and 121h (Fig. 1).
[c] Parental *S. cerevisiae* strain CEN.PK2. The rates were calculated between time points 69 and 147 h (Fig. 2A).

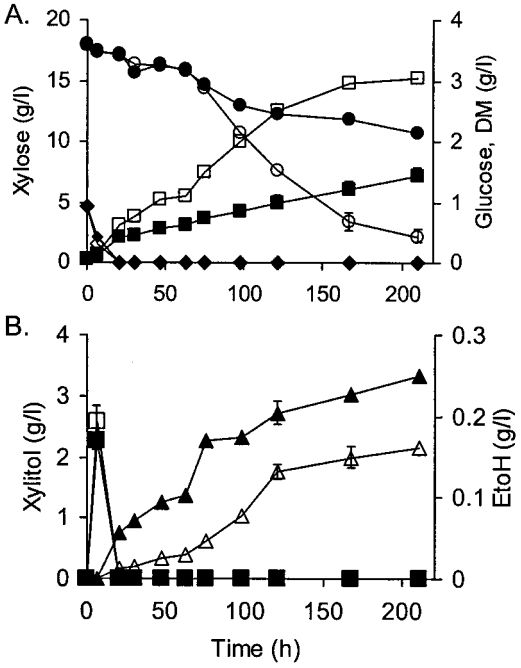

FIG. 1. Comparison of *S. cerevisiae* S150-2B strains harboring XR and XDH either from *P. stipitis* or from *S. cerevisiae* in shake flask cultures. Growth and product formation on 20 g of xylose plus 1 g of glucose liter^{-1} as a carbon source. Filled symbols represent the strain overexpressing *GRE3* and Sc*XYL2* genes, and open symbols represent the strain with *P. stipitis XYL1* and *XYL2* genes. (A) Shown are data for DM (■), glucose (♦), and xylose (●). (B) Shown are data for xylitol (▲) and ethanol (EtOH) (■). Results are based on two replications and are given in grams liter^{-1}. Points without error bars have an associated error that is <10% of the value of the point.

were calculated as C-mmol of substrate consumed or product formed by 1 g of DM per h. The DM values for shake flask cultures were obtained by using a conversion of 0.3 g of DM liter^{-1} for an OD_{600} value of 1 (unpublished data).

The nontransformed strains CEN.PK2 and ENY.WA-1A were cultured in YP medium (10 g of yeast extract liter^{-1}, 20 g of peptone liter^{-1}) with either 20 g of glucose liter^{-1} or 20 g of glucose liter^{-1} and 100 g of xylose liter^{-1}. Cells were harvested by centrifugation (3400 × *g*, 2 min at 20°C) and either frozen in liquid nitrogen (RNA samples) or moved directly to −70°C (samples for enzyme activities).

Metabolite production was analyzed from growth media by high-pressure liquid chromatography with an Aminex HPX-87H column (Bio-Rad Laboratories). The column was maintained at 35°C with an eluant of 5 mM H_2SO_4 at a constant flow rate of 0.6 ml min^{-1}. Glucose, xylose, xylitol, glycerol, acetate, and ethanol were quantified by using a combination of a refractive index and UV (λ = 210 nm) detectors connected in series.

Plasmid stability was measured by plating cells from different time points on plates without selection, growing them for 2 days, and then replicating them on plates of selection media. All of the recombinant strains contained two expression vectors, so we studied possible recombination between the plasmids. Samples were taken when growth was complete, and plasmids were extracted and transformed into *E. coli*. Plasmid DNA was extracted from bacterial transformants and analyzed by restriction enzyme digestion.

RESULTS

Recombinant strains. The XR activities of strains expressing *XYL1*, *XYL2*, *GRE3*, and *ScXYL2* in glucose-grown cultures were similar, 15 to 22 nkat (per mg of total protein), for both *GRE3*- and *XYL1*-encoded enzymes. The XDH activity varied between about 10 nkat (per mg of total protein) for ScXDH and about 90 nkat (per mg of total protein) for the XDH of *P. stipitis*. Over 90% of the cells harbored both plasmids after cultivation for 2 days without auxotrophic selection, and no recombination between the plasmids was detected.

Growth on D-xylose. When the S150-2B-derived strains containing XR and XDH either from *P. stipitis* or from *S. cerevisiae* were cultivated in shake flasks with 20 g of xylose liter^{-1} and 1 g of glucose liter^{-1} as the carbon source, glucose was consumed during the first 10 h, and then xylose consumption began. The strain with the *S. cerevisiae* XR and XDH consumed xylose slower and formed less biomass than did the strain containing the enzymes from *P. stipitis* (Fig. 1A). The control strain (data not shown) used practically no xylose, and growth on glucose and on ethanol derived from glucose resulted in 0.3 g of DM liter^{-1}, compared to 1.5 and 3.0 g liter^{-1} with strains containing *S. cerevisiae* and *P. stipitis* enzymes, respectively. The biomass yield on xylose was 20% for both overexpressing strains.

Significantly more xylitol was formed by the strain with the *S. cerevisiae* enzymes (Fig. 1B). Equal amounts of glycerol were formed (0.1 g liter^{-1}) and subsequently consumed by both strains (data not shown). Acetate (maximally 0.2 g liter^{-1}) was detected only with the strain overexpressing the *S. cerevisiae* enzymes (data not shown).

The cultures grew in a biphasic manner with a period of slow xylose consumption (up to 60 h), followed by a faster metabolic phase (from 63 to 121 h) from which the specific rates (Table 2) were calculated. The strain overexpressing the *S. cerevisiae* genes grew much slower than did the strain with the *P. stipitis* genes. The specific xylose consumption rate differed by 15%, but the specific xylitol production rate was twice as high in the strain overexpressing the *S. cerevisiae* enzymes. The xylitol yield on xylose was about 45% for the strain overexpressing the *S. cerevisiae* enzymes and about 10% for the strain expressing the *P. stipitis* enzymes.

Effect of XDH activity on xylose metabolism of strains expressing *GRE3*. XDH activity in the strains overexpressing the ScXDH-encoding gene was about 10 times lower than in the strains overexpressing the gene for *P. stipitis* XDH. Significantly higher amounts of xylitol were accumulated by the strain carrying the *S. cerevisiae* ScXDH. A CEN.PK2 strain overexpressing the *S. cerevisiae GRE3* and *P. stipitis XYL2* genes grew (Fig. 2A) and consumed xylose at the same rate as a strain carrying the *GRE3* and *ScXYL2* genes (Table 2). The strain expressing ScXDH initially produced xylitol slightly faster than did the strain with the *P. stipitis* XDH, but the final xylitol yield did not differ much, being 55 and 49%, respectively. Also, these strains showed a biphasic growth curve (Fig. 2A). Compared to the S150-2B-based strain, the growth rate of CEN.PK2-based strains was about 1.3 times higher, the specific xylose consumption rate was nearly 1.4-fold higher, and the specific xylitol production rate was over twofold higher, demonstrating the effects of different parental strains on the efficiency of xylose metabolism. Ethanol was produced only from glucose in the beginning of the cultivation, no glycerol was detected, and acetate accumulated in low quantities, maximally 0.6 g liter^{-1}.

In shake flask cultures with xylose (20 g liter^{-1}) as the sole carbon source, the biomass of the strain carrying the *P. stipitis* XDH increased more rapidly than did the biomass of the strain

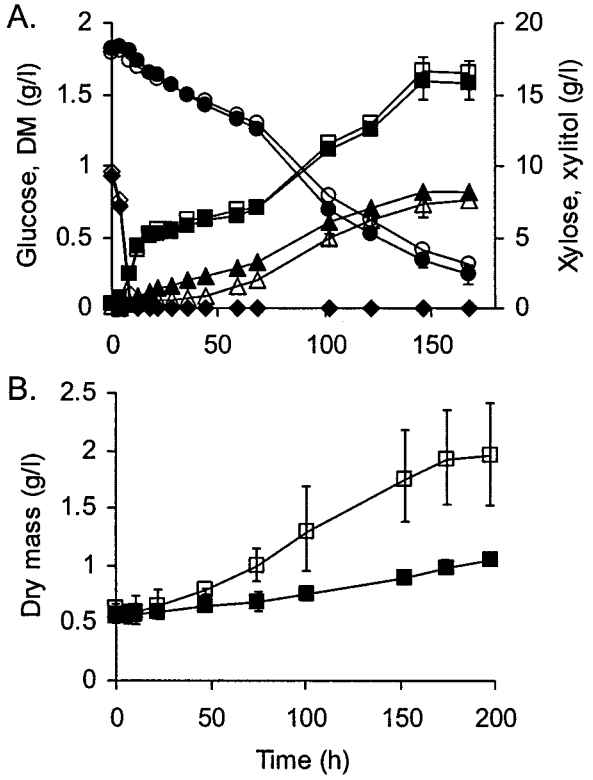

FIG. 2. Xylose metabolism with the CEN.PK2 strains overexpressing *GRE3* and *ScXYL2* (filled symbols) or *GRE3* and the *P. stipitis XYL2* (open symbols) in shake flask cultures. Points without error bars have an associated error that is <10% of the value of the point. Results are based on two replications. (A) Growth and xylose consumption and xylitol formation on 19 g of xylose plus 1 g of glucose liter^{-1} as a carbon source. DM (■), glucose (♦), xylose (●), and xylitol (▲), all in grams liter^{-1}. (B) Growth on 20 g of xylose liter^{-1} as a carbon source.

expressing ScXDH (Fig. 2B). With the latter, strain growth was almost negligible. The strain with *P. stipitis* XDH consumed more xylose than did the strain with *S. cerevisiae* XDH (16.2 versus 9.5 g in 200 h). The xylitol yield was 50% for the strain with *P. stipitis* XDH and 60% for the strain expressing the *S. cerevisiae* XDH-encoding gene. Thus, even 10-fold-higher XDH activity levels did not decrease the high xylitol yield with Gre3p. In bioreactor culture under anaerobic conditions, a small amount of xylitol (about 3.0 g liter^{-1}) was formed from 47.5 g of xylose liter^{-1} and 2.5 g of glucose liter^{-1} in 140 h by both strains, and the xylose was not consumed further. The strain with *P. stipitis* XDH maintained more biomass under anaerobic conditions than did the strain with the *S. cerevisiae* XDH (data not shown).

Transcript pattern of the endogenous xylose pathway genes. The glucose consumption and ethanol formation and subsequent consumption in cultures of nontransformed strains, CEN.PK2 and ENY.WA-1A, with and without xylose were similar (Fig. 3). Xylose metabolism and ethanol consumption began after glucose was exhausted. With the CEN.PK2 strain, biomass of the culture containing xylose and glucose continued to increase even after glucose, ethanol, and acetate consumption had stopped. The CEN.PK2 and ENY.WA-1A strains

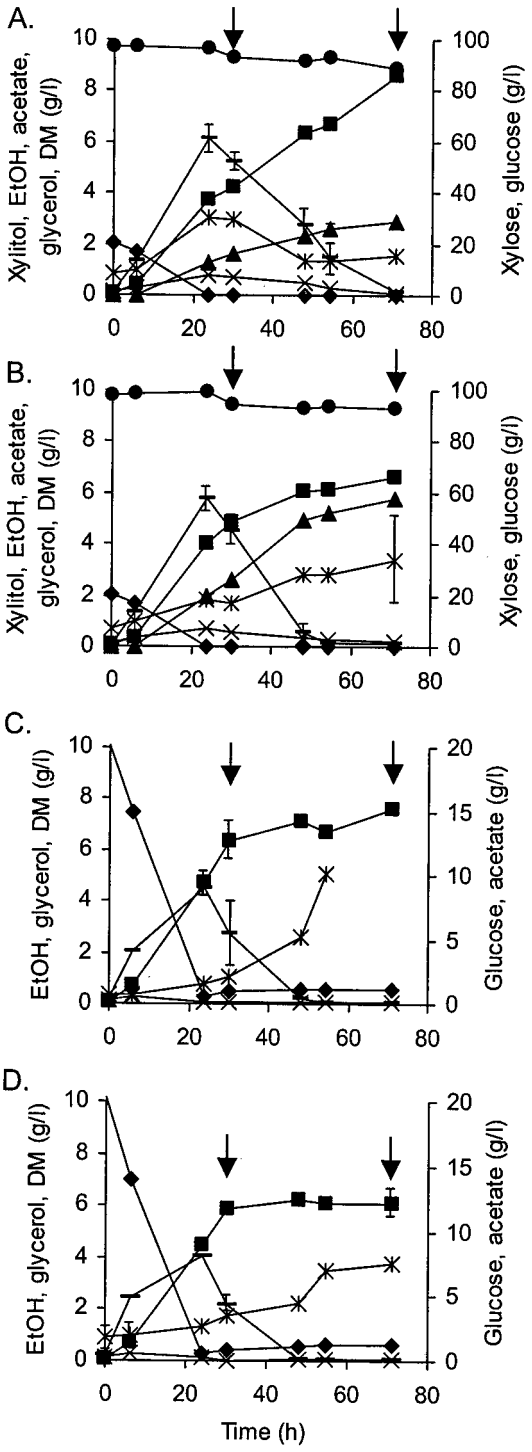

FIG. 3. Metabolism of nontransformed *S. cerevisiae* strains on glucose with and without xylose in shake flask cultures. Substrate consumption and main product formation in 100 g of xylose plus 20 g of glucose liter^{-1} (A, B) and 20 g of glucose liter^{-1} (C, D) media. Points without error bars have an associated error that is <10% of the value of the point. Results are based on two replications. Panels A and C, the CEN.PK2 strain; panels B and D, the ENY.WA-1A strain. DM (■), xylitol (▲), xylose (●), glucose (♦), ethanol (EtOH) (-), glycerol (×), and acetic acid (*), all in grams liter^{-1}. Time points for gene expression analysis are indicated by arrows.

used about 9.0 and 6.0 g of xylose liter^{-1} and produced 3.0 and 5.5 g of xylitol liter^{-1}, respectively. The ENY.WA-1A strain converted almost all of the xylose to xylitol, but the CEN.PK2 strain excreted only one-third of the xylose consumed as xylitol. This shows that *S. cerevisiae* strains differ in their ability to metabolize xylose.

RNA samples were taken at 29 h, after glucose depletion but while ethanol consumption and xylose metabolism were in progress, and at 70 h, when xylose alone was being slowly consumed. XR and XDH activities were measured at the same time.

ScXYL2 was not induced in the presence of xylose, but the expression in the presence of xylose remained two- to threefold higher than in the control culture at 70 h. The expression of *SOR1* increased 12- and 220-fold (time point, 29 h) and 21- and 3-fold (70 h) compared to that of the nontransformed control cultures (data not shown). The XDH activity was 30- and 125-fold higher at both 29 and 70 h for the ENY.WA-1A and CEN.PK2 strains, respectively (data not shown). Therefore, the presence of xylose resulted in higher XDH activity and induced the expression of the *SOR1* gene coding for SDH, which also has XDH activity.

The expression of *GRE3* was not affected by xylose in the ENY.WA-1A strain but was 2.6 times higher in the CEN.PK2 strain than in the control at 29 h (data not shown). XR activity was very similar in both cultures and was 4 and 20 times lower than the XDH activity for ENY.WA-1A and CEN.PK2, respectively (data not shown).

XKS1 was not induced in the presence of xylose, and its expression was 2 to 10 times lower than that in the control culture (data not shown). Furthermore, in the CEN.PK2 strain, the *XKS1* expression on xylulose was 1.6 times lower than in glucose-grown cells at the early growth phase (0.7 g of DM liter^{-1}, 5 h) and showed no increase during the later growth phase (2.1 g of DM liter^{-1}, 21 h; data not shown). A multicopy expression construct in which *XKS1* was controlled by an *ADH1* promoter had over 60 times higher expression than cells grown on xylulose. Thus, neither xylulose nor xylose induced *XKS1* expression.

The genes encoding transketolase (*TKL1*) and transaldolase (*TAL1*) were expressed at significantly higher levels in the presence of xylose than in the control culture. *TKL1* expression increased about 10-fold in both strains at 29 h, while *TAL1* expression increased by two- to eightfold (data not shown). At 72 h, *TKL1* expression was not much higher than in the control, but *TAL1* expression was still six- to eightfold higher than in the control.

DISCUSSION

Overexpression of the endogenous genes *GRE3* and *ScXYL2* enabled *S. cerevisiae* to grow on xylose in the presence of glucose in aerobic shake flask cultures. Relative to an *S. cerevisiae* strain expressing XR and XDH from *P. stipitis*, however, strains expressing the endogenous genes grew slower and accumulated more xylitol.

The accumulation of xylitol by the strain overexpressing *ScXYL2* could be due to low XDH activity, but the xylitol yield decreased by <10% when *ScXYL2* was replaced with the *P. stipitis XYL2*. A more likely explanation for the xylitol accumulation in the strain overexpressing the *S. cerevisiae GRE3* and *ScXYL2* is the strict NADPH specificity of Gre3p, since XRs accepting only NADPH as a cofactor cannot supply the NAD^+ needed by the XDH reaction (8). In anaerobic conditions, where NAD^+ regeneration is even lower due to lack of respiration, the strain overexpressing the endogenous enzymes was unable to utilize xylose. The NADPH specificity of Gre3p must create a severe redox imbalance, resulting in xylitol accumulation and the inability to metabolize xylose anaerobically.

The XR and XDH activities in xylose-utilizing yeasts (3, 5, 22, 36) and the XR activity in *S. cerevisiae* (4) increased when cells were grown on xylose. We detected an increase in only the XDH activity of *S. cerevisiae*. At the mRNA level, the *SOR1* gene encoding SDH was induced on xylose. Thus, the increased *SOR1* expression may lead to the higher SDH/XDH activity detected. The reason van Zyl and coworkers (44) did not detect XDH activity may be because they measured XDH activity in glucose-grown cells. Contradictory to Batt and coworkers (4) but in agreement with van Zyl and coworkers (44), we did not see an increase in the XR activity when *S. cerevisiae* was grown in the presence of xylose but detected a low level of activity with and without xylose. The differences between our results and the results of Batt and coworkers may be strain dependent.

Several putative aldose reductase (*GRE3*, *YPR1*, and *YJR096w*)- and polyol dehydrogenase (*ScXYL2*, *SOR1*, and *YDL246c*)-encoding genes exist in the *S. cerevisiae* genome, but no clear physiological functions have been attributed to the corresponding enzymes. It has been postulated that Ypr1p is involved in isoleucine catabolism and fusel alcohol formation, as it has activity with 2-methylbutyraldehyde (10). The *GRE3* gene is up-regulated in stress conditions, such as osmotic and oxidative stress, high temperature, and carbon starvation (2, 11, 30, 31). It may have a role in detoxification of methylglyoxal synthesized in response to stress (2). The transcriptional analysis of xylose and glucose cultures performed in our laboratory did not show any xylose-specific responses for these genes, except for *SOR1* (L. Salusjärvi, unpublished results).

The observation that the XDH activity and the *TKL1* and *TAL1* expression were induced in the presence of xylose, in addition to *S. cerevisiae* being able to grow on xylulose, suggests that *S. cerevisiae* has in its evolutionary past consumed xylose. On the other hand, the absence of induction on xylose and a wide substrate specificity of Gre3p indicate that the enzyme also has other roles. In the construction of recombinant, xylose-utilizing *S. cerevisiae* strains, the endogenous Gre3p and ScXDH cannot replace the *P. stipitis* XR and XDH enzymes before the redox constraints of the pathway are solved.

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