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Arabinose Utilization by Xylose-Fermenting Yeasts and Fungi

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ABSTRACT

Various wild-type yeasts and fungi were screened to evaluate their ability to ferment L-arabinose under oxygen-limited conditions when grown in defined minimal media containing mixtures of L-arabinose, D-xylose, and D-glucose. Although all of the yeasts and some of the fungi consumed arabinose, arabinose was not fermented to ethanol by any of the strains tested. Arabitol was the only major product other than cell mass formed from L-arabinose; yeasts converted arabinose to arabitol at high yield. The inability to ferment L-arabinose appears to be a consequence of inefficient or incomplete assimilation pathways for this pentose sugar.

Index Entries: L-arabinose; pentose; metabolism; ethanol; fermentation.

INTRODUCTION

Production of fuel ethanol from renewable materials offers the potential of reducing the nation's increasing dependence on imported oil and alleviating the atmospheric accumulation of carbon dioxide, the principle greenhouse gas (*1,2*). For these reasons, the US Department of Energy (DOE)/National Renewable Energy Laboratory (NREL) has established an Ethanol from Biomass project to develop economic, high-yield processes for producing fuel ethanol from renewable lignocellulosic materials. Inexpensive, abundant biogenic materials being considered as feedstocks include hardwoods, herbaceous crops, and agricultural residues.

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Biomass materials exhibit a wide range of susceptibilities to conversion because of structural and compositional differences. However, the dominant components in all types of lignocellulosic biomass are cellulose, hemicellulose, and lignin (*3*). In general, saccharification rates and yields on native lignocellulosics are low, making some type of pretreatment necessary to achieve high enzymatic hydrolysis yields. A variety of pretreatment processes exist, most based on a combination of mechanical, physical, and chemical processing steps (*4*). NREL currently favors dilute acid prehydrolysis as the pretreatment method for a commercial biomass-to-ethanol process (*5,6*). In the dilute acid process, chipped and/or milled biomass particles of nominal 1-mm size are impregnated with approx 1% (w/w) sulfuric acid (liquid basis) and then incubated at 140–160°C for a period ranging from several minutes to an hour. NREL researchers have characterized the susceptibility of many short-rotation woody and herbaceous crops and agricultural residues to dilute acid pretreatment (*7–11*). Dilute acid pretreatment hydrolyzes hemicellulose and solubilizes hemicellulosic sugars. Hemicellulose removal increases the porosity of lignocellulosic materials and improves enzymatic digestibility; cellulose conversion yields increase substantially following dilute acid pretreatment. Achieving high conversion yields of hemicellulosic and cellulosic sugars is necessary for favorable process economics because feedstock costs represent roughly one-half of total projected ethanol production costs in this type of process (*5,6,12*).

In the context of conversion of hemicellulosic sugars, fermentability of L-arabinose becomes important. The approximation is often made that hydrolyzates generated by dilute acid pretreatment contain only D-xylose because D-xylose is the most abundant hemicellulosic sugar. As a result, most studies on conversion of hemicellulose hydrolyzates focus on conversion of D-xylose. However, hemicelluloses are heteropolysaccharides containing both substituted pentosans and hexosans (*13*). Although xylan is the dominant pentosan and glucomannan the dominant hexosan, the levels of arabinan are significant in some biomass materials.

Table 1 shows the pentosan and nonglucan hexosan composition of six hardwood species and seven types of herbaceous crops (*11,14,15*). (Only nonglucan hexosan components are listed because total glucan includes glucan in the nonhydrolyzed cellulose fraction.) For all species listed, pentosans make up more than 90% of total nonglucan carbohydrate, with the principal component being xylan. As Table 1 shows, however, approximating hydrolyzate monosaccharide composition as D-xylose alone is not always justified. In particular, arabinan levels are significant in herbaceous species. Whereas arabinan makes up only 2–4% of total nonglucan carbohydrate in hardwoods, arabinan represents 10–20% of total nonglucan carbohydrate in many herbaceous crops. Microbial biocatalysts selected or developed to ferment hydrolyzates derived from materials with high arabinan content must therefore exhibit the ability to

Table 1
Composition of Hardwood and Herbaceous Feedstocks

Biomass type	Composition, wt%, dry basis						Total nonglucan hexosan and pentosan	Arabinan Fraction		Ref.
	Pentosan			Nonglucan hexosan				Arabinan to pentosan ratio, %	Arabinan to total nonglucan ratio, %	
	Xylan	Arabinan	Total	Galactan	Mannan	Total				
Hardwood species										
Silver maple	17.1	0.7	17.8	0.0	1.2	1.2	19.0	3.93	3.68	14
Sycamore	16.3	0.6	16.9	0.0	0.9	0.9	17.8	3.55	3.37	14
Black locust	16.2	0.4	16.6	0.0	1.0	1.0	17.6	2.41	2.27	14
Poplar hybrid NE388	14.6	0.3	14.9	0.3	0.5	0.8	15.7	2.01	1.91	11
Poplar hybrid N11	11.3	0.3	11.6	0.7	0.3	1.0	12.6	2.59	2.38	11
Sweetgum	17.5	0.4	17.9	0.3	0.4	0.7	18.6	2.23	2.15	11
Herbaceous species										
Switchgrass	16.1	2.2	18.3	1.2	0.0	1.2	19.5	12.02	11.28	11
Weeping lovegrass	17.6	2.6	20.2	1.7	0.0	1.7	21.9	12.87	11.87	11
Sericea lespedeza	14.5	1.6	16.1	0.9	0.0	0.9	17.0	9.94	9.41	11
Reed canary grass	9.8	2.4	12.2	0.1	0.0	0.1	12.3	19.67	19.54	15
Flatpea hay	7.4	2.0	9.4	1.5	0.1	1.6	11.0	21.28	18.18	15
Corn cobs	28.4	3.6	32.0	1.1	0.0	1.1	33.1	11.25	10.88	14
Corn stover	21.5	1.8	23.3	1.0	0.0	1.0	24.3	7.73	7.41	14

ferment L-arabinose, in addition to D-xylose, and preferably other sugars as well, to ethanol.

Numerous bacteria, yeasts, and fungi are being considered for use in processes for converting hemicellulosic hydrolyzates to ethanol (*16*). The literature suggests that xylose-fermenting bacteria are superior to xylose-fermenting yeasts and fungi in their ability to ferment arabinose. Fermentation of arabinose to ethanol at significant yields has been reported for wild-type strains of *Sarcina ventriculi* (*17*) and *Bacillus macerans* (*18*), for example, and for a recombinant strain of *Erwinia chrysanthemi* (*19*). Ethanologenic recombinant *Escherichia coli* ATCC 11303 (pLOI297) has been reported to convert arabinose to ethanol at near-theoretical yield (*20*). The recombinant strains achieve high yields because they have been genetically engineered to express the pyruvate decarboxylase gene (*pdc*) from *Zymomonas mobilis;* only a few bacteria, including *Z. mobilis* and *S. ventriculi,* naturally carry the *pdc* gene (*21*).

Although the fermentation of arabinose to ethanol at appreciable yields is possible using certain bacteria, the literature on arabinose fermentation by yeasts and fungi is inconclusive. Trace levels of ethanol production from arabinose have been reported for a fungal *Mucor* species, but close examination of the data suggests that only assimilation of arabinose occurs (*22*). Similarly, literature data support the contention that arabinose is assimilated, but not fermented by *Fusarium oxysporum* (*23,24*). There is scant definitive evidence of arabinose fermentation by fungi. A strain of *Neurospora crassa* produced a final ethanol concentration of more than 4 g/L from a medium containing 2% (w/v) D-arabinose, the non-naturally occurring form (*25*). Fermentation of L-arabinose to ethanol by fungi has been reported for a novel *Paecilomyces* species, *Paecilomyces* sp. NF1 (*26*). A strain of *Paecilomyces lilacinus* has also been reported to ferment arabinose (the optical rotation of the pentose was not specified) (*27*).

There is conflicting literature on L-arabinose fermentation by wild-type xylose-fermenting yeasts. A 1959 report suggests that L-arabinose can be fermented to ethanol by *Candida tropicalis,* although only trace levels of ethanol were detected (*28*). On the other hand, a comprehensive survey of over 400 yeasts showed that none were able to utilize L-arabinose anaerobically; however, the inability to utilize D-xylose by these same yeasts was also noted (*29*). Roughly half of these yeasts (214) were able to grow aerobically on D-xylose, whereas only about one-quarter (111) grew aerobically on L-arabinose. Assimilation of L-arabinose has been reported for *C. tropicalis* and *Pachysolen tannophilus* under both aerobic and fermentative conditions, and trace levels of ethanol production from L-arabinose have been observed for the xylulose-fermenting yeast *Schizosaccharomyces pombe* (*30*). More recent investigations indicate that L-arabinose is assimilated, but not fermented by the yeasts *Pichia stipitis* and *Candida shehatae* (*31–35*).

Since it is difficult to draw definitive conclusions from these studies, further research is warranted to characterize better the ability of xylose-fermenting yeasts and fungi to ferment L-arabinose. Therefore, the study described here was undertaken to clarify if generalizations can be made about the ability of xylose-fermenting yeasts and fungi to ferment L-arabinose to ethanol.

MATERIALS AND METHODS

Organisms

Fifteen strains were tested: eight fungal strains, six yeast strains, and a single bacterium. The fungal strains tested were: *Aspergillus oryzae* NRRL 3488; *Fusarium oxysporum* (nonpathenogenic C5); *Fusarium oxysporum* (lycopersici race 2); *Fusarium oxysporum* (dianthi from Mexico); *Penicillium chrysogenum* NRRL 811; *Paecilomyces* sp. NF1 (ATCC 20766); *Rhizopus oryzae* NRRL 5834; and *Rhizopus formosaencii* NRRL 295. The yeast strains examined were: *C. shehatae* NRRL Y-17024; *C. tropicalis* NRRL Y-11860; *P. tannophilus* NRRL Y-2460; *P. tannophilus* NRRL Y-12891; *P. stipitis* NRRL Y-7124; and *Torulopsis sonorensis* NRRL Y-7800. The bacterial strain tested was *E. chrysanthemi* C1R219. All strains designated with an NRRL-strain-type number were obtained in lypholyzed form from the US Department of Agriculture (USDA) National Center for Agricultural Utilization Research (NCAUR), formerly the Northern Regional Research Laboratory (NRRL). The *Fusarium* and *Erwinia* strains were kindly supplied on plates by R. Baker of the Department of Plant Pathology at Colorado State University. Lypholized cultures were resuspended in and then plated on yeast-peptone-dextrose (YPD) medium containing, in g/L: yeast extract, 5; peptone, 5; and glucose, 10 (and for plates, agar, 15). After growing up the cultures at 29°C, plates were stored at 4°C.

Medium

All experiments were carried out using defined minimal yeast nitrogen base (YNB) medium (Difco Laboratories) supplemented with mixtures of L-arabinose, D-xylose, and D-glucose. The concentrations depended on the experiment. The initial mixed-sugar study using *P. stipitus* was carried out at a pH of 5.5 using 77 mmol phosphate buffer (a mixture of 0.10% [w/v] monobasic potassium phosphate and 0.005% dibasic disodium phosphate). Yeast and fungal cultures were buffered at pH 4.5 with 73.5 mmol (0.1% [w/v]) monobasic potassium phosphate; the bacterial culture was buffered at pH 7.0 with 70.7 mmol phosphate buffer (a mixture of 0.033% [w/v] monobasic potassium phosphate and 0.033% dibasic sodium phosphate). All chemicals used were of reagent grade or higher purity.

Experimental Protocol

Experiments were conducted in duplicate in unbaffled 250-mL DeLong® culture flasks (Bellco Glass, Inc.). The first experiment examined the effect of sugar mixtures on fermentation by *P. stipitis*.Three sugar mixtures were examined: D-xylose and D-glucose; D-xylose and L-arabinose; and a mixture of D-xylose, D-glucose, and L-arabinose. Xylose alone was also run as a control. Total initial sugar concentrations were 30–40 g/L, and initial culture volumes were 75 mL. Flasks were incubated on a rotary shaker at 150 rpm and stoppered by venting exhaust gases through a water trap from 24 h elapsed time onward to promote oxygen limitation. In the comparative screening experiments, total initial liquid medium volume was 100 mL, but in this case flasks were stoppered with Morton closures (metal caps) rather than water traps, and cultivation was carried out at 29°C and 200 rpm. In both experiments, flasks were periodically sampled and, if necessary, base (1*N* NaOH) was added to maintain pH.

Analytical

Samples were analyzed for pH using an Orion 720A pH meter with the probe calibrated between pH 4.0 and 7.0 using buffer standards. Optical densities (OD) of yeast culture broths were determined at 600 nm using a Milton Roy Spectronic 20D spectrophotometer. Samples were diluted to be in the linear range of this instrument (absorbance below 0.6). Final dry cell weights (DCW) were determined in the initial mixed-sugar study by centrifuging duplicate 10-mL samples for 10 min at 9000 rpm in a Sorvall RC-28S refrigerated centrifuge operating between 4 and 10°C. Pellets were resuspended in phosphate buffer, recentrifuged, and then resuspended in distilled water and centrifuged again. Washed pellets were resuspended in a small quantity of distilled water, quantitatively transferred to preweighed aluminum tare dishes, and then dried to constant weight in an 80°C oven. OD values were converted to cell mass concentration (in g DCW/L) using a correlation factor developed from final dry cell mass and OD measurements.

Broth supernatants were analyzed by high-performance liquid chromatography (HPLC) using a Hewlett-Packard (HP) 1090L HPLC equipped with an HP 1047A refractive index detector. HPLC analyses were carried out using either a Bio-Rad HPX-87H organic acid analysis column operating at 65°C with a 0.01*N* sulfuric acid mobile-phase flow rate of 0.6 mL/min or a Bio-Rad HPX-87P carbohydrate analysis column operating at 85°C with a deionized-water mobile-phase flow rate of 0.6 mL/min. Calibration curves developed for each component of interest were used to quantify solute concentrations.

RESULTS

Representative xylose-fermenting microorganisms were screened to evaluate their ability to ferment L-arabinose under oxygen-limited conditions when grown in defined minimal media containing mixtures of L-arabinose, D-xylose, and D-glucose. The first experiment examined the conversion of different sugar mixtures by the yeast *P. stipitus*. Figure 1 shows averaged concentration profiles (average of duplicate flasks) for fermentation of the three sugar mixtures examined, as well as for a comparative pure-xylose control. As the figure shows, ethanol reached a concentration of 8 g/L fermenting xylose alone or the mixture of xylose and glucose, but only 3 g/L on the mixture of xylose and arabinose. In the mixture of all three sugars, ethanol accumulated to an intermediate concentration of 6 g/L. Cell mass concentrations in flasks containing xylose or a mixture of xylose and glucose reached 9 and 8 g DCW/L, respectively. Slightly lower values of 7 g DCW/L were attained in the other mixtures. Final ethanol yields based on consumed sugar (not shown) were 0.30 g/g for flasks containing xylose or the mixture of xylose and glucose. Final yields were 0.20 g/g for the other two mixtures. Low levels of xylitol were formed in flasks containing the mixture of xylose and glucose. No xylitol formation was observed when all three sugars were present, however, possibly because of lower glucose levels than in the xylose-glucose mixture.

Figure 1 shows that arabinose concentrations remained high when this sugar was present, even after other sugars were exhausted. Although there was a slight decrease in arabinose concentration after other sugars were depleted, no production of ethanol was observed. More extensive comparative screening experiments were therefore carried out to examine if the inability to ferment arabinose was a trait specific to *P. stipitis* or a more common characteristic of xylose-fermenting yeasts and fungi. The bacterium *E. chrysanthemi* was included (single flask) in the screening study to provide a comparative reference. To facilitate identification of microorganisms at least capable of aerobically utilizing arabinose, the aeration level was increased by capping flasks with Morton closures rather than water traps and incubating at a higher shaker rotation rate (200 rpm). Although all strains readily consumed glucose and xylose, arabinose utilization characteristics varied widely, as summarized in Table 2.

As Table 2 shows, the bacterium, yeasts, and a majority of the fungi consumed arabinose. No products other than cell mass (not shown) were produced from arabinose by the bacterium and arabinose-consuming fungi, whereas the primary product of arabinose metabolism by yeasts was arabitol (*see* discussion *below*). As anticipated, the bacterium *E. chrysanthemi* achieved the highest maximum rate of arabinose consumption, 0.59 g/L/h. Although a lag was observed in the onset of pentose sugar

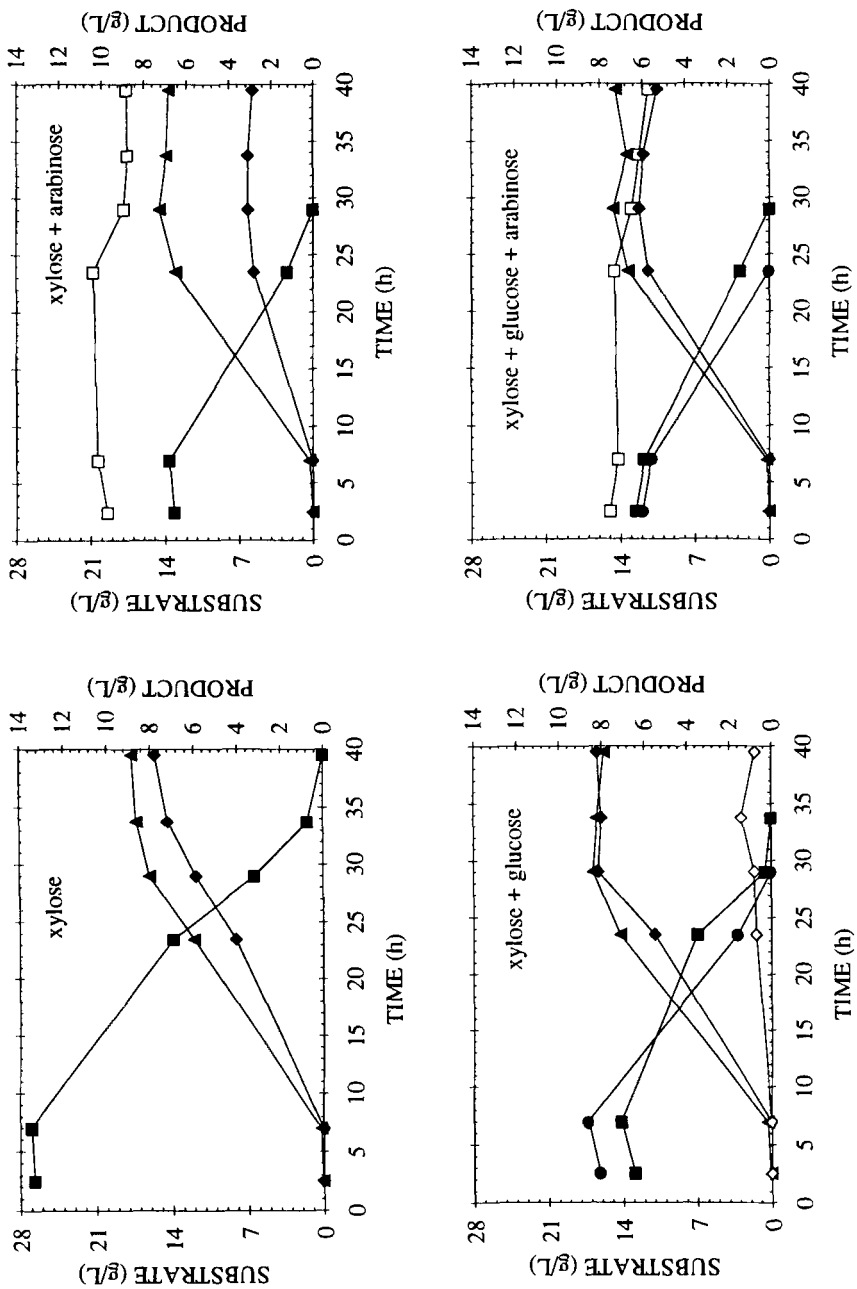

Fig. 1. Shake-flask fermentation of sugar mixtures by *P. stipitis*. Symbols represent xylose (■), arabinose (□), glucose (●), DCW (▲), ethanol (◆), and xylitol (◇).

Table 2
Arabinose Utilization Characteristics of Screened Microorganisms

Organism	Arabinose consumed	Mode of utilization[a]	Soluble product(s) other than cell mass
Bacteria			
E. chrysanthemi C1R219	Yes	Simultaneous	N.D.[b]
Yeasts			
C. shehatae	Yes	Unclear	Arabitol
C. tropicalis	Yes	Simultaneous xyl > arab	Arabitol
P. tannophilus Y-2460	Yes	Sequential	Arabitol
P. tannophilus *Y-12891*	Yes	Sequential	Arabitol
P. stipitis	Yes	Sequential	Arabitol
T. sonorensis	Yes	Sequential	Arabitol
Fungi			
A. oryzae	Yes	Sequential	N.D.
F. oxysporum nonpathogenic C5	Partial	Simultaneous xyl >> arab	N.D.
F. oxysporum sp. lycopersici	Partial	Simultaneous xyl >> arab	N.D.
F. oxysporum sp. dianthi	Partial	Simultaneous xyl >> arab	N.D.
Paecilomyces sp. NF-1	Possible	Simultaneous	N.D.
P. chrysogenum	No		
R. kazanensis (oryzae)	No		
R. formosaencii (oryzae)	No		

[a]In mixed-sugar medium; xyl = xylose; arab = arabinose.
[b]N.D. = not detected.

consumption, xylose and arabinose were utilized simultaneously at similar rates (Fig. 2). In contrast, fungi and yeasts preferentially consumed xylose at a higher rate than arabinose, regardless of whether simultaneous or sequential consumption occurred. Maximum arabinose consumption rates for yeasts were in the range of 0.20–0.30 g/L/h, and for fungi 0.05–0.10 g/L/h. Simultaneous utilization of xylose and arabinose was observed for *F. oxysporum* fungal species (Fig. 3) as well as for the yeast *C. tropicalis* (Fig. 4). *F. oxysporum* (Fig. 3) produced a small amount of ethanol (3 g/L) from xylose, but no soluble products from arabinose. In contrast, *C. tropicalis* (Fig. 4) converted xylose to xylitol and arabinose to arabitol, but produced no ethanol. Calculated arabitol yield on consumed arabinose was 1.02 g arabitol produced/g arabinose consumed, essentially equal to the theoretical maximum yield of 1.01 g/g (*see* discussion *below*). Other yeasts such as *P. stipitis* (Fig. 5) showed a sequential or

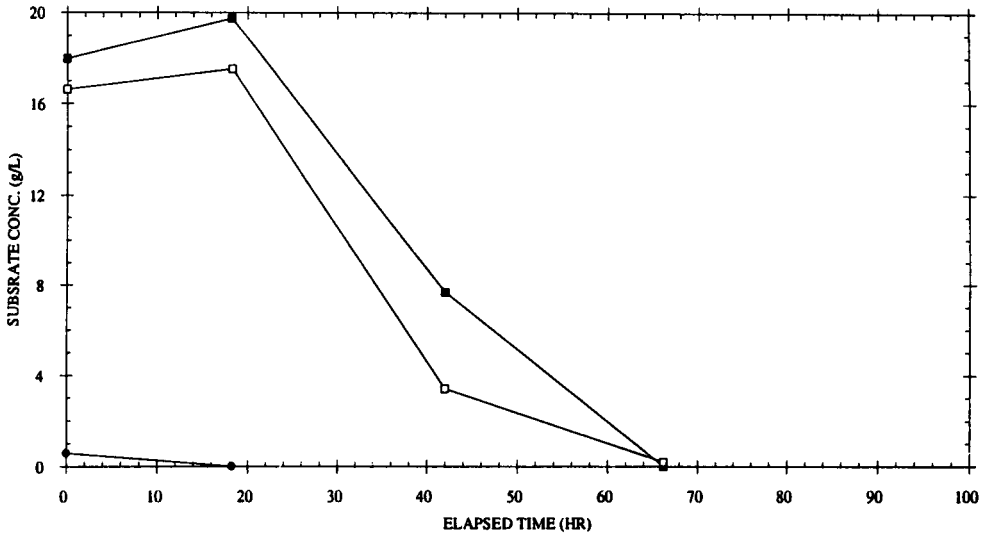

Fig. 2. Arabinose utilization by *E. chrysanthemi*. Symbols represent xylose (■), arabinose (□), and glucose (●).

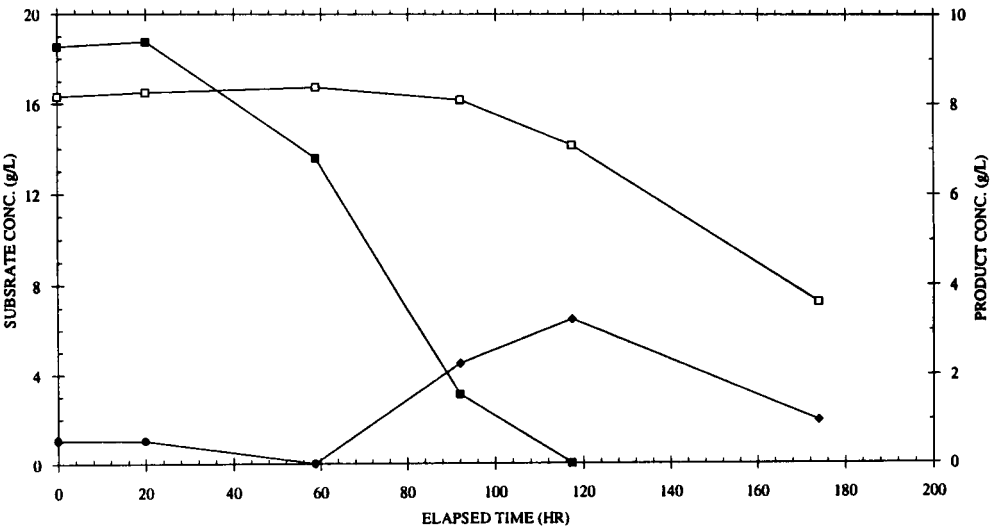

Fig. 3. Arabinose utilization by *F. oxysporum* (nonpathogenic C5). Symbols represent xylose (■), arabinose (□), glucose (●), and ethanol (◆).

diauxic pattern of pentose sugar utilization, with substantial rates of arabinose consumption occurring only after xylose concentrations fell to low levels. As Fig. 5 shows, *P. stipitis* converted xylose to ethanol at high yield and produced significant quantities of arabitol from arabinose. Optical density measurements (bacterium, yeasts) (not shown) and visual observations (fungi) indicated that cell mass was produced from xylose and arabinose by the arabinose-consuming cultures. Dry cell weight measurements were not made, however, so the extent of cell growth on pentoses is uncertain.

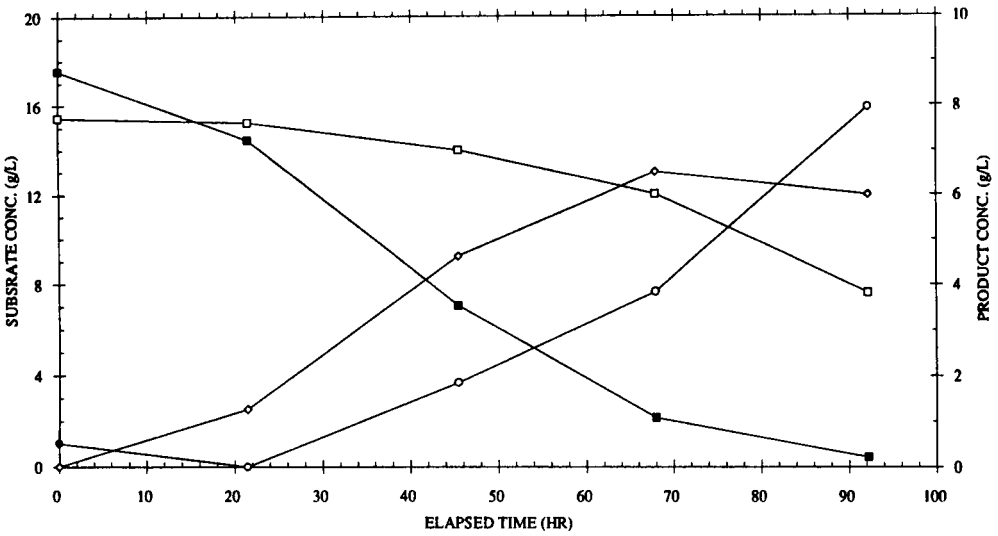

Fig. 4. Arabinose utilization by *C. tropicalis*. Symbols represent xylose (■), arabinose (□), glucose (●), arabitol (○), and xylitol (◇).

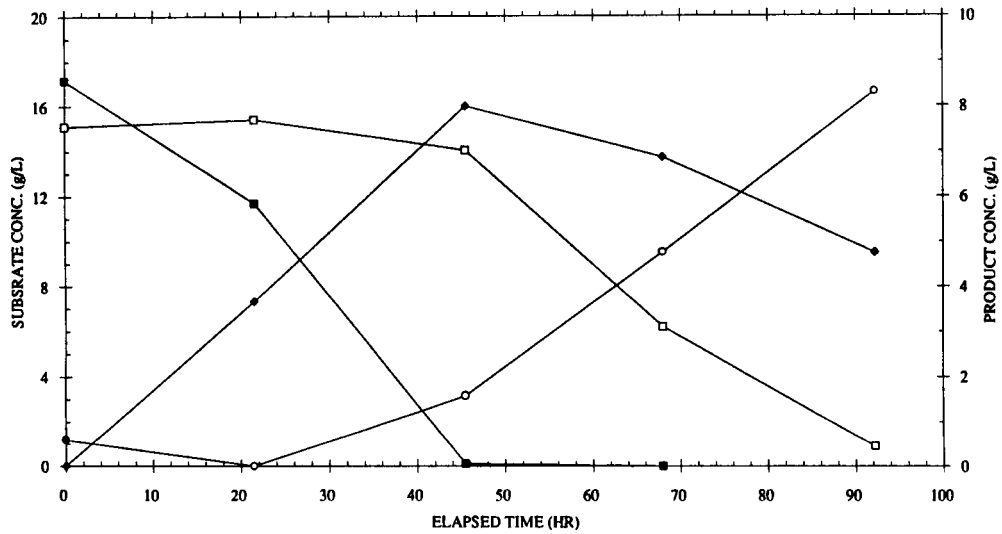

Fig. 5. Arabinose utilization by *P. stipitis*. Symbols represent xylose (■), arabinose (□), glucose (●), arabitol (○), and ethanol (◆).

DISCUSSION

Results of the comparative screening experiments suggest that many xylose-fermenting yeasts and fungi can aerobically assimilate arabinose, but are unable to ferment arabinose to ethanol. Whereas the yeasts converted xylose to cell mass, xylitol and/or ethanol, they only converted arabinose to cell mass and arabitol. In contrast, the fungi converted both xylose and arabinose primarily to cell mass, although in some cases (e.g.,

Fig. 3), some ethanol was produced from xylose. The relatively low levels of ethanol produced in these experiments (below 8 g/L in all cases) are attributed to the higher level of aeration relative to the first experiment.

The slow disappearance of ethanol observed in Figs. 3 and 5 after xylose was exhausted can be attributed to evaporation and aerobic reassimilation. A significantly higher rate of ethanol disappearance was observed with *P. stipitis* (Fig. 5) relative to *F. oxysporum* (Fig. 3), suggesting that *P. stipitis* reassimilated ethanol, since evaporation rates should be similar. The fact that arabinose consumption continued during periods of decreasing ethanol concentration supports the conclusion that these organisms are not able to ferment arabinose. The lower aeration level used and the apparent lag in the onset of arabinose utilization after exhaustion of xylose probably explain why appreciable utilization of arabinose was not observed in the first experiment using *P. stipitis*. Whereas the first experiment was continued for only 10 h after xylose was depleted (Fig. 1), the second experiment was continued for 46 h (Fig. 5).

In describing and discussing the results, it has been assumed that arabitol is formed from arabinose and that xylitol is formed from xylose, as has been documented for other pentose-assimilating yeasts (*36*). This assumption is supported by a close examination of the data. For example, Fig. 4 strongly suggests that *C. tropicalis* produces xylitol from xylose, since the concentration of arabinose remains constant during the first 20 h when only xylitol is produced. Similarly, from 70 to 90 h when only arabitol is produced, the rate of arabinose consumption is high, whereas the rate of xylose consumption is quite low, implying that this organism produces arabitol from arabinose. The profile for *P. stipitis* (Fig. 5) also supports the hypothesis that arabitol is formed from arabinose, since arabitol continues to be produced after xylose has been exhausted.

Provided this assumption is correct, the most interesting finding from these experiments is that the yeasts all produced substantial quantities of arabitol from arabinose, but no other soluble products. A review of the literature suggests that this trait is common in many yeasts. For example, screening experiments examining the ability of *S. pombe, C. tropicalis,* and *P. tannophilus* yeast strains to ferment L-arabinose identified L-arabitol as the major metabolic product (*29*). However, reported yields of arabitol on consumed arabinose were below 0.8 g/g in all cases. Thus, the calculated theoretical conversion of arabinose to arabitol achieved by the *C. tropicalis* strain examined in our studies is noteworthy.

Production of arabitol may be similar in many respects to the production of other polyhydric alcohols (polyols) produced by microorganisms, including glycerol, erythritol, mannitol, and xylitol (*37*). Many yeasts are recognized to produce pentitols from pentoses at appreciable yield; for example, xylitol from D-xylose, L-arabitol from L-arabinose, and ribitol from D-ribose (*36*). A number of processes for production of arabitol and xylitol have been developed, most based on the use of sugar- and salt-

tolerant yeasts being cultivated in high-sugar media (*38*). An example is the use of a mutant strain of *C. tropicalis* to produce xylitol from D-xylose at a yield above 95% of theoretical (1.01 g pentitol/g pentose) (*39*). Intracellular accumulation of polyols (coupled with accumulation in extracellular medium) has been shown to occur under high-sugar and/or high-salt conditions, and it has been suggested that, intracellularly, polyols function, in part, as compatible solutes that protect intracellular enzymes from inactivation or inhibition under conditions of low water activity (*37,40*). However, we used relatively low sugar and salt concentrations in our experiments, so other reasons for arabitol formation probably account for the phenomenon of arabitol production in the xylose-fermenting yeasts surveyed here.

The basis for arabitol formation is likely a direct consequence of the metabolic pathways for arabinose assimilation. Although knowledge of pentose fermentation pathways remains incomplete, microorganisms putatively convert pentoses to glycolytic intermediates that are then converted to pyruvate, via either Embden-Meyerhof-Parnas (EMP) or Entner-Doudoroff (ED) reaction pathways. Pyruvate is then fermented to products such as ethanol, lactate, and acetate; product distribution varies according to the microorganism used and the cultivation conditions employed. Many fermentation products are produced by bacteria, whereas the principal product of pyruvate fermentation by yeasts is ethanol (*21*). In order to be converted to glycolytic intermediates, pentoses, such as D-xylose and L-arabinose, are first converted to D-xylulose-5-phosphate (X-5-P) and/or D-ribose-5-phosphate (Ri-5-P) (*41,42*). These pentose phosphates are then rearranged by transaldolase- and transketolase-mediated pentose phosphate cycle (PPC) reactions to form glyceraldehyde-3-phosphate (G-3-P) and fructose-6-phosphate (F-6-P) (*43,44*), molecules that are catabolized to pyruvate by the EMP or ED pathways. Some microorganisms, notably *Lactobacillaceae,* convert X-5-P to G-3-P and acetylphosphate using phosphoketolase (*21,45*).

There are significant differences in how bacteria, yeasts, and fungi metabolize D-xylose and L-arabinose to PPC pentose phosphate precursors. In particular, many bacteria directly isomerize aldopentoses to their respective ketose forms, which are then phosphorylated. Such bacteria, including *E. coli* and *Aerobacter aerogenes* (reclassified as *Klebsiella pneumoniae*), convert D-xylose to D-xylulose and L-arabinose to L-ribulose, and then phosphorylate these molecules to X-5-P and L-ribulose-5-phosphate (Ru-5-P), respectively (*41,42,45–47*). Through the action of epimerases and isomerases, Ru-5-P can be converted to either X-5-P or Ri-5-P. Epimerization of Ru-5-P forms X-5-P. Further epimerization leads to D-ribulose-5-phosphate, which can be isomerized to form Ri-5-P. As discussed above, X-5-P and Ri-5-P are putatively converted to a mixture of G-3-P and F-6-P in the PPC, with 3 mol of pentose phosphate yielding a theoretical maximum of 2 mol of F-6-P and 1 mol of G-3-P.

In contrast to the direct isomerization pathway, yeasts, fungi, and a few bacteria assimilate pentoses using multiple-step redox processes in which aldopentoses are first reduced to their respective pentitols and then oxidized to their ketose forms prior to phosphorylation (*41,42,48–50*). Thus, D-xylose is reduced to xylitol, and then xylitol is oxidized to form D-xylulose, which is subsequently phosphorylated. Again, X-5-P can be epimerized and isomerized to Ru-5-P to provide precursors for PPC rearrangements. The pathway for L-arabinose assimilation is similar, but longer than that for D-xylose (*42,50*). L-arabinose is first reduced to form L-arabitol, then oxidized to L-xylulose, and then reduced again to form xylitol, an achiral pentitol, after which conversion to X-5-P and Ri-5-P proceeds as for D-xylose.

We hypothesize that differences in the redox pathways for L-arabinose assimilation, in which at least four enzymes are required for conversion to D-xylulose, in comparison to D-xylose assimilation, where only two are required, are the reason that high-yield fermentation of L-arabinose to ethanol remains rare in xylose-fermenting yeasts and fungi. The metabolic requirement to produce assimilatory pathway enzymes (and maintain the genes encoding for them) is greater for assimilation of L-arabinose than for D-xylose. In cases where assimilation of L-arabinose does not occur, for example for several of the fungi examined in this work, it is likely that the microorganisms do not possess one or more of the necessary assimilatory enzymes (or genes). Similarly, in cases where L-arabinose is converted principally to arabitol, as for the yeast *C. tropicalis*, it is probable that assimilatory enzymes downstream of the initial reductive conversion step are absent. In contrast, microorganisms that grow on L-arabinose, but do not ferment L-arabinose to ethanol (or other products) clearly possess a functioning assimilation pathway. In such cases, it is likely that inability to ferment L-arabinose results in part from the relatively inefficient multistep redox assimilation pathway. Maintaining proper enzyme and cofactor levels for this pathway might require more metabolic energy than can be obtained by fermentative substrate-level phosphorylation. If so, more aerobic conditions enabling energy production by oxidative phosphorylation would be needed for the pathway to function. This would explain why production of cell mass from L-arabinose is favored over production of fermentation products. Further experimentation to elucidate the details of pentose metabolism is needed to test this hypothesis, however, since other factors, such as differences in transport, regulation, and cofactor requirements, may also play an important role.

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