PRODUCTION OF ETHANOL FROM WOOD HEMICELLULOSE HYDROLYZATES

BY A XYLOSE-FERMENTING YEAST MUTANT, CANDIDA SP. XF 217

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SUMMARY
Ethanol was produced from wood chip hemicellulose hydrolyzate by a xylose-fermenting yeast mutant, Candida sp. XF 217. The rates of D-xylose consumption and ethanol production were greater under aerobic than fermentative conditions. The slow rate of fermentation under fermentative conditions could be overcome by supplementing the broth with D-xylose isomerase (glucose isomerase). The ethanol yield, as based on the sugar consumed, was approximately 90% of the theoretical value.

INTRODUCTION
Next to D-glucose, D-xylose is one of the most abundant carbohydrates derived from woods (18) and agricultural residues (9). D-Xylose resides in the hemicellulose portion of the plant cell wall. It is often in the form of branched chain heteropolysaccharides of mixed hexosans and pentosans. D-Xylose and L-arabinose are the constituents of pentosans while D-glucose, D-mannose and D-galactose are the constituents of hexosans (16).

The hemicellulose portion of cell walls can be hydrolyzed to constituent carbohydrates easily by either acid, chemical, or enzymatic hydrolysis. The hydrolysis products contain a mixture of sugars with D-xylose as the major component (2,9). Many bacteria (13) and mycelial fungi (7,17) assimilate and convert D-xylose to a variety of products including ethanol. In general, the yields of ethanol are low and the microorganisms have low ethanol tolerance.

Traditionally, pentoses were considered as being non-fermentable by yeasts. Recently, several yeasts have been found to produce ethanol from D-xylose, especially when aerobic incubation conditions are used (6,8,14). Furthermore, a mutant strain, XF217, derived from a xylose-utilizing-xylitol-producing yeast Candida sp. that produces significant
quantities of ethanol at the expense of xylitol production was isolated and characterized in our laboratory (5).

In this communication, we use XF217 to produce ethanol from hemicellulose hydrolyzate which was derived from hardwood.

MATERIALS AND METHODS

Microorganism: Stock culture of Candida sp. XF217 (5) was maintained on peptone, malt-extract, yeast-extract, glucose agar (Difco) slants.

Substrates: Wood chip hemicellulose hydrolyzate was prepared by the methods as described by Ladisch (11). Wood chips of 50% moisture were hydrolyzed with 5% (w/v) of sulfuric acid at 100°C for 4 hours. The hemicellulose hydrolyzate was then recovered from the wood chips by down-flow leaching at 80°C using water. The hydrolyzate was then neutralized by calcium oxide to a pH of 3 followed by the addition of sodium hydroxide to a pH of 6. The hydrolyzate contains the following neutral sugars (w/v): D-glucose, 0.4%; D-xylose, 4.4%; and L-arabinose, 0.5%.

Cultures: VMP media was used for yeast growth and had the following composition per liter: Yeast-extract, 3 g; malt-extract, 3 g; Bacto-peptone, 5 g; and D-xylose, 10 g. Yeast were grown aerobically in 100 ml VMP media in 250 ml Erlenmeyer flasks for 24 hours at 30°C in an incubator-shaker.

Fermentation: Shake flask experiments were conducted in 50 ml Erlenmeyer flasks, each containing 20 ml of substrates. Inocula were prepared by growing the yeasts in flasks in VMP media. After incubation, yeast cells were collected by centrifugation in a clinical centrifuge. After washing the yeast pellets with sterile water, yeasts having a cell density of 3 x 10^8 cells/ml were inoculated into substrates to carry out fermentation on a reciprocal incubator-shaker at 200 rpm and 30°C.
Aerobic Fermentation: Flask cultures used for aerobic fermentation were capped with milk filters and aluminum foil.

Anaerobic Fermentation: The fermentation was carried out in flasks with rubber stoppers; carbon dioxide produced was allowed to escape through a syringe needle.

Fermentation Using Yeasts and D-xylose Isomerase: Immobilized whole-cell preparation of Bacillus sp (Sweetzyme type Q, lot BA-117-0298, 220 IU/g, Novo Biochemical Industries, Inc.) in the amounts of 10 g per liter were added into substrate in addition to yeasts to carry out anaerobic fermentation.

Sugar Consumption and Product Formation: Sugar consumption and non-volatile products formed were analyzed and quantified by low-pressure liquid chromatography as described by Ladisch and Tsao (10). The column packing material was Aminex 50W-X4 (BioRad) in the Ca²⁺ form and the operating conditions were the same as those described earlier (1).

Other Determination: The concentration of ethanol produced was measured by gas chromatography. Sugar alcohols produced were identified and verified by paper chromatography (15) and glucose concentration was measured with a Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, CA).

RESULTS AND DISCUSSION

The fermentation of wood chip hydrolysate was conducted under aerobic and fermentive conditions. Incubation was also carried out under fermentive conditions with immobilized Bacillus sp as the xylose isomerase source (1).

Under aerobic incubation conditions, D-xylose was consumed by yeasts with the production of ethanol as a metabolic product (Fig. 1a). D-Glucose was utilized by yeasts within the first six hours of incubation, presumably to produce ethanol, while the utilization of L-arabinose was
Figure 1. Production of ethanol from wood chips. Hemicellulose and cellulose by C. ANAEROBIC + GI, B. ANAEROBIC, C. ANAEROBIC with glucose fermentation.

A. AEROBIC

B. ANAEROBIC

C. ANAEROBIC + GI

XYLOSE OR ARABINOSE (G/L)

ETHANOL (G/L)
slow. Small amounts of xylitol (0.1%) and arabinol (0.05%) were also produced in addition to ethanol. The sugar alcohols were presumably derived from D-xylose and L-arabinose, respectively. Upon exhaustion of D-xylose, the ethanol produced was utilized by yeasts. This was not observed when oxygen-limited fermentation conditions were employed. Under fermentative conditions, the rates of D-xylose consumption and ethanol production were about one-third of that under aerobic conditions. Ethanol was the fermentation product and L-arabinose was not utilized (Fig. 1b).

When xylose isomerase was present under fermentative conditions the rates of D-xylose consumption and ethanol production were similar to that under aerobic conditions (Fig. 1c). The amounts of ethanol formed remained high after the exhaustion of D-xylose.

The yields of ethanol as based on sugar consumed was 90% of the theoretical value. The results indicate that:

1. D-xylose is readily consumed by XF217 under either aerobic or fermentative incubation conditions resulting in the production of ethanol as the metabolic product.
2. The presence of oxygen enhanced the rate of D-xylose consumption and ethanol production.
3. The product, ethanol, could be utilized by yeast under aerobic incubation conditions when D-xylose was consumed.
4. When D-xylose isomerase was present, yeasts utilized D-xylose under fermentative conditions at a rate similar to that under aerobic conditions.
5. D-xylose isomerase catalyzed the isomerization of D-xylose to D-xylulose (3), and D-xylulose is a better substrate than D-xylose for yeast under fermentative conditions.

Previously, we have observed that many yeasts including Saccharomyces cerevisiae are able to ferment D-xylulose to ethanol in high yields even though they are unable to metabolize D-xylose effectively (1,3). These observations suggested D-xylulose is the better substrate for yeasts and oxygen is required to maintain the high rate of D-xylose utilization. The enhanced rate of D-xylose utilization under aerobic conditions was also observed in other D-xylose-utilizing-ethanol-producing yeasts such as Pachysolen tannophilus (14) and C. tropicalis (8).

661
Normally yeasts metabolize D-xylene to produce xylitol as metabolic products (4, 12). The use of yeast mutants such as Candida sp. XF217 could overcome this problem with respect to ethanol production from D-xylene.

In conclusion, the hemicellulose portion of cellulosic materials could be hydrolyzed easily to a mixture of D-glucose and D-xylene. These sugars can readily be converted to ethanol by yeasts such as Candida sp. XF217. The same yeasts could also be used to produce ethanol from wood processing waste.

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REFERENCES