

CONVERSION OF XYLOSE TO ETHANOL UNDER AEROBIC
CONDITIONS BY *CANDIDA TROPICALIS*

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SUMMARY

Candida tropicalis converts xylose to ethanol under aerobic, but not anaerobic, conditions. Ethanol production lags behind growth and is accelerated by increased aeration. Adding xylose to active cultures stimulates ethanol production as does serial subculture in a medium containing xylose as a sole carbon source.

INTRODUCTION

Recent reports have shown that some yeasts will ferment the ketose sugar, xylulose, after it is formed from xylose through the action of xylose (glucose) isomerase (Wang, *et al.*, 1980; Gong, *et al.*, 1981). Of 42 yeasts screened for this trait, *Candida tropicalis* fermented xylulose at a rate exceeding that attained by any others tested, including two strains of *Schizosaccharomyces pombe* and five strains of *Saccharomyces cerevisiae* (Jeffries and Choi, 1981). Moreover, unlike *S. pombe* and *S. cerevisiae*, *C. tropicalis* will readily assimilate xylose. We decided, therefore, to see if aeration would enhance the fermentation of xylulose. Since we generally

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employ mixtures of xylose and xylulose to test for xylulose fermentation, we ran control cultures containing pure xylose. Ethanol was detected in these aerobic xylose cultures.

While this manuscript was in preparation, an account of ethanol production from xylose by another yeast (*Pachysolen tannophilus*) was published (Schneider *et al.*, 1981).

MATERIALS AND METHODS

Candida tropicalis ATCC 1369, *Candida* sp. ATCC 28528 and *Candida utilis* ATCC 22023 were obtained from the American Type Culture Collection, Rockville, Md. *Kluyveromyces fragilis* was obtained from J. D. Macmillan, Rutgers University, New Brunswick, N.J. All organisms were maintained on slants of yeast malt agar (Difco) at 27°C. For liquid cultivation, cells were grown in 0.67% yeast nitrogen base (YNB, Difco) plus 7.5% (w/v) xylose (Sigma, grade II). Inocula consisted of 18-hour-old cells. These were either washed in distilled water to remove contaminating glucose and other nutrients, or 1.0 ml was subcultured directly from YNB xylose medium into 32 ml of fresh medium. In either case, the initial optical density (O.D.) was 0.3 to 0.5 at 525 nm. Standard culture conditions employed 33 ml of YNB xylose medium in a 125 ml Erlenmeyer, shaken on a rotary shaker at 200 rpm (2.5 cm radius). Anaerobic conditions were similar except that flasks were fitted with sterile No. 5 rubber stoppers and flushed aseptically with N₂ for 15 minutes after inoculation or sampling. Higher aeration rates were obtained by using the same amount of medium in either 300 ml Erlenmeyer flasks or baffled 300 ml Erlenmeyer flasks at 200 or 400 rpm.

Ethanol was determined by gas chromatography on a packed glass column (120 cm x 0.2 cm) of Chromosorb 101 at 165°C, using helium as a carrier gas and a flame ionization detector. The identity of ethanol was confirmed by use of alcohol dehydrogenase (Sigma).

Xylose was determined by the method of Nelson (1944).

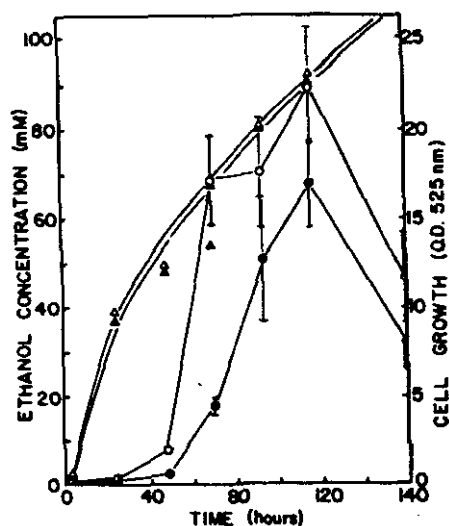
RESULTS

Under standard conditions, growth preceded rapid ethanol production. The initial growth rate was rapid, rising to an O.D. of 11 in the first 24 hours, and only trace amounts (0.3 to 1.2 mM) of ethanol were detected during this period. After 24 to 48 hours, the growth rate decreased and ethanol began to accumulate in the medium. Cultures inoculated with cells which had been previously grown in YNB xylose medium exhibited a more rapid appearance of ethanol than cultures inoculated with cells grown in YNB glucose (Fig. 1). After 4 to 5 days, ethanol concentrations decreased, presumably as a result of assimilation.

Adding xylose on day three to cultures which were actively producing ethanol increased the amount of ethanol formed and decreased the rate of its disappearance from the medium (Fig. 2). Relatively high concentrations of xylose were still present at the time of the second addition, and the rates of xylose utilization were similar before and after addition. If 15% instead of 7.5% xylose was present initially, however, the initial growth rate was slowed and the time of ethanol formation was delayed by about 24 hours, presumably as a result of sugar inhibition (data not shown).

The lag time for ethanol production could be shortened by increasing the aeration rate, but this was achieved at the expense of decreased ethanol concentrations and yields (Fig. 3). If cultures were switched from aerobic to anaerobic conditions after one day, ethanol production was effectively inhibited (Fig. 2). Maximal ethanol production might occur at O_2 concentrations lower than those tested here.

Three other yeasts (*Candida* sp. ATCC 28528, *Candida utilis* ATCC 22023, and *Kluyveromyces fragilis*) capable of xylose assimilation and xylulose fermentation were tested for their capacities to convert xylose to ethanol under the standard conditions used for *C. tropicalis* and were found to be negative.



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Figure 1. *C. tropicalis* was grown in either YNB-glucose (solid symbols) or YNB-xylose (open symbols) medium and washed prior to inoculation into fresh YNB-xylose medium. Growth (Δ , Δ) and ethanol ($\circ$, $\bullet$) were followed. Brackets show the range of values obtained in duplicate cultures.

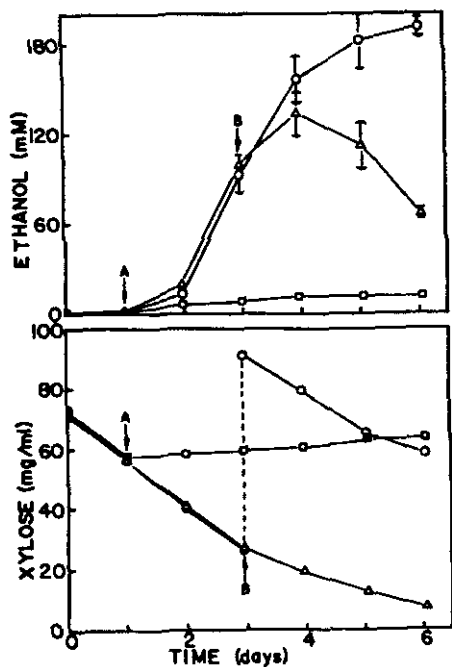


Figure 2. *C. tropicalis* was sub-cultured four times in YNB-xylose medium. Cultures were incubated under standard aerobic conditions. After 24 hours, some flasks were switched to anaerobic conditions (Δ , $\square$). After 3 days, additional xylose (7.5% sterile, solid) was added to some flasks (B, $\circ$). The remaining flasks continued under initial conditions (Δ). Brackets show the standard deviations obtained in triplicate cultures.

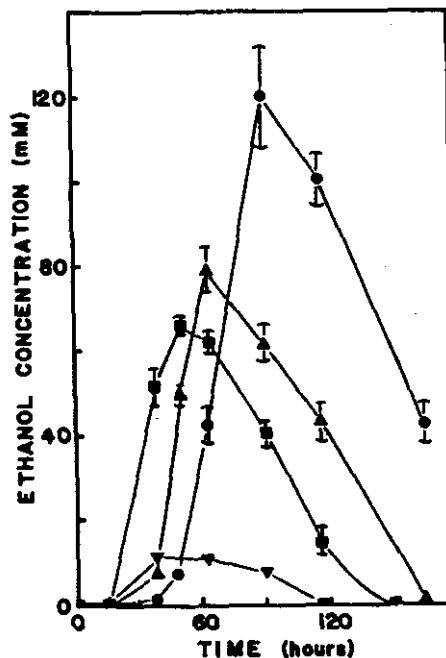


Figure 3. Flasks containing 32 ml of YNB-xylose medium were inoculated with 1.0 ml each of *C. tropicalis* prepared as in Fig. 2. Aeration conditions were as follows: 125 ml Erlenmeyer, 200 rpm ($\bullet$); 300 ml Erlenmeyer, 200 rpm ($\blacktriangle$); 300 ml Erlenmeyer, 400 rpm ($\blacksquare$); 300 ml baffled Erlenmeyer, 400 rpm ($\blacktriangledown$).

DISCUSSION

These data and the previous report by Schneider, *et al.* (1981) show that *Candida tropicalis* and *Pachysolen tannophilus* convert xylose to ethanol under aerobic conditions. Many yeasts are capable of assimilating xylose under aerobic conditions (Biely, *et al.*, 1978; Lodder, 1971). and many will ferment xylulose (Gong, *et al.*, 1981), but of 434 species tested, none will carry out a classical anaerobic fermentation of xylose (Barnett, 1976). *Schizosaccharomyces pombe* readily ferments xylulose but does not oxidize xylose, whereas *C. utitis* readily assimilates xylose but ferments xylulose only slowly (Wang, *et al.*, 1980). The three other yeasts reported herein will both assimilate xylose and ferment xylulose, but they are negative for the production of ethanol from xylose under aerobic conditions. The bases for these traits are not completely understood, but they are believed to stem from differences in predominant metabolic pathways and mechanisms of metabolic regulation (Flickinger, 1980).

In order to assimilate xylose, yeasts and fungi convert xylose to xylulose via xylitol as an intermediate. The initial reduction from xylose to xylitol generally requires NADPH, a cofactor which is most readily supplied by isocitrate dehydrogenase in the TCA cycle (Horecker, 1962). Presumably, it is this requirement which renders xylose utilization obligately aerobic.

In order to convert xylose to ethanol under aerobic conditions, it is necessary to have active Embden Meyerhoff and pentose phosphate pathways which are not repressed by air under the conditions employed. Respiratory activity in many yeasts can be impaired by the presence of excess glucose. (Sols, *et al.*, 1971). It is possible that a similar effect is responsible for the enhancement of ethanol production observed following the secondary addition of xylose.

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