Vol. 94, No. 1, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 14, 1980 Pages 248-254

FERMENTATION OF A PENTOSE BY YEASTS*

Patrick Y. Wang, Charles Shopsis and Henry Schneider

Molecular Genetics Group, Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada, K1A 0R6

Received March 27, 1980

SUMMARY

Several yeasts have been found to be able to ferment D-xylulose, a catabolite of D-xylose, and to produce ethanol thereby. The fermentation is carried out by several species which can utilise D-xylose oxidatively as well as by several which cannot do so. Xylose itself, and the other aldopentoses, are not utilised anaerobically by yeasts. Fermentation of D-xylulose by D-xylose oxidising species indicates that a control operates under conditions of low oxygen tension which prevents the catabolism of D-xylose to D-xylulose. The results are pertinent in efforts to obtain yeasts which can ferment biomass pentoses, a problem of interest in attempts to obtain a liquid fuel from a renewable resource.

INTRODUCTION

The yield of ethanol in the fermentation by yeasts of some saccharified plant polysaccharides is limited by a compositional factor. Yeats can ferment the component hexoses, but not the pentoses. Although many yeasts can utilise five-carbon sugars aerobically, none have been described which can do so anaerobically (1,2). The availability of yeasts which can ferment pentoses would be of interest because the content of five carbon sugars in plant biomass can be significant. The pentose content can approximate 40% that of the carbohydrate of some agricultural wastes (taken as pentosan plus cellulose) (3) and can exceed 30% that of some trees (3,4). Pentoses can be present also in spent sulfite liquor in amounts approximating 40% that of the hexoses (5). The most commonly occurring pentose is probably D-xylose.

*National Research Council of Canada Publication Number 18197.

0006-291X/80/090248-07$01.00/0

Part of the objective of the present study was to determine the extent to which yeasts already possess the biochemical processes necessary to ferment pentoses. It was found that critical components of such processes exist in several yeasts of different genera and species, as shown by their ability to ferment D-xylulose, a catabolite of D-xylose. This finding provides insight into factors controlling pentose utilisation under anaerobic conditions, and suggests specific avenues to obtain yeasts which can ferment pentoses.

The present investigation had its origin in the observation that D-xylulose supported growth of several yeasts when air was present (6). Among such organisms are included several which can utilize D-xylose, as well as several which can utilise neither this compound nor xylitol, the catabolite of D-xylose immediately preceeding D-xylulose (7). Growth on D-xylulose of yeasts which do not assimilate D-xylose is hypothesized (6) to depend on the presence of an enzyme acting as a D-xylulose kinase, the resultant D-xylulose 5-phosphate then entering the pentose phosphate pathway. Yeasts of this sort, Saccharomyces cerevisiae, Saccharomyces carlsbergensis and Schizosaccharomyces pombe, are used in the fermentation industry to produce ethyl alcohol. This relationship prompted investigation of the ability of such organisms, as well as that of D-xylose-oxidizing species, to ferment D-xylulose.

METHODS AND MATERIALS

Fermentations were carried out in tightly sealed, screw-cap vials, 2 ml capacity, filled with 1.7-1.8 mls of medium plus 0.1-0.2 mls of inoculum. A small air bubble was left in each vial. The filled vials were rotated continuously at 30 rpm at 30°C. Ten to fifteen vials were used for each experiment, a vial being discarded after it was opened to obtain a sample for alcohol analysis. Growth was followed turbidimetrically by measuring optical density at 600 nm.

Ethyl alcohol was determined by gas chromatography, using methanol as an internal standard, and D-xylulose was determined by the cysteine-carbazole method (8). The growth medium was 0.6% Yeast Nitrogen Base (Difco) plus 1% D-glucose or D-xylulose. Except when using Schizosaccharomyces pombe the inoculum consisted of cells grown to mid-log phase aerobically in yeast nitrogen base plus 1% glucose, washed twice in 0.1% KH_2PO_4 and resuspended in this salt solution to the volume of the original culture. The inoculum for Schizosaccharomyces pombe was prepared identically except that it was grown in 1% malt extract broth.

D-xylulose was prepared by pyridine epimerisation (9) or by enzymic isomerisation using a commercial preparation of insolubilised glucose isomerase (Maxazyme, Gist-Brocades nv, Delft, Holland) (10). Final purification was performed on an anion-exchange column in the bisulfite form (11). Purity was assayed by thin-layer chromatography and gas-chromatography coupled with mass-spectrometry of the alditol acetates (12) and of the silylated oxime (13). No evidence was found for the presence of contaminating sugars at levels exceeding 1%.

RESULTS

Ethyl alcohol was produced by Schizosaccharomyces pombe NCYC 132 growing on D-xylulose in amounts similar to that when grown on glucose. In a typical experiment (Figure 1), the maximum amount of alcohol produced was 0.45%. The rate of alcohol production was slower on D-xylulose, 2-3 days being required for attainment of the maximum, versus 36-48 hours for glucose. D-xylulose was not as efficient a growth substrate as glucose, the final optical density being lower by about 50%, a factor of interest where the objective is the production of alcohol rather than biomass.

Conversion efficiency (14) of D-xylulose to ethanol was estimated to be at least 85%. Evaluation of conversion efficiency necessitates knowledge of the metabolic pathway involved. It was computed assuming that since glucose and D-xylulose produced similar amounts of ethanol, critical aspects of their metabolic pathways must be identical. The pathway proposed for D-xylulose catabolism is initial phosphorylation to D-xylulose 5-phosphate, conversion of D-xylulose

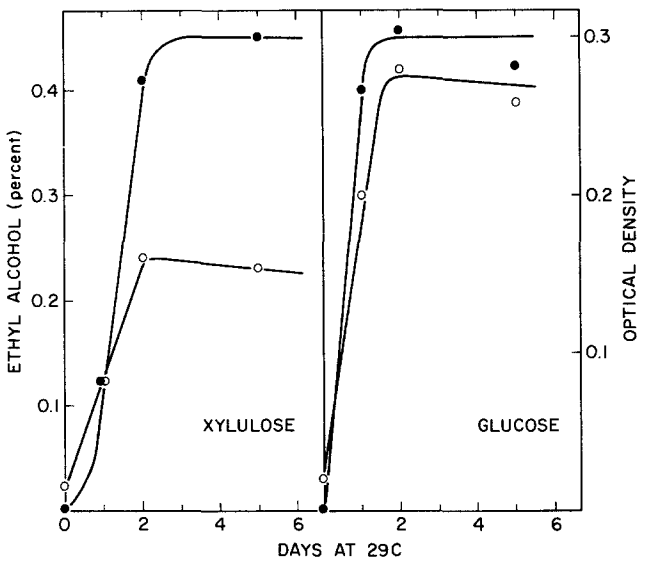

FIGURE 1 Alcohol production and growth of Schizosaccharomyces pombe on 1% D-xylulose or 1% D-glucose: left side, D-xylulose, right side, glucose; ● ethyl alcohol, o optical density at 600 nm.

5-phosphate to D-glucose 6-phosphate non-oxidatively by enzymes of the pentose shunt, followed by conversion of the D-glucose 6-phosphate to ethanol via the Embden-Meyerhof-Parnas pathway. On this basis, six moles of D-xylulose 5-phosphate produce five moles of D-glucose 6-phosphate (15), and each mole of glucose 6-phosphate leads to two of CO_2 and two of ethanol.

The value of 85% for efficiency is considered to be a minimum. All of the D-xylulose was assumed to have been utilised, whereas some small amount could have been left in the medium. Also, no account was taken of other products which might be produced during fermentation (14). The minimum efficiency computed for Schizosaccharomyces pombe is comparable with the value of 93-96% obtained in the fermentation of glucose by a brewers yeast (14).

Alcohol was produced at a lower rate by Saccharomyces cerevisiae YGSC X2180-1B growing on D-xylulose than by Schizosaccharomyces pombe, 0.17-0.19% being attained after

20-30 days. However, conversion efficiency was comparable, 85%. Growth of Saccharomyces cerevisiae on D-xylulose was also much slower than on glucose, the optical density after 20-30 days on the pentose being one third that after 2-3 days on the hexose. Saccharomyces carlsbergensis 15 exhibited behavior similar to Saccharomyces cerevisiae, but conversion efficiency was not evaluated.

Alcohol was produced as well by yeasts which utilise D-xylose only oxidatively. After 15-20 days, the alcohol concentration was 0.36% for Kluyveromyces lactis NRCC 202029 and 0.28% for Saccharomyces amurcae NRCC 2198. When D-xylose was substituted for D-xylulose under identical conditions, optical density changes associated with growth were insignificant and only traces of alcohol were produced, the amount never exceeding 1% of that from D-xylulose. Candida utilis NRCC 204023 produced a much smaller amount of ethanol when given D-xylulose than the other two xylose-oxidising yeasts tested, 0.014% after 30 days. When D-xylose was substituted, 0.002% alcohol was produced. Conversion efficiency was not evaluated for the xylose-oxidising yeasts.

DISCUSSION

The ability to ferment D-xylulose by yeasts which can utilise xylose only oxidatively indicates that when oxygen tension is low D-xylose catabolism is regulated to prevent the formation of D-xylulose. Candidates for such sites of regulation are the D-xylose transport system and the two enzymes involved in the conversion of D-xylose to D-xylulose by yeasts; D-xylose reductase (D-xylose → xylitol) and xylitol dehydrogenase (xylitol → D-xylulose) (7). The controls could be exerted at either the gene or the gene product level. The possibility

that anoxic inhibition of transport prevents fermentation has been suggested for some hexoses and glycosides in various yeasts (16). A mitochondrial factor may also be involved in transport inhibition (17).

From the viewpoint of conversion of biomass pentoses to ethanol, the results are of interest in suggesting specific experimental approaches to obtain yeasts which can ferment a pentose such as D-xylose. For yeasts which can oxidise D-xylose, one approach is to use mutagenesis to eliminate or cicumvent the controls preventing anoxic utilisation. A more general approach, because it would be applicable to yeast which oxidise pentoses as well as to those which do not, is to use genetic engineering techniques. It may be possible to introduce into yeasts appropriate genes from other organisms which can ferment pentoses.

ACKNOWLEDGEMENTS

Thanks are due to Drs. D.R. Bundle, R. Deslauriers and H.C. Jarrell for confirming the identity of the D-xylulose used. Thanks are also due to Mr. F.P. Cooper for assistance in carrying out analyses and to Mr. J.L. Labelle for assistance in preparing the D-xylulose.

REFERENCES

1. Barnett, J.A. (1976) Adv. Carbohydr. Biochem. 32, 125-234.
2. Suomalainen, H., and Oura, E. (1971) The Yeasts, Rose, A.H., and Harrison, J.S. 2, 9, Academic Press, New York.
3. Dunning, J.W., and Lathrop, E.C. (1945) Ind. Eng. Chem. 37, 24-29.
4. Rydholm, S.A. (1965) Pulping Processes, pp. 90-100, Interscience, New York.
5. Inskeep, G.C., Wiley, A.J., Holderby, J.M., and Hughes, L.P. (1951) Ind. Eng. Chem. 43, 1702-1711.
6. Wang, P.Y., and Schneider, H., in preparation.
7. Horecker, B.L. (1962) Pentose Metabolism in Bacteria, pp. 19-31, John Wiley & Sons, New York.
8. Ashwell, G. (1957) Meth. in Enz. 3, 73-105.
9. Touster, O. (1962) Meth. Carbohyd. Chem. 1, 98-101.
10. Scallet, B.L., Shieh, K., Ehrenthal, I., and Slapshak, L. (1974) Die Stärke, 26, 405-408.
11. Lindberg, B., and Slessor, K.N. (1967) Carb. Res. 5, 686-691

12. Gunner, S.W., Jones, J.K.N., and Perry, M.B. (1961) Can. J. Chem. 39, 1892-1899.
13. Petersson, G. (1974) Carbohyd. Res. 33, 47-61.
14. Harrison, J.S., and Graham, J.C.J. (1971) The Yeasts, Rose, A.H., and Harrison, J.S., 3, 283-348, Academic Press, New York.
15. Mahler, H.R., and Cordes, E.H. (1974) Biological Chemistry, 2nd Edn., p. 535. Harper and Row, New York.
16. Sims, A.P., and Barnett, J.A. (1978) J. Gen. Microbiol. 106, 277-288.
17. Mahler, H.R., and Wilkie, D. (1978) Plasmid 1, 125-133.