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Simultaneous utilization of glucose and xylose by Candida curvata D in continuous culture

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SUMMARY: Of ten, mainly oleaginous, yeasts examined for the ability to use glucose and xylose simultaneously, only one, Candida curvata D, was found which could do so. This yeast was examined further in a single-stage chemostat wherein it produced similar biomass yields, lipid contents and fatty acids on glucose plus xylose mixed in varying proportions. This oleaginous yeast would therefore be capable of growing on hydrolysed wood and straw wastes as a potential source of single cell oil.

INTRODUCTION

Simultaneous utilization of sugars by microorganisms, and especially by yeasts, is a little studied phenomenon in spite of numerous industrial processes which use mixtures of carbohydrates in the feedstock. Differing rates of fructose and glucose utilization by Saccharomyces cerevisiae have been reported (Cason et al., 1987a) though there may exist two mechanisms for multiple sugar uptake in this yeast (Cason et al., 1987b). Candida utilis, when grown on glucose, fructose and sucrose together, preferentially uses the glucose first, then sucrose and finally fructose but all three sugars appear to be consumed simultaneously in the late stages of the culture (Aker and Robinson, 1987). We have not been able to discover any studies previously carried out with a mixture of glucose and xylose, though this is not unexpected as xylose is not utilized by S. cerevisiae. However if hydrolysed waste plant material, such as wood and straw, were to be used as a substrate for yeast cultivation, mixtures of hexoses, which would be mainly glucose from the cellulose, and pentoses, mainly as xylose from the hemicelluloses, would be present in such a feedstock. In this study, we have surveyed a small number of yeasts for simultaneous utilization of these two sugars. As a principle interest of this laboratory is in the potential of single cell oil production (Ratledge, 1986), we have concentrated mainly on oleaginous yeasts in this survey. Only one yeast, Candida curvata, was found able to use both sugars simultaneously and was then examined further in chemostat culture using

varying proportions of glucose and xylose.

METHODS

Yeasts and growth in batch culture. Yeasts (see Table 1) were grown on a medium containing (g/l) glucose, 15; xylose, 15; NH_4Cl, 1.0; KH_2PO_4, 7; Na_2HPO_4, 2; $MgSO_4.7H_2O$, 0.5; yeast extract, 0.25; $CaCl_2.H_2O$, 0.1; $FeCl_3.6H_2O$, 0.08; $ZnSO_4.7H_2O$, 0.001; $MnCl_2.4H_2O$, 0.001 and $CuSO_4.5H_2O$, 0.0004; with the pH adjusted to 5.5. Medium (800 ml) was vigorously stirred in 1 litre vortex-aerated bottles at 28°C being inoculated with 10 ml of 48 h shake-flask culture of the yeast.

Continuous culture of C. curvata. A 3 l chemostat with a working volume of 2.1 l was used. The yeast was grown on the same nitrogen-limiting medium as described above except that two separate supplies were used: one with glucose (at 30g/l) and the other with xylose (also at 30g/l). Media were sterilized by filtration. Each medium was fed into the chemostat through a separate pump and inlet; the combined rate of entry of the media gave a constant dilution rate of 0.06 h^{-1}. Aeration was at 1 vol/vol/min and the pH controlled to 5.5. The initial steady-state was established for 8 days using glucose as sole carbon substrate, thereafter the proportion of glucose to xylose was changed after 5 complete changes of medium had gone through the chemostat; i.e. after approx. 84 h.

Analyses. Samples, 10 ml, were taken in duplicate, centrifuged at 5000 rpm for 5 min; the cell pellet was washed twice in water, dried overnight at 80°C in a vacuum oven and then weighed. Supernatants were analysed for sugars by paper chromatography of a 2 ml aliquot using ethyl acetate/pyridine/water (13:5:4, by vol.) as solvent. Sugars were visualized with an aniline oxalate spray (Dawson et al., 1986). Lipid contents of the cells and fatty acid analyses were carried out as previously described (Evans and Ratledge, 1983).

RESULTS AND DISCUSSION

Ten yeasts were grown on medium containing glucose and xylose and sampled after 24, 48 and 72 h. Culture supernatant solutions were examined by paper chromatography for the presence or absence of both sugars on a simple qualitative basis. Cell dry weights were also recorded. The results (Table 1) showed that nine of the ten yeasts had consumed all the glucose from the medium while leaving some or all of the xylose in the medium. With seven of these nine yeasts, some xylose still remained unused after 3 days' growth. As xylose utilization involves the induction of

phosphoketolase in these yeasts (Evans and Ratledge, 1984) this result would suggest that there had to be a period of adaptation before growth could begin on xylose. Only in Candida curvata, C. utilis and C. tropicalis was xylose all completely used after three days and only in the former yeast did xylose disappear from the medium simultaneously with the glucose.

Thus simultaneous utilization of glucose and xylose was indicated to occurring in only one yeast and to verify this, C. curvata was subsequently grown in a single-stage chemostat using nitrogen-limiting medium with the concentration of total sugars carefully adjusted so that less than 1% remained in overflow medium at the selected dilution rate of 0.06 h^{-1} (Evans and Ratledge, 1983). The culture was fed from two medium reservoirs - one with glucose the other with xylose - and the proportions of the two sugars was then changed during the course of the experiment. As C. curvata is an oleaginous yeast with a potential for single cell oil production (Ratledge, 1986), we also examined the lipid and fatty acids of this yeast during its cultivation.

The results (Table 2) taken at six steady states with different proportions of the two sugars indicated that there was no significant change in the cell yield, lipid yield or in the fatty acid profile of the total lipid of the cell. These results are similar to previous ones obtained for the same yeast grown on each sugar separately (Evans and Ratledge, 1983).

Table 2. Growth, lipid and fatty acid contents of C. curvata growing on glucose and xylose in varying proportion in continuous culture at a constant dilution rate of 0.06 h^{-1}.

Proportion (w/w) of glucose to xylose	Biomass (g/l)	Lipid content (w/w)	Rel. % (w/w) fatty acids*				
			16:0	18:0	18:1	18:2	18:3
100 : 0	10.6	27	25	14	49	8	4
80 : 20	8.4	25	23	14	48	11	4
60 : 40	8.5	23	22	15	48	11	4
40 : 60	10.7	27	23	16	48	10	3
20 : 80	8.4	24	22	17	47	10	4
0 : 100	8.2	30	22	17	45	12	4

*small amounts of 14:0 (~0.4%) and 16:1 (~0.3%) were also seen.

Table 1. Utilization of glucose and xylose by yeasts growing on both sugars simultaneously in batch culture.

Yeast	Time of growth: 24 h — Presence of residual glucose	24 h — Presence of residual xylose	24 h — Biomass (g/l)	48h — Presence of residual glucose	48h — Presence of residual xylose	48h — Biomass (g/l)	72h — Presence of residual glucose	72h — Presence of residual xylose	72h — Biomass (g/l)
Candida curvata	+	+	1.5	-	-	2.6	-	-	4.4
C. tropicalis	-	+	1.2	-	-	1.1	-	-	1.1
C. utilis	-	+	2.5	-	+	3.3	-	-	3.5
Cryptococcus albidus	+	+	0.3	-	+	3.1	-	+	4.8
Hansenula polymorpha	+	+	0.8	-	+	2.5	-	+	3.5
H. saturnus	-	+	2.7	-	+	3.1	-	+	3.9
Lipomyces starkeyi	+	+	0.0	+	+	0.2	-	+	1.2
Pichia media	+	+	0.7	-	+	1.7	-	+	2.1
Rhodosporidium toruloides	+	+	0.8	-	+	5.3	-	+	6.3
Rhodotorula glutinis	+	+	1.5	+	+	2.3	-	+	3.1

Presence (+) or absence (-) of residual glucose or xylose in culture supernatant solutions was determined by paper chromatography.

The evident attributes of C. curvata as an excellent producer of triacylglycerol lipids from a variety of substrates (Evans and Ratledge, 1983; Ratledge, 1986) can now be extended to include growth of this yeast on substrates containing mixtures of sugars - in this case glucose and xylose which would be found together in hydrolysates of such waste materials as wood and straw. Cellulosic waste materials could form a valuable feedstock for a fermentation industry but have the disadvantage of providing a mixture of hexose and pentose sugars. Waste fruit materials, such as banana pulp which has been used to support growth of C. curvata (Glatz et al., 1985), often contain mixed disaccharides and hexoses such as sucrose, glucose and fructose but little or no pentoses. Simultaneous utilization of these sugars, though, do not present the same metabolic problem to a yeast as does simultaneous use of glucose and xylose as efficient xylose utilization requires the specific induction of phosphoketolase (Evans and Ratledge, 1984). As this enzyme is still induced when C. curvata is grown on these mixed sugars (P.D. Jefferies, unpublished work) it is clear that two separate pathways for sugar utilization co-exist in this yeast. The potential of C. curvata as a producer of Single Cell Oil using mixed sugars from cellulosic waste materials is indicated from this work.

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