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A QUANTITATIVE SCREENING OF SOME XYLOSE-FERMENTING YEAST ISOLATES

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SUMMARY

A quantitative screening procedure for xylose fermentation was conducted on 56 yeast isolates. Several of the isolates were found to be markedly superior to *C. shehatae* CSIR-Y492, one of the better xylose-fermenting yeasts identified thus far. The outstanding isolate was a strain of *Pichia stipitis* which had an ethanol yield coefficient of 0.45 from xylose and which produced no detectable amounts of xylitol.

INTRODUCTION

The hemicellulose component of some lignocellulosic materials can represent up to 35% of the dry biomass, with D-xylose as the major sugar constituent (Dekker and Lindner, 1979; Rosenberg, 1980; Tsao *et al.*, 1982). The economic exploitation of lignocellulosics for ethanol production by fermentation will thus significantly be enhanced by the efficient utilization of this pentosan fraction. However, the ability to ferment xylose is not widespread among yeasts (Toivola *et al.*, 1984). Although recent attempts at screening for yeasts have identified a number of strains capable of fermenting aldopentose sugars to ethanol, in most cases the yeast strains were characterized by a slow rate of fermentation and a low ethanol yield (Maleszka and Schneider, 1982; Maleszka *et al.*, 1982; Margaritis and Bajpai, 1982; Kurtzman, 1983; Schneider *et al.*, 1983; Suihko and Dražić, 1983).

So far all the screening procedures for xylose-fermenting yeasts have been conducted in a qualitative or semi-quantitative fashion. However, Toivola *et al.* (1984) demonstrated that the standard fermentation test based on CO_2 production in a Durham tube was not a dependable criterion for assessing fermentative capacity, and reliable screening is further hampered by the fact that the degree of oxygenation can exert a profound effect on both the rate and yield of ethanol production from xylose (Du Preez *et al.*, 1984). The role of oxygen in xylose metabolism has been elucidated by Bruinenberg *et al.* (1983;

1984). Therefore to obtain a more accurate appraisal of the xylose-fermenting capabilities of 56 selected yeast isolates, we subjected these strains to a more rigorous quantitative evaluation procedure under aerated conditions.

MATERIALS AND METHODS

Microorganisms. The yeast strains were isolated by J.P. van der Walt (Council for Scientific and Industrial Research, Pretoria) from various soil sources using xylose-based enrichment techniques. A number of the *Candida* strains could not be identified to species level according to Kreger-van Rij (1984). All strains produced gas from 2% xylose within 14 days in the standard taxonomy fermentation test (Van der Walt, 1970). *Candida shehatae* CSIR-Y492, the strain used in earlier studies (Du Preez and Van der Walt, 1983; Du Preez *et al.*, 1984) was included in the study as a reference strain to enable a comparison with the other isolates under similar experimental conditions. Also included in the screening procedure were two diploid strains of the haploid *C. shehatae* CSIR-Y492, designated by the suffixes M and P, which had been created by protoplast fusion techniques described elsewhere (Johannsen *et al.*, 1985).

Culture medium. Medium CA containing 50 g/ℓ D-xylose (Merck), casamino acids, added vitamins and mineral salts, as described elsewhere (Du Preez and Van der Walt, 1983; Du Preez *et al.*, 1984) was used.

Experimental conditions. The fermentations were conducted in 500 mℓ Erlenmeyer flasks, each containing 300 mℓ medium and fitted with a cotton wool plug, which were incubated at 30°C on a rotary shaker at 150 rpm. These operating conditions were selected on the basis of previous experience with *C. shehatae* CSIR-Y492 to give a relatively high ethanol yield within a reasonably short time period. Incomplete fermentations were terminated after 48 h. The inoculum was prepared by inoculating a 150 mℓ Erlenmeyer flask containing 15 mℓ medium from a fresh slant of YM agar (Wickerham, 1951), and incubating at 30°C on a rotary shaker at 180 rpm for 24 h. A 2 mℓ volume of this culture was then transferred to a 250 mℓ shake flask containing 25 mℓ medium. After a 20 h incubation period a 10 mℓ volume of this culture was used to inoculate the fermentation flasks.

Ethanol, xylose, xylitol, culture turbidity and dry cell mass were determined as described previously (Du Preez and Van der Walt, 1983; Du Preez *et al.*, 1984).

RESULTS

The fermentation of xylose by the various strains were evaluated in terms of the final ethanol concentration, ethanol yield coefficient (Y, g ethanol/g xylose utilized), maximum volumetric rate of ethanol production (Q_p, calculated from the slope of the ethanol vs. time curve), efficiency of substrate utilization (E, g xylose utilized/g initial xylose x 100%), the maximum specific growth rate (μ_{max}), and the amount of xylitol produced. The results obtained with some of the better isolates are presented in Table 1. The highest ethanol yields of Y = 0.41 to 0.45, coupled with a 100% efficiency of xylose utilization,

TABLE 1 The parameters for xylose fermentation by various yeast isolates in 48 shake flask cultures with ca. 50 g/ℓ D-xylose. The values are the mean of two or more experiments.

Yeast isolate	E (%)	Maximum ethanol (g/ℓ)	Y	Xylitol (g/ℓ)	μ_{max} (h^{-1})	Q_p (g/ℓ.h)
C. shehatae CSIR-Y492	100	17.5	0.36	3.22	0.14	0.75
Pi. stipitis CSIR-Y633	100	21.46	0.45	0	0.14	0.92
Pi. stipitis CSIR-Y567	100	21.28	0.42	1.48	0.16	0.85
C. shehatae CSIR-57 D/1	100	20.64	0.42	0	0.13	0.80
C. shehatae CSIR-117 A/1	100	20.69	0.42	0	0.13	0.82
C. shehatae CSIR-Y492 P	100	17.42	0.35	2.74	0.12	0.78
C. shehatae CSIR-Y492 M	100	17.56	0.34	3.56	0.12	0.78
C. shehatae CSIR-Y599	100	15.44	0.33	6.62	0.17	0.75
C. shehatae CSIR-Y600	82	11.60	0.28	2.70	0.14	0.26
C. shehatae CSIR-Y601	100	12.43	0.24	9.39	0.15	0.33
C. tenuis CSIR-Y565	83	11.11	0.26	5.09	0.20	0.37
C. tenuis CSIR-Y566	95	11.06	0.24	10.94	0.17	0.40
Candida CSIR-Y579	72	13.66	0.36	0	0.14	0.47
Candida CSIR-41 D/1	100	17.28	0.37	5.88	0.15	0.75
Candida CSIR-III 43 A/4	88	16.35	0.41	2.28	0.16	0.57
Candida CSIR-III 43 A/5	97	16.63	0.33	2.91	0.10	0.49
Candida CSIR-56 D/1	100	18.28	0.40	3.63	0.21	0.60
Candida CSIR-58 D/2	91	15.76	0.38	1.74	0.14	0.58
Candida CSIR-62 A/2	100	20.09	0.41	0	0.13	0.76
Candida CSIR-62 A/3	100	18.84	0.41	0	0.12	0.63
Candida CSIR-64 A/1	65	11.24	0.39	0	0.14	0.45
Candida CSIR-64 A/2	92	16.04	0.34	1.98	0.12	0.44
Candida CSIR-76 D/1	100	17.99	0.36	3.43	0.12	0.70
Candida CSIR-83 D/2	75	14.41	0.44	2.11	0.14	0.38
Candida CSIR-89 D/1	87	15.89	0.36	0	0.14	0.48
Candida CSIR-91 D/1	100	19.18	0.40	2.05	0.22	0.73
Candida CSIR-94 D/1	100	17.06	0.36	3.16	0.13	0.73
Candida CSIR-96 D/2	100	18.82	0.40	1.74	0.22	0.73
Candida CSIR-96 D/4	100	14.98	0.32	5.41	0.14	0.46
Candida CSIR-100 D/2	100	17.13	0.36	4.90	0.21	0.76
Candida CSIR-103 D/3	89	13.68	0.32	2.85	0.13	0.41
Candida CSIR-104 D/1	100	18.16	0.38	4.30	0.25	0.73
Candida CSIR-105 D/4	68	12.05	0.37	2.65	0.19	0.71
Candida CSIR-113 D/1	77	12.19	0.29	5.21	0.15	0.36
Candida CSIR-114 A/1	100	17.76	0.36	5.19	0.22	0.55
Candida CSIR-114 A/3	62	10.68	0.37	2.00	0.20	0.33
Candida CSIR-114 D/2	64	11.77	0.40	0	0.19	0.55
Candida CSIR-116 A/2	100	18.16	0.40	3.66	0.12	0.71
Candida CSIR-117 D/2	100	17.67	0.38	3.44	0.11	0.65

were found with the strains *Pichia stipitis* CSIR-Y633 and CSIR-Y567, *Candida shehatae* CSIR-57D/1 and CSIR-117A/1, and the *Candida* isolates CSIR-62A/2 and CSIR-62A/3. It is noteworthy that with the exception of *Pi. stipitis* CSIR-Y567, none of these strains produced detectable amounts of xylitol. The sensitivity limit for xylitol determination by high performance liquid chromatography was in the order of 0.5 g/ℓ.

These ethanol yields were substantially higher than the yield

coefficient of 0.36 found with the reference strain of *Candida shehatae* CSIR-Y492, which was also the value reported earlier for the same strain and obtained within a comparable fermentation time (Du Preez *et al.*, 1984). A number of the other isolates in Table 1 gave similar or higher yield values, also with a 100% efficiency of xylose utilization.

Next to the ethanol yield, the Q_p value was the most important fermentation parameter in this screening procedure. Relatively few of the yeast strains surpassed *C. shehatae* CSIR-Y492 in this respect, however. The highest volumetric rate of ethanol production was found with *Pi. stipitis* CSIR-Y633, namely a mean value of 0.92 g/ℓ.h in comparison with the mean value of 0.75 g/ℓ.h obtained with the *C. shehatae* reference strain. High Q_p values were also found with *Pi. stipitis* CSIR-Y567 and the *C. shehatae* strains CSIR-57D/1 and CSIR-117A/1. Isolate CSIR-62A/2 had a higher ethanol yield than the *C. shehatae* reference strain with a similar Q_p value.

Thus based on the criteria of ethanol yield and rate of production, and the efficiency of substrate utilization, *Pi. stipitis* CSIR-Y633 appears to be the yeast of choice for xylose fermentation, followed by *Pi. stipitis* CSIR-Y567, *C. shehatae* CSIR-57D/1 and *C. shehatae* CSIR-117A/1. The diploid strains of *C. shehatae* CSIR-Y492 showed little difference in the fermentation parameters in comparison with the reference strain under these experimental conditions.

DISCUSSION

Previous work showed that *C. shehatae* CSIR-Y492 was capable of fermenting xylose to ethanol much more rapidly and at a slightly higher yield than *Pachysolen tannophilus* (Du Preez *et al.*, 1984). Maleszka *et al.* (1983) reported that by increasing the chromosome number of *Pa. tannophilus* above the haploid level, both the yield and rate of ethanol production from xylose was increased, but we did not make a similar observation using diploid strains derived from *C. shehatae* CSIR-Y492. However, we did find two strains of *Pichia stipitis*, which is the perfect stage of *C. shehatae* (Barnett *et al.*, 1983), as well as several *Candida* strains to be markedly superior to *C. shehatae* CSIR-Y492 in terms of the fermentation rate and especially the ethanol yield. In an extensive screening programme Toivola *et al.* (1984) identified *C. shehatae* and *Pi. stipitis* as potential candidates for the industrial fermentation of pentose sugars. Others also have reported *Pi. stipitis* to be superior to *Pa. tannophilus* in its xylose fermenting capacity with

ethanol yield coefficients ranging from 0.38 to 0.49 (Bruinenberg *et al.*, 1984; Dellweg *et al.*, 1984; Lindman and Björling, 1984). With *Pi. stipitis* CSIR-Y633 we obtained a mean ethanol yield coefficient of 0.45 with 5% D-xylose, which is equivalent to 88% of the theoretical maximum according to the stoichiometry

$$3 \text{ xylose} \rightarrow 5 \text{ ethanol} + 5 \text{ } CO_2$$

Pentose fermention by microorganisms other than yeasts has also been investigated. Although a high ethanol yield from xylose (Y = 0.42) was obtained with *Fusarium oxysporum* (Viikari *et al.*, 1984), the fermentation was deemed too slow for industrial application (Rosenberg, 1980). The use of obligate anaerobic bacteria for xylose fermentation has the advantage that the requirement for a critical dissolved oxygen tension does not have to be met in order to maximize the ethanol yield (Patel, 1984), whereas this is an important factor in yeast fermentations (Baillargeon *et al.*, 1983; Suihko and Dražić, 1983; Du Preez *et al.*, 1984; Watson *et al.*, 1984). However, xylose-fermenting bacteria generally have a low ethanol tolerance or produce undesirable by-products, such as acetate, that decrease the ethanol yield (Bruinenberg *et al.*, 1984; Patel, 1984). Xylose-fermenting yeasts suffer from a similar disadvantage in that the production of xylitol, an intermediate in the catabolism of xylose, is a typical phenomenon (Gong *et al.*, 1983) and is inversely related to the aeration rate (Baillargeon *et al.*, 1983; Du Preez and Van der Walt, 1983; Schvester *et al.*, 1983). Although xylitol production can be minimized by control of the aeration rate, this strategy is not completely successful in maximizing the ethanol yield because the ethanol yield is also decreased by increasing the aeration (Baillargeon *et al.*, 1983; Schvester *et al.*, 1983; Du Preez *et al.*, 1984; Watson *et al.*, 1984). In view of the above the discovery of yeast strains producing no detectable amounts of xylitol appears particularly promising for industrial application. Providing that the ethanol tolerance of these low xylitol producing strains is acceptable, it would seem that yeasts have distinct advantages over other microbial groups for xylose fermentation.

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