UNSTABLE PETITE AND GRANDE VARIANTS OF Candida shehatae

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SUMMARY

Two strains of Candida shehatae (ATCC 22904 and CSIR Y492) exhibit marked variability in colony size (petite, grande) and respiratory activity (tetrazolium reaction) when grown on glucose, xylose, and—especially—xylitol agar. The transitions occur in both directions at high frequency. Strains showing a negative or weak tetrazolium reaction on xylitol ferment xylose better than those showing a strong tetrazolium reaction. The type strain (ATCC 34887) shows stable colonial morphology with moderate respiratory and fermentative activities. The objective of this report is to demonstrate these variations.

INTRODUCTION

The recent description of Candida shehatae as a rapid fermenter of D-xylose (du Preez and van der Walt, 1983; du Preez et al., 1984) was a major step forward in the emergence of a practical process for the utilization of this abundant sugar. The specific rate of ethanol production by C. shehatae is about 2.5 to 3.5 times greater than the best values reported for Pachysolen tannophilus. C. shehatae attains higher ethanol concentrations than P. tannophilus when growing on xylose, but the ethanol yields are similar. Like P. tannophilus, C. shehatae uses xylose aerobically or anaerobically. Under aerobic conditions, P. tannophilus concomitantly forms and respires ethanol, (Maleszka and Schneider, 1982) resulting in lowered ethanol yields. Although it has not yet been demonstrated, C. shehatae could also exhibit concomitant ethanol formation and respiration.

One way to improve ethanol yields is to obtain a petite mutant. This is a yeast strain with deficient respiratory capacity and small colonial morphology. Selection of petite mutants was useful in the development of improved strains of Candida pseudotropicalis (Moulin et al., 1981), but it was less important in the case of Saccharomyces cerevisiae (Alexander and Detroy, 1983). Petite mutants have not been previously described in xylose-fermenting yeasts. Pachysolen tannophilus—the organism most studied in this regard—is petite-negative, i.e., petite mutants die after a few generations (Neirinck et al., 1984).

This report describes the selection and characteristics of petite and grande strains of C. shehatae and a preliminary evaluation of their fermentation characteristics. It is concluded that the colonial and respiratory variations occur in both directions with high frequencies, that colony size and respiratory activity are separate but related traits, and that selection for a stable petite may be a useful technique for further strain development.

MATERIALS AND METHODS

Strains. Three parental strains of C. shehatae were employed: ATCC 22904, ATCC 33487 and CSIR Y492. The first two were obtained from the American Type Culture Collection, Rockville, Maryland, USA; the last was obtained from J.P. van der Walt, Council for Scientific and Industrial Research, Pretoria, South
Africa. Stock cultures were maintained on Difco Yeast Malt Agar (YMA). Slants were grown out at 32°C then stored at 5°C for up to 3 months. Isolates from these parental strains were obtained by streaking on Difco Yeast Nitrogen Base w/o ammonium sulfate or amino acids (YB) supplemented with 2.27 g/l of urea (U), 2% xylitol (Xi), xylose (Xo) or glucose (G) and 1.8 g/l agar.

Culture conditions. YMA plates were incubated at 32°C for 7 d; YBXi, YBUG and YBUG plates were incubated for 5–18 d in order to distinguish grande and petite colonies. Ethanol fermentation rates were tested in triplicate cultures of YBUG broth as previously described (Jeffries, 1982, 1984). Inocula were adjusted to obtain the same initial OD for all organisms in each test (1 OD @ 525 nm=0.19 mg/ml cells). Triplicate flasks of each organism were used and the results reported as average values.

Isolation of variants. Petite and grande isolates were obtained from YMA–grown cells as small or large colony variants on YBXi agar. Petite II isolates were obtained from petite I isolates. ATCC 22984–1, 2 and 3 were successive grande variants selected from a petite variant in the preceding passage on YBUG; ATCC 22984–SU was a petite isolate showing unstable morphology after four serial passages on YBUG. CSIR Y492–1 and CSIR Y492–2 were isolated from CSIR Y492 by streaking on YBUG. Other strains were obtained as described in the text.

Variant frequencies. The frequencies of conversions from petite to grande and grande to petite colonial morphologies were estimated for 18-day old cells grown on YBUG agar. One petite and one grand colony from YBUG agar were each suspended in sterile water and plated onto triplicate plates of YBUG, YBUG and YBUG agar. After five days, plates were scored for the numbers of small and large colonies. After scoring, diameters were measured under a dissecting microscope, and two plates from each set were overlaid with tetrazolium agar to determine the frequency of respiration positive- and respiration-negative colonies. After 18 days, the remaining plates were overlaid with tetrazolium agar and the frequency of petites and respiratory competent cells were again determined for each of the three carbon sources.

Tetrazolium agar overlay. This method was performed as described by Ogur et al. (1957) using 5– to 18-day-old cultures streaked on YBUG agar with 2% Xi, Xo or G as the carbon source. Color reactions were scored on an arbitrary scale of 1+ to 4+. Respiration-deficient colonies remain white (1+, Tet−) while respiration-sufficient colonies turn red (4+, Tet+).

Assays. Cell density, ethanol, xylose, and xylitol determinations were performed as previously described (Jeffries, 1982, 1984; Verhaar and Kuster, 1980).

RESULTS

Colonial morphologies. ATCC 22984 and CSIR Y492 formed convex, smooth, entire, cream-colored colonies on YMA. ATCC 34007 formed convex, rugose, cream- to light yellow-colored colonies with an undulate edge. Within each strain, isolated colonies on YMA were essentially similar. When streaked onto YBUG, however, ATCC 22984 exhibited distinct heterogeneity in colony size and morphology (Fig. 1). Small colonies on older plates tended to be lobate and irregular, indicating colonial sectoring and reversion to more extensive growth. The colonial transformation occurred in both directions, i.e. large colony strains could be derived from small and vice versa. ATCC 22984–SU continued to give rise to large and small colonies as long as it was subcultured (eight passages). Large (1) and small (2) variants of CSIR Y492 were also obtained on