Production of Long-chain Alcohols by Yeasts

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Fourteen yeast strains from six genera were analysed for the presence of long-chain alcohols. Six strains from three genera contained long-chain alcohols, highest levels being found in *Candida albicans*. The alcohols were identified and determined by TLC, GLC and GLC-MS. The major long-chain alcohols synthesized by these organisms were saturated, primary alcohols with C₁₄, C₁₆ or C₁₈ chain length. Unsaturated long-chain alcohols were not detected. In all strains that produced long-chain alcohols, the relative proportions were C₁₆ > C₁₈ > C₁₄. Long-chain alcohol contents were higher in organisms from anaerobically, as compared with aerobically, grown cultures reaching about 650 µg (g dry wt organisms)⁻¹ in stationary-phase cultures of *C. albicans*. In cultures of *C. albicans*, synthesis of long-chain alcohols occurred only after the end of exponential growth. The alcohols were predominantly present as free alcohols. The fatty-acyl chain-length profile of the triacylglycerol and to a lesser extent the sterol/wax ester fractions from *C. albicans* reflected that of the long-chain alcohols produced by this yeast.

INTRODUCTION

Long-chain alcohols occur as components of animal, higher plant and microbial lipids as free alcohols, precursors of glycerol ether lipids and in combination with carboxylic acids as wax esters (Mahadevan, 1978; Harwood & Russell, 1984). Among micro-organisms that synthesize long-chain alcohols, bacteria have been studied most intensively (Stewart & Kallio, 1959; Raymond & Davis, 1960; Baptist *et al.*, 1963; Day *et al.*, 1970; Allen *et al.*, 1971; Naccarato *et al.*, 1972; Lloyd & Russell, 1983). Some long-chain alcohol-containing bacteria, such as mycobacteria (Ratledge, 1976), have been studied because of their medical importance. Other bacteria have been investigated for their industrial importance in, for example, degradation of oil spillage (Higgins & Gilbert, 1978). In oil-degrading micro-organisms, long-chain alcohols are produced as intermediates in terminal oxidation of alkanes, and may also be found esterified with long-chain carboxylic acids which are produced by further oxidation of the alcohols (Ratledge, 1978; Britton, 1984). When grown on carbon sources other than alkanes, some bacteria synthesize long-chain primary alcohols by reduction of CoA esters of long-chain carboxylic acids which arise either from activity of the fatty acid synthase (Day *et al.*, 1970; Naccarato *et al.*, 1972; Lloyd & Russell, 1983) or are supplied exogenously (Naccarato *et al.*, 1974; Lloyd & Russell, 1983).

Some yeasts are able to grow on alkanes (Britton, 1984) and certain of these organisms may accumulate wax esters containing long-chain alcohols. These yeasts include *Candida guilliermondii* (Muratov *et al.*, 1979) and *Rhodotorula glutinis* (Zalashko *et al.*, 1979; Zalashko & Salokhina, 1982). Reports of long-chain alcohol production by yeasts grown on non-alkane

substrates are rare. Davidova *et al.* (1978) reported that wax esters were detectable in glucose-grown *Candida tropicalis* harvested from exponential-phase cultures, although their synthesis was more prolific when this yeast was grown on alkanes. The present paper reports synthesis of long-chain alcohols by several yeasts in media containing glucose as the carbon source.

METHODS

Organisms. The yeasts used were *Candida albicans* NCYC 1467, *C. bombicola* NCYC 1449, *C. maltosa* Colworth Microbial Culture Collection (CMCC) 3152, *C. ingens* NCYC 822, *C. utilis* NCYC 168, *C. utilis* NCYC 707, *Debaryomyces hansenii* NCYC 9, *Pichia fermentans* NCYC 850, *Rhodotorula glutinis* NCYC 59, *Rh. glutinis* CMCC 2272, *Rh. rubra* NCYC 195, *Rhodotorula* sp. ATCC 20254, *Saccharomyces cerevisiae* Y 185 (a gift from J. R. Woodward, Department of Biotechnology, University of Leeds, UK) and *Saccharomycopsis (Yarrowia) lipolytica* ATCC 20225. They were maintained at 4 °C on slopes of malt extract-yeast extract-glucose-mycological peptone (MYGP) agar (Wickerham, 1951).

Experimental cultures. Organisms were grown aerobically in a medium containing (l⁻¹): glucose, 20 g; (NH₄)₂SO₄, 3.0 g; KH₂PO₄, 4.5 g; yeast extract (Lab M), 1.0 g; MgSO₄·7H₂O, 25 mg; and CaCl₂·2H₂O, 25 mg (adjusted to pH 4.5 with HCl). Growth under self-induced anaerobic conditions used the same medium except that the concentration of glucose was 200 g l⁻¹ and that of yeast extract 4 g l⁻¹. One-litre portions of medium were dispensed into 2 l round flat-bottomed flasks and sterilized by autoclaving at 6.89 × 10⁴ Pa for 1 min. Flasks used for aerobic growth were plugged with cotton wool, and those used for self-induced anaerobic growth were fitted with fermentation locks (Beavan *et al.*, 1982). Starter cultures (100 ml medium in a 250 ml flask) were inoculated with a pinhead of organisms from a slant culture, and incubated for 48 h at 30 °C on an orbital shaker (200 r.p.m.). Experimental cultures used in the survey of long-chain alcohol production were inoculated with 1 ml of starter culture; those used for further studies were inoculated with a portion of starter culture containing 10 mg dry wt organisms. Cultures were incubated aerobically as described by Patching & Rose (1969) and under self-induced anaerobic conditions as described by Beavan *et al.* (1982). The latter cultures are described as anaerobic in this paper. Growth was followed by measuring optical density at 600 nm; measurements were related to the dry wt of each yeast strain using an appropriate standard curve. At the times indicated, organisms were harvested by centrifugation at 6000 g at 4 °C and washed twice with water.

Lipid extraction. Before harvesting, 2 ml solution containing 10 mg each of cycloheximide and chloramphenicol, and where indicated 10 ml 50 mM-PCMB in methanol, were injected into the culture which was incubated for a further 15 min. Lipids were extracted from washed organisms by a modification of the Folch *et al.* (1957) procedure. Washed organisms were resuspended in 20 ml methanol and shaken in a Braun homogenizer for four periods of 30 s at speed 2 (4000 r.p.m.) with glass beads (40 g; Sigma type V; 0.45–0.50 mm diam.). The sample bottle was cooled with expanding CO₂ during homogenization. Chloroform was added to the suspension to give 2:1 (v/v) chloroform/methanol, and the mixture stirred magnetically for 2 h at room temperature (18–22 °C). The suspension was then filtered through Whatman no. 44 filter paper and the extraction procedure repeated on the residue. Extracts were pooled, washed with 0.25 vol. 0.88% (w/v) KCl and the mixture left to separate overnight at –20 °C. The lower organic phase was removed, taken to dryness using a rotary evaporator, and the residue immediately dissolved in 1 ml light petroleum (b.p. 60–80 °C). Samples were stored under nitrogen gas at –20 °C. Where indicated the extraction suspension was supplemented with 1.67 mM-PCMB.

Analysis of free and esterified long-chain alcohols. Extracts were fractionated into lipid classes by chromatography on silicic acid columns prepared by a modification of the Naccarato *et al.* (1972) procedure. Graduated glass pipettes (E-MIL; 5 ml) were plugged with glass wool and filled with 1 g SIL-LC silicic acid (325 mesh; lipid chromatography grade) (Hirsch & Ahrens, 1958). Maximum suction from a water aspirator, applied to the lower end of the column, caused slight compaction of the contents and assured even and reproducible packing. Packed columns were saturated with 20 ml light petroleum by overpressure with high-purity nitrogen gas. Lipid extracts were applied to the top of the column and eluted with 10 ml each of 4%, 7% and 10% (v/v) diethyl ether in light petroleum. A flow rate of 0.5 ml min⁻¹ was maintained by nitrogen pressure on the column, and eluates were collected in 3 ml fractions. Fractions were taken to dryness under a stream of nitrogen gas, redissolved in an appropriate volume of light petroleum and 20 µl portions spotted onto a 20 cm × 20 cm × 0.25 mm Silica Gel 60 precoated TLC plate (Merck). The plate was developed with a light petroleum (b.p. 40–60 °C)/diethyl ether/acetic acid (70:30:1, by vol.) solvent mixture (Mangold, 1969); lipids were located by spraying with 0.2% (w/v) 2',7'-dichlorofluorescein in ethanol (Griffith *et al.*, 1981) and viewed under ultraviolet (254 nm) radiation. Standards [1 mg ml⁻¹ light petroleum (b.p. 60–80 °C)] were cholesterol palmitate, tripalmitin, stearic acid, palmitic acid, hexadecanol, octadecanol and ergosterol. The method gave excellent separation of lipid classes from extracts (Fig. 1). Subsequent analysis of free long-chain alcohols used only fractions 6–9 from the silicic acid column (Fig. 1). To assay long-chain alcohols, appropriate fractions from the silicic acid column were pooled, concentrated under nitrogen gas and further separated by TLC using a solvent mixture of hexane/diethyl ether/acetic acid (30:70:1,

by vol.) (Naccarato *et al.*, 1972). Long-chain alcohols were visualized as described above. The appropriate areas of the plate were scraped off and lipids extracted using light petroleum/methanol/30% (w/v) NaCl (1:1:1, by vol.). Samples were shaken vigorously and allowed to separate. The top layer was removed and the extraction procedure repeated twice. Extracts were pooled and taken to dryness under nitrogen gas.

Long-chain alcohols were separated and identified by GLC after conversion to their trimethylsilyl (TMS) ethers. Samples were taken up in 0.5 ml pyridine, and the solution was mixed with an equal volume of bis(trimethylsilyl)trifluoroacetamide. The mixture was transferred to a 1 ml screw-top vial and heated at 70 °C for 15 min. Silylated samples were separated using a Pye Unicam PU 4500 gas chromatograph fitted with a 25 m SE30 capillary column. The injection temperature was 300 °C, and the detector temperature 350 °C. The initial column temperature was 190 °C, and this was increased after 25 min at a rate of 16 °C min⁻¹ to give a final temperature of 250 °C which was maintained for a further 10 min. The carrier gas (helium) flow rate was 1 ml min⁻¹, and the nitrogen flow rate, as a make-up gas across the detector, was 40 ml min⁻¹. TMS ethers of long-chain alcohols were identified by comparing their retention times with those of known standards and by co-chromatography with authentic standards. Peaks were analysed using a Pye Unicam CDPI computing integrator, and quantified by reference to a 1-heptadecanol internal standard added during the extraction procedure after disruption of organisms. Identification of long-chain alcohols was verified by GLC-MS using electron-impact ionization or chemical ionization. Silylated long-chain alcohols and carboxylic acids were used, and typical outputs of total ion current versus time matched the GLC traces obtained with the Pye Unicam PU 4500 gas chromatograph described above. The instrument used was a VG Analytical 70/70E, with a DB1 capillary column programmed at 150 °C for 5 min and rising to 300 °C at 30 °C min⁻¹ for 5 min and held at the upper temperature. The flow rate of the carrier gas (helium) was 1 ml min⁻¹, the resolution 1000, the electron impact 70 eV and the calibrated range 20–580.

To examine the effect of PCMB on extraction of esterified long-chain alcohols, fractions 1–3 from the silicic acid column, which contained neutral lipids, were pooled and applied to Silica Gel 60 plates. Lipids were separated using the solvent system light petroleum–diethyl ether–acetic acid (90:10:1, by vol.) (Kates, 1972), and visualized as described above. Lipid bands with mobilities similar to heptadecanyl acetate, the internal standard, were scraped off the plate, and the lipids extracted with light petroleum as described above. Samples were then dried under a stream of nitrogen gas, and saponified using a modification of the method of Pollard *et al.* (1979) by adding 5 ml 1 M-KOH in 95% (v/v) methanol, sealing and heating at 80 °C for 3 h. After cooling, samples were diluted with 5 ml methanol, and non-saponifiable lipids removed with 3 × 5 ml light petroleum. They were then prepared for GLC and analysed as described above.

Analysis of the fatty-acyl composition of lipids. Lipid extracts from organisms were dissolved in chloroform to give approximately 25 mg lipid ml⁻¹. To prepare fatty-acid methyl esters of total lipids from organisms, 1 ml lipid solution was taken to dryness under a stream of nitrogen gas. BF₃ (14%, w/v, in methanol; 3 ml) was added and the mixture was heated for 1 h at 80 °C in a sealed vial. To extract fatty-acid methyl esters, the reaction mixture was made up to 5 ml with methanol and supplemented with 5 ml each of light petroleum and 30% (w/v) NaCl. The solution was shaken vigorously and the mixture left to separate, the procedure being repeated twice more. The solution of fatty-acid methyl esters was taken down to a small volume under a stream of nitrogen gas, streaked onto a 20 cm × 20 cm × 0.25 mm Silica Gel 60 precoated TLC plate (Merck) and the plate developed with a light petroleum/diethyl ether/formic acid (75:25:0.5, by vol.) solvent mixture (Fixter *et al.*, 1986). Methyl esters were scraped off the plate, extracted with light petroleum as described above, and taken down to a small volume under a stream of nitrogen gas. Fatty-acid methyl esters were analysed using a fused silica capillary column (25 m length; SGE BP21) in a Pye Unicam GCD chromatograph fitted with an SGE on-column adaptor. The injection temperature was 240 °C and the detector temperature 280 °C. The column was maintained at 135 °C for the first 5 min, after which the temperature was raised at the rate of 8 °C min⁻¹ until it reached 180 °C. The carrier gas was hydrogen. Percentage fatty-acyl compositions were calculated using a Pye Unicam CDPI computing integrator. Individual lipid classes in total lipid extracts were separated by streaking a solution containing 10 mg lipid onto a Silica Gel 60 precoated TLC plate as above, which was developed with a solvent mixture containing light petroleum (b.p. 40–60 °C)/diethyl ether/acetic acid (70:30:1, by vol.). Lipid classes were identified as described above, bands scraped off the plates, and fatty-acid methyl esters of the lipids prepared as described above.

Chemicals. All chemicals used were AnalaR or of the highest purity available.

RESULTS

Identification of long-chain alcohols

The mobility of certain lipid fractions on silicic acid columns and on TLC (Fig. 1) suggested that their polarity was that expected of long-chain alcohols. Subsequently purification,

Fig. 1. Drawing of a TLC plate showing separation of lipid classes in each of the ten 3 ml fractions obtained by silicic acid chromatography of a lipid extract of *C. albicans* harvested from cultures after 72 h growth under anaerobic conditions. SWE, Sterol/wax esters; TG, triacylglycerols; FFA, free fatty acids; FLCA, free long-chain alcohols; S, sterols; Stds, lipid standards.

separation and identification by GLC and GLC-MS confirmed the presence of C_{14} , C_{16} and C_{18} primary alcohols in extracts of organisms. Fig. 2(a) shows the total ion-current trace from GLC-MS of a lipid extract from anaerobically grown *C. albicans* after purification by TLC. The peak at scan no. 253 was identified as 1-hexadecanol from its fragmentation pattern in electron-impact ionization MS (Fig. 2b). Peaks 36, 115 and 362 were generated by C_{12} , C_{14} and C_{18} long-chain alcohols, respectively. Peak 309 was due to the 1-heptadecanol internal standard. Other components of the extract identified were members of the isoprenoid alcohol series (peaks 111, 128, 378 and 403). This latter group of compounds was found in all lipid extracts examined by TLC, and they were also identified in extracts of yeasts that lacked the ability to synthesize long-chain alcohols.

Survey of long-chain alcohol production by yeasts

A survey was made of the ability of 14 strains of yeast to produce long-chain alcohols. Strains were selected partly on the basis of their known ability to metabolize exogenously provided lipids. All strains grew under aerobic conditions although five were unable to do so under anaerobic conditions (Table 1). While some of the strains produced saturated long-chain alcohols, none produced unsaturated long-chain alcohols. Only three yeasts, *C. albicans*, *C. utilis* NCYC 168 and *P. fermentans*, accumulated appreciable amounts of long-chain alcohols when grown either anaerobically or aerobically (Table 1). *C. maltosa*, *C. utilis* NCYC 707 and *S. cerevisiae* contained these alcohols when grown anaerobically, but little or no such alcohol after aerobic growth. In general, anaerobic conditions favoured production of long-chain alcohols. Six of the strains with ability to produce the alcohols under these conditions were examined quantitatively for content of C_{14} , C_{16} and C_{18} alcohols (Table 2). The content of C_{16} alcohols was greatest after anaerobic growth with all of the strains examined. In *C. albicans* grown under aerobic conditions, C_{18} alcohol was found in slight excess over C_{16} alcohol, although the total long-chain alcohol content was about fourfold greater in organisms from anaerobically grown cultures of this yeast. Production of all alcohols, especially C_{16} , was greatest by *C. albicans* grown under anaerobic conditions.

Fig. 2. (a) Total ion-current trace of long-chain alcohol TMS ethers in GLC-MS. Numbers against peaks are scan numbers. (b) Electron-impact ionization mass spectrum of peak scan no. 253 of (a). This peak was recognized as having been generated by hexadecanol. Long-chain alcohols were purified using extracts of *C. albicans* harvested after 168 h growth under anaerobic conditions.

Long-chain alcohol production by C. albicans under anaerobic conditions

Further studies were confined to *C. albicans* grown under anaerobic conditions. The yeast had a doubling time of 110 min, and reached stationary phase after 48 h (Fig. 3). Only traces of long-chain alcohols were detected in organisms from exponentially growing cultures. However, after cultures entered stationary phase, the contents of C_{14} , C_{16} , and C_{18} alcohols increased rapidly until about 170 h after which alcohol contents remained constant. Rates of increase in alcohol content during stationary phase were greatest for C_{16} alcohols and smallest for C_{14} alcohols (Fig. 3). Long-chain alcohols were not detected in culture filtrates except after prolonged (500 h) incubation, when the low concentrations present were probably due to limited lysis of organisms.

Table 1. *Detection of long-chain alcohols in yeasts grown for 72 h anaerobically or aerobically*

Yeast	Detection in cultures grown:					
	Anaerobically			Aerobically		
	C _{14:0}	C _{16:0}	C _{18:0}	C _{14:0}	C _{16:0}	C _{18:0}
<i>C. albicans</i> NCYC 1467	+	+	+	+	+	+
<i>C. bombicola</i> NCYC 1449	—	—	—	—	—	—
<i>C. maltosa</i> CMCC 3152	+	+	+	—	—	—
<i>C. ingens</i> NCYC 822		No growth		—	—	—
<i>C. utilis</i> NCYC 168	+	+	+	+	+	+
<i>C. utilis</i> NCYC 707	+	+	+	—	—	—
<i>D. hansenii</i> NCYC 9	—	—	—	—	—	—
<i>P. fermentans</i> NCYC 850	+	+	+	+	+	+
<i>Rh. glutinis</i> NCYC 59	—	—	—	—	—	—
<i>Rh. glutinis</i> CMCC 2272		No growth		—	—	—
<i>Rh. rubra</i> NCYC 195		No growth		+/-	+/-	+/-
<i>Rhodotorula</i> sp. ATCC 20254		No growth		—	—	—
<i>S. cerevisiae</i> Y185	+	+	+	+/-	+/-	+/-
<i>Saccharomycopsis</i> (<i>Yarrowia</i>) <i>lipolytica</i> ATCC 20225		No growth		—	—	—

Table 2. *Long-chain alcohol contents of yeasts*

Cultures were grown under anaerobic conditions (except where indicated) for 72 h. Values quoted are the means of three independent determinations $\pm$ SD.

Yeast	Long-chain alcohol content [μg (g dry wt organisms) ⁻¹]		
	C _{14:0}	C _{16:0}	C _{18:0}
<i>C. albicans</i> NCYC 1467 (aerobic)	8.76 $\pm$ 0.80	18.08 $\pm$ 0.97	26.31 $\pm$ 2.03
<i>C. albicans</i> NCYC 1467	20.59 $\pm$ 1.79	139.29 $\pm$ 10.24	34.47 $\pm$ 2.82
<i>C. maltosa</i> CMCC 3152	1.76 $\pm$ 0.35	47.91 $\pm$ 13.84	5.62 $\pm$ 1.01
<i>C. utilis</i> NCYC 168	3.30 $\pm$ 0.63	17.93 $\pm$ 3.47	3.92 $\pm$ 0.82
<i>C. utilis</i> NCYC 707	2.13 $\pm$ 0.72	27.05 $\pm$ 9.26	4.42 $\pm$ 0.98
<i>P. fermentans</i> NCYC 850	3.25 $\pm$ 0.30	28.47 $\pm$ 7.88	4.38 $\pm$ 0.68
<i>S. cerevisiae</i> Y185	0.89 $\pm$ 0.02	4.66 $\pm$ 0.48	1.35 $\pm$ 0.55

Effect of PCMB on extraction of free and esterified long-chain alcohols from C. albicans

Supplementing cultures with the lipase inhibitor PCMB (Schousboe, 1976) just before harvesting, and including this compound during the extraction procedure up to the stage at which filtered chloroform/methanol extracts were pooled, appreciably decreased the apparent content of all three alcohols (Table 3). The possibility that a proportion of the alcohols was esterified was therefore examined using unsupplemented extractions and extractions containing PCMB. Only a small proportion was found to be esterified in this fraction (Table 3).

Fatty-acyl composition of total lipids and lipid classes in C. albicans

The major fatty-acyl residues in all of the fractions analysed (Table 4) were C₁₆ and C₁₈, including both saturated and unsaturated residues. In the triacylglycerol fractions and in the total lipid extract, the proportion of C_{14:0} residues was also high, much more so than in the phospholipid, sterol/wax ester and free fatty-acid fractions.

Table 3. *Effect of PCMB supplementation on contents of free and esterified long-chain alcohols in C. albicans harvested from 120 h cultures*

PCMB was included in the culture and during the extraction procedure as indicated in the text. Values quoted are the means of three independent determinations $\pm$ SD.

Inhibitor	Fraction	Long-chain alcohol content [μg (g dry wt organisms) $^{-1}$]		
		$C_{14:0}$	$C_{16:0}$	$C_{18:0}$
None	Free	90.57 $\pm$ 5.69	252.91 $\pm$ 10.50	135.65 $\pm$ 14.86
	Wax ester	8.23 $\pm$ 0.64	7.47 $\pm$ 0.12	6.54 $\pm$ 0.16
PCMB	Free	55.77 $\pm$ 3.0	125.96 $\pm$ 16.79	61.08 $\pm$ 3.86
	Wax ester	19.47 $\pm$ 0.54	22.85 $\pm$ 0.71	12.08 $\pm$ 0.61

Table 4. *Fatty-acyl composition of total lipids and lipid classes from C. albicans grown anaerobically for 168 h*
 Δ mol $^{-1}$ values were calculated as described by Kates & Hagen (1964).

Lipid	Contents of residues (percentage of total) (means of three independent analyses $\pm$ SD)								Δ mol ⁻¹
	C _{12:0}	C _{14:0}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}	
Total	11.3 $\pm$ 1.3	18.4 $\pm$ 1.2	25.9 $\pm$ 1.3	6.7 $\pm$ 0.6	15.2 $\pm$ 1.8	7.8 $\pm$ 0.1	13.3 $\pm$ 0.9	1.7 $\pm$ 0.2	0.46
Phospholipids	1.5 $\pm$ 0.2	4.3 $\pm$ 0.5	25.4 $\pm$ 1.0	6.3 $\pm$ 0.6	18.3 $\pm$ 0.3	16.2 $\pm$ 1.8	25.2 $\pm$ 1.2	2.8 $\pm$ 0.1	0.81
Triacylglycerols	11.6 $\pm$ 1.1	20.6 $\pm$ 1.9	22.8 $\pm$ 0.8	5.3 $\pm$ 0.5	21.3 $\pm$ 1.6	7.1 $\pm$ 0.6	9.9 $\pm$ 0.3	1.3 $\pm$ 0.1	0.36
Sterol/wax esters	14.5 $\pm$ 1.7	13.3 $\pm$ 0.5	23.5 $\pm$ 1.6	6.3 $\pm$ 1.5	22.2 $\pm$ 0.8	8.7 $\pm$ 1.1	10.2 $\pm$ 1.2	1.4 $\pm$ 0.3	0.40
Free fatty acids	7.3 $\pm$ 2.0	7.4 $\pm$ 0.9	24.3 $\pm$ 0.7	7.4 $\pm$ 3.9	27.1 $\pm$ 6.0	10.3 $\pm$ 1.0	14.2 $\pm$ 0.9	2.1 $\pm$ 0.5	0.52

Fig. 3. Time-course of growth (○) of *C. albicans* and of its contents of C_{14:0} (●), C_{16:0} (□) and C_{18:0} (■) long-chain alcohols. Values quoted are the means of three independent analyses $\pm$ SD.

DISCUSSION

The present paper is the most extensive study published on long-chain alcohols in yeasts. Davidova *et al.* (1978) reported briefly that glucose-grown *C. tropicalis* synthesizes wax esters. In addition, several reports have appeared of yeast lipid fractions which, from their mobilities, could well be long-chain alcohols (Zalashko *et al.*, 1979; Watanabe & Takakuwa, 1984), although they are frequently reported as being 'unidentified'. The present report of long-chain alcohol production by *C. albicans* contrasts with that by Davidova *et al.* (1978) in that alcohol production was confined to the stationary phase of growth, suggesting that long-chain alcohols in this yeast are secondary metabolites. Naccarato *et al.* (1972, 1974) reported detection of long-chain alcohols in *Escherichia coli* harvested from stationary-phase cultures but they did not describe the time-course of alcohol production.

All of the yeasts which in the present study were shown to produce long-chain alcohols synthesized these compounds with the same chain-length profile, namely C₁₆ > C₁₈ > C₁₄. Traces of C₁₂ alcohol were also detected in some extracts. Significantly, none of the yeasts examined synthesized unsaturated alcohols. The chain-length profile is similar to the fatty-acyl residue profile reported for yeasts in general (Ratray *et al.*, 1975; Kaneko *et al.*, 1976). The fatty-acyl composition of total lipid extracts of *C. albicans*, and specifically of the triacylglycerol and sterol/wax ester fractions, was particularly rich in C₁₄ residues, much more so than most other yeasts. It is tempting to suggest that saturated long-chain alcohols arise by reduction of CoA esters of long-chain carboxylic acids that alternatively would be channelled into other neutral lipids, a route which has been demonstrated in a variety of other organisms (Riendeau & Meighen, 1985). The switch from incorporation into triacylglycerols and other neutral lipids into long-chain alcohols would appear to be associated with cessation of growth. Synthesis by reduction of long-chain carboxylic acids is the more likely route than α -oxidation to the corresponding aldehyde (Fulco, 1967) followed by reduction to the alcohol, since the latter route would yield predominantly odd-chain alcohols which were not detected in any of the yeasts studied.

Synthesis of long-chain alcohols by the yeasts studied was clearly favoured by anaerobic conditions which repress formation of mitochondria (Käppli, 1986). This might be explained

by the anaerobic conditions diverting acyl-CoA derivatives to reductive routes. Repression of mitochondrial synthesis would have been increased by the rather high concentration of glucose in the medium used for aerobic and particularly anaerobic growth. Possibly, therefore, mitochondria are, in *C. albicans*, organelles which indirectly exert some type of control over synthesis of long-chain alcohols.

No attempt was made in the present study to establish where long-chain alcohols are located in *C. albicans*. We did, however, try to establish whether and to what extent these alcohols were present in organisms in the free and esterified form. Having discovered that lipid extracts of *C. albicans* contained wax esters, it seemed possible that these esters contained residues of long-chain alcohols which might be hydrolysed during extraction of lipids from the yeast. Inclusion of the lipase inhibitor PCMB in the yeast culture and during lipid extraction, although causing a considerable decrease in the content of long-chain alcohols in extracts, did not lead to a corresponding increase in the size of the wax-ester fraction. It is likely, therefore, that long-chain alcohol residues are present in *C. albicans* in some other as yet unknown molecular form.

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