Dimorphism-associated Variations in the Lipid Composition of *Candida albicans*

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Yeast and mycelial forms of *Candida albicans* ATCC 10231, growing together in 12 h and in 96 h cultures, were separated and their lipids were extracted and characterized. The total lipid content of the yeast forms was always lower than that of the mycelial forms. In 12 h cultures the lipids from the two morphological forms consisted mainly of polar compounds, viz. phospholipids and glycolipids. In 96 h cultures both the yeast and mycelial forms accumulated substantial amounts of apolar compounds, mainly steryl esters and triacylglycerols. The mycelial forms were more active than the yeast forms in this respect. Major differences in the lipid composition between the two morphological forms involved the contents of sterols and complex lipids that contain sterols. As a rule, the yeast lipids contained much larger proportions of free sterols than the mycelial lipids. However, the mycelial lipids contained several times more sterols than the yeast forms but bound as steryl glycosides, esterified steryl glycosides and steryl esters. Steryl glycosides and esterified steryl glycosides occurred in yeast lipids only in traces, if at all. The major steryl glycoside in the mycelial forms was unequivocally identified as cholesteryl mannoside. At both phases of growth the apolar and polar lipid fractions from the mycelial forms contained higher levels of polyunsaturated fatty acids (18:2 and 18:3) but lower levels of oleic acid (18:1) than the corresponding fractions from the yeast forms. The lipid content and composition of 12 h and 96 h yeast and mycelial forms of *C. albicans* KCCC 14172, a clinical isolate, were almost identical with those of *C. albicans* ATCC 10231.

INTRODUCTION

In the last ten years the lipids of *Candida albicans* have been the subject of a considerable number of investigations. Many studies have been devoted to the relation of lipids to antibiotic resistance (Fryberg *et al.*, 1975; Yamaguchi, 1977; Koh *et al.*, 1977; Subden *et al.*, 1977; Singh *et al.*, 1979a; Mehta *et al.*, 1984); others have been concerned with changes in lipids during ageing (Combs *et al.*, 1980), starvation and germ-tube formation (Sundaram *et al.*, 1981) and with the role of sterols in amino acid transport (Singh *et al.*, 1979b). The lipids of isolated cellular fractions such as the cell wall (Bianchi, 1967) and the plasma membrane (Marriott, 1975; Yamaguchi, 1977) have also been investigated.

The comparison of lipids and their constituent fatty acids in yeast and mycelial forms of *C. albicans* is particularly important because only one of the two morphological forms is pathogenic (Balish & Phillips, 1966; Rebora *et al.*, 1973). Two studies have dealt with the lipids of cellular fractions only (Bianchi, 1967; Marriott, 1975) and another two only with the phospholipids of whole yeast cells and mycelia (Ballmann & Chaffin, 1979; Yano *et al.*, 1982). In all these studies the two morphological forms analysed were grown in separate cultures; hence, any differences recorded in the lipids of the two forms can, at least in part, be attributed to variations in culture conditions.

The main objective of the present work was to characterize and compare the lipids and their constituent fatty acids of yeast and mycelial forms of *C. albicans* grown in the same cultures.

METHODS

Organisms and growth conditions. *Candida albicans* ATCC 10231 and *C. albicans* KCCC 14172, a clinical isolate obtained from the oral cavity of a cancer patient undergoing therapy at Kuwait Cancer Control Centre, were the strains used. Stock cultures were maintained on slopes of modified Sabouraud agar medium (Difco) stored at 4 °C and subcultured routinely. The organisms were grown at 37 °C by static incubation in SSV medium (Marriott, 1975) for 96 h. Several samples were taken, at intervals, for the determination of total dry weight and the relative proportions of yeast and mycelial forms.

Separation of yeast and mycelial forms. The culture containing yeast and mycelial forms was centrifuged; the pellet was washed three times with 0.9% NaCl solution and was then mixed with 0.9% NaCl solution to form a relatively heavy suspension that was passed through two successive filters (Retsch) with pore sizes of 38 µm and 20 µm, respectively. The mycelial residue on the filters was repeatedly rinsed with 0.9% NaCl solution until it became free of yeast-like forms on microscopic examination. Yeast-like cells in the filtrate were harvested by centrifugation.

Scanning electron microscopy (SEM). Samples of the yeast and mycelial forms as well as of the mixed cultures were prepared for SEM (Ghannoum & Al-Khars, 1984). The cells were suspended in glutaraldehyde (1.5%, w/v) for 2 min, collected by centrifugation and resuspended in a fresh sample of the same fixative for 16 h at 4 °C. The samples were washed with distilled water, placed on a coverslip, left to dry and coated ready for examination in a stereoscan electron microscope (Novascan 30).

Lipid extraction and analysis. Cultures of each strain grown for 12 h and 96 h were studied. Total lipids were extracted from samples of yeast and mycelial forms as well as from the mixed culture with chloroform/methanol (2:1, v/v) and purified using established procedures (Folch *et al.*, 1957). Apolar lipid fractions were resolved by chromatography on thin layers of silicic acid using hexane/diethyl ether/acetic acid (90:10:1, by vol.) as a developing solvent (Mangold & Malins, 1960). Polar lipid fractions were resolved by two-dimensional TLC using the solvent systems chloroform/methanol/7 M-ammonium hydroxide (65:30:4, by vol.) in the first direction and chloroform/methanol/acetic acid/water (170:25:25:4, by vol.) in the second direction (Nichols, 1964).

The various lipid fractions were detected by exposure to iodine vapour or by charring at 220 °C after spraying the plates with 50% (v/v) H₂SO₄. The fractions were identified by comparing their chromatographic behaviour with that of authentic samples and by the colour reactions they gave with specific spray reagents (Stahl, 1962; Dittmer & Lester, 1964; Siakotos & Rouser, 1965). For structural studies of polar lipid fractions, samples (about 10 mg) of pure compounds, separated by preparative TLC and eluted from the adsorbent with chloroform/diethyl ether/ethanol (1:1:1, by vol.) (Kates, 1972), were subjected to acid hydrolysis (Gaver & Sweeley, 1965). Hydrolysis products soluble in chloroform were identified by TLC, GLC and mass spectrometry (Finnigan MAT 311A; double-focusing reverse near-Johnson geometry). Lipid fractions resolved by TLC and subjected to charring were quantified densitometrically using a Beckman R-112 densitometer. Sterols were also determined quantitatively by GLC using β -sitosterol as an internal standard. Hydrolysis products soluble in water and found by paper chromatography (Menzies & Seakins, 1976) to be hexoses were quantitated spectrophotometrically (Yamamoto & Rouser, 1970).

Samples of apolar and polar lipid fractions separated by preparative TLC were subjected to methanolysis (Chalvardjian, 1964). The resulting methyl esters were purified by TLC and analysed by GLC using a Pye Unicam model 204 gas chromatograph fitted with a glass column (1.83 m $\times$ 4 mm i.d.) packed with 15% DEGS on Anakrom D, 100–120 mesh. Sterols were analysed by GLC using a glass column (1.83 m $\times$ 2 mm i.d.) packed with 3% OV-17 on Chromosorb W-HP, 100–120 mesh.

RESULTS

The separation of yeast and mycelial forms by filtration led to highly pure preparations of each form (Fig. 1).

The growth curves of the two *C. albicans* strains and, for strain ATCC 10231, changes in the proportions of the two morphological forms during the incubation period are shown in Fig. 2. It is apparent that the 12 h samples taken for lipid analysis were in the exponential phase of growth whereas the 96 h cultures were in the late stationary phase. The proportions by weight of the two morphological forms showed slight fluctuations during growth. Apparently some mycelial forms were producing blastospores (e.g. after 18 h) and some blastospores were germinating again into mycelia (e.g. after 96 h) without any significant change in the total biomass. However, as the results in Fig. 2 indicate, these fluctuations involved only relatively small proportions of the two morphological forms. Therefore, the samples collected for lipid analysis may be considered satisfactorily homogeneous, as far as their ages are concerned.

Fig. 1. Scanning electron micrographs of 12 h cultures of *C. albicans* ATCC 10231. (a) Mixed culture (bar 10 µm); (b) yeast forms (bar 4 µm); (c) mycelial forms (bar 10 µm).

Fig. 2. Growth curves of *C. albicans*. △, Strain ATCC 10231; ○, strain KCCC 14172. □, percentage of yeast forms; ▽, percentage of mycelial forms (strain ATCC 10231).

Fig. 3. Typical TLC plates showing apolar lipids of yeast and mycelial forms of 12 h (a) and 96 h (b) cultures of *C. albicans* ATCC 10231. The sorbent was Silica Gel G; the solvent was hexane/diethyl ether/acetic acid (90:10:1, by vol.). The lipids were visualized by exposure to iodine vapour. SE, steryl esters; AE, alkyl esters; TG, triacylglycerols; FA, fatty acids; S, sterols; PL, polar lipids.

We did not find strain-associated variation in the total lipid content nor in the lipid composition of *C. albicans*. The TLC plates of lipids from both strains were always identical. Therefore, we concentrated on the lipids of *C. albicans* ATCC 10231 only.

The total lipid content of 12 h yeast and mycelial forms was 0.4% and 1.3%, respectively, on a dry weight basis. The total lipid content of 96 h cultures was 1.3% and 6.3% for yeast and mycelial forms, respectively.

The lipids from 12 h cultures of the two morphological forms consisted predominantly of polar compounds (Table 1). This situation changed in 96 h cultures, especially for mycelial lipids. However, the absolute content in various classes of polar lipids by no means decreased with ageing because the total lipid content of 96 h cultures of the two forms was several times higher than that of 12 h cultures. The apolar lipids from the yeast forms of 12 h cultures consisted predominantly of sterols, which were present at much lower concentrations in the mycelial forms of the same age (see also Fig. 3).

In 96 h cultures there were still more sterols in the yeast lipids than in the mycelial lipids. Yet, at this phase of growth, both morphological forms had accumulated considerable proportions of other apolar lipids, viz. steryl esters, alkyl esters, triacylglycerols and fatty acids. The mycelial forms accumulated considerably more of these lipid classes (about 60% of the total lipids) than did the yeast forms (about 40%).

The polar lipids of 12 h cultures of the two morphological forms consisted mainly of

Fig. 4. Typical TLC plates of lipids from yeast (*a, c*) and mycelial (*b, d*) forms of 12 h (*a, b*) and 96 h (*c, d*) cultures of *C. albicans* ATCC 10231. The sorbent was Silica Gel G; the solvents were (I) chloroform/methanol/7 M-ammonium hydroxide (65:30:4, by vol.) and (II) chloroform/methanol/acetic acid/water (170:25:25:4, by vol.). The lipids were visualized by charring. S, start; PI, phosphatidylinositols; PS, phosphatidylserines; PA, phosphatidic acids; PC, phosphatidylcholines; PE, phosphatidylethanolamines; PG, phosphatidylglycerols; DGD, digalactosyldiacylglycerols; CMH, ceramide monohexosides; SG, steryl glycosides; ESG, esterified steryl glycosides; MGD, monogalactosyldiacylglycerols; CL, cardiolipins; FA, fatty acids; NL, neutral lipids.

phospholipids in addition to smaller proportions of glycolipids. In the 96 h cultures this remained true only for the yeast forms, whereas in the mycelial forms glycolipids predominated (see also Fig. 4).

The major phospholipids were phosphatidylcholines, phosphatidylethanolamines and phosphatidylinositols. Smaller proportions of phosphatidylserines and phosphatidylglycerols (mainly in the yeast forms) and phosphatidic acids were also found. The major glycolipids in the yeast lipids were ceramide monohexosides that occurred in the mycelial lipids only in traces. On the other hand, the mycelial lipids contained as major glycolipids steryl glycosides and, in 96 h cultures, esterified steryl glycosides too, in addition to substantial proportions of digalactosyl-

Table 1. *Comparison of lipids from yeast and mycelial forms of C. albicans ATCC 10231*

Values are expressed as a percentage of the total lipids. Tr, traces; ND, not detected.

Lipids	12 h cultures		96 h cultures	
	Yeast forms	Mycelial forms	Yeast forms	Mycelial forms
Apolar compounds				
Hydrocarbons	Tr	Tr	Tr	Tr
Steryl esters	Tr	Tr	8.5	25.3
Alkyl esters	Tr	Tr	3.8	8.8
Triacylglycerols	Tr	Tr	6.4	16.5
Fatty acids	Tr	Tr	10.4	11.2
Sterols	14.8	Tr	9.3	Tr
Polar compounds				
Esterified steryl glycosides	ND	ND	Tr	6.5
Monogalactosyldiacylglycerols	Tr	Tr	Tr	2.7
Steryl glycosides	Tr	16.2	Tr	9.1
Ceramide monohexosides	10.3	Tr	8.8	Tr
Phosphatidylethanolamines	11.4	12.7	9.6	Tr
Phosphatidylglycerols	9.2	Tr	7.3	Tr
Phosphatidylcholines	25.6	28.8	15.0	4.1
Digalactosyldiacylglycerols	Tr	10.2	Tr	6.2
Phosphatidylserines	8.8	9.9	6.9	ND
Phosphatidylinositols	10.9	11.4	8.9	ND
Phosphatidic acids	9.0	7.3	4.8	ND

Table 2. *Identities and relative proportions (%) of sterols in total lipids* from yeast and mycelial forms*

Cultures were 12 h old.

Sterol	Yeast forms	Mycelial forms
Degradation product of ergosterol	4.5	ND
Cholesterol	ND	37.8
Zymosterol	17.0	13.7
Ergosterol	14.6	5.6
24,28-Dehydroergosterol	42.7	18.8
3- β -Hydroxy-24-methyl cholesta-5,7-diene	11.7	22.2
4,4-Dimethylzymosterol	9.5	2.0

ND, Not detected.

* Subjected to acid hydrolysis.

diacylglycerols and traces of monogalactosyldiacylglycerols. These glycolipids were only minor constituents of the yeast lipids.

Determinations based on spectrophotometric estimation of the sugar moiety (Yamamoto & Rouser, 1970) as well as on GLC analysis of the sterol moiety confirmed the densitometric quantification, and showed that steryl glycosides made up 11–15% of the total lipids from 12 h mycelial forms compared with only traces in lipids from 12 h yeast forms.

The fraction tentatively identified as steryl glycosides was isolated from mycelial lipids by preparative TLC and was subjected to acid hydrolysis. It produced a hexose and a sterol mixture in a molecular ratio of 1:1. In GLC analysis the sterol fraction showed a composition similar to that presented in Table 2. The sterol, whose retention time corresponded to that of cholesterol, was isolated by preparative GLC in a pure form (Fig. 5) and subjected to mass-spectrometric analysis. Low-resolution mass spectrometry of this compound was done at 125 °C, with direct insertion. From the MS traces the molecular ion was assumed to have an *m/e* of about 386. High resolution measurements gave values of 386.297, 386.354 (main peak) and 386.390. The

Fig. 5. Typical gas chromatogram showing the purity of cholesterol isolated from the sterol moiety of steryl glycosides by preparative GLC.

Table 3. *Fatty acid composition of apolar and polar lipid fractions from yeast and mycelial forms*

Apolar lipid fractions contained free fatty acids, triacylglycerols and steryl esters; polar lipid fractions contained phospholipids and glycolipids. Tr, traces.

Fatty acid	12 h cultures				96 h cultures			
	Yeast forms		Mycelial forms		Yeast forms		Mycelial forms	
	Apolar lipids (%)	Polar lipids (%)	Apolar lipids (%)	Polar lipids (%)	Apolar lipids (%)	Polar lipids (%)	Apolar lipids (%)	Polar lipids (%)
14:0	4.3	4.2	4.2	3.6	6.6	7.0	6.6	6.5
14:1	1.8	6.2	Tr	Tr	5.1	2.0	5.7	8.2
16:0	11.8	13.1	14.5	15.3	28.5	25.4	28.4	20.7
16:1	11.4	9.6	12.2	13.2	10.4	10.0	14.6	Tr
16:2	Tr	Tr	Tr	Tr	8.7	3.1	6.0	9.9
18:0	14.0	10.3	16.7	15.2	14.5	7.4	10.4	12.1
18:1	37.6	41.5	26.7	26.7	14.7	22.4	15.9	8.2
18:2	12.0	10.7	16.3	13.5	5.5	18.8	2.7	Tr
18:3	7.5	4.4	9.4	12.5	5.7	3.9	9.3	33.7

calculated mass of authentic cholesterol was 386.355. A low resolution run of authentic cholesterol on the mass spectrometer gave a fragmentation pattern comparable to that of the isolated compound.

The hexose moiety of the steryl glycoside fraction was identified by paper chromatographic analysis as mannose. The major steryl glycoside in the mycelial lipids was thus cholesteryl mannoside. The identities and relative proportions of individual sterols from total lipids that were subjected to acid hydrolysis are shown in Table 2. The mycelial lipids contained the same sterols as the yeast lipids, and in addition relatively high proportions of cholesterol.

The fatty acid composition of apolar and polar lipid fractions from yeast and mycelial forms is shown in Table 3. In 12 h cultures both fractions of mycelial lipids contained higher levels of polyunsaturated fatty acids (18:2 and 18:3) but lower levels of oleic acid (18:1) than the corresponding yeast fractions. These differences became even more pronounced in 96 h cultures, in which the mycelial forms contained up to 33.7% linolenic acid (18:3) in their polar lipid fraction.

DISCUSSION

In earlier studies on the lipids of *C. albicans* the two morphological forms were grown in separate cultures at various temperatures (Bianchi, 1967; Ballmann & Chaffin, 1979) and/or using media of different composition (Bianchi, 1967; Marriott, 1975; Ballmann & Chaffin, 1979; Yano *et al.*, 1982). Such factors dramatically alter the lipid composition of various organisms (for reviews see Radwan & Mangold, 1976; Wassef, 1977). To eliminate this source of error we analysed the lipids from yeast and mycelial forms growing in the same cultures.

Our results show that mycelial lipids are poorer than yeast lipids in sterols, but are much richer in complex lipids that contain sterols such as steryl glycosides and esterified steryl glycosides. Otherwise, the two morphological forms have phospholipid patterns similar to those described previously (Ballmann & Chaffin, 1979; Yano *et al.*, 1982). Steryl glycosides and esterified steryl glycosides are widely distributed in the lipids of higher plants and plant cell cultures (e.g. Radwan *et al.*, 1975) but reports of their occurrence in lipids of micro-organisms are very rare (Thompson *et al.*, 1973). Hence, the unequivocal identification of this lipid appeared to be of particular importance. By combined TLC, GLC and MS the major steryl glycoside in mycelial lipids was identified as cholesteryl mannoside.

As yet, it is not known what biological role(s) the steryl glycosides may play in the living cell. However, being polar compounds with hydrophilic (hexose) and hydrophobic (sterol) ends it is expected that such compounds would occur mainly in the cell envelope. Additional studies are still needed to explain why mycelial lipids of *C. albicans* contain sterols mainly as steryl glycosides whereas yeast lipids contain these compounds mainly in the free form. Sterols are commonly believed to stabilize cell membranes and provide them with rigidity. The mycelial forms of *C. albicans*, being filamentous, must endure greater physical stress on their membranes than the yeast forms. The question that remains is do steryl glycosides confer more rigidity on membranes than free sterols? Mycoplasmas are prokaryotes that grow as naked thin filaments; they need to be supplied with cholesterol and accumulate this compound as a glycoside in their membranes (Rose, 1976).

Our result concerning the occurrence of higher levels of polyunsaturated fatty acids in mycelial lipids than in yeast lipids basically confirms the finding of an earlier study (Yano *et al.*, 1982).

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