Mode of Action of the Polyene Antibiotic Candicidin: Binding Factors in the Wall of *Candida albicans*

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The polyene antibiotic candicidin produces a rapid efflux of K^+ ions from a suspension of *Candida albicans*. Onset of K^+ leakage depends on the culture age, stationary-phase yeasts leaking K^+ more slowly than exponential-phase yeasts. The time taken for potassium leakage to begin represents the time taken by the antibiotic to cross the cell wall and produce membrane damage. It was shown that there were factors in the cell wall of *C. albicans* that increased their total binding capacity and their affinity for candicidin during growth. An attempt was made to relate changes in the lipid content of the yeast cell with the increased time taken to produce membrane damage.

Polyene antibiotics, e.g., nystatin, amphotericin B, and candicidin, display a selective toxicity towards organisms whose membranes contain sterols. This group of antibiotics destroys the selective permeability properties of a wide variety of fungi and other eukaryotic organisms (membranes that contain sterols) but has no effect on prokaryotic organisms (membranes that do not contain sterol) (22). In common with other membrane-active antimicrobial agents, treatment of yeasts with polyenes produces leakage of small molecules. Losses from the yeast of potassium, inorganic phosphate, carboxylic and amino acids, and phosphate esters have all been reported from yeasts treated with growth-inhibitory concentrations of polyenes (22). Yeasts assimilate potassium from the environment against the concentration gradient (25). Changes in the selective permeability of the yeast cell membrane, induced by polyene antibiotics, lead to a rapid efflux of these ions into the suspending medium. Specific ion electrodes have been used to study the effects of polyenes on membrane permeability (10, 14, 21). It has been shown that the age of the *Candida albicans* culture affects the time taken for the polyene-induced potassium efflux to begin. Older yeasts leak potassium more slowly than younger yeasts (14, 21). It is thought that the time taken for the efflux of potassium to begin after polyene application represents the time taken for the antibiotic to penetrate the cell wall and affect the cell membrane. It has been reported that the bulk of the bound candicidin is associated with an un-

known component in the yeast cell wall in addition to the active site in the cell membrane (13). Addition of fatty acids to yeast suspensions prevents polyene antifungal activity (11, 15, 26). The lipid composition of the cell wall of *C. albicans* was investigated to see whether this could provide a possible explanation of the observed facts.

MATERIALS AND METHODS

Antibiotic. Candicidin (lot 681 NOF2) with a potency of 218% (manufacturer's bioassay, in which original candicidin made by S. B. Penick Ltd. was given an activity of 100%) was supplied by Pharmax Ltd., Bexley, Kent, and stored desiccated at -20°C . Aqueous solutions were prepared by diluting candicidin dissolved in dimethyl sulfoxide (Me_2SO) with 0.025 M sodium dimethyl glutarate buffer (DMG) to give a final Me_2SO concentration of less than 0.05% (vol/vol). [^{14}C]candicidin was prepared as described previously (12), dissolved in Me_2SO and diluted with 0.025 M DMG buffer.

Fresh antibiotic solutions were prepared daily. The potency of the antibiotic, as measured by microbiological assay (28), did not change during the experimental period. Aqueous solutions of the antibiotics were protected from light, to which they are sensitive, wherever practicable.

Organism and method of culture. Approximately 10^6 cells from a maintenance slope of *C. albicans* NCTC 713 grown on Sabouraud dextrose agar (Oxoid Ltd., London) for 24 h at 37°C , and stored at 4°C , were transferred to 100 ml of prewarmed sterile Sabouraud dextrose broth (SDB) (Oxoid Ltd.) in a 300-ml Erlenmeyer flask fitted with a loose cotton-wool plug and incubated at 37°C in shake culture to ensure aerobic growth. At set time intervals the yeast was harvested by centrifugation (MSE 18, Croydon; $10,000 \times g$ for 10 min) and washed once with distilled water and once with 0.025 M DMG (pH 6.0).

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Preparation of standard yeast suspension. Enough washed yeast was suspended in 0.025 M DMG, pH 6.0, to give $E_{660\text{nm}}^{1\text{cm}} = 1.0$ (Cecil 304 spectrophotometer, Cecil Instruments Ltd., Cambridge). Such suspensions were found to contain 3.7×10^7 yeasts/ml (total hemocytometer count), and on drying to constant weight at 105 C they contained 0.92 mg (dry weight)/ml.

Preparation of protoplasts of *C. albicans*. Yeasts grown in SDB for 18 h at 37 C were harvested by centrifugation (MSE 18; $10,000 \times g$ for 10 min) and washed with 0.025 M DMG, pH 8.0. Protoplasts were prepared according to the method of Schibeci et al. (27).

Protoplast formation was followed using (i) phase-contrast microscopy, (ii) lysis of protoplasts viewed under the microscope on addition of 0.1 M MgCl_2 , and (iii) the decreasing extinction of samples at 660 nm on addition of distilled water. After 3 h of Helicase treatment it was found that the yield was 95 to 100%. Protoplasts formed by this method were viable, and, on plating out on corn meal agar supplemented with 15% mannitol after serial dilution, the viable count was 85% of the total hemocytometer count. Protoplasts grown on corn meal agar produced cell walls and chlamydospores within 3 days at 37 C.

Preparation of a wall fraction from *C. albicans*. Approximately 20 g of *C. albicans* (wet weight) was plasmolyzed in 1 M sodium chloride for 1 h at 20 C. The plasmolyzed yeasts were rapidly washed in distilled water and resuspended in 25 ml of distilled water before freeze-pressing (X press, AB Biotech, Croydon) according to the method of Edebo (8). The press was operated at -25 C, and the yeast was passed through the orifice five times at a pressure of approximately 2,000 kg/cm². It was found by determining the viable count that this method disrupted 98 to 99% of the yeast cells.

The freeze-pressed cells were then differentially centrifuged using an MSE 18 8 by 50 rotor. A wall fraction was obtained by centrifugation of the freeze-pressed yeast for 5 min at $10,000 \times g$. The wall fraction was a white creamy precipitate, and this was examined by phase-contrast and electron microscopy. It was found to contain exclusively large wall fragments, which were almost complete. The fraction was freeze dried (Genevac, model CFD-1, Radcliffe, Lancs.) and stored at -20 C. The wall fraction constituted 16 to 19% of the dry weight of the yeast. The wall fraction was examined for membrane contamination by assaying the fraction for sterol (3). Negligible amounts of this known membrane component were present.

Determination of bound [¹⁴C]candidicin. Bound [¹⁴C]candidicin was determined by the method of Hammond and Kliger (12).

Detection of potassium leakage. Candidicin-induced potassium fluxes from *C. albicans* in the standard yeast suspension were monitored in situ with a Phillips IS 560K specific ion electrode as described previously (14).

Determination of yeast ergosterol. The ergosterol level of *C. albicans* was determined by the method of Breivik and Owades (3).

Determination of triglycerides in yeast cell walls. Preweighed freeze-dried wall fractions of *C. albicans* were suspended in 0.5 M KOH in 90% (vol/vol) aqueous spectroscopic ethanol using an ultrasonic probe (MSE 100-W ultrasonic disintegrator, 1.8-cm probe, 20- μm amplitude, at 20 kHz/s for 15 min at 5 C). A 1-ml amount of 0.1 M MgCl_2 was added, and the mixture was incubated for 45 min at 55 C. The mixture was centrifuged (MSE 18, $20,000 \times g$ for 15 min) and assayed for triglycerides according to the method of Eggstein (9).

Extraction, purification, and gas-liquid chromatography of lipids from cells of *C. albicans*. A 50-mg amount of freshly prepared yeast cell walls was treated with 80% (vol/vol) aqueous ethanol for 15 min at 80 C to inactivate enzymes, particularly lipase, and lipids were extracted with 10 ml of chloroform-methanol (1:1, vol/vol). The material was stirred continuously during three successive 3-h extraction periods. The extracts were pooled, evaporated, dissolved in chloroform-methanol (2:1, vol/vol), and purified by a modification of the method proposed by Pederson (24). A 25-ml beaker was submerged in a 100-ml beaker containing 90 ml of distilled water. The crude lipid extract was delivered very slowly into the submerged small beaker, allowing the extract to fall through the entire water depth. The contents of the small beaker were stirred magnetically overnight at room temperature, when 1 ml of methanol was added to dissolve the cloudy interface. The lipid sample was removed with the chloroform-methanol layer, evaporated, dissolved in 0.5 ml of chloroform, and stored at -20 C. Methyl esters of the fatty acids present in the extracted lipids were prepared by direct methanolysis using the method of Stoffel et al. (29) as modified by Kates (19). The purified lipid extract was evaporated and dissolved in 2 ml of methanolic hydrogen chloride (2.5%, made by saturation of methanol with hydrogen chloride gas) and refluxed for 1 h. The methylated fatty acids were then extracted using successive quantities of petroleum ether (bp 30 to 60 C). The petroleum ether fractions were combined and evaporated to dryness.

The methyl esters were separated by gas-liquid chromatography using a Pye 104 apparatus (Pye-Unicam, Cambridge) with a glass column (0.125 inch by 5 feet [ca. 0.2 by 152.4 cm]) packed with 10% (wt/wt) polyethylene glycol adipate on Chromosorb W. The column temperature was 109 C, and the rate of carrier gas (nitrogen) flow was 60 ml/min.

Methyl esters were identified by comparing retention times with known standards (Sigma Ltd., Kingston, Surrey).

RESULTS

Potassium leakage from *C. albicans* treated with candidicin. Standard yeast suspensions made of *C. albicans* cells known to be in the logarithmic growth phase (yeasts harvested 6 h after inoculation into SDB) and a similar suspension of yeasts known to be in the stationary phase (harvested 18 h after inoculation into SDB) were prepared in 0.025 M DMG, pH 6.0.

FIG. 1. Potassium leakage from the standard *C. albicans* suspension on treatment with 25 μ g of candididin per ml. Symbols: stationary-phase cells (■); logarithmic cells (●); stationary-phase cells treated with 1% Me_2SO (○); log-phase cells treated with 1% Me_2SO (▼); log-phase cells untreated (▲); and *C. albicans* protoplasts treated with 5 μ g of candididin per ml (□).

The potassium efflux on treatment with 25 μ g of candididin per ml at 20 C was followed with a potassium-sensitive electrode. The experiment was repeated with an equivalent suspension of *C. albicans* protoplasts. Results appear in Fig. 1. The age of the yeast culture from which the protoplasts were prepared was found to have no effect on the leakage pattern observed. Me_2SO (0.1%) alone produced negligible K^+ leakage from *C. albicans* protoplasts. Low levels of candididin, sublethal for the standard *C. albicans* suspension, produced almost immediate K^+ leakage in equivalent suspensions of *C. albicans* protoplasts (Fig. 1). The absence of a cell wall to absorb candididin raises the effective antibiotic concentration and also makes the sites for candididin binding in the cell membrane more accessible. *C. albicans* cells known to be in the exponential growth phase, when treated with lethal levels of candididin, begin to leak potassium 90 s after antibiotic application, leakage being complete within 15 min. Equivalent suspensions of yeasts from the stationary growth phase treated with candididin begin to leak potassium 4 min after antibiotic treatment, and leakage is completed within 20 min. Hence the time taken for antibiotic to penetrate the cell wall of *C. albicans* and damage the cell membrane is of the order of 90 s after antibiotic application for exponential-phase yeasts and 4 min for stationary-phase yeasts (as protoplasts undergo almost instantaneous leakage).

Consistent potassium leakage curves were produced as a result of candididin action. By taking the midpoint of such curves, i.e., the time taken for 50% of the total potassium leak-

age, a convenient index permitting comparison between different conditions was obtained. Figure 2 shows the effect of culture age upon the time taken for 50% of the potassium to leak from the standard suspension of *C. albicans* in 0.025 M DMG, pH 6.0, treated with 25 μ g of candididin per ml.

C. albicans cells from an 18-h culture grown in SDB at 37 C were made into a standard suspension, and 2,000 U per ml of a lipase known to be free of amylase and protease (type VII purified from *Candida cylindracea*; Sigma Ltd.) was added. The mixture was incubated for 1 h at 37 C, washed in 0.025 M DMG, pH 6.0, and made up to the original volume with fresh buffer. Figure 3 shows the potassium leakage induced by candididin from lipase-treated and untreated *C. albicans* cells. Candididin elicits a more rapid K^+ efflux from stationary-phase yeasts after lipase treatment.

Binding of [^{14}C]candididin to cell walls derived from *C. albicans* cells of differing ages. *C. albicans* was grown in SDB at 37 C and harvested by centrifugation at 12 and 24 h. Cell wall fractions were prepared from the yeasts in both growth phases. A 30-mg portion of freeze-dried, 12- and 24-h walls was suspended in 6 ml of 0.1 M DMG (pH 6.0) and 4 ml of [^{14}C]candididin to make a final antibiotic concentration of 40 μ g/ml. The mixture was incubated at 25 C for 1 h, and the walls were centrifuged (MSE 18, 10,000 $\times g$ for 10 min) and washed with 0.1 M DMG (pH 6.0) until the supernatant gave no extinction at 380 nm. The washing procedure was kept uniform (using a Gallenkamp bench shaker, setting 10 for 10 min). The

FIG. 2. Effect of culture age on the time taken for 50% K⁺ leakage from *C. albicans* after treatment with 25 μ g of candididin per ml (20 C, pH 6.0).

FIG. 3. Potassium leakage from 18-h *C. albicans* cells treated with 25 μ g of candididin per ml (■); leakage induced by 25 μ g of candididin per ml from 18-h *C. albicans* cells pretreated with 2,000 U of lipase per ml (●); leakage from *C. albicans* treated with 1% Me₂SO (○); and leakage from lipase-treated yeasts + 1% Me₂SO (□) (pH 6.0, 20 C).

[¹⁴C]candididin levels were determined for the bound candididin, the nonbound candididin, and the washings. From the amount of non-bound candididin it was estimated that the 12-h

cell walls bound 48.8% of the antibiotic applied and the 24-h cell walls bound 61.4% of the antibiotic applied. After six washings, 12-h cell walls retained 3% of the [¹⁴C]candididin applied, whereas after six washings 24-h walls retained 13.7% of the applied [¹⁴C]candididin. Table 1 shows how this [¹⁴C]candididin was released with washing. Candididin appears to be bound much more loosely by 12-h cell walls than by older walls; i.e., candididin can be washed more easily from younger cell walls. Chemical analysis showed that there was negligible sterol in either of these wall fractions, suggesting that a nonsteroid, candididin-binding component is present in the cell wall of *C. albicans*, which with time increases in total binding capacity and degree of binding affinity.

Effects of fatty acids on [¹⁴C]candididin uptake by *C. albicans*. The effects of fatty acids on [¹⁴C]candididin uptake by *C. albicans* were studied by adding sufficient acetic, caprylic, capric, lauric, palmitic, stearic, oleic, linoleic, or α -linolenic acids (Sigma Ltd.) (dissolved in 1 ml of absolute ethanol) to the standard *C. albicans* suspension to give a final fatty acid concentration of 0.4 mM, and the pH was adjusted to 4.5. The suspensions were treated with 20 μ g of [¹⁴C]candididin per ml, and the amount of [¹⁴C]candididin bound was determined after 1 h (when binding to *C. albicans* is complete; 12). The results appear in Table 2. When the standard *C. albicans* suspension is treated with 20 μ g of [¹⁴C]candididin at pH 4.5, 4.3 μ g of candididin becomes bound per mg (dry weight) of yeast. The addition of fatty acids having less than 12 carbon atoms has little or no effect on candididin uptake, but addition of longer-chain fatty acids can prevent candididin uptake by intact yeasts (Table 2). The effect on antibiotic uptake increases with fatty acid chain length. However, more significant are changes in the degree of saturation upon candididin uptake. In the C₁₈ series, stearic acid (saturated) prevents 20%, oleic acid (one double bond) 25%, linoleic

TABLE 1. Removal of [¹⁴C]candididin from isolated cell wall fractions of *C. albicans* by a uniform washing procedure with 0.1 M DMG (pH 6.0) of cell walls treated with 13.3 mg of candididin per g

Condition	mg of candididin bound/g of cell wall	
	12-h walls	24-h walls
Initially bound	6.45	8.105
After 1 washing	1.65	5.11
After 2 washings	1.05	3.60
After 3 washings	0.80	3.05
After 4 washings	0.60	2.60
After 5 washings	0.40	2.20
After 6 washings	0.20	1.82

TABLE 2. Effect of the addition of 0.4 mM fatty acids on the uptake of [14 C]candidin by *C. albicans* treated with 20 μ g of antibiotic per ml

Aliphatic fatty acid	Systematic name	No. of carbon atoms	Candici- din bound (μ g/mg of yeast, dry wt)
Acetic	<i>n</i> -Ethanoic acid	2	4.36
Caprylic	<i>n</i> -Octanoic acid	8	4.21
Capric	<i>n</i> -Decanoic acid	10	3.98
Lauric	<i>n</i> -Dodecanoic acid	12	3.98
Palmitic	<i>n</i> -Hexadecanoic acid	16	3.61
Stearic	<i>n</i> -Octadecanoic acid	18	3.49
Oleic	<i>cis</i> -9-octadecenoic acid	18 (1=)	3.01
Linoleic	<i>cis,cis</i> -9,12-octadecadienoic acid	18 (2=)	2.20
α -Linolenic	All- <i>cis</i> -9,12,15-octadecatrienoic acid	18 (3=)	1.60

acid (two double bonds) 49%, and α -linolenic acid (three double bonds) 64% of antibiotic uptake.

Changes in ergosterol content of *C. albicans* during growth. Approximately 10^6 yeasts from an overnight culture were inoculated into 100 ml of prewarmed SDB in a 300-ml Erlenmeyer flask and sampled at 6-h intervals, and total ergosterol was determined by the method of Breivik and Owades (3). Figure 4 shows that the total ergosterol content of *C. albicans* increases with the age of culture.

Changes in the triglyceride content of the cell wall of *C. albicans* during growth. Cell wall fractions of *C. albicans* were prepared every 3 h after inoculation into SDB at 37 C. The triglyceride level of each of these fractions was determined by the method of Eggstein (9). Figure 5 shows that wall triglycerides increase with culture age.

A purified lipid extract was prepared from these wall fractions, which were methylated and separated by gas-liquid chromatography. The components in the extracted lipids were identified by measuring retention times (R_t) and comparing these values to the R_t of standards and by the addition of known amounts of lipid standards to the lipid extract and examining the trace for coincidence. Table 3 shows the distribution of fatty acids in cell walls of *C. albicans* of increasing culture age. There is an increase in the total amount of fatty acids present, an increase in the chain length, and an

increase in the degree of unsaturation with age of culture.

DISCUSSION

The susceptibility of *C. albicans* to polyene antibiotics, as measured by K^+ leakage patterns, decreases progressively throughout the yeast growth cycle, the loss in susceptibility

FIG. 4. Changes in total ergosterol levels of *C. albicans* during growth in SDB at 37 C.

FIG. 5. Changes in wall triglycerides of *C. albicans* during growth in SDB at 37 C.

TABLE 3. Fatty acid content of cell walls of *C. albicans* (mg/100 mg, dry wt) determined by gas-liquid chromatography^a

Fatty acid	Chain length & degree of saturation	Age of cell wall			
		6 h	12 h	18 h	24 h
Caprylic	8	0.216	0.23	+	0.27
Capric	10	—	0.46	0.17	0.27
Lauric	12	+	0.41	0.40	+
Myristic	14	+	0.51	0.23	+
Palmitic	16	1.08	1.51	2.40	3.51
Palmoleic	16 (1=)	+	—	+	+
Margaric	17	—	+	+	+
Stearic	18	1.944	1.04	2.05	1.75
Oleic	18 (1=)	1.998	0.81	1.25	1.75
Linoleic	18 (2=)	+	0.58	1.41	2.65
Linolenic	18 (3=)	—	+	0.64	2.97

^a +, Trace; —, none found.

being greatest when the culture reaches the stationary phase (10, 14). Polyene uptake also alters with culture age, reflecting changes in the permeability properties of the yeast (23). Electron microscopy has shown that the cell wall of *C. albicans* increased by 6% during growth (13). Such small increases in cell wall thickness cannot account for the reduction in polyene susceptibility or the doubling of the lag phase before the onset of candidin-induced K⁺ leakage (Fig. 1). If the yeast cell wall acts only as a simple diffusion barrier, the time taken for candidin to cross the wall (0.15 to 0.2 μ m in diameter) and damage the cell membrane would be much shorter than was observed.

An alternative explanation would be the suggestion of qualitative and/or quantitative changes in cell wall components as the culture ages and that these changes prevent the antibiotic penetrating the cell wall and reaching the active site in the membrane. If there is a binding site for polyene antibiotics in the yeast cell wall, it cannot be to sterols, since they are not present in the yeast cell wall (16).

Kinsky et al. (20) stated that interaction could occur between polyenes and alcohol groups other than sterols, particularly to cetyl alcohols. These authors also reported that interaction could occur to a lesser extent with cerebrosides. The absorption and antifungal activity of polyene antibiotics are inhibited by the presence of fatty acids. Inhibition increases with fatty acid chain length and unsaturation (11, 15, 26). The presence of long-chain unsaturated fatty acids in the medium prevented a large proportion of added candidin binding to the yeast. The fatty acids either combined with the yeast surface, masking sterol sites in the

cell membranes, or, alternatively, the antibiotic may have combined with fatty acid micelles in the medium.

Treatment of *C. albicans* with lipase reduces the wall triglyceride level of 24-h yeasts without any apparent effect on yeast morphology or viability. Lipase-treated, 24-h yeasts showed a reduction in the time lag before the onset of K⁺ leakage; i.e., this treatment reduces the effect of the candidin binding factor in the cell wall.

It was thought that if the fatty acid component of the cell wall increased in concentration or changed in the degree of saturation during yeast growth, then fatty acids or fatty acid esters could conceivably be the factors in the wall of *C. albicans* protecting membrane sterol sites from damage. The bulk of lipids in yeasts is located in membranes of one sort or another. Reports on the lipid content of cell walls of yeast range from 1 to 12% of the wall dry weight (16).

Dyke (7) demonstrated that the lipid found in the cell wall of yeasts was a bona fide component and not a cytoplasmic contaminant. Hurst (17) suggests that lipids contribute to the stiffness of the wall. The lipid is firmly bound to the cell wall (2). The lipid composition of yeasts is very susceptible to variations in growth conditions (17). Several workers have observed changes in the lipid composition, notably in the total amount of lipid, the degree of lipid saturation, and the relative proportions of different phospholipids during growth (4, 6, 16, 18). Total yeast lipid and degree of unsaturation are affected by deficiency of nitrogen, phosphate, or carbon source and changes in O₂ tension or temperature.

In the experiments described in this paper *C. albicans* was grown aerobically in batch culture at 37 C, and the growth conditions were not fully defined. The limiting factors for growth would be nitrogen, carbon, and possibly phosphate sources. Figure 5 shows that the total triglyceride content of the cell wall of *C. albicans* increases markedly with culture age. Figure 6 shows this increase superimposed upon changes in the potassium leakage pattern of *C. albicans* with age of culture. It will be seen that the two phenomena follow closely.

Gas-liquid chromatography analysis of lipid extracts from cell wall fractions of *C. albicans* of increasing culture age revealed that the fatty acids in the wall triglycerides also change in composition (Table 3). The proportion of fatty acids increases in total amount, chain length, and degree of unsaturation during growth in batch culture.

The sterol content of *C. albicans* increases

FIG. 6. Correlation of changes in the $T_{50\%} K^+$ leakage (○) and total wall triglycerides (●) of *C. albicans* during growth in SDB at 37 C.

with the age of culture (Fig. 4). Increased sterol levels in yeasts result in greater susceptibility to polyene antibiotics (1, 5, 12), suggesting that older *C. albicans* cells should be more susceptible to candididin. As older yeast cells are more resistant to membrane damage caused by candididin (as revealed by potassium leakage), it suggests that the wall factor has a greater effect in preventing membrane damage than the presence, in the membrane, of additional sterol as potential binding sites.

The changes in the fatty acid composition of the wall of *C. albicans* conform to the criteria laid down for the proposed candididin-binding wall factor. Fatty acids are nonsteroid, have known polyene affinities, and increase in concentration during growth in batch culture. It has also been shown that not only does the triglyceride content of the cell wall increase, but there is also a tendency towards the production of longer-chain unsaturated fatty acids known to have greater polyene affinities.

This evidence leads us to make the tentative suggestion that the binding site in the cell wall of *C. albicans* may be to fatty acids or fatty acid esters and that this binding capacity must be

satisfied before candididin can bring about its lethal effect by binding to sterol in the cell membrane.

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LITERATURE CITED

1. Athar, M. A., and H. I. Winner. 1971. Development of resistance by *Candida* species to polyene antibiotics. *J. Med. Microbiol.* 4:505-516.
2. Bartnicki-Garcia, S. 1968. Cell wall chemistry, morphogenesis and taxonomy of fungi. *Annu. Rev. Microbiol.* 22:87-108.
3. Breivik, B. W., and J. L. Owades. 1957. Spectrophotometric semi-micro determination of ergosterol in yeast. *J. Agric. Food Chem.* 5:360-363.
4. Brown, C. M., and A. H. Rose. 1969. Fatty acid composition of *C. utilis* as affected by growth temperature and dissolved oxygen tension. *J. Bacteriol.* 99:371-378.
5. Bulder, C. J. 1971. Anaerobic growth, ergosterol content and sensitivity to polyene antibiotics of the yeast *Shizosaccharomyces japonica*. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 37:353-358.
6. Dawson, P. S., and B. M. Craig. 1966. Lipids of *C. utilis*—changes with growth. *Can. J. Microbiol.* 12:775-785.
7. Dyke, K. 1964. The chemical composition of the cell wall of the yeast *Nadsonia elongata*. *Biochim. Biophys. Acta* 82:374-384.
8. Edebo, L. 1966. Disintegration by freeze-pressing. II. Effect on fungi. *Acta Pathol. Microbiol. Scand.* 52:361-368.
9. Eggstein, M. 1966. A new method for the determination of triglycerides in blood and tissue. *Klin. Wochenschr.* 44:268-274.
10. Gale, E. F. 1974. The release of potassium ions from *C. albicans* in the presence of polyene antibiotics. *J. Gen. Microbiol.* 80:451-465.
11. Ghosh, A., and J. Ghosh. 1963. Factors affecting the absorption of nystatin by *C. albicans*. *Ann. Biochem. Exp. Med.* 23:101-112.
12. Hammond, S. M., and B. N. Kliger. 1974. Polyene resistant *C. albicans*; a proposed nutritional influence. *Microbios* 10:97-101.
13. Hammond, S. M., and B. N. Kliger. 1974. Studies on the role of the cell wall of *C. albicans* in the mode of action of polyene antibiotics. *Proc. Soc. Gen. Microbiol.* 1:45.
14. Hammond, S. M., P. A. Lambert, and B. N. Kliger. 1974. The mode of action of polyene antibiotics; induced potassium leakage in *C. albicans*. *J. Gen. Microbiol.* 81:325-330.
15. Hickey, R. 1952. The antagonism between the antifungal antibiotic ascocin and some long chain fatty acids. *Arch. Biochem. Biophys.* 46:331-336.
16. Hunter, K., and A. H. Rose. 1971. Yeast lipids and membranes, p. 211-270. In A. H. Rose and J. S. Harrison (ed.), *The yeasts*, vol. 2. Academic Press Inc., London.
17. Hurst, H. 1952. Electron diffraction study of the structure and orientation of the lipids in yeast and bacterial cell walls. *J. Exp. Biol.* 29:30-52.
18. Jollow, D., G. M. Kellerman, and A. W. Linane. 1968. The biogenesis of mitochondria. III. The lipid composition of aerobically and anaerobically grown *S. cerevisiae* as related to the membranes of the cell. *J. Cell Biol.* 37:221-230.

19. Kates, M. 1964. Simplified procedure for hydrolysis of lipids. *J. Lipid Res.* 5:132-135.
20. Kinsky, S., S. Luse, and L. L. M. Van Deenen. 1966. Interaction of polyene antibiotics with natural and artificial membrane systems. *Fed. Proc.* 25:1503-1520.
21. Lambert, P. A., and S. M. Hammond. 1973. Potassium leakage; first indication of membrane damage in micro-organisms. *Biochem. Biophys. Res. Commun.* 54:796-799.
22. Lampen, J. O. 1966. Polyene antibiotics and membrane function. *Symp. Soc. Gen. Microbiol.* 16:111-130.
23. Lampen, J. O., P. Arnow, Z. Borowska, and A. Laskin. 1962. Location and role of sterol at nystatin binding sites. *J. Bacteriol.* 84:1152-1164.
24. Pederson, T. A. 1962. Lipid formation in *Cryptococcus terricola*. III. Extraction and purification of lipids. *Acta Chem. Scand.* 16:374-382.
25. Rothstein, A., and M. Bruce. 1958. The efflux of potassium from yeast in a potassium-free medium. *J. Cell. Comp. Physiol.* 51:439-455.
26. Sarachek, A., and P. Higgins. 1972. Effect of ergosterol, palmitic acid and related simple lipids on the recovery of *C. albicans* from UV radiation. *Arch. Mikrobiol.* 82:38-54.
27. Schibeci, A., J. Rattray, and D. Kidby. 1973. Isolation and identification of yeast plasma membranes. *Biochim. Biophys. Acta* 311:15-25.
28. Simpson, J. 1963. Microbiological assay using large plate methods, p. 88-124. *In* K. Kavanagh (ed.), *Analytical microbiology*. Academic Press Inc., New York.
29. Stoffel, W., F. Chu, and E. H. Ahrens. 1959. Analysis of long chain fatty acids by GLC-micromethod for the preparation of methyl esters. *Anal. Chem.* 31:307-308.