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Fatty Acid and Sterol Composition of *Mucor genevensis* in Relation to Dimorphism and Anaerobic Growth

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Fatty acid and sterol content and composition were determined for the dimorphic mold, *Mucor genevensis*, grown under a variety of experimental conditions. Fatty acids account for 6 to 9% of the dry weight of aerobically grown mycelium, and 70 to 80% of these are unsaturated. The organism contains γ-linolenic acid which is characteristic for *Phycomycetes*, and in sporangiospores this compound represents 40% of the total fatty acids. Of the sterols found in mycelium, 80% is ergosterol, and stigmasterol was positively identified as one of the minor components. In anaerobically grown yeastlike cells, sterol content is less than 10% of the level found in aerobically grown cells, and fatty acids amount to less than 2% of the dry weight. These fatty acids are predominantly short chain and less than 10% are unsaturated. Yeastlike cells obtained under aerobic conditions by growth in the presence of phenethyl alcohol have fatty acid and sterol compositions characteristic of aerobically grown mycelium. It is concluded that the dimorphology of the organism is not directly related to lipid composition.

Yeastlike development of dimorphic species of *Mucor* can be induced by a variety of conditions. Generally, it has been found that high glucose and carbon dioxide concentrations and anaerobic conditions favor yeastlike morphology (3). Certain chemicals such as phenethyl alcohol (21) and an unidentified compound in peptone (8) also favor the development of yeastlike cells.

The cell walls of yeastlike anaerobically grown *Mucor rouxii* have been shown to differ in mannan composition from the cell walls of aerobically grown mycelia (5). However, in *Saccharomyces cerevisiae*, anaerobic growth is known to affect greatly the lipid composition of the cells, especially the unsaturated fatty acid content (11). Therefore the possibility was considered that the dimorphology of *Mucor* species may be related to lipid composition. Accordingly, the fatty acid and sterol compositions of a dimorphic species, *M. genevensis*, were compared under conditions of anaerobic and aerobic growth and aerobic growth in the presence of phenethyl alcohol.

This communication reports that anaerobic growth leads to considerable alteration in lipid content and composition compared with aerobically grown cells. The total sterol and fatty acid content decreases in anaerobically cultured cells, but the most significant change is the reduction in unsaturated fatty acid level. However, the morphology of the organism is not directly related to these changes in lipid composition since aerobic yeastlike cells, induced by growth in the presence of phenethyl alcohol, have a lipid composition which is characteristic of aerobically grown mycelium.

MATERIALS AND METHODS

Organism. *M. genevensis* NRRL 1407 was kindly supplied by C. W. Hesseltine of the Northern Utilisation Research and Development Division, Peoria, Ill.

Production of sporangiospores. The culture was maintained on cornmeal-agar slopes (Difco) at 4 C and transferred every 6 weeks. For the production of sporangiospores, tomato juice-agar slopes (commercial tomato juice diluted 1:1 with water and containing 2.5% agar; reference 9) were inoculated with a small piece of mycelium cut from the cornmeal stock culture. After growth at 30 C for 7 days, sufficient sterile distilled water was added to each slope to completely immerse the mycelium. After standing at room temperature for 1 hr, the mycelial surface was gently agitated with a glass rod, and the resulting suspension was poured through a filter funnel plugged with cotton. The filtrate containing the spores was centrifuged, and the spores were washed once with sterile distilled water by centrifugation. The spores were resuspended in distilled water, counted in a hemocytometer, and either stored

at 2 C or used for lipid extraction.

Liquid culture medium. The standard glucose synthetic medium (2%GSy) contained, per liter: glucose, 20 g; KH_2PO_4, 0.5 g; $(NH_4)_2SO_4$, 1.5 g; NaCl, 0.1 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; ferric citrate $\cdot 3H_2O$, 0.0054 g; and 5 ml of a trace metal solution. The trace metal solution contained, per 5 ml: $CuSO_4 \cdot 5H_2O$, 0.2 mg; KI, 0.5 mg; $ZnSO_4 \cdot 7H_2O$, 0.5 mg; $Na_2MoO_4 \cdot 2H_2O$, 0.5 mg; $Na_2B_4O_7 \cdot 10H_2O$, 0.5 mg; and $MnSO_4 \cdot 4H_2O$, 1.0 mg. For anaerobic growth, 0.5% (w/v) Difco yeast extract was added to the medium (2%GSy0.5YE).

Growth conditions. For aerobic growth, approximately 10^6 spores were added to 400 ml of 2%GSy medium in a 1-liter conical flask, and the culture was agitated at 30 C on a gyratory shaker at 200 rev/min for 14 to 16 hr. Where indicated, phenethyl alcohol was added to a concentration of 0.1% (v/v) before autoclaving. Cells were harvested by filtration and washed with chilled distilled water. Dry weights of yeastlike cells, which could be uniformly dispersed, were determined after drying samples at 110 C for 24 hr. Dry weights of mycelium for lipid analysis were calculated from the wet weight after determining the ratio of wet weight to dry weight of a sample from the same mycelial mat.

For anaerobic growth, approximately 2.6×10^7 spores were added to 900 ml of 2%GSy0.5YE medium in a 1-liter conical flask fitted with a rubber stopper and glass inlet and outlet tubes. After inoculation, all joints were sealed with molten paraffin wax, and the medium was flushed for 1 hr with commercial nitrogen freed from oxygen by the method of Meites and Meites (13). The culture was stirred with a magnetic stirrer at 30 C for 16 to 20 hr. The culture was harvested by filtration at 4 C; the cells were washed with cold, distilled water, and resuspended in distilled water. Samples were taken immediately for dry weight and lipid analysis. In the case of anaerobic samples, not more than 5 to 6 min elapsed between breaking the seal of the anaerobic apparatus and the commencement of hydrolysis for lipid analysis.

Extraction of fatty acids and sterols. In preliminary experiments, the hydrolysis procedure was examined to find conditions which resulted in maximal extraction of the lipids. The washed cells (50 to 500 mg, dry weight), together with 4 mg of pentadecanoic acid per g, dry weight, as an internal standard, were hydrolyzed under the following conditions: (i) A 20-ml amount of 50% (w/v) KOH per sample was added and saponification was allowed to proceed for 2 to 6 hr in a boiling-water bath. (ii) A 20-ml amount of 3 N HCl per sample was added, and hydrolysis was carried out for 2 hr in a boiling-water bath; subsequently, 10 g of solid KOH was added, and hydrolysis was continued for a further 4 hr. Hydrolyzed samples were extracted with two 100-ml portions of diethyl ether to yield the nonsaponifiable sterol-containing fraction. The residue was acidified to *p*H 1.0 with concentrated HCl, and the extraction was repeated with diethyl ether to give a fraction containing the fatty acids.

Sterol analysis. The ergosterol content of the nonsaponifiable extract was estimated spectrophotometrically by the method of Shaw and Jeffries (16). The remainder of this fraction was evaporated to dryness, dissolved in benzene or ethyl acetate, and analyzed by gas-liquid chromatography on a stainless-steel column (5 ft by 0.25 inches) of 3.8% silicone rubber UCCW-982 on Diaport S, 80 to 100 mesh (Hewlett-Packard Co.), at a column temperature of 250 C, or on a column of 3.6% JXR Silicone on Gas Chrom Q, 120 mesh, (Applied Science Laboratories), at a column temperature of 260 C. For thin-layer chromatography of the nonsaponifiable fraction, plates of silica gel G impregnated with 5% $AgNO_3$ were prepared as described by Morris (14). Development in the first dimension was with chloroform and in the second dimension with hexane-ethyl acetate (80:20). Compounds of interest were revealed after spraying with either Lieberman-Burchard reagent (7) or 50% aqueous H_2SO_4 and heating at 100 C for 10 min.

Fatty acid analysis. The fraction containing fatty acids was dried over Na_2SO_4, the ether was removed by evaporation, and the fatty acid residue was methylated by refluxing with anhydrous methanol containing HCl for 60 min at 50 C. The methyl esters were partitioned into hexane after evaporation of the methanolic HCl and then separated by gas-liquid chromatography on a column (5 ft by 0.25 inches) of 25% polyethylene glycol adipate on Diatomite CO (80 to 100 mesh), operated at 180 C. The esters were identified by their retention times (10) relative to methyl ester standards (Applied Science Laboratories Inc.). Carbon chain lengths of the unsaturated fatty acids were confirmed by gas-liquid chromatography after hydrogenation. For the hydrogenation, approximately 40 mg of fatty acids in 8 ml of methanol was mixed with 50 mg of Pt_2O in a microhydrogenator. Reduction was followed by hydrogen consumption and checked by nuclear magnetic resonance spectroscopy (loss of ethylene proton signal on reduction) with nuclear magnetic resonance spectrometer (model R-10, Perkin-Elmer Corp.) operated at 60 Mhertz.

Purification of 18:3 fatty acid component. Thin-layer plates of silica gel G impregnated with 5% $AgNO_3$ were prepared as described by Morris (14). The developing system was diethyl ether-light petroleum (boiling point, 40 to 60 C), 2:3 (v/v). After development, marker spots were detected by spraying with 50% aqueous H_2SO_4; corresponding regions from the unsprayed area of the plate were selected and eluted. Purity was checked by both gas-liquid and thin-layer chromatography.

RESULTS

Hydrolysis method for optimal extraction of lipid components. Preliminary studies indicated that the apparent fatty acid content of mycelium varied with hydrolysis time in KOH (Table 1). Accordingly, conditions were sought for maximum lipid extraction. It was found that a preliminary acid hydrolysis followed by 4 hr of heating in alkali gave satisfactory results and that further heating did not increase the yield of fatty acid or ergosterol. All of these procedures resulted in estimation of the fatty acid components in the same proportions. Under these conditions, the pentadecanoic acid was added after

TABLE 1. *Hydrolysis method for optimal extraction of lipid components*

Expt no.	Method of hydrolysis	Total time of hydrolysis (hr)	Ergosterol (mg/g dry wt)	Total fatty acid (mg/g dry wt)	Unsaturated fatty acid (%)
1	50% (w/v) KOH	2	2.00	33.0	81
	50% (w/v) KOH	6	2.17	57.3	80
2	50% (w/v) KOH	6	1.29	33.4	82
	3N HCl for 2 hr, then 50% (w/v) KOH for 4 hr	6	2.06	51.3	83

the acid hydrolysis. This procedure eliminated errors due to selective destruction of the standard, a process which has been found to occur in some experiments when the standard was added before acid hydrolysis. Two different samples of mycelium grown to stationary phase in 2%GSy medium were used in this study. It was subsequently found that fatty acid content of mycelium falls upon the aging of cultures in stationary phase, and it is believed that the difference between fatty acid content of the two cultures after 6 hr of hydrolysis in KOH represents a real difference in fatty acid content.

Experiments on the efficiency of fatty acid extraction from the yeastlike form of the organism showed, in contrast to similar experiments on the mycelial form, that a preliminary acid hydrolysis did not lead to increased lipid yields. Hence a preliminary acid hydrolysis was routinely included when extracting mycelium or spores, but it was omitted in the case of yeastlike cells.

Relationship of culture age to lipid content and composition. It is known that the lipid composition of fungi may vary with the growth phase of the culture (18). Consequently it was of importance to establish the limits of any variation in lipid content of *M. genevensis* before comparison of mycelial and yeastlike forms were made. The variation of sterol and fatty acid content during growth of a mycelial culture is shown in Table 2. During the transition from exponential growth to stationary phase, there is a decline in both total and unsaturated fatty acid content of the cells. This decline is accounted for almost entirely by the loss of the long-chain saturated and polyunsaturated fatty acids, whereas the content of monounsaturated acids showed slight increases over this period.

The distribution of sterols from an exponentially growing culture is shown in Fig. 1a. Ergosterol accounts for more than 80% of the total sterol in this phase of growth. In stationary-phase mycelium (Table 2), there is a substantial increase in the content of this sterol, and stigmasterol, which in exponential phase is a minor component, increases to 40% of the total sterol present.

In experiments described below, lipid composition was compared while cultures were still in the exponential phase of growth.

Effect of growth conditions on morphology and lipid composition. It is known that anaerobic growth of *M. rouxii* is stimulated by thiamine and nicotinic acid (4). To obtain satisfactory growth of *M. genevensis* under anaerobic conditions, it is necessary to supplement the medium with yeast extract as a source of vitamins and perhaps other undetermined growth factors.

When aerobic mycelial cells, grown in the presence or absence of yeast extract, were compared with anaerobically grown yeastlike cells in respect to their lipid content, it was found that there are considerable differences between the aerobic and anaerobically cultured cells (Tables 2 and 3). The fatty acid content of anaerobically grown cells is lower than that of aerobically grown cells, and less than 10% of the fatty acids are unsaturated. In the anaerobic cells there is

TABLE 2. *Fatty acid and ergosterol content during the aerobic growth cycle*[a]

Incubation time (hr)	Growth phase	Cell density at harvest (mg dry wt/ml)	Ergosterol (mg/g dry wt)	Total fatty acid (mg/g dry wt)	Unsaturated fatty acid (%)	Per cent distribution of fatty acids by wt[b]									
						10:0	12:0	14:0	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3
24	Exponential	0.10	1.15	91	70	0.1	0.2	2.1	23.1	1.7	0.5	0.0	3.7	20.0	20.0	28.7
30	Exponential	0.68	1.69	89	75	0.1	0.2	1.8	19.2	1.8	1.0	0.7	2.2	22.7	18.1	32.2
40	Stationary	2.39	2.78	58	80	0.3	0.4	3.0	14.6	8.1	0.7	1.7	1.4	40.3	12.7	16.8

[a] *Mucor genevensis* was incubated at 30 C in 2%GSy medium.

[b] In naming the fatty acids, the convention used is number of carbon atoms: number of double bonds.

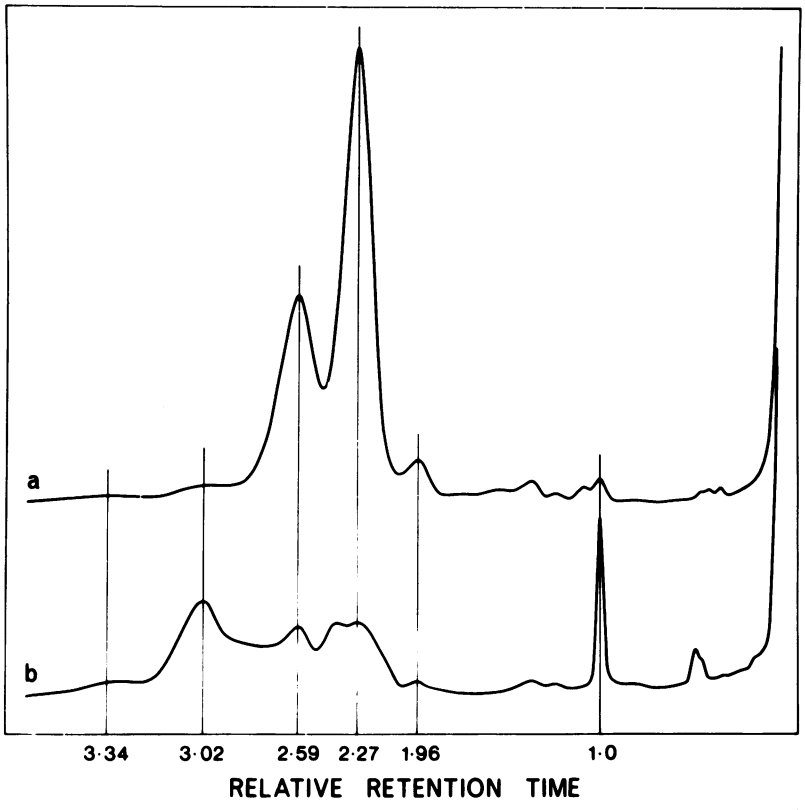

FIG. 1. *Gas-liquid chromatograms of eluates from the JXR silicone column of the nonsaponifiable lipid fraction from (a) aerobic mycelium, and (b) anaerobic yeastlike cells. Retention times were measured relative to squalene. Components with relative retention times of 2.27 and 2.59 were identified as ergosterol and stigmasterol, respectively, and components with retention times of 1.96 and 3.02 co-chromatograph with cholesterol and β-sitosterol.*

also a preponderance of short-chain fatty acids as well as two unidentified components which elute ahead of 18:0 and between 18:2 and 18:3 in the gas-liquid chromatogram. The position of these two peaks does not change after hydrogenation, and they have not been characterized further. It has also been found consistently that mycelium grown aerobically in the presence of yeast extract contains a lower amount of fatty acid than mycelium grown in the absence of yeast extract, although the composition of the fatty acids is not altered.

The gas-liquid chromatography analyses of the nonsaponifiable fraction from aerobically grown mycelium and anaerobically grown yeastlike cells are shown in Fig. 1. The components were identified by their retention times relative to squalene and by the comparison of these times with standard compounds. The identification of ergosterol and stigmasterol has been confirmed by gas-liquid chromatography before and after formation of trimethyl silane derivatives and by two-dimensional thin-layer chromatography on $AgNO_3$-impregnated plates. The identification of the other components is tentative. The peak which runs with cholesterol is not observed when the acid hydrolysis step is omitted.

It can be seen that ergosterol and stigmasterol are the major components of the aerobically grown mycelium with smaller amounts of cholesterol and β-sitosterol. In the anaerobically grown cells, the proportion of ergosterol is markedly decreased whereas the amount of squalene is increased.

The possibility that yeastlike morphology is linked with these changes in lipid components was examined in aerobically grown cells by using phenethyl alcohol to induce yeastlike growth. There is little difference in fatty acid composition when these cells are compared with mycelium (Table 3), although their total fatty acid content is lower. A small decrease in ergosterol content is also found, but there is no change in the relative proportion of the sterols.

TABLE 3. *Effect of growth conditions on morphology and lipid content of Mucor genevensis*

Growth conditions	Morphology[a]	Cell density at harvest (mg dry wt/ml)	Ergosterol (mg/g dry wt)	Total fatty acid (mg/g dry wt)	Unsaturated fatty acid (%)	Distribution of fatty acids by wt (%)[b]											
						10:0	12:0	14:0	16:0	16:1	17:0	17:1	a	18:0	18:1	18:2	c	18:3
Aerobic 0.5% YE	M	0.65	3.07	54	71	0.2	0.5	4.0	19.9	5.8	0.5	0.4		3.9	19.4	16.8		28.7
Anaerobic 0.5% YE	Y	0.89	<0.3	12	6.3	7.2	18.8	30.2	16.4	1.7	0.0	0.0	4.6	10.7	4.6	0.0	5.8	0.0
Aerobic 0.1% phenethyl alcohol	Y	0.20	1.42	42	80	0.0	0.2	3.1	13.0	1.3	0.0	0.0		3.2	30.3	15.6		33.0
Aerobic sporulation agar	Spores		3.0	52	83	0.0	0.8	1.6	14.4	2.2	0.0	1.8		0.7	22.8	16.0		40.5

[a] M and Y represent mycelial and yeastlike forms, respectively.

[b] Compounds "a" and "b" occur in the chromatogram of anaerobically grown cells.

The ergosterol and fatty acid composition of spores has been included in Table 3 for interest. It can be seen that their lipid composition differs little from that of aerobic mycelium.

Identification of γ-linolenic acid component of aerobic cells. A component which comprised approximately 30% of the fatty acids of aerobic cells was isolated by preparative thin-layer chromatography. Its retention time in gas-liquid chromatography was greater than linoleic acid but less than α-linolenic acid (Δ9,12,15). Hydrogenation suggested the presence of three double bonds, and the product from this reaction cochromatographed with stearic acid. Mass spectrometry and nuclear magnetic resonance measurements on this compound are consistent with it being γ-linolenic acid (Δ6,9,12).

DISCUSSION

M. genevensis was selected for this study, from a number of facultatively anaerobic *Mucor* species, including *M. rouxii*, for two reasons. It was found to have the shortest division time under our experimental conditions and to be more readily transformed into the yeastlike form by agents such as phenethyl alcohol (21) than the more widely studied species *M. rouxii*.

M. genevensis is similar in fatty acid composition to other members of the genus *Mucor* (17) and contains γ-linolenic acid, which is thought to be characteristic of *Phycomycetes* (17, 18). We also found that, in this rapidly growing fungus, the fatty acid content of mycelium in exponentially growing cultures remains constant. However, as the culture ages in stationary phase, the fatty acid content falls, presumably because lipid reserves are consumed. A fall in fatty acid content of fungi with increasing culture age is not unusual (18) but, in contrast to Shaw (19), we find that the content of 18:2 and 18:3 unsaturated fatty acids are decreased, whereas the content of 18:1 acid is relatively unaffected. This suggests that in *M. genevensis* the polyunsaturated fatty acids are storage compounds.

The fatty acid content and composition of freshly harvested sporangiospores are similar to those of actively growing mycelium, except for a slightly higher proportion of γ-linolenic acid in the spores, again suggesting that this is a storage fatty acid. This report is at variance with the results of Sumner and Morgan (20), who found that freshly harvested spores of several species of *Mucor* contain less lipid and less unsaturated fatty acid than the corresponding mycelia. Their analysis of lipid content is based on a difference in weight before and after extracting spores and mycelium rather than by direct analysis of the extracted lipids. In addition, we find that a preliminary acid hydrolysis is necessary to obtain maximal recovery of lipid from mycelium and that this treatment does not alter the relative distribution of extracted fatty acids. This conclusion agrees with an earlier report concerning the importance of a preliminary acid hydrolysis in lipid extraction (15).

The fatty acid content of anaerobically cultured cells is lower than that of aerobically grown cells, and the composition is notably altered. The anaerobically grown cells contain predominantly short-chain saturated fatty acids. Under similar circumstances in yeast, there is also a predominance of short-chain saturated fatty acids (11, 12). Anaerobically grown *M. genevensis* has a low level of ergosterol with an increase in the proportion of squalene, and this is also the situation in anaerobically grown yeast (11). In this regard, Andreasen and Stier (1, 2) have shown that anaerobic growth of *S. cerevisiae* requires ergosterol and unsaturated fatty acids, and Bloomfield and Bloch (6) have shown that oxygen is required for the synthesis of unsaturated fatty acids in this organism. Although the requirements for anaerobic growth of *M. genevensis* have not been determined, it may be assumed that the organism is similar to yeast at least in the requirement for oxygen in the synthesis of unsaturated fatty acids.

Phenethyl alcohol has previously been shown to promote yeastlike development of *M. genevensis* (21), a fact which we substantiate in this study. The fatty acid and ergosterol content of these aerobically grown yeastlike cells is essentially the same as in mycelium and is in marked contrast to that from anaerobically grown yeastlike cells. It therefore appears that there is no direct relationship between lipid content and composition and morphology. However, if anaerobically grown yeastlike cells had been compared only with aerobically grown mycelium, such a relationship might have been inferred. This situation is unfortunately the case where a number of parameters of anaerobically grown yeastlike cells of *M. rouxii* from stationary-phase culture have been compared with those of actively growing aerobic mycelium, with the implication that the difference in mannan and protein composition between the two types of cells is morphologically significant (5).

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LITERATURE CITED

1. Andreasen, A. A., and T. J. B. Stier. 1953. Anaerobic nutrition of *Saccharomyces cerevisiae*. I. Ergosterol requirement for growth in a defined medium. J. Cell. Comp. Physiol. **41**:23-36.
2. Andreasen, A. A., and T. J. B. Stier. 1954. Anaerobic nutrition of *Saccharomyces cerevisiae*. II. Unsaturated fatty acid requirement for growth in a defined medium. J. Cell. Comp. Physiol. **43**:271-281.
3. Bartnicki-Garcia, S. 1968. Control of dimorphism in *Mucor* by hexoses: inhibition of hyphal morphogenesis. J. Bacteriol. **96**:1586-1594.
4. Bartnicki-Garcia, S., and W. J. Nickerson. 1961. Thiamine and nicotinic acid: anaerobic growth factors for *Mucor rouxii*. J. Bacteriol. **82**:142-148.
5. Bartnicki-Garcia, S., and W. J. Nickerson. 1962. Isolation, composition and structure of cell walls of filamentous and yeastlike forms of *Mucor rouxii*. Biochim. Biophys. Acta **58**:102-119.
6. Bloomfield, D. K., and K. Bloch. 1960. The formation of Δ^9-unsaturated fatty acids. J. Biol. Chem. **235**:337 345.
7. Cook, R. P., and J. B. M. Rattray. 1958. Methods of isolation and estimation of sterols, p. 117-143. *In* R. P. Cook (ed.), Cholesterol. Academic Press Inc., New York.
8. Elmer, G. W., and W. J. Nickerson. 1970. Nutritional requirements for growth and yeastlike development of *Mucor rouxii* under carbon dioxide. J. Bacteriol. **101**:595-602.
9. Haidle, D. W., and R. Storck. 1966. Control of dimorphism in *Mucor rouxii*. J. Bacteriol. **92**:1236-1244.
10. James, A. T. 1960. Qualitative and quantitative determination of fatty acids by gas-liquid chromatography, p. 1-59. *In* D. Glick (ed.), Methods of biochemical analysis, vol. 8. Interscience, New York.
11. Jollow, D., G. M. Kellerman, and A. W. Linnane. 1968. The biogenesis of mitochondria. III. The lipid composition of aerobically and anaerobically grown *Saccharomyces cerevisiae* as related to the membrane systems of the cells. J. Cell Biol. **37**:221-230.
12. Light, R. J., W. J. Lennarz, and K. Bloch. 1962. The metabolism of hydroxystearic acids in yeast. J. Biol. Chem. **237**:1793-1799.
13. Meites, L., and T. Meites. 1948. Removal of oxygen from gas streams. Anal. Chem. **20**:984-985.
14. Morris, L. J. 1964. Specific separations by chromatography on impregnated absorbents, p. 295-319. *In* A. T. James and L. J. Morris (ed.), New biochemical separations. D. van Norstrand and Co. Ltd., London.
15. Ratledge, C., and R. K. Saxton. 1968. Quantitative extraction of lipid and fatty acids from a *Candida* species. Anal. Biochem. **26**:288-294.
16. Shaw, H. W. C., and J. P. Jefferies. 1953. The determination of ergosterol in yeast. III. Corrections for irrelevant absorption in solutions of ergosterol. Analyst **78**:519-523.
17. Shaw, R. 1965. The occurrence of γ-linolenic acid in fungi. Biochim. Biophys. Acta **98**:230-237.
18. Shaw, R. 1966. The fatty acids of Phycomycete fungi, and the significance of the γ-linolenic acid component. Comp. Biochem. Physiol. **18**:325-331.
19. Shaw, R. 1966. The polyunsaturated fatty acids of microorganisms. Adv. Lipid Res. **4**:107-174.
20. Sumner, J. L., and E. D. Morgan. 1969. The fatty acid composition of sporangiospores and vegetative mycelium of temperature adapted fungi in the order Mucorales. J. Gen. Microbiol. **59**:215-221.
21. Terenzi, H. F., and R. Storck. 1969. Stimulation of fermentation and yeast-like morphogenesis in *Mucor rouxii* by phenethyl alcohol. J. Bacteriol. **97**:1248-1261.