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Lipid Composition of the Zoospores of *Blastocladiella emersonii*[1]

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The zoospores of *Blastocladiella emersonii*, when derived from cultures grown on solid media, contain about 11% total lipid. This lipid was separated chromatographically on silicic acid into neutral lipid (46.6%), glycolipid (15.8%), and phospholipid (37.6%). Each class was fractionated further on columns of silicic acid, Florisil, or diethylaminoethyl-cellulose, and monitored by thin-layer chromatography. Triglycerides were the major neutral lipids, mono- and diglycosyldiglycerides were the major glycolipids, and phosphatidylcholine and phosphatidylethanolamine were the major phospholipids. Other neutral lipids and phospholipids detected were: hydrocarbons, free fatty acids, free sterols, sterol esters, diglycerides, monoglycerides, lysophosphatidylcholine, lysophosphatidylethanolamine, phosphatidic acid, phosphatidylserine, and phosphatidylinositol. Palmitic, palmitoleic, stearic, oleic, γ-linolenic, and arachidonic acids were the most frequently occurring fatty acids. When *B. emersonii* was grown in ^{14}C-labeled liquid media, lipid again accounted for 11% of both mature plants and zoospores released from them. The composition of the lipid extracted from such plants and spores was also the same; however, it differed markedly from that of the lipid in spores harvested from solid media, consisting of 28.3% neutral lipid, 12.0% glycolipid, and 59.7% phospholipid. The major lipids were again triglycerides for neutral lipids, mono- and diglycosyldiglycerides for glycolipids, and phosphatidyl choline and phosphatidylethanolamine for phospholipids.

Except for a paper by Katsura (21), cited by Gay et al. (12), which we have not seen, apparently the only available data on the lipid content of fungal zoospores, as estimated by direct chemical analysis, are to be found in two recent reports on the water mold *Blastocladiella emersonii* (5). The first of these (37) established changes in the quantity of lipid/cell during endogenous metabolism of swimming zoospores; the second (36) provided a preliminary description of changes in lipid composition after zoospore germination. We have been investigating the lipids in certain organelles in these zoospores (for a review of their structure, see reference 40), for which the composition of total cell lipid constitutes an essential reference point. In this communication, we characterize the whole spore lipid of *B. emersonii*, and provide some comparative information about the lipid content of the plants from which such spores are derived.

[1] Dedicated to Frederick K. Sparrow, Jr., in the year of his retirement as Professor of Botany in the University of Michigan, and in honor of his distinguished service to the field of mycology.

MATERIALS AND METHODS

Production of zoospores on solid media. The original strain of *B. emersonii* (5) was grown on peptone-yeast extract-glucose (Difco; PYG) agar by inoculating with 4×10^5 spores per standard petri plate and culturing at 22 C in the dark. Zoospores were obtained about 24 h later from first-generation plants by flooding each plate with 5 ml of water and filtering 15 min later. After population densities were established with a model B Coulter counter, the zoospores were sedimented at $1{,}000 \times g$ for 5 min. Under these conditions, the yield was approximately 4×10^9 zoospores per 100 plates.

Production of plants and zoospores in liquid media. PYG broth cultures were prepared, inoculated, and induced to release zoospores at 22 C, by the method of Myers and Cantino (26). [1,2-^{14}C]sodium acetate (New England Nuclear Corp.; 50 μCi) was added 9 h after inoculation, the final concentration being 5×10^{-4} M. For studies of zoosporangial lipid, thalli were harvested either just before zoospore cleavage or after zoospores had been cleaved but not released (ca. 23 h after inoculation); for studies of zoospore lipid, spores were collected about 1 h later.

Extraction of lipid. Spore pellets were washed with water, sedimented, sonically treated (30 s, 80 W), and extracted at room temperature with chloro-

form-methanol (2:1, vol/vol) overnight. Additional extractions did not increase yields. The spore homogenate was filtered through a coarse, fritted-glass Büchner funnel, and the filtrate was evaporated to dryness under N_2. Nonlipid contaminants were removed with Sephadex G-25 by the method of Rouser and Fleischer (30).

Column chromatography. Lipids were fractionated into neutral, glyco-, and phospholipids on activated silicic acid (100 mesh, Mallinckrodt Chemical Co., St. Louis, Mo.). Neutral lipids were separated further on silicic acid after removal of free fatty acids (FFA; 9), or on 7% hydrated Florisil (6). Glycolipids were separated into individual components with Florisil (27), and phospholipids were fractionated on diethylaminoethyl (DEAE)-cellulose (Sigma Chemical Co., St. Louis, Mo.) by the method of Rouser et al. (31).

Thin-layer and paper chromatography. Thin-layer chromatography (TLC) was used to check the purity of column fractions. Neutral lipids were separated with petroleum ether-diethyl ether-acetic acid (80:20:1, vol/vol) by using ITLC-SG chromatography media (Gelman Instrument Co., Ann Arbor, Mich.). Phospholipids were chromatographed on the same media with isopropanol-ammonium hydroxide (100:7, vol/vol); glycolipids were separated with the same solvent on ITLC-SA media. Phospholipids were also chromatographed two-dimensionally on Redi-Coats (Supelco Inc., Bellefonte, Pa.) by using chloroform-methanol-ammonium hydroxide (60:25:5, vol/vol) in the first direction and chloroform-acetone-methanol-acetic acid-water (3:4:1:1:0.5, vol/vol) in the second direction.

Lipid components were visualized with ultraviolet light, I_2 vapor, or a saturated solution of K_2CrO_4 in 70% (vol/vol) H_2SO_4 (34). Specific sprays included $SbCl_3$ for sterols and sterol esters (34), 0.2% ninhydrin in butanol for free amino groups, Dragendorff reagent for the detection of choline-containing compounds (34), and the reagent of Dittmer and Lester (8) for P. Glycolipids were detected with orcinol (34), phenol-H_2SO_4 (15), or diphenylamine (34).

Descending chromatography of water-soluble hydrolysis products was carried out on Whatman no. 1 paper. Glycerol phosphate esters were resolved with phenol-water (100:38, vol/vol) (45), and the phosphate groups were detected by the salicylsulfonic acid-$FeCl_3$ procedure (41) or with acid molybdate (17). Glycolipid hydrolysis products were chromatographed with *N*-propanol-ammonium hydroxide-water (6:3:1, vol/vol) or ethyl acetate-pyridine-water (12:5:4, vol/vol) (19). Ammoniacal $AgNO_3$ was used to detect carbohydrates. The hydrolysis products were also studied by TLC. Phosphate-impregnated Chromagram sheets (Eastman; 6061 silica gel) were prepared, spotted, and multideveloped, and sugars were located thereon, all by the method of Welch and Martin (43).

Hydrolysis procedures. Glycerol phosphate esters were prepared by deacylating the phospholipids in 0.2 N methanolic NaOH for 15 min at room temperature (20). The solution was neutralized with Dowex-50 (H^+) after partitioning with chloroform and water. The water-soluble hydrolysis products were concentrated almost to dryness under a stream of N_2 at 40 C, and used for chromatography. Phospholipids were also deacylated by mild alkaline hydrolysis at 0 C (45). Glycolipids were hydrolized in 2 N HCl for 2 h at 100 C. The hydrolysate was extracted three times with petroleum ether; the HCl was removed under a stream of N_2 or by drying the samples over KOH pellets.

Analytical procedures. Lipid-P was determined after digestion of samples with 10 N H_2SO_4 by a modification of Bartlett's method (1). Total N was assayed with the microprocedure of Sloane-Stanley (35) or by direct nesslerization with commercial (Harleco dry pack) Folin Wu (11) reagent. Acyl esters were estimated by the ferric hydroxamate method (28) with tripalmitin as a standard. Glycerol analyses (16) were based on the determination of formaldehyde produced by oxidation of glycerol with periodate by using α-glycerol phosphate as a standard. Total hexoses were determined with anthrone (44) or the phenol-sulfuric acid method (10). Glucose was also analyzed enzymatically (Glucostat, Worthington Biochemical Corp., Freehold, N.J.), and total hexosamines were estimated by a modification (9) of the Elson-Morgan reaction.

Gas chromatography. Fatty acid methyl esters of the total lipid and the neutral, glyco-, and phospholipid fractions were prepared by saponification and extraction of fatty acids (9); the latter were methylated with BF_3 (25). Methyl esters were examined with a Packard gas chromatograph model 7300 equipped with a flame ionization detector. The methyl esters were separated on a column (0.32 by 200 cm) packed with 15% Lac-2R-446 on Chromosorb W (80 to 100 mesh) operated at 187 C. The carrier gas was He; the injector port temperature was 193 C; and the detector temperature was 225 C. The esters were identified by their retention times relative to methyl ester standards.

Materials. Phospholipid standards were prepared from egg yolks. The phospholipids were extracted with chloroform-methanol (1:1, vol/vol), separated from neutral lipids by silicic acid chromatography, and then fractionated on DEAE-cellulose. The phospholipids were purified further by TLC and compared with published data on egg yolk phospholipids (29). Some organic reagents and most solvents were redistilled before use. Methyl esters were obtained from Supelco Inc., Bellefonte, Pa.

RESULTS

Characterization of total lipid in spores produced on solid media. Lipid extracts were fractionated on silicic acid columns (Table 1). Neutral, glyco-, and phospholipid accounted for 46.6, 15.8, and 37.6%, respectively, of the total lipid. The latter constituted 11% of the dry weight of the zoospore.

Phospholipid. The phospholipid was fractionated further on DEAE-cellulose, and the

TABLE 1. *Lipid composition of zoospores produced on solid media*[a]

Fraction[b]	Percentage[c]
Neutral lipid	46.6 ± 2.2
Glycolipid	15.8 ± 2.4
Phospholipid	37.6 ± 2.5

[a] Percentages were established gravimetrically after the lipid had been fractionated on silicic acid columns (2 by 8 cm).

[b] Fractions were eluted successively with 150 ml of chloroform, 100 ml of acetone, and 150 ml of methanol.

[c] Means and standard deviations for four experiments.

purity of each fraction was verified by TLC (Fig. 1). Peak I contained phosphatidylcholine (PC; R_f 0.26; all R_f values listed in this report are average values for many runs) and lysophosphatidylcholine (LPC; R_f 0.12). Both spots were molybdate positive and gave reactions for choline. Phosphatidylethanolamine (PE; R_f 0.72) and lysophosphatidylethanolamine (LPE; R_f 0.45) were found in peaks II and III, respectively. Both spots were ninhydrin and molybdate positive, as was phosphatidylserine (PS; R_f 0.00), the only compound in peak V. Peak IV was due to oxidation products of PE. Peak VI contained two components, phosphatidic acid (PA; R_f 0.78) and phosphatidyl inositol (PI; R_f 0.25); both spots for peak VI were molybdate

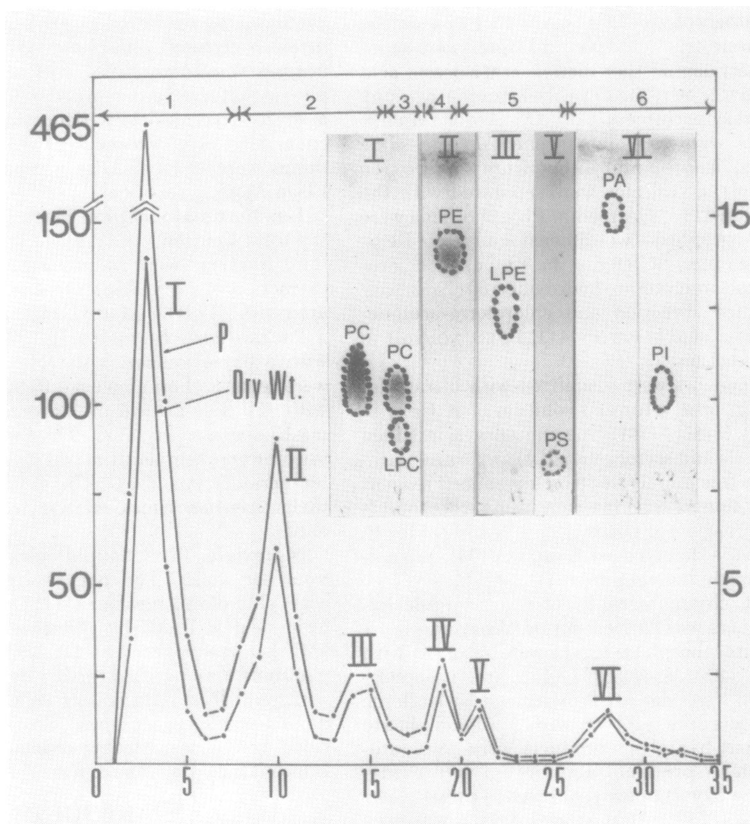

FIG. 1. *Representative elution patterns for milligrams dry weight of lipid (right axis) and micrograms of total P (left axis) obtained by column chromatography of total phospholipid on DEAE-cellulose. The column (2 by 20 cm) was loaded with 1,190 μg of phospholipid-P (obtained by silicic acid chromatography), and 50-ml fractions (horizontal axis) were collected at a rate of 3 ml/min; 1,163 μg of phospholipid-P (97.7%) were recovered. The elution sequence 1 to 6 (top) was as follows: chloroform-methanol (9:1, vol/vol), chloroform-methanol-acetic acid (7:3:0.002, vol/vol), methanol, chloroform-acetic acid (3:1, vol/vol), acetic acid, chloroform-methanol-ammonium hydroxide (32:8:1, vol/vol). The insert shows the results of TLC of compounds in peaks I to VI, phospholipid being visualized by H_2SO_4 charring. Both peaks I and VI contained two phospholipid components. Phosphatidyl choline (PC) and phosphatidic acid (PA) were in the peak fractions for I and VI, respectively, whereas lysophosphatidyl choline (LPC) and phosphatidyl inositol (PI) occurred in the corresponding shoulders.*

positive and ninhydrin negative. The quantitative composition of the total phospholipid is shown in Table 2.

The phospholipids were also characterized by determining their molar ratios of P:acyl esters:glycerol:N (Table 3). Theoretical and actual values agreed closely. The high acyl ester content of LPC was due to contamination with PC.

Glycerol phosphate esters obtained by deacylation of the phospholipids were examined by paper chromatography. Seven compounds were detected, each one reacting positively to the acid molybdate spray for P. The R_f values of the glycerylphosphoryl derivatives corresponded with published data (20) and those for our standards prepared from egg yolks. Two compounds (glycerylphosphorylserine and glycerylphosphorylethanolamine) were ninhydrin positive; two inositol-containing glycerylphosphoryl esters were detected.

Glycolipid. This lipid class was separated into five fractions on Florisil; seven different spots were derived therefrom by TLC (Fig. 2). Fraction I contained one component (R_f 0.86) which represented 10% of the total glycolipid as determined gravimetrically. Fractions II and III also contained one component each, both having an R_f of 0.55; they represented 38 and 32% of the total glycolipid, respectively. Another 12% of the glycolipid was present in fraction IV in the form of three components with R_f values of 0.31, 0.20, and 0.08. Fraction V had one component (R_f 0.88); it represented 8% of the glycolipid.

Results obtained with spray reagents suggested that all the foregoing substances were orcinol and diphenylamine positive except IVa and IVb; the latter were weakly orcinol and molybdate positive. The components in fractions II, III, and IVa were also ninhydrin positive.

TABLE 2. *Composition of zoospore phospholipid*

Component	Percentage[a]
Phosphatidylcholine	55.0 ± 3.0
Lysophosphatidylcholine[b]	6.3 ± 2.6
Phosphatidylethanolamine	22.1 ± 2.6
Lysophosphatidylethanolamine	6.3 ± 1.7
Phosphatidylserine	3.0 ± 1.0
Phosphatidic acid	3.0 ± 1.0
Phosphatidylinositol	4.3 ± 1.7

[a] Means and standard deviations for three experiments based on the amount recovered after column chromatography.

[b] Determined by P analysis after preparative TLC of column fractions containing lysophosphatidylcholine and phosphatidylcholine (see insert, Fig. 1).

TABLE 3. *Molar ratios for zoospore phospholipids*[a]

Compound	P:acyl esters:glycerol:N	
	Actual	Theoretical
PC	1.00:1.93:0.96:0.85	1:2:1:1
LPC	1.00:1.47:0.95:0.97	1:1:1:1
PE	1.00:2.13:1.09:0.97	1:2:1:1
LPE	1.00:1.08:1.00:1.13	1:1:1:1
PS	1.00:2.00:0.83:0.90	1:2:1:1
PA	1.00:1.96:1.01	1:2:1
PI	1.00:2.11:1.00	1:2:1

[a] The phospholipids used to establish molar ratios were taken either from column fractions (where purity was verified by TLC) or directly from TLC plates.

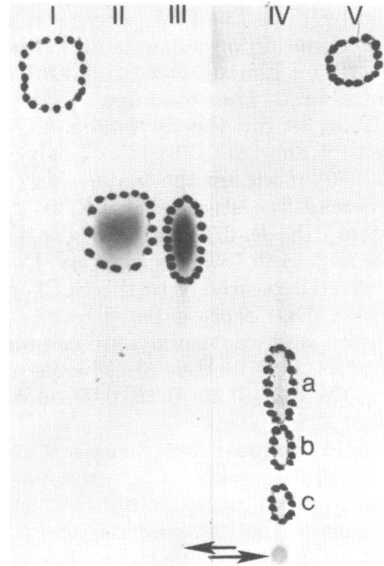

FIG. 2. *Glycolipid fractions collected by Florisil column chromatography (1 by 15 cm) and resolved further by TLC. Fractions I to V were obtained by successive elutions with 50 ml of chloroform-acetone (1:1, vol/vol), acetone, 95% acetone, 90% acetone, and methanol. The chromatograms were visualized by H_2SO_4 charring.*

The glycolipid fraction (15.8% of the total lipid [Table 1]) contained 6.8% of the P in the total lipid extract. Analyses by phenol-sulfuric acid and anthrone methods, with glucose as a standard, suggested that 23% of the glycolipid was carbohydrate. However, judging by enzymatic assays with glucose oxidase, only 6% of this carbohydrate was actually glucose.

Thin-layer and paper chromatography of the acid hydrolysis products indicated that, in addition to glycerol, several substances that be-

haved like carbohydrates were present in the glycolipid. Fractions I and V appeared to contain monoglycosyl diglycerides judging from R_f values (Fig. 2) and the fact that each released only one carbohydrate after hydrolysis and TLC. Fractions II and III behaved like diglycosyl diglycerides chromatographically (Fig. 2), and each released two components, one of them being ninhydrin positive. Three carbohydrates were produced by Fraction IV, one of which was ninhydrin positive.

Neutral lipid. This group of lipids was resolved by TLC into 13 spots (Fig. 3). Neutral lipid was also column fractionated through two different media (Table 4), both yielding comparable results when assayed gravimetrically and monitored by TLC. The FFA, whether removed before fractionation on silicic acid or fractionated directly on Florisil, constituted 8% of the total neutral lipid. They migrated to or near the solvent front, as did the hydrocarbons, which accounted for another 13 to 14%. Triglycerides (TG; R_f 0.89) made up the major class (28 to 30%), whereas free sterols (FS; R_f 0.84) and sterol esters (SE; R_f 0.79 and 0.73) constituted 12 to 13% and 15 to 18%, respectively. Both FS and SE reacted positively to the $SbCl_3$ spray. Diglycerides (DG) represented 8 to 9% of the neutral lipid and contained four components (R_f 0.65, 0.58, 0.45, and 0.38), whereas monoglycerides (MG; R_f 0.30, 0.22, 0.09, and 0.00) represented 12%.

Fatty acid composition. Zoospores contain at least 20 fatty acids. The principal ones (Table 5) were palmitic, palmitoleic, stearic, oleic, γ-linolenic, and arachidonic acids. Nineteen fatty acids were detected in the neutral lipid fraction, palmitic and oleic acids accounting for over 50% of them. Eight fatty acids were found in the glycolipid fraction, palmitic, oleic, and γ-linolenic being the major ones, and arachidonic being conspicuously absent. The phospholipid fraction contained 12 fatty acids of which the major ones were palmitic, oleic, γ-linolenic, and arachidonic.

Lipid composition of sporangia and zoospores produced in liquid media. The proportions of neutral, glyco-, and phospholipid in zoospores derived from liquid cultures were very different than those for zoospores from agar media (Table 6, column 3 versus Table 1), even though the total lipid in the two kinds of zoospores was the same, i.e., about 11%. On the other hand, the proportions of these three lipid classes were about the same in the mature (i.e., post cleavage) zoospore-producing parent plants (column 2, Table 6) as they were in the zoospores themselves (column 3, Table 6); however, the lipid composition characteristic for both zoospores and postcleavage plants was

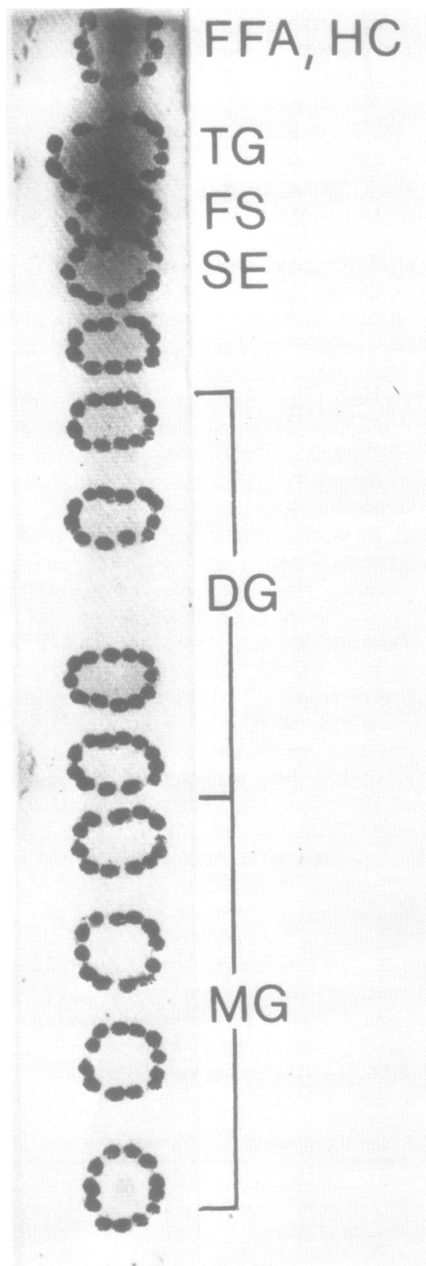

FIG. 3. *Components in the neutral lipid fraction separated by TLC and visualized by H_2SO_4 charring. Neutral lipids are, from top to bottom: free fatty acids (FFA), hydrocarbons (HC), triglycerides (TG), free sterols, (FS), sterol esters (SE), diglycerides (DG), and monoglycerides (MG).*

different than that of precleavage plants (column 1, Table 6). These conclusions were substantiated by the distribution of radioactivity in the three lipid classes (columns 4, 5, and 6, Table 6) derived from precleavage plants, postcleavage plants, and zoospores from liquid cultures containing [^{14}C]acetate.

The three lipid classes derived from zoospores also resembled those from postcleavage plants when characterized further by TLC. The results obtained by similarly separating the labeled neutral, glyco-, and phospholipid via TLC and then scanning the chromatograms for radioactivity are also delineated (Fig. 4). Most of the radioactivity in the neutral lipid fraction was associated with TG (A-I). FS (A-II) and SE (A-III) were also major components, whereas small amounts of label were associated with the MG and DG. Monoglycosyl diglycerides (B-I) and diglycosyl diglycerides (B-II) were the major components labeled in the glycolipid fractions; peaks B-III and B-IV are mixtures of glycolipids that were not resolved completely in this solvent system. PC (C-III) and PE (C-I) were the major labeled compounds in the phospholipid fraction, LPE (C-II) and LPC (C-III) also being present. Minor phospholipids were PA, PI, and PS.

TABLE 4. *Composition of zoospore neutral lipid*[a]

Fraction	Percentage	
	By silicic acid	By Florisil
Hydrocarbons	14.0 ± 2.0[b]	13
Free fatty acids	8.3 ± 1.1	8
Triglycerides	29.8 ± 2.1	28
Free sterols	12.3 ± 1.7	13
Sterol esters	15.0 ± 1.0	18
Diglycerides	9.0 ± 1.4	8
Monoglycerides	11.6 ± 1.0	12

[a] Determined gravimetrically after fractionations on either silicic acid or 7% hydrated Florisil.

[b] Means and standard deviations based on three determinations.

TABLE 5. *Principal fatty acids in zoospore lipid*

Fatty acid[a]	Percentage of fatty acid in			
	Total lipid	Neutral lipid	Glycolipid	Phospholipid
16:0 (palmitic)	28	19	38	39
16:1 (palmitoleic)	4	4	5	3
18:0 (stearic)	9	9	3	2
18:1 (oleic)	32	35	23	14
18:3 (γ-linolenic)	12	6	18	23
20:4 (arachidonic)	7	5	0	13

[a] The first and second numbers represent length of carbon chain and number of double bonds, respectively.

TABLE 6. *Composition of lipid in plants and zoospores produced in liquid media*[a]

Fraction	Composition by weight (%)			Distribution of ^{14}C[b] (%)		
	Precleavage plants	Postcleavage plants	Spores	Precleavage plants	Postcleavage plants	Spores
Neutral lipid	17.7	25.2	27.2	16.0	23.2	29.4
Glycolipid	13.1	12.0	12.4	15.3	11.7	11.6
Phospholipid	69.2	62.8	60.4	68.7	65.1	59.0

[a] Total lipid was extracted and separated as before (see Table 1) on silicic acid columns.

[b] Counted with a Tracerlab Versa/matic scaler.

DISCUSSION

The wall-less, motile zoospores of *B. emersonii* possess an elaborate, tightly organized, membrane-bound corps of organelles; one especially prominent component is a cluster of discrete lipid globules partially wedged into a structure which, until its function and chemical composition has been at least partially characterized, is being called "SB matrix" (40). These zoospores can develop along four different macrocylic pathways (4), as well as a microcyclic pathway (18), thereby producing plants which give rise to new generations of swarm cells. Against this background, we wish to comment briefly about the extractable lipid produced by this fungus.

Both zoospores and sporulating cells contain neutral lipids, glycolipids, and phospholipids, which vary in amount and relative proportions depending on the developmental stage. Additionally, we have shown in this report that the proportions of these three lipid classes in zoospores derived from plate-grown cultures of ordinary colorless (OC) plants differed from the corresponding amounts in zoospores derived from liquid cultures; this observation is consistent with accounts showing that other microorganisms also change in lipid composition as they adjust to different environmental conditions (2, 23). With *B. emersonii*, the temperature, pH, and composition of the media used for the two kinds of cultures were similar; however, population densities—especially when considered in relation to the amounts of oxygen apparently available—were quite different. We

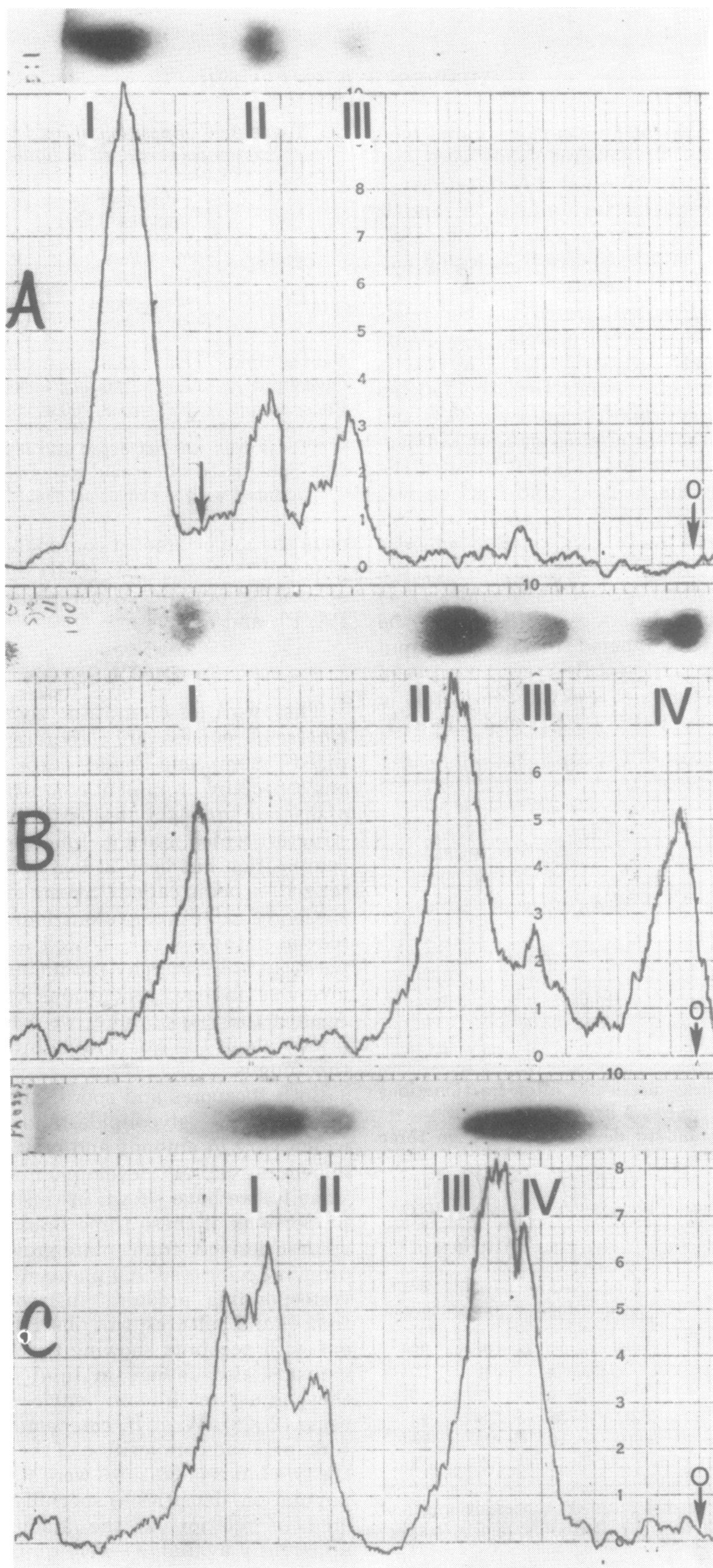

FIG. 4. *Separable components in the ^{14}C-labeled neutral lipid (A), glycolipid (B), and phospholipid (C) extracted from postcleavage plants of B. emersonii and resolved by TLC, and the profiles for distribution of radioactivity in these components. The arrow (in A) indicates a shift to a 2.5-fold reduction in amplification of the signal; O represents the origin. Tracings were made with a Tracerlab 4-pi scanner.*

think this difference may account for the corresponding dissimilarities in lipid composition of the zoospores; our reasoning is as follows.

We observed (Table 6), as did Smith and Silverman (36), that there was about 45 to 50% more neutral lipid in zoospores than in sporulating plants when both were derived from liquid cultures. The transition (precleavage plants → postcleavage plants → free swimming zoospores) was associated (Table 6) with a shift in neutral lipid from 16.8% through 24% to 28.3%, respectively. When grown in well-aerated liquid cultures, OC plants increase exponentially in weight up to about the time of sporogenesis (13, 22); the corresponding increases in respiration and other evidence for oxidative activity (22, 24), including the reutilization of lactic acid (3), suggest that there was a continuously increasing demand for O_2 up to the end of their generation time. In plate cultures, on the other hand, neither forced aeration nor agitation was employed; the demand for oxygen may have existed, but its availability was undoubtedly restricted by various diffusion processes and perhaps other factors; hence, the average O_2 uptake per plant was probably very much reduced, whereas lactic acid simultaneously accumulated in the medium. This information and the fact that the population density of the plants in plate cultures (ca. 2×10^5 thalli/ml after submersion in flooding water) was about five times greater than that in liquid cultures (ca. 0.4×10^5 plants/ml) lead us to suspect that exogenous oxygen deficiencies and corresponding shifts toward fermentative metabolism prior to and during sporogenesis may have been responsible, at least in part, for the increased proportion of neutral lipids in *B. emersonii* grown on solid media.

The composition of the extractable lipid in spores derived from plate cultures was also different than that in spores derived from liquid cultures. In the former, the major lipids were PC (20.3%), TG (13.5%), and diglycosyldiglycerides (DGD; 13.3%), whereas PE, FS plus SE, and lysophosphatides (LP) accounted for 6.7, 12.2, and 4.9% of the lipid, respectively. In the latter, PC and PE were the most abundant components, and lesser amounts of TG, DGD, LP, FS, and SE were also present, the phosphatides constituting 62% of the total lipid. These results are consistent with the phospholipid contents of zoospores derived from liquid cultures as reported by Smith and Silverman (36) and Suberkropp and Cantino (37), i.e., 55% and up to 85% of the extractable lipid, respectively. The latter value is not out of line because it was unquestionably inflated by its inclusion of glycolipids; the fractionation techniques used at that time separated the total lipid into only two classes, neutral lipids and polar lipids.

Although the quantity of sterols and SE in the zoospores of *B. emersonii* seems to exceed the amount in most other Phycomycetes (42), the fatty acid composition of the *B. emersonii* zoospores does resemble that of other terrestrial phycomycetes (2, 7, 14, 33, 39). In addition, γ-linolenic acid—once thought (33) to be both characteristic of and limited to the phycomycetes, but recently found (32) to occur in other fungi—was associated predominantly with polar lipids in the zoospores we analyzed, as it was in the mixture of variously aged thalli harvested from multiple generation *B. emersonii* cultures by Sumner (38). However, the average degree of saturation among the fatty acids we extracted from zoospores was greater (an approximate estimate can be derived from the data in Table 5) than the average value obtained by Sumner (38) for plants. This apparent difference between spores and plants of *B. emersonii* could, of course, be due simply to differences in culture conditions rather than stages in ontogeny; on the other hand, our results do agree with Sumner's (38) observations in that more polyunsaturated fatty acids were associated with polar lipids than with neutral lipids.

The glycolipid in *B. emersonii* seems to be of an unusual nature. It is also the most homeostatic of the three lipid classes, apparently being stabilized at a level of about 12 to 15% of the total lipid whatever the developmental stage—whether the glycolipid is derived from zoospores or sporangia, mature or immature, or extracted from plate-grown or liquid-grown cultures. This conclusion contrasts sharply with the results of Smith and Silverman (36), who concluded that glycolipid accumulated during sporulation. Although they did not specify how many spores were released by their sporulating plants, the information provided suggests that they may have been microcyclic plants similar to the unispored plantlets studied by Hennessy and Cantino (18). The physiological changes occurring after induction of sporogenesis in lag-phase (microcyclic) germlings and log-phase (macrocyclic) plants are known to show similarities but also some differences (18). Perhaps the latter includes lipid composition.

Our unpublished data lead us to believe that the glycolipids are associated with specific organelles in *B. emersonii*, which could account for the stability of this class of lipids. A more

detailed study is underway to identify the nature of these glycolipids and their possible relationships to certain functional aspects of the zoospore of *B. emersonii*.

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