Lipid Composition of 30 Species of Yeast[1]

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ABSTRACT

The detailed composition of cellular lipid of more than 23 species of yeast has been determined quantitatively by thin-chrography on quartz rods, a method previously used for estimating cellular lipids of seven species of yeast. That data was fortified by neutral and phospholipid quantitations on 30 species of yeast cells. Most of the test organisms contained 7-15% total lipid and 3-6% total phospholipid per dry cell weight, except for the extremely high accumulation of triglycerides in two species of *Lipomyces*. Qualitatively, 30 species of yeast cells contained similar neutral lipid constituents (triglyceride, sterol ester, free fatty acid, and free sterol) and polar lipid components (phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, cardiolipin, and ceramide monohexoside) without minor constituents. Based on the quantitative composition of neutral lipids, the 30 species of yeast were divided into two groups, the triglyceride predominant group and the sterol derivative group. These groupings were fairly well overlapped from the standpoint of the distribution characteristics of fatty acid. The relative polar lipid compositions also grossly resembled each other. Only one exception of polar lipid composition in yeast cells was found in *Rhodotorula rubra* species which contained phosphatidyl ethanolamine as the most abundant phospholipid. Fatty acid distribution patterns in yeast cells consistently coincided with other reports concerning fatty acid composition of yeast cells. Correlation of lipid composition and classification of yeasts are suggested and discussed.

INTRODUCTION

Despite considerable interest in the production of fat yeast, comparatively few detailed investigations concerning the nature of yeast lipids have appeared in the literature. Especially there is a lack of quantitative data on the distribution of the lipids in the cells and on the lipid productivity of many species of yeast. Previously a simple method for estimating the cellular lipids of yeast was established in this laboratory (1). YM-agar plate culture was used for the production of yeast cells. YM-medium is the most generally used for the preservation and maintenance of yeast strains. Moreover, this culturing method is less laborious and gives the average value about various growth stages of cells.

The principal purpose in this series of investigations is to acquire basic information about lipid components in as many yeast species as possible. Work with the pyrophosphatidic acid of *Cryptococcus neoformans* in this laboratory (2,3) also suggested that there are significant relationships between yeast taxonomy and distribution of pyrophosphatidic acid in yeast species (4).

This paper reports the lipid compositions and contents of 23 species of yeast cells and compares them with previously published compositions of seven species.

EXPERIMENTAL PROCEDURES

Yeast Strains

In addition to the seven species of yeast in a preceding report (1), the following 23 species were used in this study.

Ascosporogenous yeast: *Saccharomyces rosei* W-70, *Saccharomyces carlsbergensis* BH1-3, *Saccharomyces rouxii* MS1-3, *Kluyveromyces polysporus* EC12-4, *Debaryomyces hansenii* MY-45, *Debaryomyces nilssonii* Z-9-6, *Pichia membranaefaciens* IV-5-1, *Pichia farinosa* WH3-1, *Hansenula anomala* WH16-2, *Lipomyces lipoferus* 0673, *Saccharomycodes ludwigii* Shin 3-5.

Ballistospologenous yeast: *Sporobolomyces salmonicolor* WF-174.

Asporogenous yeast: *Cryptococcus laurentii* Z-6-5, *Torulopsis colliculosa* J-5, *Torulopsis candida* MYA-3, *Candida Krusei* WF-16, *Candida toropicalis* Shin 1-3, *Candida mycoderma* WF-8, *Candida pulcherrima* 33C, *Trigonopsis variabilis* S-3-9, *Trichosporon cutaneum* KC4-3, *Rhodotorula glutinis* H3-9-1, *Rhodotorula rubra* AY-2, *Rhodotorula rubra* Np. 2-17-4B. All cultures were obtained from the

[1]A part of this investigation has been reported at the 14th conference of the Japan Oil Chemists' Society, Nagoya, Japan, October 1975.

TABLE I

The Amount and Composition of the Lipids in 30 Species of Yeast[a]

Species	TL (% of DC)	PL (% of DC)	PL (% of TL)	Lipid productivity[b] (mg)
Ascosporogenous yeast				
Schiz. pombe	9.1 ± 0.1[c]	4.0 ± 0.5[c]	42.7 ± 4.2[c]	10.0
Sacch. cerevisiae	7.2 ± 0.3[c]	3.7 ± 0.4[c]	52.0 ± 5.0[c]	18.5
Sacch. rosei	8.5 ± 0.3	4.8 ± 0.0	56.8 ± 1.8	21.1
Sacch. carlsbergensis	7.1 ± 0.2	4.4 ± 0.1	61.7 ± 0.7	13.2
Sacch. rouxii	8.1 ± 0.2	4.1 ± 0.1	49.9 ± 1.1	17.3
K. polysporus	10.7 ± 0.4	5.5 ± 0.3	51.6 ± 1.3	10.9
Schw. occidetalis	9.0 ± 0.6[c]	4.8 ± 0.2[c]	53.4 ± 2.2[c]	14.1
Deb. hansenii	7.0 ± 0.4	4.2 ± 0.1	59.5 ± 3.2	16.0
Deb. nilssonii	6.7 ± 0.3	3.9 ± 0.2	57.9 ± 0.3	19.5
P. membranaefaciens	11.3 ± 0.4	5.0 ± 0.2	43.6 ± 2.9	29.2
P. farinosa	8.7 ± 0.1	4.4 ± 0.1	50.3 ± 1.9	31.4
H. anomala	8.6 ± 0.1	4.6 ± 0.1	52.6 ± 1.3	23.8
L. starkeyi	32.3 ± 2.3[c]	5.3 ± 1.8[c]	15.5 ± 4.9[c]	51.7
L. lipoferus	25.8 ± 1.5	3.8 ± 0.3	14.4 ± 0.1	78.0
S'codes. ludwigii	9.6 ± 0.1	4.7 ± 0.2	48.0 ± 1.4	12.6
Ballistospologenous yeast				
Sp. salmonicolor	13.4 ± 0.3	4.0 ± 0.1	29.8 ± 1.3	39.8
Asporogenous yeast				
Cr. neoformans	9.8 ± 0.9[c]	6.0 ± 0.9[c]	63.1 ± 8.3[c]	17.2
Cr. laurentii	11.5 ± 0.2	3.1 ± 0.2	26.7 ± 1.4	46.6
T. colliculosa	8.9 ± 0.2	5.1 ± 0.1	57.8 ± 0.3	21.2
T. candida	8.9 ± 0.2	2.9 ± 0.1	32.2 ± 0.5	29.0
C. utilis	10.6 ± 1.8[c]	4.0 ± 0.3[c]	37.8 ± 5.5[c]	23.9
C. krusei	7.3 ± 0.1	4.1 ± 0.1	55.5 ± 0.4	23.5
C. tropicalis	6.3 ± 0.0	3.4 ± 0.0	53.2 ± 0.1	19.6
C. mycoderma	11.5 ± 0.0	5.0 ± 0.1	43.1 ± 0.3	27.0
C. pulcherrima	7.1 ± 0.1	4.8 ± 0.2	67.7 ± 2.1	15.2
Kl. apiculata	6.6 ± 0.2[c]	2.4 ± 0.4[c]	37.3 ± 3.8[c]	4.6
Trig. variabilis	8.7 ± 0.4	5.5 ± 0.3	63.6 ± 4.2	20.7
Tr. cutaneum	13.3 ± 0.9	5.3 ± 0.3	39.5 ± 0.6	33.3
Rh. glutinis	11.2 ± 1.3	4.6 ± 0.0	41.3 ± 4.8	30.7
Rh. rubra AY-2	14.8 ± 0.3	3.9 ± 1.3	25.4 ± 2.5	40.9
Rh. rubra Np. 2-17-4B	18.6 ± 0.1	3.0 ± 0.3	16.5 ± 1.2	60.0

[a]Abbreviations: DC, dry cell weight; TL, total amount of lipid; PL, total amount of phospholipid. The figures in this table are the mean values ± SD of 5-10 repeated analyses of lipid samples. The yeast cultures were repeated 2-4 times.

[b]Lipid productivity (mg) is expressed as the total amount of cellular lipid which has been obtained from cells grown on a unit of cultural medium (40 ml YM-agar medium, 12 cm diameter petri dish). These data have about ± 10 percent error for mean values.

[c]These data had been reported in a previous report (1).

culture collection maintained at the Faculty of Engineering, Yamanashi University.

Cell production was substantially the same as described previously (1). The YM-culture medium contained per liter: 10 g glucose, 3 g yeast extract, 3 g malt extract, 10 g peptone, and 20 g agar. Yeast cells freshly grown on the slope of the agar medium for 48 hr at 30 C were inoculated on the surfaces of agar plates. After cells had grown to cover the surfaces of the plates, they were harvested.

Total Cellular Lipid Preparation

The total lipid of the yeast was obtained from fresh cells according to the previous paper (1). A 5 g sample of the wet cells was suspended in 20 ml of a chloroform:methanol mixture (1:2 v/v) together with 40 ml of glass beads (0.40-0.50 mm diameter) and the mixture was homogenized for 4 min at 4000 cpm in a cell homogenizer (Braun Model MSK). The disrupted cell suspension was filtered through a glass filter (4G). The residual cell debris were stirred on the magnetic stirrer with 50 ml of a chloroform:methanol mixture (2:1 v/v) for 30 min at a room temperature under an atmosphere of nitrogen and filtered. This stirring and filtering process was repeated three more times. The combined chloroform-methanol filtrate was washed with a 0.9% NaCl solution to remove the nonlipid contaminants according to Folch's method (5).

Quantitative Analysis

The membrane filter (Sartorius MF-100) was

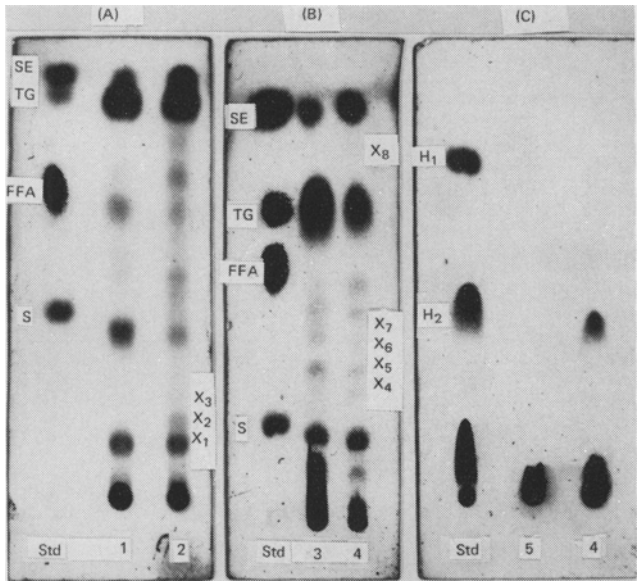

FIG. 1. Thin layer chromatograms of neutral lipids of five yeast species. Plate: silica gel sintered plate. Developing solvent systems: (A) petroleum ether:ethyl ether:gracial acetic acid (80:30:1, v/v); (B) petroleum ether:ethyl ether:gracial acetic acid (90:10:1 v/v); (C) *n*-hexane. Detection: charring at 140 C for 20 min after spraying conc. sulfuric acid. Abbreviations: Std, standard sample; H_1, saturated hydrocarbon (squalane); H_2, unsaturated hydrocarbon (squalene); SE, sterol ester (cholesterol palmitate); TG, triglyceride (tripalmitin); FFA, free fatty acid (oleic acid); S, sterol (cholesterol); X_1-X_8, (unidentified lipids). Samples: 1, *C. krusei*; 2, *T. colliculosa*; 3, *H. anomala*; 4, *Deb. nilssonii*; 5, *P. membranaefaciens*.

used for the determination of the amount of dry cells. The total amount of lipids was estimated gravimetrically. The phosphorus content in a total lipid was determined by the method of King (6). The total amount of phospholipids was calculated by multiplying the phosphorus value by 25.

Thin Layer Chromatography (TLC)

Lipids were chromatographed on silica gel sintered plates (Yamato Kagaku, Tokyo, Japan) and silica gel precoated plates (Merck, Darmstadt, West Germany). Solvent systems and detecting reagents were as described previously (7). Relative neutral lipid composition was determined by thinchrography, a quantitative TLC using a new apparatus which was equipped with silica gel sintered rods (0.9 x 150 mm) and a hydrogen flame ionization detector (Iatroscan TH-10, Iatron, Tokyo). The solvent system was petroleum ether:ethyl ether:acetic acid (90:10:1 v/v) (8). Relative phospholipid composition was determined with quantitative two-dimensional TLC combined with phosphorus estimation (9,10). Developed chromatograms were stained by iodine vapor, and detected spots were scraped from thin layer plates and digested in 0.6 ml $HClO_4$ at 180 C for 2 hr. After addition of 0.2 ml of 5% ammonium molybdate and 0.2 ml of reducing reagent, the total volume was adjusted to 5 ml with distilled water. The reaction mixtures were heated for 7 min in boiling water and centrifuged at 3000 rpm for 10 min. The optical density of the supernatant at 830 nm was estimated.

Fatty Acid Composition

Fatty acid methyl esters were prepared by the HCl-methanol method (11). Gas liquid chromatography was carried out by the use of Hitachi K-53 and 063 gas chromatographs. The column packings used were 10% diethyleneglycolsuccinate (170 C) and 3% SE-30 (70-250 C, 5 C/min) on acid washed Gas Chrom W (60-80 mesh). The flow rate of the N_2 carrier gas was 30 ml/min at 170 C. The injection port and the detector were operated at 320 C. Each peak was identified by comparing the retention time with those of the authentic methyl esters of even numbered straight chain saturated acids (C_{12}-C_{18}), palmitoleic acid, oleic acid, and linoleic acid (Nippon Oil & Fat). All of the samples were chromatographed through the polar and nonpolar columns. Fatty acid composition was expressed as percentage of peak area.

Chemicals

The organic solvents were of reagent grade and freshly distilled before use. Other organic

TABLE II

Neutral Lipid Composition of 30 Yeast Species[a]

Species	S (%)	FFA (%)	TG (%)	SE (%)	Unidentified NL[b]
Ascosporogenous yeast					
Schiz. pombe	4.2 ± 0.6	4.0 ± 0.9	54.4 ± 5.0	28.9 ± 1.7	2
Sacch. cerevisiae	3.5 ± 0.6	2.5 ± 1.6	29.9 ± 6.3	52.9 ± 5.2	2
Sacch. rosei	6.6 ± 0.9	T[c]	14.4 ± 1.4	76.4 ± 2.0	5
Sacch. carlsbergensis	11.1 ± 1.6	10.7 ± 0.4	19.2 ± 0.7	57.1 ± 1.7	5
Sacch. rouxii	8.9 ± 1.5	3.0 ± 1.2	47.0 ± 2.5	38.1 ± 3.6	5
K. polysporus	6.7 ± 0.3	2.8 ± 1.0	26.0 ± 1.7	54.8 ± 5.2	6
Schw. occidentalis	3.0 ± 1.5	7.2 ± 0.1	76.0 ± 5.5	4.9 ± 2.7	3
Deb. hansenii	13.8 ± 1.7	10.0 ± 1.8	65.8 ± 5.2	1.8 ± 0.8	4
Deb. nilssonii	21.2 ± 2.0	2.1 ± 0.5	41.2 ± 1.0	34.4 ± 3.0	5
P. membranaefaciens	3.8 ± 0.9	4.4 ± 1.3	70.4 ± 6.7	6.5 ± 0.6	4
P. farinosa	25.9 ± 2.5	3.5 ± 1.3	59.6 ± 4.3	4.5 ± 1.0	4
H. anomala	11.9 ± 4.0	T	74.0 ± 1.1	7.6 ± 4.0	5
L. starkeyi	2.4 ± 0.7	3.2 ± 0.8	93.4 ± 1.8	T	5
L. lipoferus	1.8 ± 0.2	7.6 ± 0.6	85.4 ± 0.6	T	4
S'codes. ludwigii	3.9 ± 1.9	3.8 ± 1.0	36.1 ± 5.7	46.1 ± 4.7	5
Ballistospologenous yeast					
Sp. salmonicolor	7.2 ± 1.9	15.9 ± 1.3	66.0 ± 6.2	5.0 ± 1.0	2
Asporogenous yeast					
Cr. neoformans[d]	3.6 ± 0.1	1.2 ± 0.2	90.7 ± 0.3	3.1 ± 0.1	4
Cr. laurentii	6.3 ± 1.5	67.2 ± 1.3	21.3 ± 1.5	2.5 ± 0.8	3
T. colliculosa	6.7 ± 1.5	1.7 ± 0.9	39.0 ± 1.6	46.6 ± 2.8	8
T. candida	3.1 ± 0.9	1.1 ± 0.4	92.4 ± 2.3	1.0 ± 0.2	5
C. utilis	3.1 ± 1.4	2.2 ± 0.6	87.6 ± 3.4	2.3 ± 3.0	3
C. krusei	19.4 ± 2.0	4.5 ± 1.0	71.1 ± 1.1	4.9 ± 2.3	5
C. tropicalis	18.4 ± 0.8	4.8 ± 0.8	69.7 ± 1.7	7.0 ± 0.8	5
C. mycoderma	6.5 ± 1.1	1.6 ± 0.2	70.9 ± 2.5	1.3 ± 0.2	4
C. pulcherrima	30.3 ± 2.5	T	62.5 ± 2.9	2.0 ± 0.7	4
Kl. apiculata	7.1 ± 1.7	29.3 ± 3.4	51.2 ± 3.8	4.8 ± 1.4	3
Trig. variabilis	27.7 ± 1.6	14.3 ± 1.2	32.0 ± 2.8	6.9 ± 0.6	4
Tr. cutaneum	1.8 ± 0.6	T	93.4 ± 2.1	2.5 ± 1.4	4
Rh. glutinis	6.7 ± 1.2	7.0 ± 1.4	79.7 ± 3.7	4.5 ± 1.0	3
Rh. rubra AY-2	3.6 ± 0.3	8.0 ± 0.8	84.8 ± 1.7	1.7 ± 0.3	3
Rh. rubra Np. 2-17-4B	1.3 ± 0.1	6.6 ± 0.3	90.9 ± 0.3	0.8 ± 0.1	3

[a]The figures are obtained from the same experiments as in Table I. Abbreviations: S, sterol; FFA, free fatty acid; TG, triglyceride; SE, sterol ester; NL, neutral lipid.

[b]The number of unidentified lipids detected on the area between the origin and sterol ester on the silica gel thin layer chromatograms (Fig. 1).

[c]T<1%.

[d]That was obtained from liquid cultured cells (10).

and inorganic reagents, of analytical grade or of the highest quality commercially available, were used without further purification.

RESULTS AND DISCUSSION

Lipid Productivity and Lipid Content

The lipid productivity by yeast is expressed as the total amount of lipid obtained from cells grown on a unit of cultural medium. The total amount of cellular lipid per dry cell weight, the total amount of cellular phospholipid per dry cell weight, and the cellular lipid production for 30 species of yeast are summarized in Table I. The cells belonging to ascosporogenous yeasts contained relatively constant levels of total lipid (about 7-11%), but two strains of yeast of the so-called fat yeast, *L. starkeyi* and *L. lipoferus*, contained 32% and 26%, respectively. Lipid abundance in asporogenous yeasts ranged from 7 to 18%. A similar range of total phospholipids in ascosporogenous yeasts was found. Table I also shows that the high production of lipid by *L. starkeyi* and *L. lipoferus* was due largely to the deposit of neutral lipids.

Neutral Lipid Composition

Typical chromatograms for cellular neutral lipids of yeast on silica gel plate are shown in Figure 1. The neutral lipid composition of 30 species of yeast cells is presented in Table II. Each of them was found to contain at least four kinds of neutral lipids: triglyceride, sterol ester, free sterol, and free fatty acid. For many

TABLE III

Phospholipid Composition of 30 Yeast Species[a]

Species	PC	PE	PI	PS	CL	PA	PG	LPC	pyro-PA	A[b]	D[b]	G[b]	Unidentified[c] polar lipids
Ascosporogenous													
Schiz. pombe	50.6 ± 0.4	13.1 ± 0.5	13.8 ± 0.7	12.3 ± 0.4	5.5 ± 0.9	T[d]	-[e]	-	-	-	-	-	
Sacch. cerevisiae	42.0 ± 1.5	25.3 ± 1.1	15.8 ± 0.4	7.0 ± 0.6	9.4 ± 0.6	T	-	-	-	-	-	-	
Sacch. rosei	39.2 ± 0.6	21.8 ± 0.2	18.3 ± 0.4	7.3 ± 0.6	9.2 ± 0.4	1.0 ± 0.5	-	-	-	3.2 ± 0.2	-	-	E
Sacch. carlsbergensis	34.1 ± 0.8	25.4 ± 0.4	16.4 ± 0.4	8.5 ± 0.5	8.3 ± 0.2	1.1 ± 0.1	-	T	-	5.2 ± 0.4	-	-	E
Sacch. rouxii	44.2 ± 1.7	16.6 ± 0.6	15.4 ± 0.7	8.5 ± 1.0	7.6 ± 0.8	1.7 ± 0.3	-	T	-	5.5 ± 0.1	-	T	E
K. polysporus	42.3 ± 2.6	14.8 ± 0.1	18.0 ± 1.3	7.4 ± 1.0	5.3 ± 0.7	1.6 ± 0.4	-	-	-	8.0 ± 0.8	-	T	E
Schw. occidentalis	48.2 ± 3.0	16.3 ± 0.3	10.1 ± 1.0	11.9 ± 0.1	7.4 ± 0.6	T	4.1 ± 1.8	1.2 ± 1.1	-	-	-	-	
Deb. hansenii	41.5 ± 1.5	15.6 ± 0.3	9.1 ± 0.6	11.8 ± 0.3	8.7 ± 0.8	2.9 ± 0.4	3.5 ± 0.2	T	-	5.1 ± 0.5	T	-	E
Deb. nilssonii	33.2 ± 0.3	20.1 ± 0.3	17.7 ± 0.8	11.5 ± 1.1	7.4 ± 1.1	1.3 ± 0.6	-	-	-	7.5 ± 0.3	-	1.2 ± 0.3	E, F
P. membranaefaciens	37.7 ± 1.0	17.1 ± 0.4	16.1 ± 0.7	6.9 ± 0.8	6.6 ± 0.2	1.3 ± 0.6	-	-	-	13.1 ± 1.7	-	1.1 ± 0.1	B, E, F
P. farinosa	25.3 ± 0.7	17.6 ± 0.3	16.5 ± 0.8	12.6 ± 0.3	6.0 ± 0.4	3.5 ± 0.3	-	-	-	17.8 ± 0.2	-	T	E, F
H. anomala	39.8 ± 1.8	15.8 ± 0.2	11.0 ± 0.6	13.2 ± 0.5	8.6 ± 0.7	T	-	-	-	7.8 ± 0.9	-	-	E, F
L. starkeyi	43.1 ± 0.6	25.4 ± 0.5	11.7 ± 0.8	10.9 ± 0.5	5.1 ± 0.1	1.3 ± 0.4	-	1.9 ± 0.6	-	-	-	-	
L. lipoferus	37.7 ± 0.7	19.9 ± 0.4	15.8 ± 0.5	9.4 ± 0.9	3.2 ± 0.6	2.2 ± 1.5	-	-	-	10.8 ± 2.4	T	-	E
S'codes. ludwigii	46.7 ± 1.5	13.7 ± 1.4	7.5 ± 0.7	18.6 ± 0.6	8.1 ± 1.0	T	-	-	-	4.6 ± 1.0	-	-	E
Ballistospologenous yeast													
Sp. salmonicolor	28.9 ± 0.7	30.4 ± 1.4	11.5 ± 0.8	12.6 ± 0.6	7.5 ± 0.2	2.7 ± 0.5	-	-	T	6.5 ± 0.6	-	-	B, C, E, F
Asporogenous yeast													
Cr. neoformans[f]	48.5 ± 1.0	27.5 ± 0.5	7.2 ± 0.5	7.8 ± 0.5	3.5 ± 0.5	T	T	2.5 ± 0.5	T	T	T	-	
Cr. laurentii	30.0 ± 1.0	13.1 ± 0.4	10.9 ± 1.0	4.2 ± 0.4	7.9 ± 0.2	10.8 ± 0.5	-	-	1.1 ± 0.3	18.7 ± 0.6	3.2 ± 1.1	-	C, E, F
T. colliculosa	34.4 ± 0.2	27.4 ± 0.3	17.1 ± 0.4	5.9 ± 0.4	9.4 ± 0.3	1.2 ± 0.6	T	T	-	2.7 ± 0.4	T	-	B, E
T. candida	28.5 ± 0.5	16.1 ± 0.2	16.1 ± 0.2	13.5 ± 0.8	7.9 ± 0.4	4.3 ± 1.1	3.8 ± 0.5	-	-	8.6 ± 0.9	-	1.3 ± 0.9	E, F
C. utilis	31.7 ± 0.6	27.3 ± 0.4	12.7 ± 0.4	12.1 ± 0.5	8.0 ± 0.2	2.7 ± 0.3	2.1 ± 0.9	T	-	2.6 ± 0.1	-	-	
C. krusei	33.4 ± 0.4	17.1 ± 0.2	19.9 ± 0.8	9.0 ± 0.5	6.1 ± 0.1	2.0 ± 0.3	T	T	-	11.0 ± 0.2	-	T	E
C. tropicalis	30.9 ± 0.6	19.2 ± 0.4	14.9 ± 1.1	13.9 ± 1.0	8.3 ± 0.2	3.3 ± 1.0	1.2 ± 0.2	-	-	7.3 ± 0.6	T	T	
C. mycoderma	35.2 ± 0.2	17.3 ± 0.9	16.7 ± 0.8	6.6 ± 0.5	7.6 ± 0.3	1.5 ± 0.8	1.4 ± 0.4	T	-	11.3 ± 0.7	-	1.6 ± 0.4	E, F
C. pulcherrima	41.3 ± 0.5	17.5 ± 0.4	13.0 ± 0.9	10.9 ± 0.7	6.2 ± 0.3	T	T	T	-	9.2 ± 0.8	-	-	F
Kl. apiculata	30.6 ± 2.7	16.5 ± 0.5	8.2 ± 1.4	10.6 ± 0.4	24.6 ± 0.6	T	-	5.7 ± 0.9	-	-	3.6 ± 0.8	-	
Trig. variabilis	41.1 ± 0.7	17.8 ± 0.1	11.5 ± 0.4	9.9 ± 0.4	6.6 ± 0.1	1.6 ± 0.4	-	-	-	10.5 ± 0.8	-	1.0 ± 0.5	B, E, F
Tr. cutaneum	28.9 ± 0.4	15.1 ± 0.5	10.6 ± 0.2	11.6 ± 0.2	5.8 ± 0.6	6.6 ± 0.1	-	1.8 ± 0.3	1.6 ± 0.3	13.4 ± 0.4	-	-	B, E, F
Rh. glutinis	40.2 ± 1.5	28.7 ± 2.6	11.9 ± 1.7	4.7 ± 1.7	7.6 ± 0.4	2.8 ± 0.5	-	-	T	-	3.8 ± 0.2	-	E
Rh. rubra AY-2	15.6 ± 1.2	28.7 ± 2.6	7.1 ± 0.5	16.4 ± 0.9	11.8 ± 0.3	6.4 ± 0.7	-	-	2.4 ± 1.0	9.7 ± 1.4	-	T	B, C, E, F
Rh. rubra Np. 2-17-4B	6.5 ± 0.3	29.8 ± 0.3	7.4 ± 0.6	15.7 ± 0.2	13.8 ± 0.4	13.4 ± 0.1	-	-	2.8 ± 0.3	10.7 ± 0.5	-	-	B, C, E, F

[a]The figures are obtained from the same experiments as in Table I. Abbreviations: PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PI, phosphatidyl inositol; PS, phosphatidyl serine; CL, cardiolipin; PA, phosphatidic acid; PG, phosphatidyl glycerol; LPC, lysophosphatidyl choline; pyro-PA, pyro phosphatidic acid.

[b]These lipids are unidentified phospholipids. Their chromatographic characteristics are described in Figure 2 and in the text.

[c]Those unidentified lipids were negative to the molybdenum blue reagent (5).

[d]T<1%.

[e]-, not detected;

[f]That was obtained from liquid cultured cells (10).

TABLE IV

Fatty Acid Composition of 23 Species of Yeast[a]

Species	C_{10}	C_{12}	C_{14}	C_{15}	$C_{16:0}$	$C_{16:1}$	C_{17}	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	C_{20}	C_{22}	C_{24}
Ascosporogenous yeast														
Sacch. rosei	T[b]	1.7 ± 0.2	3.7 ± 0.2	T	14.7 ± 0.9	47.5 ± 3.5	-[c]	1.0 ± 0.3	33.3 ± 2.5	-	-	-	-	-
Sacch. carlsbergensis	-	T	2.8 ± 0.2	T	6.2 ± 0.2	50.8 ± 0.9	-	1.2 ± 0.3	37.6 ± 0.3	-	-	-	-	-
Sacch. rouxii	-	T	T	-	13.6 ± 0.2	16.4 ± 0.6	T	3.4 ± 0.1	31.6 ± 0.2	34.4 ± 0.2	-	-	-	-
K. polysporus	T	2.8 ± 0.6	11.0 ± 0.0	T	14.6 ± 0.4	51.3 ± 3.7	-	1.3 ± 0.3	21.0 ± 1.5	-	-	-	-	-
Deb. hansenii	-	-	T	T	18.9 ± 1.0	1.0 ± 0.1	2.0 ± 0.0	10.5 ± 0.7	43.7 ± 1.3	18.7 ± 0.6	4.7 ± 0.1	2.8	T	T
Deb. nilssonii	T	T	1.3 ± 0.1	T	10.7 ± 0.1	33.1 ± 0.4	T	2.5 ± 0.1	35.3 ± 0.3	17.0 ± 0.1	-	-	-	-
P. membranaefaciens	-	-	T	T	12.3 ± 1.0	14.8 ± 1.7	T	T	40.9 ± 0.3	23.6 ± 1.5	7.7 ± 0.9	T	-	-
P. farinosa	-	-	T	T	25.5 ± 1.5	1.5 ± 0.1	T	1.3 ± 1.1	23.9 ± 0.1	46.4 ± 1.7	-	T	T	T
H. anomala	-	-	T	T	17.2 ± 2.0	1.3 ± 0.1	T	3.8 ± 0.1	34.7 ± 0.9	35.6 ± 0.9	7.5 ± 0.2	-	-	-
L. lipoferus	-	-	T	T	37.3 ± 1.0	4.3 ± 0.3	-	6.7 ± 0.3	48.3 ± 1.3	3.2 ± 0.3	-	T	T	T
S'codes. ludwigii	T	T	1.0 ± 0.5	-	13.4 ± 1.1	3.0 ± 0.7	-	T	80.2 ± 2.6	-	-	T	-	-
Ballistospologenous yeast														
Sp. salmonicolor	-	-	T	T	24.0 ± 4.7	T	T	6.1 ± 2.7	35.9 ± 2.1	36.3 ± 9.1	3.4 ± 1.6	-	-	-
Asporogenous yeast														
Cr. laurentii	-	-	T	T	17.2 ± 0.2	T	T	8.5 ± 0.4	37.5 ± 0.1	36.5 ± 0.5	-	T	T	T
T. colliculosa	T	1.2 ± 0.1	3.4 ± 0.2	-	13.9 ± 0.3	46.5 ± 1.0	-	T	34.5 ± 1.0	-	-	-	-	-
T. candida	-	-	T	T	27.9 ± 1.4	3.7 ± 0.3	1.8 ± 0.0	9.0 ± 0.1	42.5 ± 2.0	11.9 ± 0.3	2.7 ± 0.5	-	-	T
C. krusei	-	T	T	T	15.0 ± 0.2	6.2 ± 0.2	T	1.1 ± 0.1	48.2 ± 2.0	14.9 ± 0.6	13.7 ± 1.4	T	T	-
C. tropicalis	-	T	T	1.2 ± 0.0	21.8 ± 0.2	5.4 ± 0.2	2.6 ± 0.1	9.4 ± 0.6	28.6 ± 0.5	26.2 ± 0.2	4.4 ± 0.3	-	-	T
C. mycoderma	-	-	T	T	14.1 ± 0.6	17.1 ± 0.8	2.4 ± 0.1	2.3 ± 0.2	41.1 ± 1.4	18.6 ± 1.0	4.7 ± 0.7	T	-	-
C. pulcherrima	-	-	1.4 ± 0.1	T	21.0 ± 0.1	7.4 ± 0.6	T	1.5 ± 0.2	41.4 ± 2.0	25.6 ± 0.6	1.4 ± 0.3	-	-	-
Trig. variabilis	-	-	T	T	19.0 ± 0.5	34.4 ± 1.6	1.5 ± 0.1	T	22.9 ± 1.8	21.5 ± 1.6	-	-	-	-
Trich. cutaneum	-	-	T	T	24.0 ± 4.7	T	T	6.1 ± 2.7	35.9 ± 2.1	36.3 ± 9.1	3.4 ± 1.6	-	-	-
Rh. glutinis	-	T	T	T	12.3 ± 1.0	T	T	1.9 ± 0.6	31.6 ± 2.2	53.1 ± 1.9	-	T	-	T
Rh. rubra AY-2	-	-	T	T	22.2 ± 1.6	T	T	4.2 ± 0.8	61.0 ± 0.7	10.2 ± 0.8	2.7 ± 0.4	-	-	-
Rh. rubra Np. 2-17-4B	-	-	2.0 ± 0.2	-	23.8 ± 3.7	T	-	2.3 ± 0.1	56.9 ± 0.1	11.5 ± 1.8	3.2 ± 1.3	-	-	-

[a]The figures are the average percentages of relative peak area ± SD obtained from the same experiments as in Table I.

[b]T<1%.

[c]-, not detected.

species, triglyceride made up the major component (50-90% of the total amount of neutral lipid), whereas sterol ester, free sterol, and free fatty acid constituted 2-5%, 3-7%, and trace to 5%, respectively. All of the strains of *Saccharomyces, K. polysporous,* and *T. colliculosa* tested did not fit the above generalization. They contained sterol derivatives as a main neutral lipid (50-80%). *Cr. laurentii* and *Kl. apiculata* contained abnormally high levels of free fatty acids. These results suggest that extensive lipolytic activities (lipase and/or phospholipase) had been functioning in the course of cell growth.

Most of the test organisms except *Pichia, Lipomyces, C. mycoderma,* and *Kl. apiculata* contained hydrocarbon as the minor constituents of neutral lipids. Additionally, the following minor components were detected commonly in most of the test strains: X_1 and X_3 were located on silica gel thin layer chromatogram between the original point and the free sterol; X_4-X_7 were situated between the free sterol and the free fatty acid (Fig. 1). One or two of those spots were stained positively with sterol reagents. Further fractionation of sterols and sterol derivatives will be reported elsewhere.

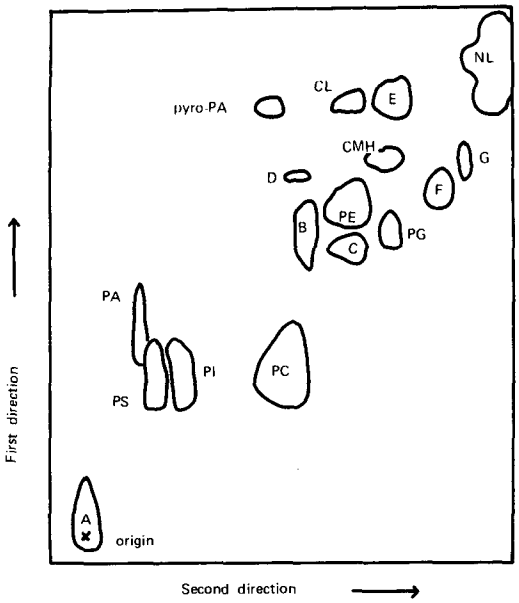

FIG. 2. A typical illustration of a thin layer chromatogram of the polar lipids of yeast. Plate: silica gel sintered plate. Developing solvent systems: first direction, chloroform:methanol:water (65:25:4 v/v); second direction, chloroform:methanol:conc. ammonium hydroxide (65:35:5 v/v). Abbreviations: A, B, C, D, E, F, and G, unidentified lipids; the others, see footnotes to Table III. A, D, and G were phosphorus positive spots. Spot A was also positive for anthrone reagent. Spot C was colored yellow with ninhydrin reagent. B, E, and F were detected with sulfuric acid-charring.

Polar Lipid Compositions

A typical illustration of the two dimensional thin layer chromatogram for yeast cellular lipids is presented in Figure 2. Relative quantitative phospholipid composition is listed in Table III. Most of the yeast species examined in this study possessed similar polar lipid patterns. Phosphatidyl choline constituted the most abundant component, and phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, cardiolipin, phosphatidic acid, and ceramide monohexoside were present. These polar lipid distribution patterns in the yeast cells agreed consistently with those patterns reported by Letters (12). The only exceptions observed were in two strains of *Rh. rubra* which contained phosphatidyl ethanolamine as the most abundant phospholipid in the cells. The specific distribution of pyrophosphatidic acid in six species of yeast was reported previously and was discussed in relation to yeast taxonomy. Phosphatidyl glycerol was also detected in some species. Other unusual distributions of phospholipids were recognized. An unidentified glycophospholipid (A), which remained almost at the starting point on silica gel thin layer chromatograms for several kinds of developing systems, was about 20% per total phospholipid in *Pichia* and *Cr. laurentii*. It is considered likely to be a derivative of the mannosylinositol phosphorylceramide complex or of inositol phosphorylceramides (13). Cardiolipin was contained in relatively large amounts in *Kl. apiculata*. Phosphatidic acid deposits in *Cr. laurentii* and *Rh. rubra* Np. 2-17-4B suggest intense phospholipase activity. From its chromatographic behavior, IR spectrum, and staining behavior, we propose that spot H probably is the *N*-methyl derivative of phosphatidyl ethanolamine (14), and that spot I may be lyso-*bis*-phosphatidic acid (15,16).

Fatty Acid Composition

Relative fatty acid composition of 23 species of yeasts is given in Table IV. Fatty acid distribution patterns in yeast cells closely resembled each other and consisted of analogous series of saturated and unsaturated even numbered aliphatic acids ranging in chain length from C_{10} to C_{24}. Their fatty acid patterns also overlap with others (17). The amounts of C_{16} plus C_{18} acids accounted for over 95% of the total fatty acid in all species again. The species tested in this study were arbitrarily divided into two groups based on fatty acid constitution: one in

which more than 50% of the lipids had chain lengths less than 16 carbons, and one in which more than 50% of the lipids had carbon chain lengths greater than 18 carbons. The three strains of *Saccharomyces, K. polysporus* and *Kl. apiculata* were placed in the first group. All of the remaining 25 species were included in the second group. All of the species of *Saccharomyces, K. polysporous* contained sterol derivatives as the main neutral lipid (50-80%). This property closely coincided with the remarkable distribution pattern of fatty acids in these groups.

Generally speaking, with a few exceptions, there were no significant differences in lipid pattern (neutral lipid, phospholipid, and fatty acid compositions) of yeasts grouped according to plant, animal, or human sources. Furthermore, these data aid in selection of the yeast species needed for raw material for preparation of certain lipids or lipid related material. For example, *Cr. neoformans* and *Rh. rubra* are suitable strains for the preparation of pyrophosphatidic acid (2).

Subsequent studies of additional new species of yeast are in progress to clarify the relationship between lipid composition and taxonomy or the phylogenetic evolution of yeast.

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