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Determination of Cellular Fatty Acid Compositions of Various Yeasts by Gas-Liquid Chromatography

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The cellular fatty acid composition of 51 cultures of various species of yeasts was determined by gas-liquid chromatography. Analysis was done with a fused-silica gas-liquid chromatography capillary column, with resolution of all components including mono-, di-, and tri-unsaturated 18-carbon acids. The cultures were placed into one of four distinct gas-liquid chromatography groups on the basis of large quantitative differences in fatty acids. Group I contained only *Saccharomyces* species and group II only *Torulopsis glabrata*. Most *Candida* species were placed into group III, and group IV contained only basidiomycetous yeasts.

In our laboratory we have developed a rapid and sensitive gas-liquid chromatography (GLC) procedure to determine the cellular fatty acid composition of various bacteria including species of *Pseudomonas, Achromobacter, Flavobacterium*, and *Legionella* (5, 6). Data from these studies show that total cellular fatty acids provide useful information for rapidly distinguishing closely related bacteria. In a similar study, Gangopadhyay et al. (2) reported that cellular fatty acid data were also useful for distinguishing various species of yeasts. In our study, we extended the studies of Gangopadhyay et al. (2) to include additional species of yeast and used the relatively new fused-silica capillary column for increased GLC resolution of cellular fatty acids (8).

MATERIALS AND METHODS

Fungi. Fifty-one isolates belonging to the genera *Candida, Torulopsis, Cryptococcus, Filobasidiella, Rhodotorula, Trichosporon*, and four other related genera, *Saccharomyces, Pichia, Kluyveromyces*, and *Yarrowia*, were selected for study. All were stock cultures maintained in the Department of Microbiology, Meiji College of Pharmacy, Tokyo, Japan, and are listed with their sources in Table 1.

Preparation of samples. Cells for fatty acid analysis were incubated at 27° for 48 h on slants of Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.). Cells were removed from the slants by addition of 0.5 ml of sterile distilled water and by gentle scraping. The turbid cell suspension was transferred to a test tube (18 by 150 mm), 4 ml of 5% NaOH in 50% methanol was added, and the tube was sealed with a Teflon-lined screw cap and placed in a boiling water bath (100°C) for 1 h. The saponified material was cooled to room temperature, and the pH was adjusted to 2.0 or less by the addition of 6 N HCl. The free fatty acids were then methylated by adding 4 ml of 10% boron trichloride-methanol reagent (Applied Science Laboratories, State College, Pa.) and heating for 5 min in an 80 to 85°C water bath. The mixture was cooled to room temperature, and the methyl esters were extracted by shaking with two successive 10-ml portions of a 1:1 mixture of diethyl ether-hexane. The ether-hexane layers were combined in a 50-ml beaker, and the volume was reduced to approximately 0.5 ml under a gentle stream of flowing nitrogen gas. Approximately 80 mg of Na_2SO_4 was added to remove residual moisture, and the sample was transferred to a screw-capped tube (13 by 100 mm). The final volume was reduced to 0.1 ml and analyzed by GLC or stored at −20°C until analysis.

GLC. Fatty acid methyl ester samples were analyzed on a fused-silica capillary column (50 m by 0.2 mm inside diameter) with OV-101 as stationary phase. The column was obtained from Hewlett-Packard Corp., Avondale, Pa., and installed in a Hewlett-Packard series 5880A gas chromatograph equipped with a flame ionization detector. The injector temperature was maintained at 275°C and the detector at 300°C. Hydrogen was used as a carrier gas at a flow rate of 0.6 ml/min. The sample size was 0.3 μl, which was split 30:1 to give 0.01 μl of sample on the column. For analysis of samples, the column temperature was programmed at 6°C/min from 130 to 270°C. The final column temperature was maintained at 270°C for 5 min and then cooled to the initial temperature (130°C) for subsequent samples. Identification of fatty acids from yeasts was made by comparing their GLC retention times to those of authentic standards (Applied Science, State College, Pa.), by hydrogenation of unsaturated acids (5), and by combined GLC-mass spectrometry (7). Quantitation of peak areas was accomplished with a Hewlett-Packard series 5880 microprocessor.

RESULTS

The chromatogram in Fig. 1 of a standard fatty acid methyl ester mix illustrates the high resolving characteristics of the fused-silica capillary

TABLE 1. Cellular fatty acid composition of various species of yeasts

GLC group	Species, strain[a]	Fatty acids (% of total)[b]												Ratios	
		12:0	14:0	16:1	16:0	a-17:0	17:0	18:2	18:3	$18:1^{\Delta 9}$	$18:1^{\Delta 11}$	18:0	24:0	16:1/16:0	16:0/18:0
I	*Saccharomyces cerevisiae*, M-6001	1	1	39	13	—	—	—	—	37	2	5	—	3.0	2.6
	Saccharomyces cerevisiae, AJ-4001	—[c]	—	34	13	—	—	—	—	40	2	7	—	2.6	1.9
	Saccharomyces cerevisiae, CBS-1171	—	—	35	11	—	—	—	—	40	1	8	—	3.2	1.4
	Saccharomyces uvarum, IFO-0565	—	—	34	7	—	—	—	—	50	2	5	—	4.9	1.4
	Saccharomyces uvarum, CBS-1513	—	1	48	11	—	—	—	—	30	2	3	—	4.4	3.7
	Saccharomyces rosei, CBS-817	—	2	36	14	—	—	12	—	32	—	4	—	2.6	3.5
	Saccharomyces rosei, IFO-0428	—	1	38	13	—	—	9	—	30	—	4	—	2.9	3.3
II	*Torulopsis glabrata*, IFO-0622	—	—	26	2	—	—	—	—	51	12	5	—	13.0	0.4
	Torulopsis glabrata, M-4001	—	—	35	3	—	—	—	—	46	7	6	—	11.7	0.5
	Torulopsis glabrata, M-4012	—	—	34	2	—	—	—	—	44	8	6	—	17.0	0.3
	Torulopsis glabrata, M-4002	—	1	35	5	1	—	—	—	36	9	7	—	7.0	0.7
III	*Candida albicans*, M-1012	1	1	8	16	2	1	20	2	33	1	8	2	0.5	2.0
	Candida albicans, M-1445	1	1	8	12	1	1	17	1	44	1	6	1	0.7	2.0
	Candida albicans, M-1447	—	1	10	14	1	1	16	2	46	2	5	1	0.7	2.8
	Candida albicans, M-2084	—	—	8	14	2	—	16	1	44	3	6	2	0.6	2.3
	Candida stellatoidea, M-1016	—	1	4	16	—	1	28	3	29	—	13	2	0.3	1.2
	Candida tropicalis, M-1017	1	1	10	20	1	1	15	2	40	—	8	1	0.5	2.5
	Candida tropicalis, M-2504	—	—	10	15	—	—	15	2	45	2	8	1	0.7	1.9
	Candida tropicalis variant M-1519	1	2	10	22	—	—	9	2	40	2	10	1	0.5	2.2
	Candida lusitaniae, CBS-4413	—	1	6	19	—	—	21	6	35	—	5	1	0.3	3.8
	Candida parapsilosis, M-1015	—	—	4	22	3	2	10	5	40	—	8	1	0.2	2.8
	Candida guilliermondii, M-1023	—	—	4	10	1	1	15	6	51	—	5	3	0.4	2.0
	Candida guilliermondii, CBS-566	—	—	9	16	1	—	13	6	45	1	3	3	0.6	5.3
	Candida guilliermondii, IFO-0679	—	—	7	19	2	—	18	5	43	1	3	1	0.4	6.3
	Candida guilliermondii, M-2052	—	—	11	24	2	—	10	3	39	—	4	2	0.5	6.0
	Candida pseudotropicalis, M-1004	—	1	12	20	—	—	26	13	24	—	4	—	0.6	5.0

	Candida pseudotropicalis, M-2063	—	—	8	11	—	—	6	4	55	—	4	—	0.7	2.8
	Candida pseudotropicalis, CBS-607	—	—	12	15	—	—	30	6	28	—	4	—	0.8	3.8
	Kluyveromyces fragilis, AJ-4060	—	—	13	16	—	—	25	7	32	—	5	—	0.8	3.2
	Kluyveromyces fragilis, AJ-5287	—	1	10	19	—	—	31	6	27	—	6	—	0.5	3.2
	Candida krusei, M-1005	—	—	7	15	1	—	17	15	40	—	2	1	0.5	7.5
	Candida rugosa, IFO-0750	—	1	16	17	4	1	11	4	41	1	4	—	0.9	4.3
	Pichia pastoris, CBS-704	—	—	6	14	—	—	18	4	51	—	4	—	0.4	3.5
	Pichia pinus, CBS-744	—	—	11	12	—	—	38	10	18	3	2	—	0.9	6.0
	Candida lipolytica, CBS-599	—	—	12	13	1	—	15	—	49	2	3	1	0.9	4.3
	Candida lipolytica, B-2389	—	—	10	14	—	—	27	—	39	—	2	2	0.7	7.0
	Candida lipolytica, B-2394	—	—	8	14	—	—	25	—	38	1	3	3	0.6	4.7
	Yarrowia lipolytica, CBS-2070	—	—	10	12	2	—	20	—	43	3	2	4	0.8	6.0
IV	*Cryptococcus neoformans*, CBS-132	—	—	—	19	—	—	32	—	38	—	6	1	0	3.2
	Cryptococcus neoformans, CDC-551	—	—	—	18	—	—	22	—	47	—	9	2	0	2.0
	Filobasidiella neoformans, NIH-B-3501	—	—	—	18	—	—	28	—	46	—	3	2	0	6.0
	Filobasidiella neoformans, NIH-444	—	1	—	18	—	—	26	—	49	—	3	2	0	6.0
	Cryptococcus terreus, CBS-1895	—	—	1	18	—	1	27	—	37	—	11	2	0.1	1.6
	Cryptococcus gastricus, CBS-1927	—	—	—	11	—	—	53	—	28	—	3	1	0	3.7
	Rhodotorula glutinis, IFO-0389	—	—	1	17	—	—	21	9	42	—	2	1	0.1	8.5
	Rhodotorula minuta, IFO-0387	—	—	—	10	1	2	46	—	31	—	3	1	0	3.3
	Rhodotorula rubra, IFO-0382	—	—	—	12	—	—	17	6	54	—	3	1	0	4.0
	Trichosporon beigelii, IFO-1198	—	1	—	24	—	—	39	8	14	—	7	2	0	3.4
	Trichosporon beigelii, IFO-1200	—	1	—	22	—	—	39	8	15	—	7	2	0	3.1
	Candida curvata, CBS-570	—	4	2	16	—	—	29	7	36	—	2	—	0.1	8.0
	Candida curvata, IFO-732	—	2	1	18	—	—	26	6	37	—	5	2	0.1	3.6

[a] The strains are coded by abbreviations for the following institutes: M, Meiji College of Pharmacy, Tokyo, Japan; AJ, Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Japan; CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; IFO, Institute for Fermentation, Osaka, Japan; CDC, Centers for Disease Control, Atlanta; NIH, National Institutes of Health, Bethesda, Md.

[b] Numbers to left of colon refer to carbon atoms, number to right refers to number of double bonds; a, an anteiso branched-chain acid.

[c] —, Not detected or less than 1%.

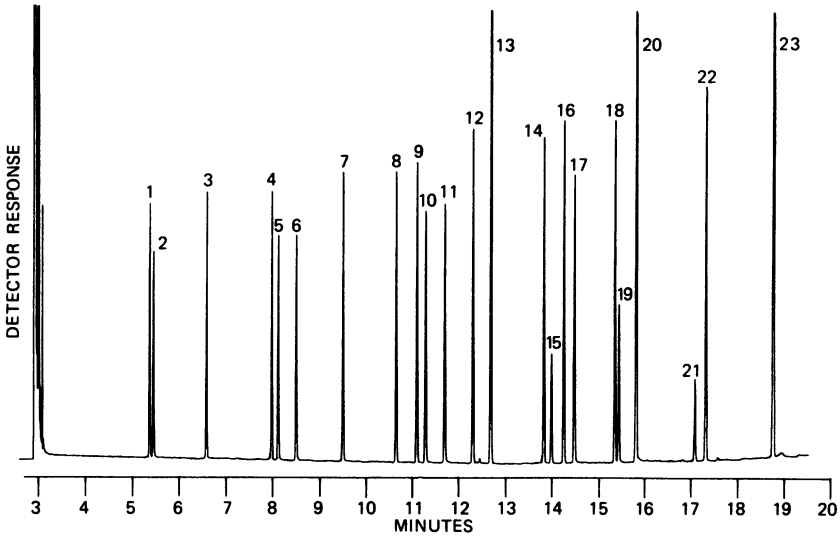

FIG. 1. Gas chromatogram of a fatty acid methyl ester standard analyzed on an OV-101 fused-silica capillary column (50 m by 0.20 mm). Peak identification: 1, undecylic (11:0); 2, 2-hydroxycapric (2-OH-10:0); 3, lauric (12:0); 4, tridecylic (13:0); 5, 2-hydroxylauric (2-OH-12:0); 6, 3-hydroxylauric (3-OH-12:0); 7, myristic (14:0); 8, anteiso-pentadecylic (a-15:0); 9, pentadecylic (15:0), 10, 2-hydroxymyristic (2-OH-14:0); 11, 3-hydroxymyristic (3-OH-14:0); 12, palmitoleic (16:1); 13, palmitic (16:0); 14, anteiso-margaric (a-17:0); 15, *cis*-9,10-methylenehexadecanoate (17:0 CYC); 16, margaric (17:0); 17, 2-hydroxypalmitic (2-OH-16:0); 18, oleic ($18:1^{\Delta 9}$); 19, *cis*-vaccenic ($18:1^{\Delta 11}$); 20, stearic (18:0); 21, *cis*-11,12-methyleneoctadecanoate (19:0 CYC); 22, nondecylic (19:0), 23, arachidic (20:0).

column. With this column, a total of 51 cultures of various species of yeasts were examined and placed into one of four distinct GLC groups on the basis of cellular fatty acids. All cultures contained saturated and unsaturated straight-chain acids as major components (Table 1). No hydroxy or cyclopropane acids were detected, and only 16 strains contained small amounts (1 to 4%) of an anteiso branched-chain acid (a-17:0). The yeasts in GLC groups I and II contained relatively large amounts of oleic ($18:1^{\Delta 9}$) and palmitoleic (16:1) acids with moderate to small amounts of palmitic (16:0) and stearic (18:0) acids (Fig. 2). Members of these two groups were distinguished from the other yeasts tested by their relatively large content of 16:1 acid compared with other acids (Table 1). The ratios of 16:1 to 16:0 acids in groups I and II were 2.0 or greater but in groups III and IV this ratio was less than 1.0. The ratio of 16:1 to 16:0 acids in the group I yeasts (which contained only species of *Saccharomyces*) was approximately 5:1 or less, whereas in the group II yeasts (which contained only *Torulopsis glabrata*) the ratio was 7:1 or greater. A further distinction between the two groups was the relative amounts of 16:0 and 18:0 acids. In group 1, the ratio of 16:0 to 18:0 was 1.4 or greater, but in group II this ratio was less than 0.8 (Fig. 2, Table 1). Group II also contained greater amounts of *cis*-vaccenic acid ($18:1^{\Delta 11}$) than did other yeasts. In group I, the two isolates of *S. rosei* differed from *S. cerevisiae* and *S. uvarum*, which others consider to be synonymous (1), by the presence of moderate amounts of linoleic acid (18:2) (Table 1).

Of the 51 cultures studied, 27 were placed in group III. In general, group III yeasts contained oleic (18:1) as the major acid followed by 18:2, 16:0, and 16:1 (Fig. 3B). However, there was considerable overlap in the fatty acid composition between strains of diverse phylogeny in this group (Table 1). The quantitative data in Table 1 are a true indication of culture differences since the reproducibility of all cultures reprocessed through the entire procedure was usually within 5% and never exceeded 10% of the initial values. *Yarrowia lipolytica* (and its anamorph *C. lipolytica*) differed from other species in group III by the absence of the 18:3 acid (Table 1).

A characteristic feature of group IV yeasts was the absence or presence of only small amounts (1 to 2%) of the 16:1 acid (Fig. 3B, Table 1). As noted above, the 16:1 acid was present in large amounts in groups I and II and was present at concentrations of 4% or greater in all group III cultures. Thus, the absence or presence of only small amounts of the 16:1 acid clearly distinguished group IV, which consisted

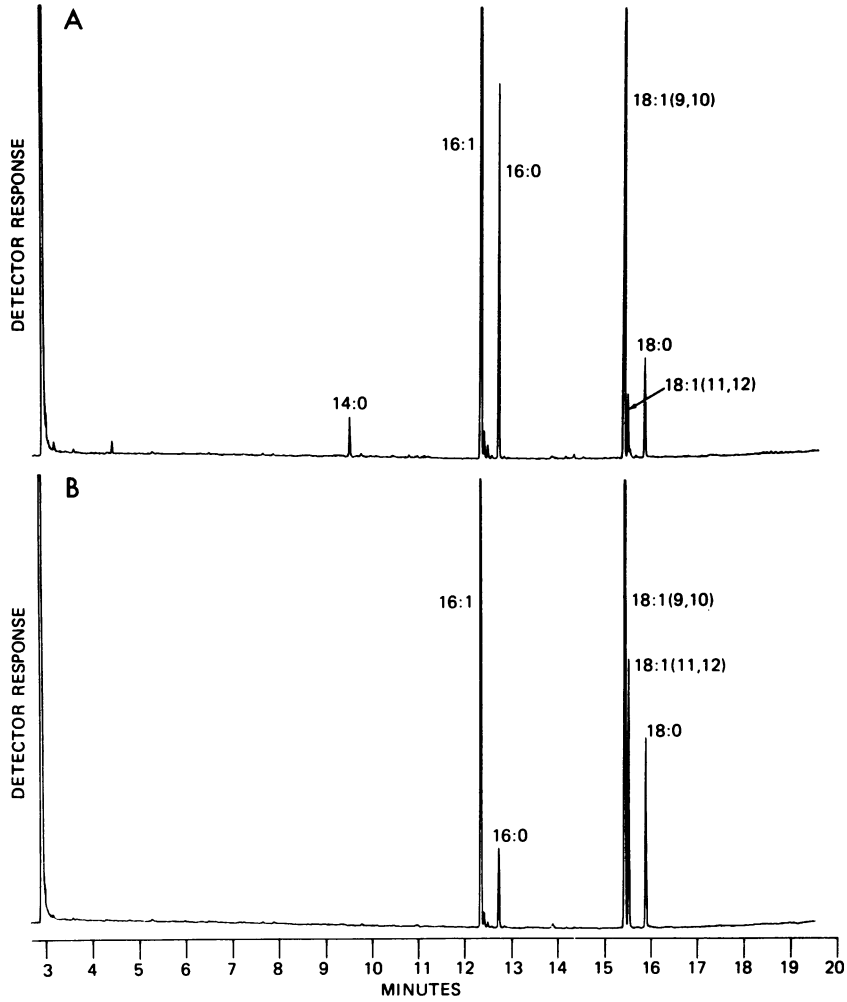

FIG. 2. Gas chromatogram of esterified fatty acids from saponified whole yeast cells analyzed on an OV-101 fused-silica capillary column. The top chromatogram (A) is a culture of *S. cerevisiae*, which is representative of the yeast species in GLC group I; the bottom chromatogram (B) is *T. glabrata*, which represents GLC group II. See the legend to Fig. 1 for peak designations and Table 1 for GLC groups.

solely of basidiomycetous fungi; all isolates in groups I, II, and III represent ascomycetous yeasts (4, 9).

DISCUSSION

Use of the fused-silica capillary GLC column allowed for accurate quantitative determination of the cellular fatty acid composition of yeasts. Each of the 51 cultures tested contained significant amounts of mono-, di-, or tri-unsaturated 18-carbon acids which were completely resolved. In addition, baseline resolution was obtained with the two mono-unsaturated 18-carbon positional isomers oleic (Δ9 octadecenoic) and *cis*-vaccenic (Δ11 octadecenoic) acids. On packed columns, one or more of these acids would coelute or would appear as shoulders on the leading or tailing edge of other peaks in the chromatogram (5, 8).

There are several differences between our data and those reported previously by Gangopadhyay et al. (2). Those authors reported the presence of small amounts (1 to 3%) of 2-hydroxylauric acid (2-OH-12:0), 2-hydroxymyristic acid (2-OH-14:0), and a 19-carbon acid in *C. albicans*. In addition, they reported moderate amounts (7 to 12%) of capric (10:0) and myristic (14:0) acids in *C. guilliermondii* and small to moderate amounts (2 to 12%) of lauric acid (12:0) in all yeasts except *C. tropicalis* and *T.*

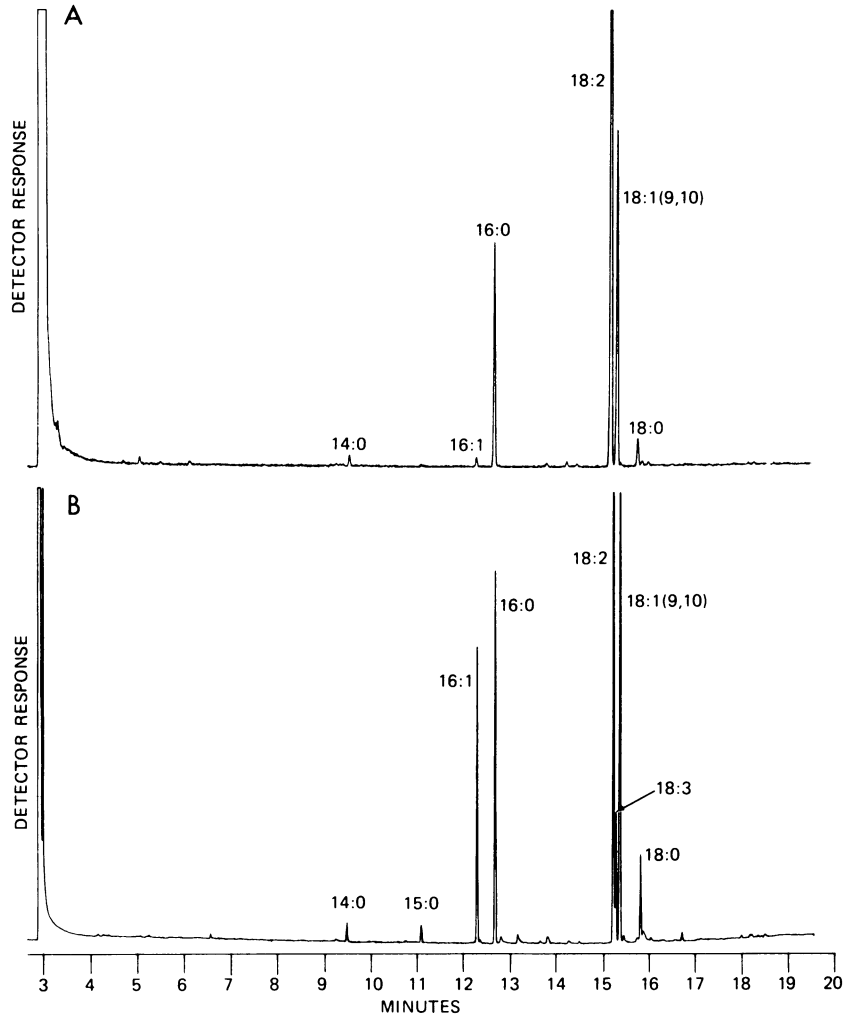

FIG. 3. Gas chromatogram of esterified fatty acids from saponified whole yeast cells analyzed on an OV-101 fused-silica capillary column. Top chromatogram (A) is a culture of *Cryptococcus gastricus*, which is representative of the yeasts in GLC group IV; bottom chromatogram (B) is a culture of *Candida pseudotropicalis*, which represents GLC group III. See the legend to Fig. 1 for peak designations and Table 1 for GLC groups.

glabrata. However, after extensive testing with our procedures as well as with the acid hydrolysis procedure of Gangopadhyay et al. (2), we were unable to obtain similar results. No hydroxy or 19-carbon acids were detected in either of our four cultures of *C. albicans*, no 10:0 acid was observed in four cultures of *C. guilliermondii*, none of our 51 cultures contained more than 1% of 12:0 acid, and only 1 of 51 cultures contained more than 2% of the 14:0 acid. Other than possible strain differences, there are no obvious reasons to account for the discrepancies since both studies used cultures grown for 48 h on Sabouraud dextrose agar. In preliminary studies we found essentially no differences between the fatty acid compositions of cells grown on two different lots of this medium; also, no major differences were observed in cells grown for 36, 48, or 72 h. Over the years we have observed that most commercial supplies of diethyl ether contain small amounts of a preservative agent (2,6-di-*tert*-butyl-4-methylphenol) which coelutes with lauric acid (12:0) on a nonpolar GLC column (Moss, unpublished observations). However, this possibility would account only for the differences in lauric acid; thus, additional cultures must be examined to resolve these questions.

In summary, the cellular fatty acid data did not permit us to distinguish phenotypically simi-

lar species of medical importance, e.g., *C. albicans* and *C. tropicalis*, but certain yeasts such as *T. glabrata* had distinctive fatty acid profiles. Basidiomycetous yeasts were separable as a group from ascomycetous species, but their fatty acid composition so overlapped as to preclude any further classification. Many of these phenotypically similar yeasts have been shown to be distinguishable by serological and biochemical data (10, 11), guanine plus cytosine ratios (12), antigenic structures (13, 14), proton magnetic resonance spectroscopy of mannan (3, 10), and respiratory quinones (15). The ease and rapidity of the preliminary taxonomic grouping of yeast cultures by GLC analysis of their cellular fatty acids suggest that this procedure may be of value for diagnostic centers handling a large number of cultures. Further studies with additional isolates and species are required to determine whether the fatty acid composition of certain ascomycetous species remains unique.

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