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Multivariate Analyses of Cellular Carbohydrates and Fatty Acids of *Candida albicans*, *Torulopsis glabrata*, and *Saccharomyces cerevisiae*

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Quantitative data of major cellular carbohydrates distinguished *Candida albicans* or *Torulopsis glabrata* from *Saccharomyces cerevisiae* but not *C. albicans* from *T. glabrata*. Multivariate analyses of both carbohydrate and fatty acid variables (I. Brondz, I. Olsen, and M. Sjöström, J. Clin. Microbiol. 27:2815–2819, 1989), however, differentiated all three species.

The taxonomic distinction between *Candida* sp. strain Berkhout and *Torulopsis* sp. strain Berlese is based on the presence or absence, respectively, of pseudohyphae. Evidence has accumulated that these criteria are not always adequate for species designations (5). Yarrow and Meyer (27), reviewing the taxonomic status of members of the genus *Torulopsis*, proposed that the diagnosis of *Candida* be amended from "pseudomycelium formation by all or most strains of all species and varieties" to "pseudohyphae absent, rudimentary or well developed," so that the genus *Candida* could include the genus *Torulopsis*. This proposal has raised both taxonomic and nomenclatural issues (4, 13).

Cell wall carbohydrates have increasingly been used for taxonomic distinction between yeasts, particularly at the generic level (8, 15, 18, 21, 22). In the present study, carbohydrates of whole-cell methanolysates from *Candida albicans* and *Torulopsis glabrata* were used to reevaluate the rationale for merging these species into the same genus. *Saccharomyces cerevisiae* was included for comparison.

The reference and clinical strains of *C. albicans*, *T. glabrata*, and *S. cerevisiae* examined in this study are listed in Table 1. The organisms were obtained directly from the American Type Culture Collection (ATCC), Rockville, Md.; the Centraalbureau Voor Schimmelcultures (CBS), Delft, The Netherlands; and the Yeast Division, National Institute of Public Health (NIPH), Oslo, Norway. They were cultured in duplicate on different days for 48 h in 1-liter flasks of chemically defined medium containing, per liter of distilled, deionized water, 10 g of glucose, 2 g of yeast extract, 0.53 g of $CaCl_2 \cdot 2H_2O$, 0.5 g of NaCl, 0.7 g of $MgSO_4 \cdot 7H_2O$, 2 g of KH_2PO_4, 1.2 g of $(NH_4)_2SO_4$, and 8 mg of $FeCl_3 \cdot 6H_2O$ (3). Yeast cells were harvested by centrifugation, washed three times in deionized, distilled water, lyophilized, and stored dry under N_2 at −20°C.

Lyophilized whole cells, 1 mg, were methanolyzed in 1 ml of 2 M hydrochloric acid in anhydrous methanol for 24 h at 95°C (1). The methanolysate was dried with a stream of N_2 and derivatized in a mixture of acetonitrile and trifluoroacetic acid (10% [vol/vol]; Fluka, Buchs, Switzerland) at 90°C for 3 min.

A gas chromatograph (model 8700; The Perkin-Elmer Corp., Norwalk, Conn.) was used for gas chromatography. The column that was used (HP Ultra Performance Column Ultra 1; Hewlett-Packard Co., Avondale, Pa.) was 25 m by 0.20 mm (inside diameter). Helium served as the carrier gas and was used at a flow rate of 2.0 ml/min. The temperature of the injector was 200°C, and that of the flame ionization detector was 275°C. The program was as follows. The temperature was held for 1 min at 90°C and was then increased from 90 to 275°C (with an increase of 6°C/min). The attenuator was set at 16. The paper speed was 5 mm/min. The sample (0.2 μl) was delivered as a splitless injection. The identities of the methanolyzed and derivatized carbohydrates were confirmed by cochromatography of authentic standards and gas chromatography-mass spectrometry (1, 2). From each duplicate sample two independent derivatizations were prepared. Three injections were made for each derivative. The quantities of the substances, expressed in relative percent, were calculated from the area under each peak and were corrected with the molar response factor (1). The sum of identified substances was considered as 100%. Reference substances used for identification of unknown carbohydrates were D-(+)-glucose and D-(+)-mannose, which were purchased from Sigma Chemical Co., St. Louis, Mo.

Two projection methods were used for statistical analysis: principal component analysis (PCA) (9, 26) and partial least-squares discriminant analysis (7, 24). The complexities

TABLE 1. Yeast species, strains, and samples examined in this study

Species	Strain	Source
C. albicans	ATCC 18804[T]	Brosio interdigitalis
	NIPH 1007/83	Coxarthrosis pus swab
	NIPH 1001/83	Throat swab
	NIPH 990/83	Expectorate
T. glabrata	CBS 138[T]	Feces
	NIPH 1052/83	Vaginal swab
	NIPH 1048/83	Cervical swab
	NIPH 943/83	Throat swab
S. cerevisiae	ATCC 18824[T]	
	NIPH 1598/83	Throat swab
	NIPH 1437/83	Vaginal swab
	NIPH 1370/83	Expectorate

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TABLE 2. Distribution of major carbohydrates in whole-cell methanolysates of yeasts[a]

Species	Strain	Sample no.	Relative % of[b]:		
			Mannose	Glucose	Galactose
C. albicans	ATCC 18804^T	1	23.7	51.8	24.5
	NIPH 1007/83	2	24.1	51.2	23.7
	NIPH 1001/83	3	35.0	46.3	18.4
	NIPH 990/83	4	26.3	53.0	20.7
T. glabrata	CBS 138^T	5	27.7	51.8	20.5
	NIPH 1052/83	6	23.4	55.5	21.1
	NIPH 1048/83	7	24.0	56.0	20.0
	NIPH 943/83	8	34.5	50.0	15.5
S. cerevisiae	ATCC 18824^T	9	49.5	34.5	16.0
	NIPH 1598/83	10	42.0	39.1	18.9
	NIPH 1437/83	11	52.0	33.3	14.7
	NIPH 1370/83	12	45.0	38.2	15.8

[a] Incubation time, 48 h; incubation temperature, 26°C.

[b] Means are given (n = 12); variations between runs were 3% or less.

of both models were determined by cross-validation (23, 25). Soft independent modeling of class analogy was also applied (14, 24).

The distributions of carbohydrates in whole-cell methanolysates from *C. albicans*, *T. glabrata*, and *S. cerevisiae* are shown in Table 2 and Fig. 1. Carbohydrates constituted 80 to 85% of the material, while fatty acids were only 1 to 10% of the material. Three major carbohydrates were identified in all three species: mannose, glucose, and galactose. *C. albicans* and *T. glabrata* could be distinguished from *S. cerevisiae* on the basis of the quantitative distribution of these carbohydrates, while no distinction could be made between *C. albicans* and *T. glabrata*. Glucose was most abundant in *C. albicans* and *T. glabrata*, while *S. cerevisiae* contained the most mannose. While major carbohydrates did not distinguish between *C. albicans* and *T. glabrata*, differences appeared when their total fingerprints on the gas chromatograms were compared. Discriminating peaks were most abundant in the area with retention times (R_ts) of 12 to 24 min (Fig. 1).

The carbohydrate variables given in Table 2 were subjected to PCA. The two PCA axes, designed t_1 and t_2, described 35 and 16%, respectively, of the total variance in the data from *C. albicans* or *T. glabrata* versus *S. cerevisiae* (Fig. 2a). The projection of t_1 against t_2 showed that t_1 clearly separated the *S. cerevisiae* class (samples 9 to 12) from the *C. albicans* (samples 1 to 4) and *T. glabrata* (samples 5 to 8) classes. No distinction could be made between the *C. albicans* and *T. glabrata* classes. From the corresponding variable-related projection (Fig. 2b), it was evident that only variables 1 (mannose) and 2 (glucose) contributed to the separation between *C. albicans* or *T. glabrata* and *S. cerevisiae*.

The carbohydrate variables and the fatty acid variables of the same strains (same batch) (3), i.e., $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$ acids, were all subjected to PCA (Fig. 2c). The two PCA axes, t_1 and t_2, described 49 and 33%, respectively, of the total variance in the data. The projection t_1 against t_2 showed that t_1 clearly separated *C. albicans* (samples 1 to 4) from *T. glabrata* (samples 5 to 8) and that t_2 separated *C. albicans* or *T. glabrata* from *S. cerevisiae* (samples 9 to 12). From the corresponding variable-related projection (Fig. 2d), it was evident that variables 4 and 6 ($C_{16:1}$ and $C_{18:2}$, respectively) contributed the most to the separation between *C. albicans* and *T. glabrata*, while variables 9 and 10 (mannose and glucose, respectively) contributed the most to the distinction between *C. albicans* or *T. glabrata* and *S. cerevisiae*. Class models were determined for the *C. albicans* and *T. glabrata* samples, and borders for these models were drawn. None of the samples

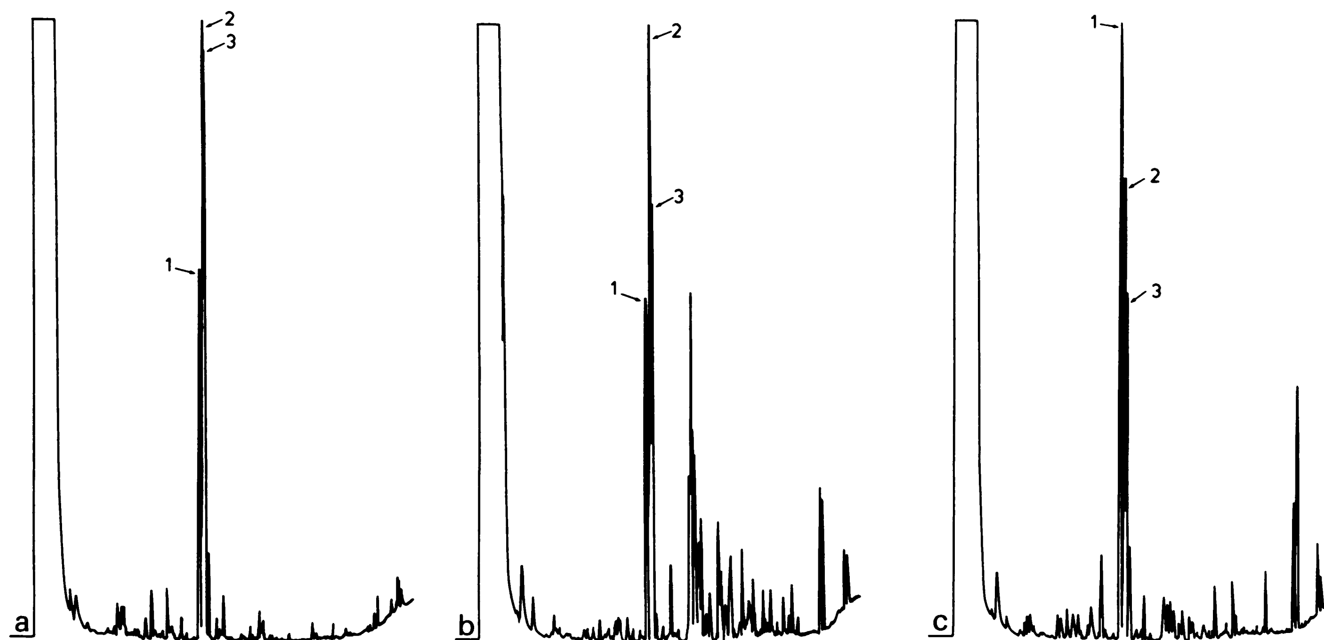

FIG. 1. Gas chromatograms from methanolysates of whole cells of *C. albicans* ATCC 18804^T (a), *T. glabrata* CBS 138^T (b), and *S. cerevisiae* ATCC 18824^T (c). The column temperature was held for 1 min at 90°C and was then increased from 90 to 275°C at a rate of 6°C/min. Mannose (R_t, 10.972 min) (peak 1), glucose (R_t, 11.164 min) (peak 2), and galactose (R_t, 11.244 min) (peak 3) are shown.

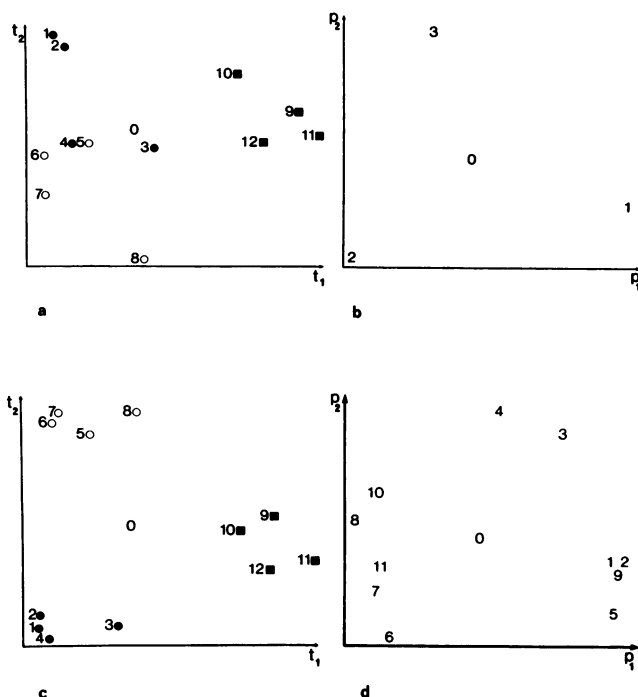

FIG. 2. (a) Projection of the two first principal components t_1 and t_2 showing the sample distribution after subjection of carbohydrate variables to PCA. Strains of *C. albicans* (●; samples 1 to 4), *T. glabrata* (○; samples 5 to 8), and *S. cerevisiae* (■; samples 9 to 12) are presented. Samples: 1, ATCC 18804^T; 2, NIPH 1007/83; 3, NIPH 1001/83; 4, NIPH 990/83; 5, CBS 138^T; 6, NIPH 1052/83; 7, NIPH 1048/83; 8, NIPH 943/83; 9, ATCC 18824^T; 10, NIPH 1598/83; 11, NIPH 1437/83; 12, NIPH 1370/83. (b) Projection of the variable loadings p_1 and p_2 from the PCA showing the contribution of the carbohydrate variables to the t_1 and t_2 projections. Variables: 1, mannose; 2, glucose; 3, galactose. (c) Projection of t_1 against t_2 after subjection of the fatty acid variables from Brondz et al. (3) and the carbohydrate variables of the present study to PCA. *C. albicans* (●; samples 1 to 4), *T. glabrata* (○; samples 5 to 8), and *S. cerevisiae* (■; samples 9 to 12) are presented. For further details on samples, see description of panel a. Fatty acid variables were $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$; and carbohydrate variables were mannose, glucose, and galactose. (d) Projection of the variable loadings p_1 and p_2 from the PCA showing the contribution of fatty acid variables (3) and carbohydrate variables of the present study to the t_1 and t_2 projection given in panel c. Variables: 1, $C_{10:0}$; 2, $C_{12:0}$; 3, $C_{14:0}$; 4, $C_{16:1}$; 5, $C_{16:0}$; 6, $C_{18:2}$; 7, $C_{18:1}$; 8, $C_{18:0}$; 9, mannose; 10, glucose; 11, galactose.

of *C. albicans* (samples 1 to 4) or *T. glabrata* (samples 5 to 8) fell within the 95% confidence limits of the *S. cerevisiae* samples (samples 9 to 12) (Fig. 3).

In this study we demonstrated that glucose is most abundant in whole cells of *C. albicans* and *T. glabrata*, followed by mannose and galactose. The cell wall of *C. albicans* is mainly composed of β-glucan and mannoprotein (16). The mannan polysaccharide is the most important antigenic component (17), while the glucan polymers, although more abundant, are immunologically less active.

Major cellular carbohydrates such as glucose, mannose, and galactose could not be used to make taxonomic distinctions between *C. albicans* and *T. glabrata*, although carbohydrates are increasingly used for differentiation between yeast genera (8, 15, 18, 21, 22). When considered separately, the major carbohydrate patterns therefore supported the proposed merger of *C. albicans* and *T. glabrata* into the

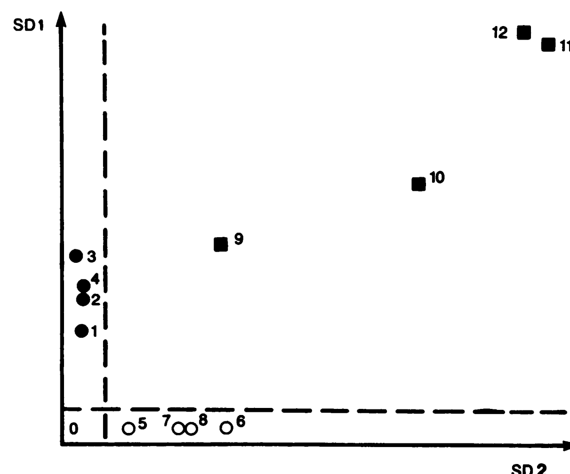

FIG. 3. Residual standard deviation for each sample when fitted to the *C. albicans* (●; samples 1 to 4) (SD1) and *T. glabrata* (○; samples 5 to 8) (SD2) class models. Dashed lines mark approximate class borders based on *F* tests ($P = 0.05$). Samples 9 to 12 (■) belong to *S. cerevisiae*. See legend to Fig. 2a for further details on samples.

same genus (27). The quantitative distribution of carbohydrates distinguished between *C. albicans* or *T. glabrata* and *S. cerevisiae*, supporting the allocation of *S. cerevisiae* to a more distant genus.

Clear differences appeared between all three species when their total fingerprints on the gas chromatograms, which included fatty acids, were compared. We have demonstrated previously (3) that the major and minor cellular fatty acids in the same batches of the strains that we examined differ quantitatively and qualitatively. The value of fatty acids for use in the taxonomy of microorganisms is firmly established, not only in bacteria but increasingly in yeasts (3, 6, 10–12, 19, 20).

PCA based on the quantitative distribution of major cellular carbohydrates distinguished *S. cerevisiae* from *C. albicans* and *T. glabrata*, while no distinction could be made between *C. albicans* and *T. glabrata*. The number of variables for PCA was small. To arrive at a more reliable conclusion concerning the taxonomic relationships of the yeasts studied here, the carbohydrate variables were combined with the fatty acid variables obtained previously (3). *C. albicans*, *T. glabrata*, and *S. cerevisiae* could then be distinguished easily (Fig. 2 and 3). Differences between species do not exclude relatedness on the generic level. Therefore, additional studies should be made that include the type species of the genera *Candida* and *Torulopsis*.

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