Journal of Chromatography, 533 (1990) 152–158
Biomedical Applications
Elsevier Science Publishers B V , Amsterdam

CHROMBIO 5478

Note

Fatty acid contents of the yeast and mycelial phase of *Candida albicans*

ILIA BRONDZ[a,*]

Research Department, National Institute of Occupational Health, Umeå (Sweden)

and

INGAR OLSEN

Department of Microbiology, Dental Faculty, University of Oslo, Oslo (Norway)

(First received March 5th, 1990, revised manuscript received July 16th, 1990)

A common cause of fungal infection in man is the dimorphic yeast *Candida albicans*. This organism is capable of growing as an ellipsoidal bud [blastospore or yeast (Y) form] or as an elongate hypha [mycelial (M) form]. The growth phenotype depends on environmental conditions and the growth history of the cells. Although both cell forms can be seen in tissue, the elongating hypha penetrates tissue most easily, leaving in its path lateral colonies of budding cells that in turn give rise to new penetrating hyphae. The hyphal form of *Candida* is therefore often considered the most pathogenic of the dimorphic phases (but not the sole pathogenic form). The higher pathogenicity of hyphae is in part related to a better ability to adhere to tissue cells [1,2], which is promoted by cell wall components [2]. Hyphae have a two- to three-fold higher chitin content than blastospores have [3–6]. Quantitative differences have also been observed in the glucan, mannan and protein composition of yeast and mycelial cell walls [5–10]. In some reports the lipid content of hyphae was found to be higher than that of blastospores [11,12], whereas other investigations revealed essentially no difference [10,13].

The present study was undertaken to compare the fatty acid contents of whole-cell alcoholysates of blastospores and hyphae from *C. albicans*. We have previously used fatty acids of whole-cell alcoholysates for taxonomic distinction between *C. albicans*, *Torulopsis glabrata* and *Saccharomyces cerevisiae* [14].

[a] Present address: Norwegian Plant Protection Institute, Box 70, N-1432 Ås-NLH, Norway

0378-4347/90/$03 50

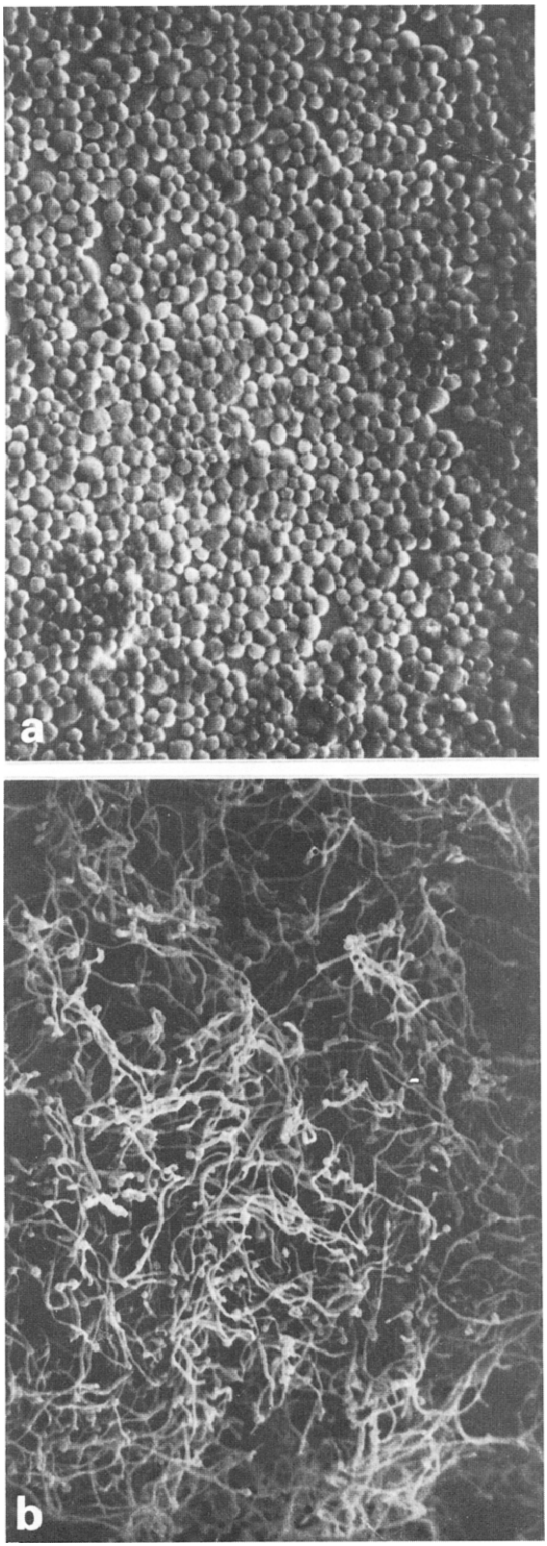

Fig. 1 Scanning electron microscopic photo showing the yeast phase preparation (a) and the mycelial phase preparation (b) of *C albicans*

EXPERIMENTAL

Yeast cultures

C. albicans, with a pronounced tendency to form hyphae, was isolated from a clinical lesion of oral candidosis. The isolate was identified by fermentation and assimilation tests [15] and by germ tube formation in serum. It was maintained on Sabouraud's dextrose agar.

Blastospores were grown to early stationary phase at 25°C in 100 ml of Lee medium [16] on a gyrotory shaker (New Brunswick Scientific Company, New Brunswick, NJ, U.S.A.) at 200 rpm [17]. Cells were collected after 22 h by centrifugation. Harvested cells were washed two times in ice-cold deionized, distilled water and resuspended in water. The suspension was aerated for 3 h at 4°C under shaking (200 rpm) and then stored at 4°C for 16–22 h. The starved cells, 1 mg wet weight per ml, were inoculated in prewarmed (25°C) Lee medium and incubated in the shaker at 25°C for 27 h. This procedure gave blastospores (Fig. 1A). Starved cells, kept at 4°C, were also suspended in prewarmed (37°C) Lee medium, 1 mg wet weight per ml, and incubated under shaking for 27 h at 37°C. This procedure gave hyphae (Fig. 1B). Cultures were produced in duplicate on different days. After checking the purity, both cell phases, which were comparable to those produced *in vivo,* were harvested by centrifugation, washed two times in deionized, distilled water and lyophilized over phosphorous pentoxide. Lyophilized cells were stored at -20°C under nitrogen

Alcoholysis and transesterification

To obtain a general view of the fatty acid composition of blastospores and hyphae, whole cells were examined. Lyophilized cells were alcoholyzed with 2 *M* hydrochloric acid in anhydrous methanol, ethanol, propanol or butanol for 24 h at 85°C [14]. The alcoholysates were dried on an ice bath under a stream of nitrogen and then extracted with 1 ml of hexane.

Gas chromatography

Gas chromatography was performed as reported previously [14]. A Model 8700 gas chromatograph (Perkin-Elmer, Norwalk, CT, U.S.A.) was used together with an HP OV-1 glass capillary column (25 m × 0.20 mm I.D.). This column was recommended by the manufacturer for analysis of fatty acid methyl esters. Helium served as carrier gas at a flow-rate of 1.5 ml/min. The temperature of the flame ionization detector was 275°C. The temperature was held for 1 min at 90°C and then increased from 90 to 275°C at 4°C/min with the attenuator set at 16. Paper speed was 5 mm/min. The sample (0.2 μl) was delivered as a splitless injection. The alcoholyzed fatty acids were identified by comparing their retention times with those of authentic standards and by cochromatography. From each alcoholysate, six runs were made on the gas chromatograph. The quantity of substances, expressed as percentage, was calculated from the area under each peak. The sum of identified substances was taken as 100%.

Reference substances and chemicals

The reference substances used included ethyl, propyl and butyl esters of capric, lauric, myristic, pentadecanoic, palmitic, heptadecanoic and stearic acids as well as ethyl esters of palmitoleic, oleic, linoleic and linolenic acids. All esters were purchased from Sigma (St. Louis, MO, U.S.A.). Bacterial acid methyl esters mix 4-7080, gas–liquid chromatography standard mixture GLC 70 4-7044, AOCS oil reference mixture RM-1 4-7020, rapeseed 4-7019 and NHI mixtures 4-7010, 4-7011, and 4-7013 were obtained from Supelco (Bellefonte, PA, U.S.A.). Ethanol was provided by Systembolaget (Umeå, Sweden) and methanol, propanol, butanol, hexane and diphosporous pentoxide by Merck (Rahway, NJ, U.S.A.).

Preparation of 99.95% ethanol

Ethanol, 99.95%, was prepared as described previously [14]

RESULTS

The distribution of fatty acids in whole-cell methanolysates of the yeast and the mycelial phase of *C. albicans* is shown in Table I and in Fig. 2A and C. Three saturated and three unsaturated fatty acids were detected. Saturated fatty acids tended to be highest in the Y phase and unsaturated fatty acids in the M phase. The predominant acids in both morphological forms were $C_{16\,0}$ and $C_{18\,1}$. In the yeast phase $C_{18\,2}$ and $C_{18:0}$ were higher than in the mycelial phase, whereas $C_{14\,0}$ and $C_{16:1}$ were most abundant in the mycelial phase. Use of ethanol, propanol or butanol instead of ethanol during alcoholysis did not demonstrate volatile fatty acids in any of the cell phases (Fig. 2B). The ethyl derivatives had higher boiling points than the methyl derivatives and could be easily separated from the solvent. Ethyl derivatives of low-molecular-mass compounds appeared between solvent and $C_{14.0}$.

TABLE I

FATTY ACIDS IN WHOLE-CELL METHANOLYSATES OF *CANDIDA ALBICANS*

Data are means of twelve runs with two extracts obtained from cells grown at different days (S D 3%)

Cell form	Distribution (% of total)					
	$C_{14\,0}$	$C_{16\,1}$	$C_{16\,0}$	$C_{18\,2}$	$C_{18\,1}$	$C_{18\,0}$
Yeast	3 0	15 1	29.2	18 3	26 9	7 9
Mycelial	3 5	18 9	29.6	14 1	28 2	6.0

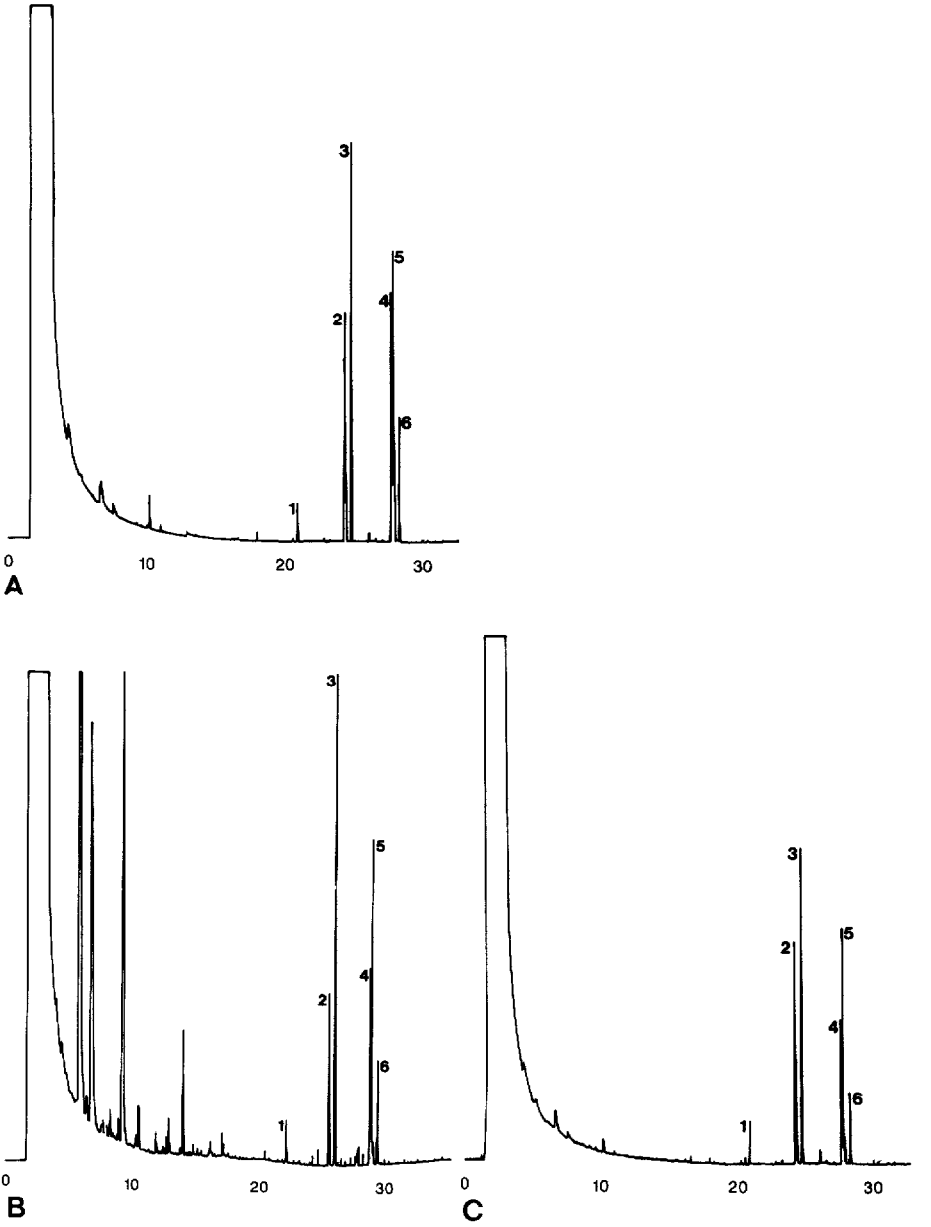

Fig 2 Gas chromatograms of methanolysate (A) and ethanolysate (B) showing the fatty acid distribution of the yeast phase of *C albicans* cells In a gas chromatogram of a methanolysate, the fatty acid distribution of the mycelial phase is shown Temperature programme hold for 1 min at 90°C, then increased from 90 to 275°C at 4°C/min. Flame ionization detector, 275°C. Splitless injection. Paper speed, 5 mm/min Sample (0 2 μl) injected in hexane Attenuation, 16 Peaks 1 = $C_{14\,0}$; 2 = $C_{16\,1}$, 3 = $C_{16\,0}$, 4 = $C_{18\,2}$, 5 = $C_{18\,1}$, 6 = $C_{18\,0}$

DISCUSSION

The ability of *C. albicans* to form hyphae, by which invasion of tissue is initiated *in vivo* [1], is strain-variable. A strain with a high tendency to form hyphae, recently isolated from a clinical lesion of candidosis, was therefore selected for the chemical analyses. Such an isolate was considered more representative of the *in vivo* conditions than a laboratory reference strain.

Whereas the yeast form was developed at 25°C, the mycelial form was induced at 37°C. In other respects the growth conditions were similar for both morphological phases. Since *Candida* have been found to be temperature-insensitive with respect to fatty acid composition [18], and both temperatures were within the optimum range for growth of this yeast [1], it was justified to compare the fatty acid contents of the yeast and the mycelial form from the same organism.

Six long-chain fatty acids were detected in both phases, three of which were saturated and three unsaturated. The concentration of saturated fatty acids tended to be highest in the yeast form, and that of unsaturated fatty acids in the mycelial form. Furthermore, $C_{14:0}$ and $C_{16:1}$ were most abundant in the M phase of the yeast, while $C_{18:2}$ and $C_{18:1}$ were highest in the Y phase. $C_{18:3}$ acid was not well resolved. Unsaturated fatty acids of microorganisms are more likely to play a structural role than saturated ones, the latter being claimed to be more often incorporated into storage products [19]. Distribution of fatty acids within the cells also reflects the biological cell activity. The present results were quite similar to those of Sadamori [12] who found the content of fatty acids to be higher in the M form than in the Y form of *C. albicans* strain IFO 1385; he also reported that the composition of $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ was highest in the Y form, whereas the composition of $C_{16:0}$ and $C_{16:1}$ acids was highest in the M form. It was suggested that the lipid composition of the M form was immature due to increased lipid biosynthesis.

If the lipid content in the M form is higher than in the Y form, it may have consequences to virulence since hyphae, having a higher adhesive capacity to tissue cells than blastospores [2], are initiators of *Candida* invasion [1]. Lipids seem to be involved in the adhesion of yeasts to tissue. Thus, ceramide monohexosides and ceramide dihexosides isolated from the total lipids of the yeast form of *C. albicans* and steryl glycosides isolated form the mycelial phase inhibited adherence of yeasts to buccal epithelial cells by 48, 49 and 54%, respectively [20]. The total lipids extracted from a membrane-free preparation of yeast cell walls and from whole epithelial cells blocked adherence by 57 and 53%, respectively.

We did not detect the volatile fatty acids $C_{10:0}$ and $C_{12:0}$, which in a previous study on *C. albicans* [14] were found to be present after ethanolysis, propanolysis or butanolysis but not after methanolysis. This was probably due to differences in the medium composition. The presently used Lee medium is a mixture of amino acids [16], while the previously used medium was essentially a salt medium sup-

plemented with glucose and yeast extract [14]. Major factors determining the fatty acid composition of yeasts are the carbon and nitrogen substrates used [21].

ACKNOWLEDGEMENT

We thank Nordisk Ministerråd for financial support: Nordisk Forskningskurs Stipend 35/90.

REFERENCES

1 F C. Odds (Editor), *Candida and Candidosis,* Baillière Tindall, London, 2nd ed., 1988
2 I Olsen, *Acta Odontol Scand*, 48 (1990) 45
3 F. W Chattawy, M R Holmes and A J E Barlow, *J Gen Microbiol*, 51 (1968) 367
4 M Hrmová and L Drobnica, *Mycopathologia,* 76 (1981) 83
5 D S Schwartz and H W. Larsh, *Mycopathologia,* 70 (1980) 67.
6 P. A Sullivan, C. Y Yin, C. Molloy, M D Templeton and M. G Shepherd, *Can. J Microbiol*, 29 (1983) 1514
7 W L Chaffin and D. M Stocco, *Can J. Microbiol.*, 29 (1983) 1438
8 M V Elorza, A. Murgui and R. Sentandreu, *J Gen Microbiol*, 131 (1985) 2209
9 P K Gopal, M. G Shepherd and P A. Sullivan, *J Gen. Microbiol*, 130 (1984) 3295.
10 H. Yamaguchi, *J. Gen Appl Microbiol*, 20 (1974) 217
11 D. E. Bianchi, *Antonie van Leeuwenhoek J Microbiol Serol*, 33 (1967) 324
12 S. Sadamori, *Hiroshima J Med. Sci*, 36 (1987) 53
13 S Sundaram, P A Sullivan and M G Shepherd, *Exp Mycol*, 5 (1981) 140
14 I Brondz, I Olsen and M Sjöstrom, *J Clin. Microbiol.*, 27 (1989) 2815
15 N J W Kreger-van Rij, *The Yeasts A Taxonomic Study,* Elsevier, Amsterdam, 3rd ed., 1984.
16 K L Lee, H R Buckley and C Campbell, *Sabouraudia,* 13 (1975) 148
17 M V. Elorza, A Marcilla and R Sentandreu, *J Gen Microbiol*, 134 (1988) 2393
18 M J Hall and C Ratledge, *Appl Environ Microbiol*, 33 (1977) 577
19 H Lechevalier and M P Lechevalier, in C Ratledge and S G. Wilkinson (Editors), *Microbial Lipids,* Vol 1, Academic Press, New York, 1988, pp 869–902
20 M A Ghannoum, K Abu El-Teen and S. S. Radwan, *Mykosen,* 30 (1987) 371
21 J B M. Rattray, in C Ratledge and S G Wilkinson (Editors), *Microbial Lipids,* Vol 1, Academic Press, New York, 1988, pp 555–697