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Comparative Study of Lipid Composition of *Candida albicans* in the Yeast and Mycelial Forms

Shinsuke SADAMORI

Department of Fixed Partial Prosthodontics, Hiroshima University School of Dentistry, Hiroshima 734, Japan

(Director: Prof. Taizo HAMADA)

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ABSTRACT

Candida albicans was grown in the yeast and mycelial forms in the same conditions except keeping the temperature of the medium at 25°C for the yeast form and 37°C for the mycelial form, respectively, and the lipid composition of the organism in the two forms was studied comparatively. The contents of fatty acids, phospholipids, sterols (except zymosterol) and squalene of the mycelial form of the fungus per DNA were found to be significantly higher than those in the yeast form. Furthermore, in the yeast form the relative composition of the fatty acids which are considered to be the further metabolites of the nascent palmitic acid, *e.g.* C18:0, C18:1, C18:2, and C18:3, were higher than those in the mycelial form, whereas that of fatty acids such as C16:0 and C16:1 were higher in the mycelial form. These results seem to suggest an immaturity in lipid composition of the mycelial form compared to that in the yeast form. Essentially a similar result was obtained with [2-^{14}C]acetate added in culture media. Such an immaturity might be due to the increased biosynthesis of lipids in the mycelial form of *C. albicans*.

Candida albicans is a dimorphic fungus which exists either in the yeast form or in the mycelial form. The significance of the mycelial form of *C. albicans* in denture stomatitis was described by Budtz-Jørgensen[3]. The transformation of yeast-mycelium form occurs under a variety of conditions[9], one of which is the biotin insufficiency as reported by Yamaguchi[14]. Since the function as a cofactor for acetyl-CoA carboxylase is one of the important functions of biotin, it may be surmised that lipid metabolism may possibly be related to yeast-mycelium transformation. However, controversial reports have so far been published concerning the relation between dimorphism and lipid composition. Thus, Gordon et al[6] reported that in *Mucor genevensis* the dimorphology of the organism is not directly related to the lipid composition, whereas Manocha[7] suggested a possible role of the cellular lipid composition in the dimorphic behaviour of *Paracoccidioides brasiliensis*. Later, Yano et al[15] seemed to have confirmed the Manocha's observation in *C. albicans*. However, they used different culture media to grow either the yeast form or the mycelial form of the fungus. Since it is well known that lipid metabolism is influenced by the nature of medium, the results obtained by such studies seem to have left some ambiguities in the interpretation of data, *i.e.* the observed difference is due to the difference of phenotypes or to that of culture media.

In this experiment we attempted a comparative study of lipid composition of the two forms of *C. albicans* using a single synthetic medium to culture both forms of the fungus.

MATERIALS AND METHODS

Organism and culture media. *C. albicans* Institute for Fermentation Osaka (IFO) 1385 was used throughout the present studies. The organism was maintained on Sabouraud glucose (2%) agar slants by transfer every 4 weeks at 4°C. PYG (1.0% polypeptone, 0.5% yeast extract, 2.0% glucose) broth (medium A) was used for cultivation of inocula, which yielded a pure yeast phase growth. A synthetic medium containing methionine (medium B) was prepared by modifying the composition of the media reported by Yamaguchi[14], Tani et al[13] and Yano et al[15]. Medium B contained KH_2PO_4 (170 mg), $MgSO_4 \cdot 7H_2O$ (13 mg), KCl (43 mg), $FeCl_3 \cdot 6H_2O$ (0.25 mg), Na_2HPO_4 (450 mg), and biotin (10 ng) per 100 ml.

Cell preparation. After preculture in medium cells were harvested in early stationary phase by centrifuging for 2 min at 1500 × *g* and washing three times with distilled water to remove the ingredients of the medium. Cells were inoculated into medium B to give the final concentration of 4.65×10^4 cells/ml. The organism grew in the yeast form when incubated at 25°C and in the mycelial form at 37°C. Shaking of the medium was performed at a rate of 50 osc/min in both cases. Cells were harvested in the early stationary phase by centrifuging for 5 min at 1500 × *g* and washed with distilled water. One half of the harvested cells was subjected to lipid analysis and the rest to DNA analysis.

Lipid extraction. Prior to lipid extraction cells were digested by a lytic enzyme (Zymolyase-60000). A typical reaction mixture contained the fungal cells (about 20 mg dry wt./ml), Zymolyase-60000 (0.3 mg/ml), 80 mM Tris-HCl (pH7.4) and 10 mM dithiothreitol. The mixture was incubated at 37°C for 1 hr. Lipids were extracted from the digested cells according to the method described by Bligh and Dyer[2].

Thin layer chromatographic analysis of lipids. Thin layer chromatography of lipid was performed on thin layer plates (Kiesel gel G, Merck Co., Germany) impregnated with rhodamine 6G using solvent system diethyl ether:glacial acetic acid:n-hexane,25:2:73[11]. Detection of lipid was performed by ultraviolet light.

Quantitation of DNA. DNA was extracted from the digested cells according to the method of Schneider[10] and the amount of DNA was determined by the diphenylamine reaction.

Phospholipid. Phospholipid was decomposed with 60% perchloric acid to give inorganic phosphorus[4] and its content was determined by the method of Fiske and Subbarow[5].

Saponifiable lipid analysis. For determination of acyl chain composition, the lipid extracted was transmethylated with 15% boron trifluoride methanol complex in a sealed ampoule at 80°C for 20 min[1].

Nonsaponifiable lipid analysis. The lipid extract was hydrolyzed with 20% methanolic KOH for 2.5 hr under an atmosphere of nitrogen[8]. The hydrolysate was extracted with petroleum ether and the solvent was evaporated off by blowing nitrogen gas at room temperature.

Gas-liquid chromatography. Gas-liquid chromatography was performed using an instrument (Shimadzu Co., Kyoto, Japan, model GC-6A) equipped with a hydrogen flame detector. The column used for nonsaponifiable lipid was 1% OV-1 (3.0 mm × 1 m) and 1.5% OV-17 (3.0 mm × 1 m). The column temperature adopted was 225°C, and the temperature of the injection point was maintained 20°C higher than that of the column. Sterol samples were applied as free alcohols and fatty acid samples were applied as methyl esters. The column used for fatty acid analysis was 10% EGSS-X (3.0 mm × 3.0 m). The temperature of the column was 185°C and that of the injection point 215°C.

Incubation with [2-^{14}C]acetate. [2-^{14}C]Acetate dissolved in water (5 μCi/ml) was added to the medium B (20 ml). The organism was grown at 25°C for 27 hr (in the yeast form) and at 37°C for 9 hr (in the mycelial form). Incubation was terminated by adding 2 ml of 50% trichloroacetic acid to the mixture at the end of incubation. Then the mixture was filtered through glass microfibre filter to collect cells, which were subsequently treated with Zymolyase. Lipids were then extracted as described above.

Radio gas-liquid chromatographic analysis of radioactive fatty acids.

Some of the authentic fatty acid methyl esters were added to the radioactive lipid sample as reference materials and the mixtures were subjected to radio gas-liquid chromatography (Yanagimoto Co., Kyoto, Japan, model RD-1).

The column used for fatty acid analysis was 10% EGSS-X (3.0 mm × 3.0 m). The column temperature was 185°C and that of the injection point 210°C. The pressure of carrier gas (He) was 1.5 kg/cm^2. The temperature of the oxidizing furnace was 700°C, and the flow rate of scavenge gas was 80 ml/min and that of Q-gas (He+1% isobutane) 70 ml/min. Reference fatty acids injected were C16:0, C16:1, C18:0, C18:1, and C18:2.

Chemicals. [2-^{14}C]Acetate was purchased from Radiochemical Centre, Amersham, England and Zymolyase was obtained from Seikagaku Kogyo Co., (Tokyo, Japan). Ergosterol, squalene and fatty acid methyl esters were the products of Sigma Chemical Co. (St. Louis, MO). Zymosterol was a gift from Dr. H. Katsuki in Kyoto University. Other chemicals used were commercially available.

RESULTS

Growth

C. albicans (IFO-1385) grew in the yeast form when cultured at 25°C with a mean generation time of 6 hr and reached a stationary phase at 36 hr. In contrast, when the fungus was cultured at 37°C, it grew in the mycelial form (about 80-90%) with a mean generation time of 2 hr (Fig. 1). The DNA content increased exponentially during the initial 10 to 11 hr period, after which it leveled off and the yeast form began to increase.

Lipid composition

Table 1 summarizes the lipid content of the yeast and mycelial forms of *C. albicans* at the late logarithmic phase, and Fig. 2 and 3 show typical gas-liquid chromatograms of fatty acids and sterols, respectively. (Identification of fatty acids was performed by mass spectrometric analysis; data not shown). Thin layer chromatography of the lipids obtained from either form of the fungus showed that the major components were phospholipid and sterols, whereas triacylglycerol was contained only in a trace amount. The content of the total phospholipid in the mycelial form was substantially higher than that of the yeast form. The contents of the individual fatty acids are shown in Fig. 4. As shown in the figure, fatty acids C16:0, C16:1, C18:0, C18:1, and C18:2, were all higher in the mycelial form than those in the yeast form. The contents of ergosterol and lanosterol were also elevated in the mycelial form compared to those in the yeast form, whereas zymosterol content was lower in the mycelial form. The most striking difference was observed with the squalene content in the mycelial form, which was about ten times as high as that of the yeast form.

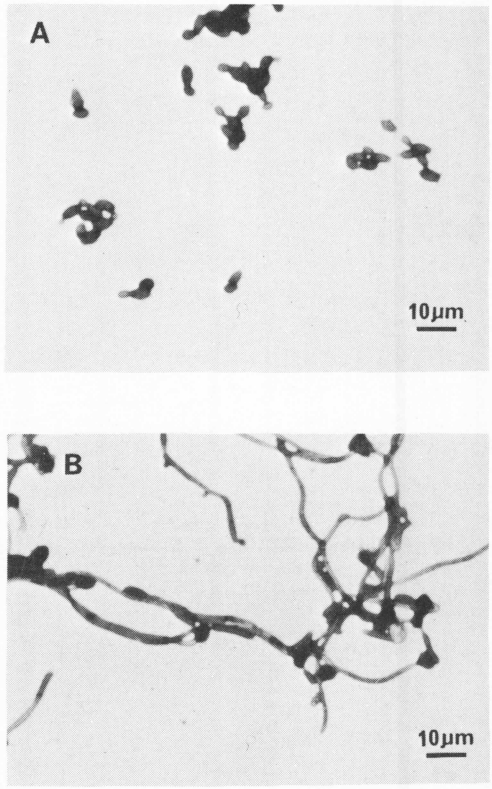

Fig. 1. Optical microscopic observations of *Candida albicans*.
(A): Typical yeast form cells of *Candida albicans* cultured at 25°C in medium B for 27 hr.; (B): A typical mycelial form of *Candida albicans* cultured at 37°C in medium B for 9 hr.

Table 1. The content of various lipids in the yeast and mycelial forms of *Candida albicans*[a]

Lipids	Yeast form nmol/μg of DNA	Mycelial form nmol/μg of DNA
Phospholipid	8.2 ± 0.4	19.2 ± 2.2
Triacylglycerol	trace	trace
Squalene	0.18 ± 0.07	1.62 ± 0.40
Ergosterol	2.37 ± 0.21	6.36 ± 0.59
Zymosterol	0.41 ± 0.06	0.26 ± 0.14
Lanosterol	0.06 ± 0.01	0.44 ± 0.06

[a]Values are means of 6 determinations ± S.E.

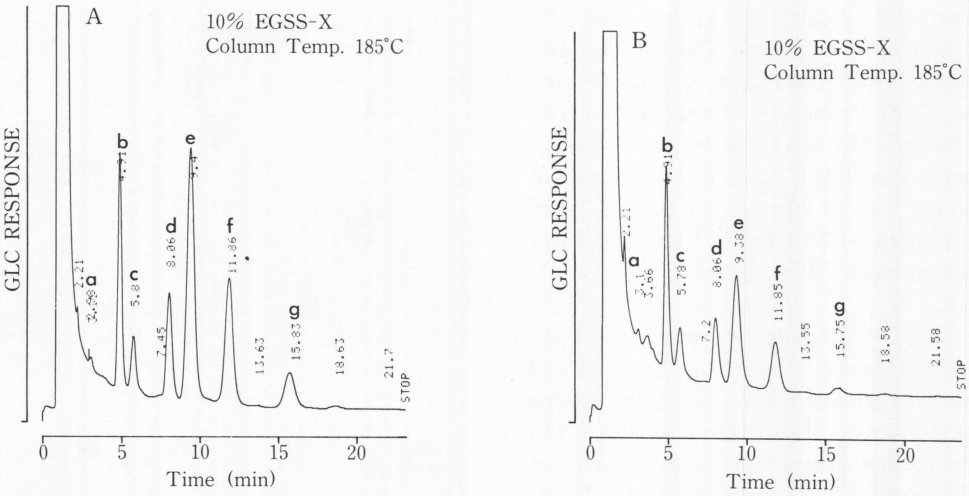

Fig. 2. Gas-liquid chromatograms of fatty acids of the yeast (A) and mycelial (B) forms of *Candida albicans*. a, C14:0; b, C16:0; c, C16:1; d, C18:0; e, C18:1; f, C18:2; g, C18:3.

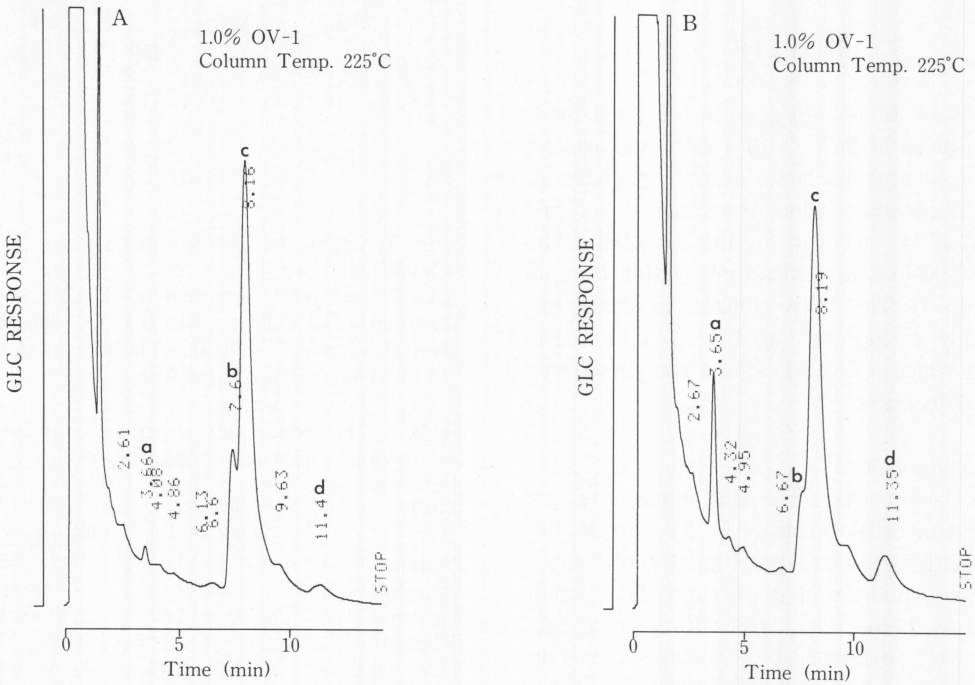

Fig. 3. Gas-liquid chromatograms of nonsaponifiable lipids of the yeast (A) and mycelial (B) forms of *Candida albicans*. a, squalene; b, zymosterol,; c, ergosterol; d, lanosterol.

Typical profiles of the fatty acids in the two forms are shown in Fig. 2 and the percentages of the individual fatty acids are in Fig. 5. The values for C18:0, C18:1, C18:2, and C18:3 fatty acids were the same or lower in the mycelial form compared to those in the yeast form. In contrast, the percentages of both C16:0 and C16:1 fatty acids were higher in the mycelial form compared to the yeast form.

Incorporation of [2-^{14}C]acetate into fatty acids

Figure 6 shows the radio gas-liquid chromatograms of the saponifiable lipids obtained from the two forms of the organism grown in the

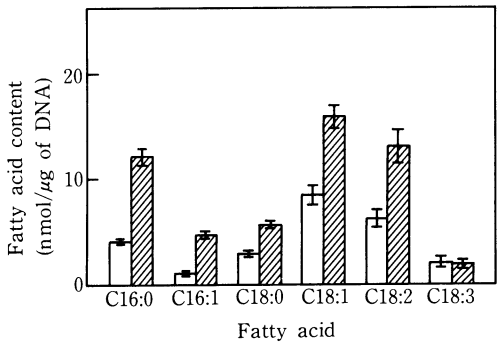

Fig. 4. The content of fatty acids in total lipids of the yeast and mycelial forms of *Candida albicans*. Open bars: yeast form; hatched bars: mycelial form. Data represent the mean of 6 determinations ± S.E.

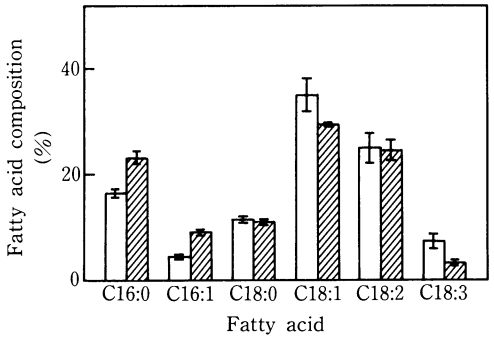

Fig. 5. The percentage composition of fatty acids in total lipids of the yeast and mycelial forms of *Candida albicans*. Open bars: yeast form; hatched bars: mycelial form. Data represent the mean of 6 determinations ± S.E.

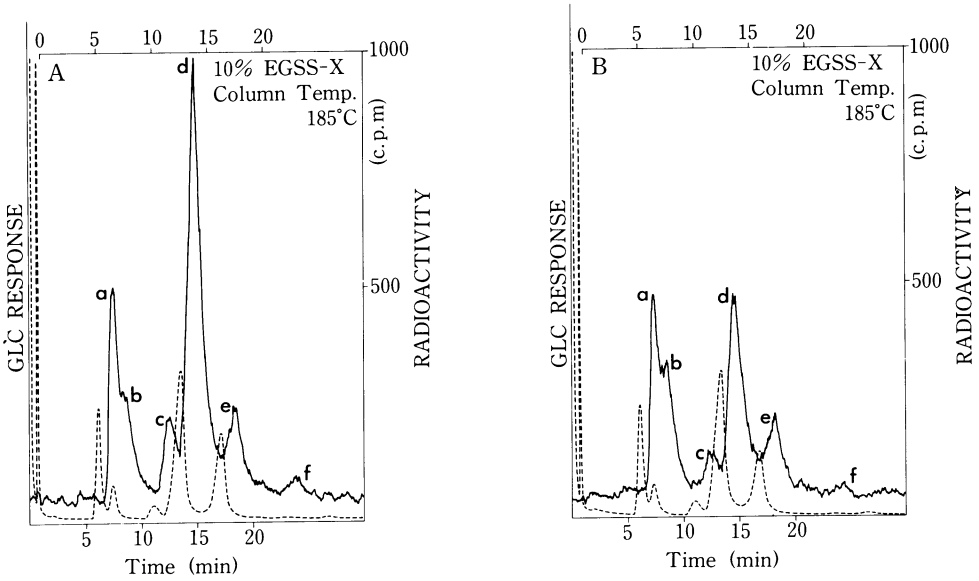

Fig. 6. Radio gas-liquid chromatograms of the radioactive fatty acids of the yeast (A) and mycelial (B) forms of *Candida albicans* incubated with [2-^{14}C]acetate. a, C16:0; b, C16:1; c, C18:0; d, C18:1; e, C18:2; f, C18:3. Time scale for mass peak is dotted line and that for radioactivity solid line. Reference fatty acids (C16:0, C16:1: and C18:0, C18:1, C18:2) were added to the sample before injection.

medium B containing [2-^{14}C]acetate. As shown in Fig. 6, apparently the fatty acids which have longer chain than palmitic acid and/or contain double bonds except the fatty acid C16:1 were relatively higher in the yeast form, whereas the fatty acids C16:0 and C16:1 were relatively higher in the mycelial form, confirming the abundance of the fatty acids which were elongated and/or unsaturated except palmitoleic acid in the yeast form and those in the nascent type or that which is metabolically closer to it (palmitoleic acid) in the mycelial form of the organism.

DISCUSSION

Fatty acid biosynthesis in the yeast was shown to be catalyzed by the fatty acid synthethase complex[12] and the end product of this enzyme complex is either palmitic acid or stearic acid depending on the ratio of acetyl-CoA/malonyl-CoA

in the medium. It seemed that some fraction of the stearic acid may be formed by the fungal elongation system from palmitic acid. Thus, stearic acid, palmitoleic acid, oleic acid, linoleic acid and linolenic acid are considered to be further metabolites of palmitic acid. In the yeast form the relative composition of C18:1, C18:2, C18:3 fatty acids were the same as C18:2 or higher than those in the mycelial form, whereas the relative amounts of fatty acids C16:0 and C16:1 were higher in the mycelial form than in the yeast form. This seems to suggest that in the yeast form the fatty acids which are elongated from C16 and/or unsaturated except palmitoleic acid (therefore considered to be the mature type) were more abundant than in the mycelial form. Since squalene and lanosterol are the precursors of ergosterol in the fungus, the increase of their content in mycelial form cells seems also to reflect the immaturity of sterol synthesis. These results, taken together with the fact that the content of fatty acids in the mycelial form was much higher than that in the yeast form, seem to suggest that in the mycelial form lipid biosynthesis is elevated compared to that in the yeast form. This would produce an "immature type" of fatty acid (nascent and/or metabolically close to its *de novo* form, *i.e.* fatty acids C16:0 and C16:1) or squalene. Essentially similar results were obtained in the study of [2-^{14}C]acetate incorporation into cells.

These results were, however, opposite to those obtained by Manocha[7] who studied the lipid composition of *P. brasiliensis*. This organism also grows in yeast and mycelial forms at 37°C and 22°C respectively. From his data it was clear that in the mycelial form "mature type" of fatty acids were predominant, while in the yeast form "immature type" of fatty acids were abundant. Since the factor which was varied to grow cells either in the yeast form or in the mycelial form of *C. albicans* and *P. brasiliensis* was only the culture temperature, the observed difference in lipid metabolism of the yeast form and the mycelial form of the organisms seems to be due either to the difference in morphology, to that of temperature, or to both. If the first alternative is true, then the present results, and those of Manocha, seem to contradict each other. That is, the "mature type" of fatty acids are abundant in yeast form in *C. albicans*, whereas they are so in the mycelial form in *P. brasiliensis*. However, if the second alternative is true, both results can be reconciled in that cells grown at lower temperature produce "mature type" of fatty acids regardless of morphology. Which of these alternative explanations is correct cannot be answered at present because the family of organisms used were different and the methods of lipid analysis employed were different. Extensive further work thus is needed to solve this problem. An experimental approach such as carried out in the present experiment, *i.e.* varying only one factor to perform yeast-mycelium transformation, should be helpful.

Finally, the lipid content was expressed as weight per DNA in the present experiment. Since the amount of DNA per cell should be constant both forms of the fungus, the value thus expressed should be proportional to the amount of lipid per fungal cell.

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