Fatty Acids of Triglycerides and Phospholipids from a Thermotolerant Strain of *Candida tropicalis* Grown on *n*-Alkanes at 30 and 40 °C

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INTRODUCTION

The factors responsible for heat resistance in thermophilic organisms have been extensively studied but without conclusive results. Certain biochemical differences have been found between thermophilic and mesophilic micro-organisms: ribosomes (Bubela & Holdsworth, 1966), ribosomal RNA (Friedman, Axel & Weinstein, 1967) and certain cytoplasmic proteins (Farrell & Campbell, 1969) show a correlation between heat stability and maximum growth temperature. Lipids become more saturated at higher growth temperatures (Kates & Baxter, 1962; Brown & Rose, 1969; Cullen, Phillips & Shipley, 1971) and the degree of saturation of membrane lipids could, perhaps, determine the maximum and minimum growth temperatures for micro-organisms (Heilbrunn, 1924; Gaughran, 1947). Marr & Ingraham (1962), however, did not agree with this proposal. With *n*-alkane-utilizing yeasts, which contain a wide range of fatty acids varying in both unsaturation and carbon chain length depending on the composition of the *n*-alkane substrate, there is perhaps greater opportunity for the organism to modify its fatty acid composition than in other yeasts. We have therefore examined the fatty acids of a thermotolerant, hydrocarbon-utilizing yeast to see what changes occur between different growth temperatures.

METHODS

Organisms and growth. *Candida tropicalis* used in this work, is a thermotolerant strain that grows at 40 °C. It was grown in batch culture in a stirred fermenter on a basal salts medium similar to that of Jenkins, Raboin & Moran (1972) with a C₁₃ to C₁₈ fraction of *n*-alkanes as carbon source. The pH was maintained at 5.5 throughout growth.

Extraction and fractionation of lipids. Lipids were extracted from freeze-dried yeast as described by Thorpe & Ratledge (1972) and, after removing non-lipid material by passage of the crude lipid down a column of Sephadex G-25 according to Wuthier (1966), were fractionated into neutral and polar lipids on a column of silicic acid by using chloroform (5 column volumes) to elute neutral lipids and methanol (5 column volumes) to elute polar lipids (Rouser, Kritchevsky & Yamamoto, 1967).

Further lipid separation was by thin-layer chromatography: neutral lipids were separated and quantitated as described by Thorpe & Ratledge (1972). Polar lipids were separated by using the solvent system chloroform + methanol + acetic acid + water (25:15:4:2, by vol.). Lipids were detected by exposure of plates to I₂ vapour (Mangold & Malins, 1966) or spraying with alcoholic Rhodamine 6G solution (Wagner, Hörhammer & Wolff, 1961). Phospholipids were detected by a molybdenum blue reagent (Dittmer & Lester, 1964) and a

Table 1. *Fatty acids and lipids of Candida tropicalis grown on n-alkanes*

(a) Lipid compositions						
Relative percentage composition of lipid						
Growth temp. (°C)	Total lipid (% dry wt)	Sterol esters + hydrocarbons	Triglycerides	Unknown*	Sterol + partial glycerides	Phospholipids
40	14.9	6.4	10.9	9.6	5.1	68
30	15.5	8.1	5.2	8.4	7.3	71

(b) Fatty-acid composition				
Growth temp (°C)				
Fatty acid	40		30	
	Triglycerides	Phospholipids	Triglycerides	Phospholipids
Mole % of fatty acids				
7:0	—	—	—	0.1
8:0	—	—	—	0.2
9:0	—	—	—	0.3
10:0	—	—	2.9	1.8
11:0	—	—	0.7	3.6
12:0	—	—	—	0.1
12:1	—	—	—	0.4
13:0	1.0	0.2	8.1	1.7
14:0	5.3	1.7	4.3	1.5
15:0	15.9	8.8	9.5	6.4
15:1	0.1	0.4	—	0.4
16:0	31.4	18.6	21.8	15.6
16:1	6.5	14.2	9.5	11.8
17:0	17.3	6.3	12.4	7.0
17:1	14.8	27.0	14.2	18.4
18:0	3.6	12.7	10.2	13.8
18:1	2.9	4.4	2.8	4.3
18:2	0.9	5.7	2.9	7.2
19:0	—	—	0.7	3.1
18:3	—	—	—	1.9
Total saturated acids (%)	73.8	48.3	70.6	55.9
Hypothetical mean melting-point (°C)	46.1	32.8	42.5	34.1

* Probably an oxidation product of a polyunsaturated glyceride (see text).

ninhydrin spray was used to detect lipids having a free amine group (Skipski, Peterson & Barclay, 1962).

Fatty-acid methyl esters were prepared directly from lipids by using a modified form of the method of Marinetti (1962). Gas-liquid chromatography of the esters was with a Hewlett Packard 7620A gas chromatograph, equipped with an integrator, with a column containing 10% diethylene glycol succinate on Chromosorb W.

Hypothetical mean melting-point. Because comparing lipids with different fatty-acid compositions is difficult, and the previously adopted concept of calculating the number of double bonds/mole of acid (Kates & Baxter, 1962) does not allow for changes in fatty-acid chain length, we have calculated from the relative percentages of the individual fatty acids and their respective melting-points a mean melting-point for the fatty-acid mixture: $\Sigma \text{m.p. of acid} \times \text{relative abundance}/100$.

RESULTS

The lipids of *Candida tropicalis* were determined after growth at 30 and 40 °C. Although there were only small changes in total lipid contents and in the contents of neutral and polar lipids between samples of the yeast grown at the two temperatures, the amount of triglyceride in the neutral-lipid fraction was halved at the lower growth temperature with a corresponding increase in the sterol and sterol-ester content (Table 1). The change in amount of triglyceride was reflected in the change in fatty-acid distribution: a higher proportion of shorter-chain fatty acids (C14 and below) and saturated acids were produced at the lower growth temperature with the result that a calculated hypothetical mean melting point for the mixture of acids was decreased by about 4 °C. The fatty acids within the total phospholipids also changed in distribution on lowering the growth temperature, but the decreases in unsaturated acids were compensated for by the increases in shorter-chain acids so that the hypothetical mean melting-points of the fatty-acid mixtures remained almost unchanged.

In the neutral lipids of this yeast an appreciable amount of an unknown material was detected. This material, which had an R_F value about 0.7 of that of $R_{\text{triglyceride}}$, was indicated by i.r. and u.v. spectroscopy to be an oxidation product of a polyunsaturated glyceride and may therefore be an artefact arising during harvesting of the yeast and lipid extraction. No change in its percentage proportion of the total lipid was seen at the two growth temperatures.

Only minor changes in phospholipid composition occurred in the yeast grown at 40 °C and at 30 °C. Phosphatidyl ethanolamine and phosphatidyl choline were the predominant phospholipids with smaller amounts of two phospholipids tentatively identified by spray reagents and R_F values, as phosphatidyl serine (or lysophosphatidyl ethanolamine) and lysophosphatidyl serine.

DISCUSSION

Candida tropicalis, like other yeasts, usually contains only about 30 % of the total fatty acids from phospholipids as saturated acids, though in the triglyceride fraction the content of saturated acids may be up to 60 % (Thorpe & Ratledge, 1972; Thorpe, 1973). With the present thermotolerant strain of *C. tropicalis* there was an unusually high content of saturated acids in both these lipid fractions even at the lower growth temperature of 30 °C. If this reflects a genuine decreased ability to synthesize unsaturated acids, conformation of the membrane, with its high content of phospholipids, might only reach optimum dynamics at elevated temperatures where a high content of saturated acids would then be beneficial to its structural and functional roles. This may therefore be an explanation for the maximum growth temperature of this yeast being higher than is usually encountered. Greshnykh, Grigorian, Dikanskaja, Diatlovickaja & Bergelsen (1968), however, have shown in another strain of this yeast, not designated as thermotolerant, that when grown on *n*-alkanes at 38 °C the concentration of linoleic acid was only 12 % of the total fatty acids, whereas at 28 °C its concentration was 33 %. There are no other reports, as far as we are aware, concerning the effects of temperature on alkane-utilizing yeasts and so it is not possible to judge if our results indicate some intrinsic abnormality of metabolism within this present strain *C. tropicalis*.

Mobilization of triglycerides, if these are considered as reserve storage materials, will be easier for this yeast to accomplish at 40 °C than at 30 °C because a radical change in the fluidity (i.e. melting-point) of the triglycerides apparently cannot be made on lowering the growth temperature. The yeast, however, does attempt to circumvent this constraint by

producing more shorter-chain acids, which have somewhat similar physical properties to the longer-chain unsaturated acids (Meyer & Bloch, 1963), though the quantity is insufficient to have much effect on the calculated hypothetical mean melting point.

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