Temperature adaptation in yeasts: the role of fatty acids

M. SUUTARI,* K. LIUKKONEN and S. LAAKSO

Helsinki University of Technology, Department of Chemical Engineering, Laboratory of Biochemistry and Microbiology, SF-02150 Espoo, Finland

(Received 2 January 1990; revised 12 March 1990; accepted 2 April 1990)

Studies on the yeasts *Candida oleophila*, *Candida utilis*, *Lipomyces starkeyi*, *Rhodosporidium toruloides* and *Saccharomyces cerevisiae* revealed the existence of three different temperature adaptation responses involving changes in fatty acid composition. These conclusions were drawn by determining the growth rates, total cellular fatty acid content, fatty acid composition, degree of unsaturation, and the mean chain length of fatty acids over a range of growth temperatures. Within temperatures permitting growth, there were no changes in the major fatty acids of any of the yeasts, but the absolute amounts and relative compositions of the fatty acids did alter. In *S. cerevisiae* there were temperature-induced changes in the mean fatty acid chain length, whereas in *R. toruloides* there were changes in the degree of unsaturation. *C. oleophila*, *C. utilis* and *L. starkeyi* showed both responses, depending on whether the growth temperature was above or below 20–26 °C. Below 20–26 °C temperature-dependent changes were observed in the mean chain length whereas above 20–26 °C there were changes in the degree of unsaturation.

Introduction

Fatty acids play an important role in determining the physicochemical properties of membrane lipids. Therefore micro-organisms have different species-specific fatty acids (McElhaney, 1976). Analysis of these characteristic fatty acids can sometimes be used for strain identification purposes (Lechevalier & Lechevalier, 1989). However, the relative amounts of the fatty acids may be altered by changes in external conditions such as temperature (Cronan, 1978). Less is known as to whether these temperature-induced changes are essential for survival and whether the capability of an organism to adapt to extreme temperature really reflects its predetermined ability to alter the relative fatty acid composition of membrane lipids (Neidleman, 1987). The mechanisms of temperature adaptation probably involve many cellular functions and components, but membrane fluidity and its maintenance by changes in fatty acid composition can hardly be ignored. How the fatty acid changes are related to the regulation of membrane-bound enzymes has been reviewed by Brenner (1984), Cronan & Gelmann (1975) and Sandermann (1978).

More extensive information on microbial temperature adaptation mechanisms would also be valuable in many modern areas of biotechnology ranging from medical microbiology and immunology to food hygiene, pro-

cessing and fermentation. In addition, interest in microbes as producers of speciality lipids is rapidly increasing (Ratledge & Boulton, 1985). These considerations led us to study the temperature-dependent changes of fatty acids in several different types of yeast. The yeasts contain a type I fatty acid synthetase and O₂-dependent desaturases (Fulco, 1974; Bloch & Vance, 1977; Wakil *et al.*, 1983), and when grown in the presence of excess O₂ could offer a wide spectrum of changes in membrane fatty acids. Measurements of fatty acid composition were made over a wide temperature range in order to examine the full extent of thermal dependence. The gathering of systematic data allows results to be presented which not only reveal the individuality of yeasts as modifiers of their fatty acid profiles but also general responses which can be related to temperature adaptation.

Methods

Strains and growth conditions. The yeasts used were *Candida oleophila* VTT-C-78088 (VTT, Technical Research Centre of Finland), *Candida utilis* VTT-C-84147, *Rhodosporidium toruloides* VTT-C-82132, *Saccharomyces cerevisiae* B I Lh 306 and *Lipomyces starkeyi* DSM 70295 (DSM, German Collection of Micro-organisms and Cell Cultures). The strains were stored in 10% (v/v) glycerol at –60 °C and grown in 250 ml

Erlenmeyer flasks containing 50 ml wort broth base. The flasks were shaken at 240 r.p.m. on a Gallenkamp orbital shaker/incubator (model INR 200 010V). For preparation of inocula cells were grown to late exponential growth phase (150–200 Klett units); 1 ml of this culture was transferred into a shake flask containing the same medium and allowed to grow to the exponential phase. This culture (1 ml) was inoculated into four parallel flasks to be used for analyses. All these stages were carried out at each temperature studied. Growth was monitored with a Klett–Summerson colorimeter (filter no. 66). The fatty acid composition was determined in early exponential (50 Klett units), exponential (100 Klett units), late exponential (150 Klett units) and stationary (> 200 Klett units) phases. The total fatty acid content of cells was determined in the exponential phase.

To determine growth temperature minima and maxima the yeasts were grown on agar in individual cuvettes in a plate-type temperature gradient incubator (Gradiplate). The medium used was wort broth base containing 0.25% (v/v) glycerol solidified with 2% (w/v) agar.

Analyses. To determine the fatty acid composition samples (5 ml) of culture suspensions were centrifuged for 10 min (5000 g). The supernatant was removed and the cells were resuspended in 5 ml of tap-water. Centrifugation was repeated, the water was removed and cells were frozen under a stream of N₂ and stored (–20 °C) for 1–10 d. Cells were suspended in an excess (1 ml) of saponification reagent containing 3.7 M-NaOH in 49% (v/v) methanol. Samples were flushed with N₂, mixed, kept at 100 °C in closed tubes for 5 min, mixed and returned to a 100 °C water-bath for 25 min. The sample was cooled down to room temperature and 4 ml of methylation reagent was added. The methylation reagent was 3.3 M-HCl in 48% (v/v) methanol. The sample was mixed, held at 80 °C in a water-bath for 10 min and cooled down to room temperature. Fatty acid methyl esters were extracted in 1.5 ml hexane/methyl tert-butyl ether solution (1:1, v/v). The sample was shaken vigorously for 10 min and the lower phase was removed with a Pasteur pipette. The sample was washed by shaking for 5 min with 3 ml 0.3 M-NaOH. The sample was centrifuged (5000 g) for 20 min and the upper phase was removed. The sample was then dried with anhydrous Na₂SO₄, flushed with nitrogen and analysed by GLC. To determine the cellular lipid content the rest of the yeast culture suspension was harvested and washed with water as described above. The yeast cells were lyophilized and weighed, an internal standard (heptadecanoic acid methyl ester, Sigma) was added and the fatty acid composition was determined as described above. The major fatty acids were identified from their GLC peak retention times relative to fatty acid methyl ester standards (Sigma). GLC was done using a Hewlett-Packard model 5890A gas chromatograph equipped with a flame ionization detector, a capillary inlet system and a model 7673A high-speed automatic liquid sampler with a 10 µl syringe. The GLC conditions were as follows: HP-FFAP (25 m × 0.2 mm × 0.3 µm) column; carrier gas helium, column flow-rate approx. 1.0 ml min⁻¹; total hydrogen flow-rate to the detector 40 ml min⁻¹; make-up gas, helium, flow-rate 30 ml min⁻¹; septum purge flow-rate 1–2 ml min⁻¹; split ratio 1:20; column inlet pressure 150 kPa; injector temperature 250 °C; detector temperature 250 °C; oven temperature was programmed from 70 °C to 200 °C at the rate of 25 °C min⁻¹. Peak areas were measured using a Hewlett-Packard model 3396A integrator.

Calculations. The growth rate was determined from the slope of the growth curve in the exponential growth phase. The relative fatty acid composition was estimated as a percentage of the total peak area and expressed as a mean value from early exponential and exponential growth phases. The absolute amount of the individual fatty acids was calculated as a percentage of the cell dry weight. The total fatty acid content of the cells was defined as the sum of cellular fatty acid methyl esters. The degree of unsaturation (Δmol^{-1}) in the lipid fraction was calculated as $\Delta\text{mol}^{-1} = [1.0 (\%\text{monoene}) + 2.0 (\%\text{diene}) + 3.0$

(%triene)]/100. The mean fatty acid chain length was expressed as the ratio of C₁₆/C₁₈ acids. The standard deviation ($n = 8$) was <0.9%.

Chemicals. Components of growth media and chemicals were obtained from Merck, unless otherwise stated.

Results

Effect of temperature on growth

The temperatures used in shake flask cultivations appeared to cover the temperature range permitting growth of the yeasts (Table 1). The minimum growth temperature used (10 °C) exceeded the actual minima by 3–8–10 centigrade degrees, as determined by the Gradiplate technique. Similarly, the highest temperatures were less than 4 centigrade degrees below the maxima. Each of the yeasts grew over a temperature range (ΔT) of 27.5–36.0 centigrade degrees.

Fig. 1 illustrates the growth rates as a function of temperature. Below 25 °C the correlations were linear and positive. At higher temperatures the rates declined in a pronounced fashion. The fatty acid compositions during both the increasing and the declining phases were investigated.

Table 1. Correlation of shake-flask temperatures with the real growth temperature ranges as determined by the Gradiplate technique

Yeast	Temperatures in shake-flask experiments (°C)	Gradiplate determinations		
		$T_{\min}$ (°C)	$T_{\max}$ (°C)	ΔT (°C)
<i>C. utilis</i>	10, 12, 15, 20, 26, 30, 35, 40	6.3	39.7	33.4
<i>C. oleophila</i>	10, 12, 15, 20, 26, 30	<0	34.3	>34.3
<i>L. starkeyi</i>	10, 15, 20, 26, 30	5.4	33.1	27.7
<i>R. toruloides</i>	10, 15, 20, 26, 30	<0	33.9	>33.9
<i>S. cerevisiae</i>	10, 12, 15, 20, 25, 30, 35	2.8	39.3	36.5

Fig. 1. Growth rates as a function of culture temperature. Δ , *C. oleophila*; $\bullet$, *C. utilis*; $\blacktriangle$, *L. starkeyi*; $\square$, *R. toruloides*; $\blacksquare$, *S. cerevisiae*.

Fig. 2. Temperature-induced variations in the fatty acid profiles expressed as relative (a, c, e, g, i) and absolute (b, d, f, h, j) contents. (a, b) *S. cerevisiae*; (c, d) *R. toruloides*; (e, f) *C. utilis*; (g, h) *L. starkeyi*; (i, j) *C. oleophila*. □, Palmitic acid; ●, palmitoleic acid; ▲, oleic acid; ■, linoleic acid; △, linolenic acid.

Effect of growth phase on fatty acid composition

The yeasts contained three to five characteristic fatty acids which accounted for more than 90% of the total amount of fatty acids (Fig. 2). The major fatty acids were palmitic (16:0), palmitoleic (16:1), oleic (18:1), linoleic (18:2) and linolenic acid (18:3). The relative compositions of major fatty acids changed less than 10% with the growth phase except in *L. starkeyi*. The ratio of C_{16}/C_{18} fatty acids did not change, except in *L. starkeyi*, in which the proportion of C_{16} acids increased. The degree of unsaturation also changed only in *L. starkeyi*, in which it decreased from the early exponential to the late stationary phase. This can be explained by the ability of *L. starkeyi* to accumulate palmitic and oleic acids in favour of palmitic acid upon cessation of growth (Suzuki & Hasegawa, 1974). Minor fatty acids were detected in all the yeasts (<2.8% of the total), but they showed little growth-phase- or temperature-dependent changes.

Effect of growth temperature on the content of palmitic and palmitoleic acids

Palmitic acid was the only major saturated fatty acid present in all the strains studied and its content varied only slightly with the growth temperature (Fig. 2). Its amount, expressed as relative content varied most in *C. oleophila* (4.0%); when expressed with respect to cell dry weight it varied most in *R. toruloides* (2.7%). There was a general trend towards an increased relative amount of palmitic acid with increasing growth temperature. There

were, however, two exceptions. The palmitic acid content in *C. utilis* showed a definite increase at 40 °C, i.e. near the maximum growth temperature, while the content of oleic acid decreased concomitantly. The lipid-accumulating yeast, *L. starkeyi*, showed a tendency to have a lower relative palmitic acid content with decreasing temperature. However, below 15 °C an increase in its absolute amount was found.

The absolute palmitoleic acid content increased with decreasing temperature in *C. oleophila*, *C. utilis*, *L. starkeyi* and *S. cerevisiae*. In relative, but not in absolute terms the palmitoleic acid also increased somewhat near the upper temperature limit of *C. oleophila*, *C. utilis* and *L. starkeyi*. In *R. toruloides* palmitoleic acid was only present in amounts less than 0.6%.

Effect of growth temperature on the content of oleic, linoleic and linolenic acids

Oleic acid was the only unsaturated fatty acid present in all the strains investigated (Fig. 2). Temperature-induced variations in the proportion of oleic acid were species-specific. In *C. oleophila*, *C. utilis* and *R. toruloides* oleic acid content varied maximally by 10% and 7% in relative and absolute terms, respectively. In *S. cerevisiae*, which contained only three major fatty acids, the relative oleic acid content varied between 28.1–38.6%. However, the absolute amounts varied by only 2.5% (between 15.9–18.4%). In *L. starkeyi* the relative and absolute amounts of oleic acid increased with decreasing temperature.

Fig. 3. Changes in the degree of unsaturation (a) and the ratio of C_{16}/C_{18} fatty acids (b) as a function of growth temperature in the exponential growth phase. Δ , *C. oleophila*; $\bullet$, *C. utilis*; $\blacktriangle$, *L. starkeyi*; $\square$, *R. toruloides*; $\blacksquare$, *S. cerevisiae*.

Fig. 4. Cellular fatty acid content at different growth temperatures in exponentially growing yeast cultures. Δ , *C. oleophila*; $\bullet$, *C. utilis*; $\blacktriangle$, *L. starkeyi*; $\square$, *R. toruloides*; $\blacksquare$, *S. cerevisiae*.

The relative amount of linoleic acid in *C. utilis* and *R. toruloides* increased almost linearly with the growth temperature. *L. starkeyi* behaved similarly with the exception of the highest temperature (around 30 °C). In *C. oleophila* a similar positive correlation was also seen up to 20 °C. At higher temperatures (between 20–30 °C) the correlation became negative. Linoleic acid was not detected in *S. cerevisiae*.

The relative and absolute amounts of linolenic acid in *C. utilis* and *L. starkeyi* were constant at low growth temperatures (up to 20 °C) but decreased with further increases in temperature. In *C. utilis* these reductions were accompanied by a concomitant increase in linoleic acid. In *R. toruloides* linolenic acid was apparently replaced by linoleic acid and to a lesser extent by oleic acid with increasing temperature when expressed in terms of relative fatty acid proportions.

S. cerevisiae and *C. oleophila* did not contain trienoic acids over the whole temperature range studied.

Effect of temperature on the fatty acid content, and the degree of fatty acid unsaturation and mean chain length

In *S. cerevisiae* (Fig. 3a) the degree of unsaturation did not vary as a function of the temperature of the growth medium. The fatty acid content of this yeast (Fig. 4) and the C_{16}/C_{18} ratio (Fig. 3b) increased with decreasing temperature. In *R. toruloides* changes in the degree of unsaturation were significant. This value changed from

Fig. 5. Summary scheme of the lipid-related responses involved in temperature adaptation of different yeasts.

1.57 to 1.73 with decreasing temperature while the fatty acid content decreased. The mean fatty acid chain length in this organism was constant over the temperature range studied. In *C. utilis* and *L. starkeyi* the dependence of the fatty acid content and the degree of unsaturation on temperature showed biphasic behaviour with a minimum and maximum at 20–26 °C, respectively. Both above and below this temperature range the fatty acid content increased. *C. oleophila* behaved similarly to *C. utilis* and *L. starkeyi* below 26 °C. The trends of changes in the degree of unsaturation were the opposite to those found for fatty acid content. Fig. 3(b) shows that in *C. oleophila*, *C. utilis* and *L. starkeyi* the ratio of C_{16}/C_{18} fatty acids increased with increasing temperature above 20 °C. Below 20 °C an opposite correlation was seen in *C. utilis*.

Discussion

This study identified three responses to temperature of the fatty acid composition of yeasts (Fig. 5).

The first response was seen in *S. cerevisiae*, which reacted to temperature changes by regulating the palmitoleic acid content. As a result the mean chain length of fatty acids (Fig. 3b) changed with temperature as demonstrated also by Okuyama *et al.* (1979). The degree of unsaturation, often proposed to vary with temperature in *S. cerevisiae*, was virtually unaltered. This had also been suggested by Hunter & Rose (1972).

The second response was seen in *R. toruloides*, which reacted to increasing temperature by lowering the degree of fatty acid unsaturation (Fig. 3a) and increasing the fatty acid content (Fig. 4). Regulation by alteration of the mean chain length can be ruled out, because the level of palmitic acid remained unchanged and the palmitoleic acid content was low over the whole temperature range. High temperatures might favour lipid accumulation in *R. toruloides*.

C. utilis demonstrated a third type of adaptation because, depending on the temperature range, it could switch from one response to another. Below 20–26 °C the ratio of C₁₆/C₁₈ fatty acids and the fatty acid content decreased with increasing temperature, analogous to the response seen in *S. cerevisiae*. According to Davis *et al.* (1981) and Coolbear *et al.* (1983) a shortening of the fatty acid chain length and insertion of the first double bond has a much greater effect on lipid fluidity than does insertion of subsequent double bond(s). This might explain the increasing monoenoic acid content as the temperature is lowered. McMurrough & Rose (1971) have also suggested that the maintenance of high proportions of polyunsaturated fatty acids is not a prerequisite for the growth of *C. utilis* at suboptimal temperatures. However, above 20–26 °C the degree of unsaturation decreased and the fatty acid content increased with temperature similarly to the response of *R. toruloides*.

L. starkeyi showed a biphasic temperature adaptation behaviour similar to that of *C. utilis*. In addition, the fatty acid content (Fig. 4) increased substantially below 20 °C at the expense of palmitic and oleic acids. In *C. oleophila* the absolute monoenoic acid content and fatty acid content (Fig. 4) decreased with increasing temperature. However, above 20 °C only the degree of unsaturation (Fig. 3a) decreased significantly.

This study revealed a much wider range of adaptive responses than previously expected. The temperature range from 20–26 °C appeared to be critical in determining the mode of response in those yeasts showing a biphasic behaviour, but this cannot be explained from the present data. Neither the growth rate maxima nor dissolved O₂ concentration coincided with this temperature range. A deeper understanding of the mechanisms involved in these responses requires further information

on fatty acid changes at the level of membrane phospholipids and/or stored triacylglycerols. This work is in progress in our laboratory.

We thank Mrs U. Ahman for her technical assistance. The financial support of the Technology Development Centre (TEKES) is gratefully acknowledged.

References

- BLOCH, K. & VANCE, D. (1977). Control mechanisms in the synthesis of saturated fatty acids. *Annual Review of Biochemistry* **46**, 263–298.
- BRENNER, R. R. (1984). Effect of unsaturated acids on membrane structure and enzyme kinetics. *Progress in Lipid Research* **23**, 69–96.
- COOLBEAR, K. P., BERDE, C. B. & KEOUGH, K. M. W. (1983). Gel to liquid-crystalline phase transitions of aqueous dispersions of polyunsaturated mixed-acid phosphatidylcholines. *Biochemistry* **22**, 1466–1473.
- CRONAN, J. E., JR (1978). Molecular biology of bacterial membrane lipids. *Annual Review of Biochemistry* **47**, 163–189.
- CRONAN, J. E. & GELMANN, E. P. (1975). Physical properties of membrane lipids: biological relevance and regulation. *Bacteriological Reviews* **39**, 232–256.
- DAVIS, P. J., FLEMING, B. D., COOLBEAR, K. P. & KEOUGH, K. M. W. (1981). Gel to liquid-crystalline transition temperatures of water dispersions of two pairs of positional isomers of unsaturated mixed-acid phosphatidylcholines. *Biochemistry* **20**, 3633–3636.
- FULCO, A. J. (1974). Metabolic alterations of fatty acids. *Annual Review of Biochemistry* **43**, 215–241.
- HUNTER, K. & ROSE, A. H. (1972). Lipid composition of *Saccharomyces cerevisiae* as influenced by growth temperature. *Biochimica et Biophysica Acta* **260**, 639–653.
- LECHEVALIER, H. & LECHEVALIER, M. P. (1989). Chemotaxonomic use of lipids – an overview. In *Microbial Lipids*, pp. 869–902. Edited by C. Ratledge & S. G. Wilkinson. London: Academic Press.
- MCLEHANEY, R. N. (1976). The biological significance of alterations in the fatty acid composition of microbial membrane lipids in response to changes in environmental temperature. In *Extreme Environments – Mechanisms of Microbial Adaptation*. Edited by M. R. Heinrich. New York: Academic Press.
- McMURROUGH, I. & ROSE, A. H. (1971). Effects of temperature variation on the fatty acid composition of *Candida utilis*. *Journal of Bacteriology* **107**, 753–758.
- NEIDLEMAN, S. L. (1987). Effects of temperature on lipid unsaturation. *Biotechnology and Genetic Engineering Reviews* **5**, 245–268.
- OKUYAMA, H., SAITO, M., JOSHI, V. C., GUNSBERG, S. & WAKIL, S. J. (1979). Regulation by temperature of the chain length of fatty acids in yeast. *Journal of Biological Chemistry* **254**, 12281–12284.
- RATLEDGE, C. & BOULTON, C. A. (1985). Fats and oils. In *Comprehensive Biotechnology*, vol. 3, pp. 983–1003. Edited by H. W. Blanch, S. Drew & D. I. C. Wang. Oxford: Pergamon Press.
- SANDERMANN, H., JR (1978). Regulation of membrane enzymes by lipids. *Biochimica et Biophysica Acta* **515**, 209–237.
- SUZUKI, T. & HASEGAWA, K. (1974). Lipid molecular species of *Lipomyces starkeyi*. *Agricultural and Biological Chemistry* **38**, 1371–1476.
- WAKIL, S. J., STOOPS, J. K. & JOSHI, V. C. (1983). Fatty acid synthesis and its regulation. *Annual Review of Biochemistry* **52**, 537–579.