Membrane phospholipids in temperature adaptation of *Candida utilis:* alterations in fatty acid chain length and unsaturation

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Abstract The effect of decreasing environmental temperature on membrane phospholipids (PL), the neutral lipid (NL) composition, and their fatty acid profiles was studied in exponentially growing *Candida utilis* from 40 to 10°C, at intervals of 5–9 centigrade degrees. According to the results, fatty acid unsaturation increased with decreasing growth temperature from 40 to 26–20°C, due to the increase in PL containing the most unsaturated fatty acid, linolenic acid (mp. −11.3 to −12.8°C). Concomitantly, an equal (phosphatidylcholine, PC; phosphatidylethanolamine, PE) or more pronounced (phosphatidylinositol and phosphatidylserine, PI + PS) decrease occurred in fatty acids with lower unsaturation and, consequently, the cellular fatty acid content decreased as the temperature was reduced from 40 to 26–20°C. In addition, when the temperature decreased within the lower growth temperature range from 26–20 to 10°C, the fatty acid chain length also shortened in PL, due to the increase in palmitoleic acid (mp. 0°C), and equal (PC and PE) or more pronounced (PI + PS) decrease in other acids. Concomitantly, triacylglycerols accumulated as the temperature decreased from 26–20 to 10°C. Thus, the results showed that *C. utilis* can adapt cellular membranes to decreases in the environmental temperature so that fatty acid unsaturation increases down to 26–20°C, and at temperatures below that the fatty acid chain length also shortens.—**Suutari, M., A. Rintamäki, and S. Laakso.** Membrane phospholipids in temperature adaptation of *Candida utilis:* alterations in fatty acid chain length and unsaturation. *J. Lipid Res.* 1997. **38:** 790–794.

Supplementary key words phospholipids • neutral lipids • temperature adaptation • *Candida utilis* • fatty acids

In *Candida utilis,* fatty acid unsaturation increased and the fatty acid content decreased when the environmental temperature was reduced from 40 to 26–20°C. This was due to the increase in the most unsaturated linolenic acid, and a more pronounced decrease in acids of lower unsaturation (1, 2), the amounts of which are altered temperature-dependently by membrane-bound desaturases (3, 4). In addition, at decreasing temperatures below 26–20°C towards 10°C, the fatty acid chain length shortened, due to the increase in palmitoleic acid content (1, 2). The latter has been related to temperature regulation of the ratio of 16 to 18 carbon acids during fatty acid biosynthesis in the cytosol by the type I multienzyme complex fatty acid synthetase (FAS I) (5). Moreover, either fatty acid unsaturation or the ratio of 16 to 18 carbon acids were altered in *C. utilis* with the decreasing growth temperature range from 30 to 10°C, depending on other growth conditions, such as the amount of carbon source (5–8). On the other hand, the low growth temperature has also been observed to induce accumulation of storage triacylglycerols (TG) in yeasts and fungi (1, 9–11). As a result, despite the central role of membrane phospholipid fatty acids in temperature adaptation, it is not known to what extent the phenomena described above represent storage TG accumulation or, on the other hand, changes in phospholipids of cellular membranes for the maintenance of appropriate fluidity properties. Therefore, fatty acid compositions were determined in major lipid classes of *C. utilis* at close temperature intervals.

MATERIALS AND METHODS

The yeast, *Candida utilis* VTT-C-84147 (VTT, Technical Research Centre of Finland) was stored and culti-

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Abbreviations: DG, diacylglycerols; DUS, degree of fatty acid unsaturation; FFA, free fatty acids; NL, neutral lipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, phospholipids; PS, phosphatidylserine; r.p.m. rounds per minute; TG, triacylglycerols; VVT, Technical Research Centre of Finland.

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vated at temperatures of 10, 15, 20, 26, 35 and 40°C as described previously (1). Yeast cells were collected at the late exponential growth phase by centrifuging (5000 *g*) for 10 min at 4°C, washed with cold tap water (10 ml/<60 mg of cell dry wt), and the lipid composition was analyzed as described earlier (11). Washed cells (<60 mg of cell dry wt) were immediately resuspended in 5 ml of 80% (v/v) ethanol, and incubated at 80°C for 15 min, to inactivate lipolytic enzymes. The ethanol extract was separated by centrifuging as above, and cells were lyophilized. Duplicate samples of lyophilized cells (50 mg) were suspended in 1 ml of sodium phosphate buffer (20 mM, pH 7.0) in a microcentrifuge tube (1.5 ml) containing 1.5 g glass beads (diam. 0.425–0.6 mm, Sigma). Cells were disrupted by using a Retch mixer mill (model MM2) at 1800 r.p.m. for 15 min. Lipids were extracted with 19 ml of chloroform–methanol 2:1 (v/v) in 100-ml erlenmeyer flasks by shaking at 25°C for 3 h at 200 r.p.m. (B. Braun model Certomat WR). The cell debris was pelleted by centrifuging (5000 *g*, 10 min), the supernatant combined with the previously obtained ethanol extract, and the pooled sample was dried in a rotary evaporator. Lipid classes were separated by silica gel G thin-layer chromatography (TLC); neutral lipids were developed in petroleum ether–diethylether–acetic acid 80:30:1 (by vol.), and phospholipids in chloroform–methanol–acetic acid–water 60:35:10:5 (by vol.). Plates were sprayed with rhodamine 6GO (0.01% w/v, Chroma); lipid classes were detected under ultraviolet radiation, and identified by comparing their R_f values with those of authentic standards (Sigma). Lipid spots were scraped from thin-layer plates, and their fatty acids were immediately saponified, methylated, extracted as methyl esters, and analyzed by gas–liquid chromatography (GLC) according to a method described earlier (1). The extraction and storage of samples at −20°C occurred under a nitrogen atmosphere. Fatty acid composition was calculated as a percentage of the total peak area on a molar basis. For quantitative analysis of individual lipid classes, a known amount of heptadecanoic acid methyl ester (Sigma) was added as an internal standard prior to the fatty acid analysis. The fatty acid chain length was expressed as the molar ratio of C_{16} to C_{18} fatty acids. The degree of fatty acid unsaturation was calculated as $\Delta mol^{-1} = \Sigma[n \times (\%\text{ fatty acids containing n double bonds})/100]$. All values are the means of two independent determinations.

Chemicals

The components of growth media and chemicals used were purchased from Merck unless otherwise stated.

RESULTS

Lipid composition

In *C. utilis,* the content of saponifiable fatty acids altered between 6.0 and 7.1% of the cell dry weight within the growth temperature from 40 to 10°C (1), and consisted mainly of palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic acids. The amounts of phospholipids (PL) and neutral lipids (NL) varied between 65.1–78.4% (43.1–55.6 mg/g dry wt) and 21.6–34.9% (15.3–23.1 mg/g dry wt), respectively, over the temperature range 40 to 10°C (**Table 1**). The major PL classes were phosphatidylcholine (PC, 17.0–25.7 mg/g dry wt), phosphatidylethanolamine (PE, 11.3–16.1 mg/g dry wt), and the combined phosphatidylinositol and phosphatidylserine fraction (PI + PS, 6.1–13.4 mg/g dry wt); cardiolipin was detected in amounts less than 4.9 mg/g dry wt. NL mainly consisted of triacylglycerols (TG, 3.1–13.7 mg/g dry wt), diacylglycerols (DG, 2.2–8.2 mg/g dry wt), and free fatty acids (FFA, 5.5–11.6

TABLE 1. Effect of growth temperature on amounts of phospholipids, neutral lipids, and individual lipid classes in *C. utilis*

	Phospholipids				Neutral Lipids				
Temperature	PC	PE	PI + PS	PL	TG	DG	FFA	NL	Total Fatty Acids
°*C*	*mg/g dry wt (% total fatty acids)*				*mg/g dry wt (% total fatty acids)*				*mg/g dry wt*
10	21.6 (32.7)	11.3 (17.1)	6.1 (9.2)	43.1 (65.1)	13.7 (20.7)	2.2 (3.4)	7.2 (10.8)	23.1 (34.9)	66.2
15	17.0 (25.8)	16.1 (24.4)	10.7 (16.2)	45.3 (68.7)	6.0 (9.2)	8.2 (12.4)	6.4 (9.8)	20.6 (31.3)	65.9
20	24.8 (37.8)	11.5 (17.5)	7.0 (10.6)	48.2 (73.4)	3.3 (5.0)	2.7 (4.0)	11.6 (17.6)	17.5 (26.6)	65.7
26	18.0 (29.9)	15.0 (24.9)	10.0 (16.6)	44.3 (73.5)	3.1 (5.1)	5.1 (8.5)	7.8 (12.9)	16.0 (26.5)	60.3
35	25.7 (36.3)	13.6 (19.2)	12.3 (17.3)	55.6 (78.4)	4.3 (6.1)	5.6 (7.8)	5.5 (7.7)	15.3 (21.6)	70.9
40	23.0 (32.4)	11.7 (16.4)	13.4 (18.9)	52.5 (73.9)	4.1 (5.1)	8.0 (11.2)	6.5 (9.1)	18.5 (26.1)	71.0

The standard deviation in parallel samples (n = 2) was ±2.2 mg/g dry weight (±4.2%) for individual lipid classes. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI + PS, combined phosphatidylinositol and phosphatidylserine; TG, triacylglycerols; DG, diacylglycerols; FFA, free fatty acids; PL, phospholipids; NL, neutral lipids.

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TABLE 2. Effect of growth temperature on fatty acid composition of total lipids, phospholipids, and individual phospholipid classes in *C. utilis*

Lipid Group	Temperature	16:0	18:0	16:1	18:1	18:2	18:3	C_{16}/C_{18}	DUS
	°C	*mg/g dry wt (% total fatty acids)*						*mol ratio*	*Δmol^{-1}*
Phospholipids	10	6.2 (14.4)	0.2 (0.5)	6.9 (16.0)	8.1 (18.8)	10.6 (24.7)	11.0 (25.6)	0.4	1.6
	15	5.9 (13.0)	0.1 (0.2)	4.3 (9.5)	10.4 (22.9)	14.6 (32.2)	10.1 (22.2)	0.3	1.6
	20	6.5 (13.5)	0.1 (0.2)	2.8 (5.8)	12.8 (26.5)	15.4 (31.9)	10.7 (22.2)	0.2	1.6
	26	6.1 (13.7)	0.3 (0.7)	1.6 (3.7)	10.4 (23.5)	16.7 (37.7)	9.1 (20.6)	0.2	1.6
	35	10.7 (19.3)	0.6 (1.1)	0.9 (1.6)	12.4 (22.4)	23.9 (43.1)	6.9 (12.5)	0.3	1.5
	40	10.8 (20.6)	0.5 (1.0)	1.1 (2.1)	13.7 (26.1)	24.0 (45.8)	2.3 (4.4)	0.3	1.3
Phosphatidylcholine	10	1.3 (5.9)	0.2 (0.9)	4.2 (19.5)	2.6 (12.2)	5.0 (23.1)	8.3 (38.4)	0.3	1.9
	15	1.0 (5.8)	0.1 (0.8)	1.8 (10.7)	3.2 (18.5)	5.4 (31.5)	5.6 (32.8)	0.2	1.9
	20	2.2 (8.8)	0.1 (0.5)	1.4 (5.7)	6.0 (24.2)	8.3 (33.5)	6.8 (27.3)	0.2	1.8
	26	1.4 (7.8)	0.4 (2.0)	0.6 (3.4)	3.6 (20.1)	7.2 (39.9)	4.8 (26.9)	0.1	1.8
	35	3.5 (13.6)	0.6 (2.5)	0.4 (1.7)	4.9 (19.2)	12.6 (49.1)	3.6 (14.0)	0.2	1.6
	40	3.4 (14.9)	0.5 (2.0)	0.5 (2.3)	6.0 (26.0)	11.6 (50.6)	1.0 (4.2)	0.2	1.4
Phosphatidylethanolamine	10	1.2 (10.9)	tr	2.0 (17.5)	2.2 (19.7)	3.8 (33.7)	2.1 (18.2)	0.4	1.6
	15	1.0 (6.1)	tr	2.0 (12.1)	3.0 (18.6)	6.0 (37.4)	4.2 (25.8)	0.2	1.8
	20	0.8 (6.9)	tr	0.9 (8.0)	2.9 (25.2)	4.5 (38.9)	2.4 (21.0)	0.2	1.7
	26	1.0 (6.6)	tr	0.8 (5.2)	2.8 (18.4)	6.6 (44.3)	3.8 (25.4)	0.1	1.9
	35	1.2 (9.0)	tr	0.4 (2.8)	2.8 (20.6)	7.2 (52.6)	2.1 (15.1)	0.1	1.7
	40	1.2 (9.9)	tr	0.5 (4.2)	2.5 (21.8)	6.9 (59.1)	0.6 (4.9)	0.2	1.6
Phosphatidylinositol and phosphatidylserine	10	2.8 (46.3)	0.1 (1.2)	0.3 (5.6)	1.5 (25.4)	1.2 (20.1)	0.1 (1.5)	1.1	0.7
	15	3.3 (31.1)	0.1 (0.6)	0.4 (3.6)	3.7 (34.4)	2.7 (25.5)	0.5 (4.8)	0.6	0.9
	20	3.0 (42.2)	0.1 (1.3)	0.2 (3.0)	2.3 (32.7)	1.3 (18.2)	0.2 (2.5)	0.9	0.8
	26	3.1 (31.3)	0.2 (2.0)	0.2 (2.3)	3.5 (34.5)	2.6 (25.6)	0.4 (4.3)	0.6	0.9
	35	5.5 (44.4)	0.3 (2.4)	0.1 (0.8)	3.5 (28.5)	2.8 (22.7)	0.1 (1.2)	0.9	0.8
	40	5.9 (43.8)	0.3 (2.2)	0.1 (0.7)	4.2 (31.3)	2.9 (21.3)	0.1 (0.7)	0.9	0.8

The standard deviation in parallel samples (n = 2) was ±0.4 mg/g dry weight (±1.0%) for the fatty acid analysis. 16:0, palmitic acid; 18:0, stearic acid; 16:1, palmitoleic acid; 18:1, oleic acid; 18:2, linoleic acid, 18:3, linolenic acid; C_{16}/C_{18}, ratio of sixteen carbon to eighteen carbon fatty acids; DUS, degree of fatty acid unsaturation; tr, trace.

mg/g dry wt). In addition, small amounts of lysophosphatidylcholine and sterol esters were detected.

Fatty acid profiles of major membrane PL classes, PC and PE, were similar, in contrast to that of PI + PS (**Table 2**). In PC and PE, 16-carbon palmitic (PC, 5.8–14.9%; PE, 6.1–10.9%) and palmitoleic (PC, 1.7–19.5%; PE, 2.8–17.5%) acids, and 18-carbon oleic acid (PC, 12.2–26.0%; PE, 18.4–25.2%) were present in nearly equal proportions. However, the relative amount of linoleic acid (PC, 23.1–50.6%; PE, 33.7–59.1%) was lower, and that of linolenic acid (PC, 4.2–38.4%; PE, 4.9–25.8%) higher in PC than in PE. In contrast, in PI + PS, the proportions of palmitic (31.1–46.3%) and oleic (25.4–34.5%) acids were higher, and that of palmitoleic acid (<5.6%) lower than in PC and PE. The relative amounts of polyunsaturated linoleic (18.2–25.6%) and linolenic (<4.8%) acids were the lowest in PI + PS. The proportions of major fatty acids were almost the same in NL classes TG and DG (**Table 3**). In contrast, the fatty acid profile of the FFA pool was similar to that of PC; that is, relative amounts of palmitic and oleic acids were lower, and those of linoleic and linolenic acids higher than in TG and DG. In the PL, fatty acid unsaturation was highest in PC and PE, and lowest in PI + PS, whereas the mean fatty acid chain length was the shortest in PI + PS. In NL, the overall fatty acid chain length was greater, and unsaturation higher in the FFA pool than in acids esterified to TG and DG.

Effect of temperature on lipid composition

The lipid content decreased in *C. utilis* when the growth temperature was reduced to 26–20°C, but increased at temperatures below that (Table 1). This was due to the decrease in PL with PI + PS over the entire decreasing growth temperature range, whereas at reduced temperatures below 26–20°C a more pronounced increase occurred in NL, because of an increase in TG.

In PL, temperature-dependent changes in fatty acids esterified to PC and PE were similar (Table 2). Within the upper decreasing growth temperature range (40 to 26–20°C) fatty acid unsaturation increased, due to the increase in linolenic acid, whereas fatty acids with lower degree of unsaturation decreased or remained unaltered. At the same time, within the lower decreasing growth temperature range (26–20°C towards 10°C) the fatty acid chain length shortened, as 16-carbon palmitoleic acid increased and 18-carbon unsaturated fatty acids decreased or remained unaltered, except that linolenic acid increased in PC. In PI + PS, the amount of linolenic acid was the highest at 26–20°C, and that of palmitoleic acid increased on decreasing the temperature towards 10°C, whereas a more pronounced de-

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TABLE 3. Effect of temperature on fatty acid composition of neutral lipids and individual neutral lipid classes

Lipid Group	Temperature	16:0	18:0	16:1	18:1	18:2	18:3	C_{16}/C_{18}	DUS
	°C	mg/g dry wt (% total fatty acids)						mol ratio	Δmol^{-1}
Total lipids	10	11.5 (17.3)	0.8 (1.2)	10.3 (15.5)	14.2 (21.7)	14.4 (21.7)	15.1 (22.8)	0.5	1.5
	15	9.1 (13.8)	0.5 (0.8)	6.0 (9.1)	15.8 (24.0)	20.4 (31.0)	14.1 (21.4)	0.3	1.6
	20	8.8 (13.4)	0.4 (0.6)	3.8 (5.8)	17.3 (26.3)	21.0 (31.9)	14.6 (22.2)	0.2	1.6
	26	8.7 (14.4)	1.0 (1.7)	2.8 (4.1)	14.5 (24.0)	21.8 (36.1)	11.9 (19.7)	0.2	1.6
	35	14.0 (19.7)	1.4 (2.0)	1.2 (1.7)	17.4 (24.5)	28.9 (40.8)	8.0 (11.3)	0.3	1.4
	40	15.0 (21.2)	1.2 (1.7)	1.6 (2.3)	19.8 (28.0)	30.0 (42.4)	3.2 (4.5)	0.3	1.3
Neutral lipids	10	5.3 (22.7)	0.6 (2.6)	3.4 (14.6)	6.1 (26.2)	3.8 (16.3)	4.1 (17.6)	0.6	1.3
	15	3.2 (15.6)	0.4 (2.0)	1.7 (8.3)	5.4 (26.3)	5.8 (28.3)	4.0 (19.5)	0.2	1.5
	20	2.3 (13.1)	0.3 (1.7)	1.0 (5.7)	4.5 (25.6)	5.6 (31.8)	3.9 (22.2)	0.2	1.6
	26	2.5 (15.8)	0.6 (3.6)	0.8 (5.1)	4.0 (25.0)	5.2 (32.7)	2.9 (17.9)	0.3	1.5
	35	3.3 (21.3)	0.8 (5.2)	0.3 (1.9)	5.0 (32.3)	5.0 (32.3)	1.1 (7.1)	0.3	1.2
	40	4.2 (22.8)	0.7 (3.8)	0.5 (2.7)	6.1 (32.6)	6.0 (32.6)	0.9 (4.9)	0.3	1.2
Triacylglycerols	10	3.6 (26.6)	0.4 (2.7)	2.0 (14.2)	4.4 (31.9)	2.1 (15.1)	1.3 (9.5)	0.7	1.1
	15	1.3 (21.8)	0.2 (3.9)	0.5 (8.4)	1.8 (30.2)	1.3 (22.2)	0.8 (13.3)	0.4	1.2
	20	0.8 (25.1)	0.1 (4.0)	0.1 (4.0)	1.5 (46.0)	0.6 (16.9)	0.1 (4.0)	0.4	1.0
	26	0.7 (23.9)	0.5 (15.8)	0.1 (4.5)	0.9 (27.5)	0.7 (23.9)	0.1 (4.5)	0.4	0.9
	35	0.9 (20.3)	0.6 (14.0)	0.2 (5.2)	1.7 (39.9)	0.7 (15.4)	0.2 (5.2)	0.3	0.9
	40	1.0 (24.3)	0.2 (4.8)	0.1 (2.9)	1.3 (32.5)	1.3 (33.1)	0.1 (2.4)	0.4	1.1
Diacylglycerols	10	0.8 (34.0)	0.2 (7.3)	0.3 (13.7)	0.7 (30.3)	0.2 (7.3)	0.2 (7.3)	0.9	0.8
	15	1.5 (18.1)	0.2 (2.1)	0.7 (8.1)	2.2 (26.7)	2.3 (28.7)	1.3 (16.2)	0.4	1.4
	20	0.6 (23.7)	0.2 (6.2)	0.3 (10.8)	0.9 (35.0)	0.5 (18.0)	0.2 (6.2)	0.5	1.0
	26	1.0 (20.5)	0.1 (2.1)	0.2 (3.0)	1.5 (30.0)	1.6 (30.4)	0.7 (14.0)	0.3	1.4
	35	1.6 (28.0)	0.2 (3.8)	0.1 (2.4)	1.8 (32.0)	1.7 (31.3)	0.1 (2.4)	0.4	1.0
	40	2.0 (25.6)	0.5 (6.4)	0.4 (4.6)	2.1 (26.7)	2.3 (28.8)	0.6 (7.9)	0.4	1.1
Free fatty acids	10	0.9 (11.9)	tr	1.1 (15.9)	1.0 (14.4)	1.5 (21.1)	2.6 (36.7)	0.4	1.8
	15	0.4 (6.4)	tr	0.5 (8.0)	1.4 (21.8)	2.2 (34.1)	1.9 (29.7)	0.2	1.9
	20	0.9 (7.6)	tr	0.6 (5.0)	2.1 (17.8)	4.5 (38.9)	3.6 (30.7)	0.1	1.9
	26	0.8 (9.9)	tr	0.5 (6.2)	1.6 (21.0)	3.0 (38.1)	1.9 (24.8)	0.2	1.8
	35	0.8 (14.7)	tr	tr	1.5 (22.8)	2.6 (48.3)	0.8 (14.1)	0.2	1.6
	40	1.2 (18.4)	tr	tr	2.7 (41.5)	2.4 (37.3)	0.2 (2.8)	0.1	1.3

Abbreviations and standard deviations as in Table 2.

crease occurred in contents of the other acids and, thus, the amount of PI + PS decreased on reducing the temperature to 10°C. As a result, fatty acid unsaturation increased in phospholipids when the growth temperature was lowered to 26–20°C, while at reduced temperatures below 26–20°C towards 10°C the fatty acid chain length shortened.

In NL classes, TG, DG and FFA, temperature-dependent changes in fatty acids were similar to those of PL, although more fluctuation occurred (Table 3). The proportion of linolenic acid increased at lowered temperatures to 26–20°C, and that of palmitoleic acid towards 10°C, whereas other acids may decrease. Consequently, the fatty acid chain length shortened when the temperature decreased, and fatty acid unsaturation was the highest at 26–20°C.

DISCUSSION

The data presented show that *C. utilis* can adapt its cellular membranes to decreases in the environmental temperature so that fatty acid unsaturation increases in major membrane phospholipid classes, PC and PE, down to 26–20°C, whereas at temperatures below that the fatty acid chain length also shortens. This was done in such a manner that biosynthesis of linolenic acid, via oleic and linoleic acids (12), increased at decreased temperatures to 26–20°C, concomitantly with an equal decrease in palmitic and linoleic acids (Table 2). Similarly, the linolenic acid content increased in *Lipomyces starkeyi* within the higher decreasing growth temperature range from 30 to 20°C, whereas fatty acids of the lower unsaturation decreased (11). In addition, at lowered temperatures below 26–20°C the amount of palmitoleic acid and the sum of contents of palmitic and palmitoleic acids increased in PC and PE of *C. utilis,* while 18-carbon unsaturated fatty acids decreased proportionally (Table 2). This is in accord with the reported increase in biosynthesis of palmitic acid, the precursor of palmitoleic acid, by FAS I at low growth temperatures (5). Therefore, within the high growth temperature range above 26–20°C desaturases forming polyunsaturated linolenic acid were mainly affected, whereas at temperatures below that there was also a significant increase in biosynthesis of 16-carbon palmitic acid by FAS I, and its desaturation to palmitoleic acid. In earlier

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studies on non-oleaginous *C. utilis* (13), the amount of linolenic acid increased at reduced growth temperatures under the high oxygen tension and in carbon-limited continuous culture, whereas the proportion of palmitoleic acid increased and, thus, the fatty acid chain length shortened in nitrogen-limited continuous culture and after the temperature shift (6–8). Thus, despite the fact that the latter changes in fatty acids were reported to occur in total lipids, it is tempting to assume that they at least partially represent changes occurring in membrane phospholipids, similarly as in this study (Table 2). Therefore, regulation of the degree of fatty acid unsaturation by desaturases forming linolenic acid, and the amount of palmitic acid by FAS I, and its further desaturation to palmitoleic acid may represent alternative routes in *C. utilis* for the maintenance of physicochemical properties of membrane lipids in the temperature adaptation. In addition to this, as the third mode of response, the relative contributions of PC and PE containing palmitoleic and linoleic acids increased in *L. starkeyi* on lowering the temperature from 20 to 10°C (1, 11).

The lipid-related responses to alterations in the environmental temperature seemed to be more complicated in *C. utilis* than generally thought for yeasts. The increase in fatty acid unsaturation at decreasing temperatures above 26–20°C, due to the increase in the lowest melting point linolenic acid (m.p. −11.3 to −12.8°C), is in accord with the previous understanding on fatty acid-related temperature adaptation (2). However, when the temperature was reduced below 26–20°C the increase in palmitoleic acid (m.p. 0°C) increased the melting point of fatty acids in PC and PE from those of linoleic (m.p. −6.5 to −5.0°C) and linolenic acids. Measurements of melting points of fatty acids and gel to liquid-crystalline phase transition temperatures of PL have shown that the replacement of a fatty acid in PL often changes the phase transition temperature of PL in the same direction as the melting point of fatty acid is changed (2). In addition, concomitant with the increase in fatty acid unsaturation on reducing the temperature, the fatty acid chain length was also shortened at lowering temperatures below 26–20°C. This change cannot be related to the increasing oxygen solubility, and thus desaturation efficiency on lowering the temperature, or to decreasing growth rate, which is highest at 35°C. (1). Despite this, effects of oxygen solubility and the growth rate may not be ignored, and the increase in TG content and concomitant decrease in PI + PS, observed on approaching 10°C, may be at least partially due to the decrease in the growth rate (14). Similarly, the degree of fatty acid unsaturation seemed to be temperature regulated by oxygen-dependent desaturases under the high oxygen tension, whereas the chain length was altered when oxygen tension was low (6–8). In conclusion, several alternative lipid-related responses possibly occur in yeasts for the adaptation to changes in the environmental temperature. The one in use may depend on other growth conditions, such as the amount of soluble oxygen, carbon or nitrogen source.

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