The Effect of Temperature on Lipid Classes and Their Fatty Acid Profiles in *Lipomyces starkeyi*

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ABSTRACT: The effect of environmental temperature on fatty acid contents of major lipid classes was determined in *Lipomyces starkeyi* at 30, 20, and 10°C. When the temperature was reduced from 30 to 20°C, the linolenic acid content increased in phosphatidylcholine but fell with further reduction to 10°C. The relative contribution of phosphatidylcholine and phosphatidylethanolamine that contained the next lowest-melting fatty acids, palmitoleic and linoleic acids, increased on lowering the temperature from 20 to 10°C and, concomitantly, the combined phosphatidylinositol and phosphatidylserine fraction decreased, and triacylglycerols were accumulated.
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KEY WORDS: Fatty acids, *Lipomyces starkeyi*, neutral lipids, phospholipids, temperature.

Lipomyces starkeyi can accumulate large amounts of lipids in intracellular oil globules, depending on factors such as NH_4^+, K^+, Ca^{2+}, Zn^{2+}, Fe^{3+}, and Mn^{2+} concentrations, the amount of carbon source, growth rate, and temperature (1–5). The optimal temperature for lipid accumulation was 25.5–29.5°C (4). However, the low growth temperature also induces triacylglycerol (TG) biosynthesis and affects its fatty acid profile (6,7). On the other hand, when the environmental temperature changes, the fatty acid composition of phospholipids is also altered, to maintain membrane physicochemical properties (8,9). In cellular lipids of *L. starkeyi*, fatty acid unsaturation increased on reducing the temperature to 20–15°C, due mainly to the increase in linolenic acid. Further reduction in the temperature to 10°C increased amounts of other acids, whereas linolenic acid content and, consequently, fatty acid unsaturation fell (6). To clarify whether these changes in fatty acids occurred in the phospholipids and thus represented temperature adaptation related to cellular membranes, or because of TG accumulation, fatty acid profiles of individual lipid classes were determined in *L. starkeyi* at 30, 20, and 10°C.

The yeast, *L. starkeyi* DSM 70295 (DSM, German Collection of Microorganisms and Cell Cultures; Braunschweig, Germany), was stored at –60°C and cultivated at temperatures of 10, 20, and 30°C as described previously (6). The late exponential growth phase cells were separated, washed with tap water, resuspended in 5 mL of 80% (vol/vol) ethanol, and incubated at 80°C for 15 min, to inactivate lipolytic enzymes (10). The ethanol extract was separated and the cells were lyophilized. These cells (50 mg) and 1.5-g glass beads (diameter 0.425–0.6 mm; Sigma; St. Louis, MO) were solubilized in 1 mL sodium phosphate buffer (20 mM, pH 7.0) in a 1.5-mL microcentrifuge tube, and cells were disrupted by the Retch mixer mill (model MM2; Haan, Germany; 1800 rpm; 15 min). Lipids were extracted in 19 mL of chloroform/methanol (2:1, vol/vol) by shaking at 25°C for 3 h at 200 rpm. The extract was separated, combined with the ethanol extract, and dried in a rotary evaporator (Rotavapor RE 111; Büchi; Slawil, Switzerland). Extractions and storage of lipid samples occurred under nitrogen atmosphere. Neutral lipids (NL) (petroleum spirit/diethylether/acetic acid; 80:30:1, vol/vol/vol) and phospholipids (PL) (chloroform/methanol/acetic acid/water; 60:35:10:5, by vol) were separated by silica gel G thin-layer chromatography. Lipid classes were detected under ultraviolet radiation after spraying with rhodamine 6GO (0.01% wt/vol; Chroma; Köngen/Neckar, Germany), and identified by comparing their R_f values to those of standards (Sigma). Lipid spots were removed, an internal standard (heptadecanoate; Sigma) was added, and fatty acids were analyzed by gas–liquid chromatography as described earlier (6). All values are the means of two independent determinations.

Lipid composition. In *L. starkeyi*, the fatty acid content accounts for 7–14% of the cell dry weight and mainly consists of palmitic, palmitoleic, oleic, linoleic, and linolenic acids (6). Their amounts in PL and NL were 27.3–42.3 mg/g dry weight (19.7–56.7%) and 32.0–111.5 mg/g dry weight (42.9–80.3%), respectively (Table 1). The major PL classes of *L. starkeyi* (11)—phosphatidylcholine (PC), phosphatidylethanolamine (PE), and the combined phosphatidylinositol and phosphatidylserine fraction (PI + PS)—were present in nearly equal amounts, whereas cardiolipin and lysophosphatidylcholine accounted for less than 2.2 mg/g dry wt (3.1%). NL mainly consisted of TG and free fatty acids (FFA). In addition, diacylglycerols (DG) and sterol esters were detected. Within the lipid classes, the proportion of 16-carbon palmitic acid generally increased at all temperatures in the order: PC < FFA < PE < TG < PI + PS, whereas palmitoleic acid was present nearly in equal amounts in all subgroups (Table 2). The relative amount of 18-

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TABLE 1
The Effect of Growth Temperature on Lipid Classes of *Lipomyces starkeyi*[a]

Temperature (°C)	Phospholipids (mg/g dry wt)				Neutral lipids (mg/g dry wt)				Total lipids (mg/g dry wt)
	PC	PE	PI + PS	PL	TG	DG	FFA	NL	
10	7.9 (5.7)	11.1 (8.0)	6.4 (4.6)	27.3 (19.7)	90.8 (65.4)	1.7 (1.2)	19.1 (13.7)	111.5 (80.3)	138.8
20	13.7 (17.1)	10.4 (12.8)	10.5 (13.1)	35.8 (44.5)	31.2 (38.8)	3.8 (4.7)	9.7 (12.0)	44.6 (55.5)	80.4
30	16.4 (21.9)	10.9 (14.6)	12.0 (16.1)	42.3 (56.7)	25.3 (33.8)	2.3 (3.1)	4.5 (6.0)	32.0 (42.9)	74.6

[a]PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PL, phospholipids; TG, triacylglycerols; DG, diacylglycerols; FFA, free fatty acids; NL, neutral lipids; standard phosphatidylinositol deviation (n = 2) 2.2 mg/g dry weight, 4.2%; numbers in parentheses indicate % total lipids.

TABLE 2
The Effect of Temperature on the Fatty Acid Composition of Individual Lipid classes in *Lipomyces starkeyi*[a]

Lipid group	Temperature (°C)	16:0[b]	16:1[b]	18:1[b]	18:2[b]	18:3[b]	C_{16}/C_{18}	DUS (Δmol^{-1})
Total lipids	10	26.0 (18.9)	11.3 (8.2)	64.9 (47.0)	29.6 (21.4)	1.6 (1.2)	0.37	1.02
	20	18.9 (24.1)	3.1 (3.9)	29.6 (37.8)	16.0 (20.6)	6.3 (8.0)	0.38	1.07
	30	24.9 (34.3)	3.6 (4.9)	20.3 (27.9)	20.7 (28.1)	0.4 (0.6)	0.65	0.91
Phospholipids	10	3.5 (13.2)	3.2 (12.1)	9.2 (34.7)	10.2 (38.4)	0.2 (0.6)	0.34	1.25
	20	8.9 (25.2)	1.4 (4.0)	12.0 (33.7)	9.5 (26.7)	3.4 (9.5)	0.42	1.20
	30	11.7 (28.7)	2.3 (5.7)	10.4 (25.6)	15.5 (38.0)	0.4 (0.9)	0.53	1.10
Neutral lipids	10	22.5 (20.2)	8.1 (7.3)	55.7 (49.9)	19.3 (17.3)	1.5 (1.3)	0.38	0.96
	20	10.0 (22.3)	1.6 (3.7)	19.0 (42.5)	7.0 (15.8)	2.9 (6.5)	0.36	0.97
	30	13.3 (41.3)	1.2 (3.9)	9.9 (30.9)	5.2 (15.6)	0.1 (0.1)	0.81	0.70
Phosphatidylcholine	10	0.4 (5.0)	0.9 (11.3)	2.0 (24.9)	4.4 (54.9)	0.2 (2.0)	0.20	1.52
	20	1.3 (9.5)	0.6 (4.5)	3.4 (24.7)	4.9 (35.7)	3.3 (24.0)	0.16	1.73
	30	2.4 (14.4)	1.1 (6.4)	3.1 (19.0)	9.2 (56.1)	0.4 (2.3)	0.26	1.45
Phosphatidylethanolamine	10	1.3(11.7)	1.3 (11.7)	4.4 (39.9)	4.0 (35.6)	trace	0.31	1.23
	20	2.0 (19.2)	0.6 (5.6)	4.8 (46.5)	2.9 (27.7)	trace	0.33	1.08
	30	2.1 (19.5)	0.9 (8.6)	3.9 (36.0)	3.8 (34.8)	trace	0.40	1.14
Phosphatidylinositol and phosphatidylserine	10	1.7 (27.0)	0.8 (12.5)	2.5 (39.8)	1.3 (20.7)	trace	0.65	0.94
	20	5.4 (51.2)	0.2 (1.5)	3.6 (33.8)	1.4 (13.5)	trace	1.11	0.62
	30	6.8 (56.9)	0.2 (1.7)	3.0 (25.1)	2.0 (16.2)	trace	1.42	0.59
Triacylglycerols	10	21.0 (23.1)	6.2 (6.8)	50.3 (55.4)	8.0 (8.8)	1.5 (1.6)	0.43	0.85
	20	7.0 (22.5)	0.9 (2.8)	15.3 (48.9)	3.8 (12.2)	1.1 (3.6)	0.34	0.87
	30	10.9 (43.1)	0.9 (3.5)	8.0 (31.6)	3.3 (12.9)	0.1 (0.1)	0.89	0.61
Free fatty acids	10	1.1 (5.7)	1.8 (9.3)	4.8 (24.9)	11.1 (58.3)	0.1 (0.1)	0.18	1.51
	20	1.6 (16.1)	0.8 (7.8)	2.3 (23.9)	2.7 (27.7)	1.8 (18.5)	0.32	1.43
	30	1.1 (24.6)	0.4 (8.1)	1.1 (23.9)	1.8 (39.1)	0.1 (0.1)	0.49	1.11

[a]16:0, Palmitic acid; 16:1, palmitoleic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid; C_{16}/C_{18}, the ratio of sixteen to eighteen carbon fatty acids; DUS, the degree of fatty acid unsaturation; $\Delta mol^{-1} = \Sigma[n \times (\%$ fatty acids containing n double bonds)/100]; standard deviation (n = 2) 0.4 mg/g dry wt, 1.0%; numbers in parentheses indicate % total lipids.
[b]mg/g dry wt.

carbon monoene, oleic acid, increased in lipids in the order: PC, FFA < PI + PS < PE, TG, while the proportion of linoleic acid altered as follows: TG < PI + PS < PE < FFA, PC. Linolenic acid was present in significant amounts only in the PL classes in PC, and in the NL classes in TG and FFA. As a result, fatty acid chainlength and unsaturation were the greatest in PC and FFA, intermediate in PE, and the lowest values occurred in PI + PS and TG. Therefore, the highest amount of the most unsaturated and lowest-melting fatty acids occurred in major membrane PL classes, PC and PE, whereas saturated palmitic acid and, consequently, the shortest fatty acids, were present in PI + PS. Generally, desaturases are known to use either coenzyme A (CoA)- or PL-bound fatty acids as substrates (12,13). In *Mortierella ramanniana* var. *angulispora*, the desaturase used PC-bound linoleic acid as a substrate for the formation of linolenic acid. Thereafter, linolenic acid was removed by phospholipase and incorporated into DG and TG (13). In *L. starkeyi*, the same mechanism may occur due to the presence of linolenic acid only in PC. This mechanism is further supported by the fact that in *L. starkeyi* the accumulated TG contained mainly palmitic and oleic acids

(Table 2). Such fatty acids are typically accumulated to TG that are synthesized as CoA derivatives before their transacylation to phosphatidic acid, which is the precursor of TG *via* DG before the biosynthesis of major PL (12,14,15).

The effect of temperature on lipid composition. When the environmental temperature was lowered, the lipid content increased in *L. starkeyi*, due to TG accumulation and concomitant increase in FFA, whereas PL decreased to a lesser extent with PC and PI + PS (Table 1). Similarly, and earlier, the increase in TG content has been related to the reduction in PI + PS, and the growth rate (3,16). The latter decreases on lowering the temperature (6). At decreased temperature to 10°C, the TG content of those containing palmitic and oleic acids mainly increased, although levels of palmitoleic, linoleic, and linolenic acids also increased (Table 2). In PL, the amount of PC esterified with linolenic acid increased within the decreasing growth temperature range from 30 to 20°C, whereas a more pronounced decrease occurred in palmitic or linoleic acid-containing PL. Consequenly, when the temperature decreased to 20°C, the proportion of oleic acid increased in all PL, as did linolenic acid in PC, whereas other acids would decrease. Instead, at reduced temperatures below 20°C to 10°C, PL classes that contained palmitic, oleic, or linolenic acids decreased and the proportions of palmitoleic and linoleic acids increased. Thus, the degree of fatty acid unsaturation increased in PC when the growth temperature was reduced from 30 to 20°C, and in PE and PI + PS from 20 to 10°C. The reduced desaturation of linoleic to linolenic acid at lowered temperatures from 20–15 to 10°C (6) (Table 2) could be a result of the decrease in the activity of a desaturase, as has been observed to occur for stearoyl-CoA desaturase in *Fusarium oxysporum* (17). The decrease in linolenic acid was caused by the increase in the next lowest-melting fatty acids. However, the reduction in the desaturation of linoleic to linolenic acid at 10°C does not necessarily need to be the determinant of the lower growth temperature extreme of *L. starkeyi*, the value of which is as low as 5.4°C (6).

The results presented show that, within the higher decreasing growth temperature range from 30 to 20°C, membrane physicochemical properties were maintained in *L. starkeyi* by increasing the linolenic acid content in PC and, concomitantly, PL with less unsaturated fatty acids was reduced. However, when the temperature was further reduced to 10°C, the efficiency to desaturate linoleic acid to linolenic acid fell, and instead, the relative contribution of PL containing the next lowest-melting fatty acids, palmitoleic and linoleic acids, increased. In addition, TG accumulation was induced when the environmental temperature decreased to 10°C, in connection with the decrease in PI + PS and the growth rate. Therefore, *L. starkeyi* maintained membrane physicochemical properties by different changes in PL and their fatty acids within the high and low growth temperature ranges, as has been oberved to occur in several bacteria (9). In addition, TG accumulation was clearly induced by the lowered growth temperature.

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