Temperature Shifts in Regulation of Lipids Accumulated by *Lipomyces starkeyi*

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The effect of temperature on the accumulating triglycerides of *Lipomyces starkeyi* was studied in 10-L fermentation experiments. The temperature during the growth, lipid accumulation and postaccumulation phases was altered. The cellular lipid content, the glucose conversion efficiency and the specific lipid production rate were highest when the growth phase temperature was 28°C, instead of 16–18°C. The temperature of the accumulation phase had an influence on the lipid quality at the end of the accumulation. Oleic acid content increased from 52 to over 60% when the accumulation phase temperature was decreased from 28 to 15°C and, concomitantly, palmitic acid decreased from 33 to 26%. The degree of fatty acid unsaturation was the highest (0.75 Δmol^{-1}) when the accumulation phase temperature was the lowest (15°C) and *vice versa*. The temperature shift after the lipid accumulation phase affected neither the composition nor the amount of the accumulated lipids. In conclusion, the temperature profile for the highest lipid yield with the most desired composition should be: (i) a growth phase temperature that gives the maximum growth rate and (ii) an accumulation phase temperature that produces the desired ratio of palmitic to oleic acid.

KEY WORDS: Accumulation of lipids, lipids, *Lipomyces starkeyi*, temperature.

After cell multiplication has finished, oleaginous yeasts accumulate triglycerides upon exhaustion of nitrogen or phosphate and in excess of carbon. Consequently, the intracellular adenosine monophosphate pool is decreased, and isocitrate dehydrogenase of the tricarboxylic acid cycle is blocked. The excess carbon flows *via* citrate to acetyl-CoA in the presence of the adenosinetriphosphate:citrate lyase (1,2), and further to fatty acids synthesized by fatty acid synthetase I. End products, saturated palmitic and stearic acids may be desaturated to palmitoleic and oleic acids before transacylation to form phosphatidylglycerol, the precursor of phospholipids and triacylglycerols (3,4). The unique fatty acid composition of microbial lipids is determined by the precise control of the chainlength, unsaturation and glyceride composition, all of which are known to be temperature-sensitive (4–8).

Oleaginous microorganisms could be an interesting alternative to conventional plant and animal lipid sources. They can accumulate lipids up to 80% of biomass relatively quickly and independently of climatic conditions, and could even use prosessing wastes and by-products as carbon sources (9,10). In addition, the unique composition of microbial lipids can be affected by several environmental factors, one of the most important being temperature (7,11). However, increased understanding of the control of fatty acid and lipid biosynthesis is needed for economical production of fats and oils of special composition for edible and industrial purposes, as well as for new areas of technology (9,11). Therefore, the regulation of the fatty acid profile and content of *Lipomyces starkeyi* was studied by changing the growth, lipid accumulation and postaccumulation temperatures.

MATERIALS AND METHODS

Strain and media. The yeast *Lipomyces starkeyi* DSM 70295 (DSM, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) was stored in 10% glycerol at −60°C. The inocula were grown on wort broth base. In fermentation experiments, the medium contained the following constituents (% wt/vol): 3 + 2 D-glucose, 0.01 $CaCl_2 \cdot H_2O$, 0.05 NH_4Cl, 0.15 yeast extract (Difco Labs, Detroit, MI), 0.7 KN_2PO_4, 0.25 $Na_2HPO_4 \cdot 2H_2O$, 0.15 $MgSO_4 \cdot 7H_2O$, 0.008 $FeCl_3 \cdot 6H_2O$, 0.001 $ZnSO_4 \cdot 7H_2O$, 0.000007 $MnSO_4 \cdot H_2O$, 0.00001 $CuSO_4 \cdot 5H_2O$, and 0.0000063 $Co(NO_3)_2$ (12).

Fermentation experiments. To prepare inocula, the yeast was grown to the late exponential growth phase (150–200 Klett units) in a 250-mL Erlenmeyer flask containing 50 mL wort broth base. A 1-mL portion of the yeast suspension was transferred into a similar shaker flask, and allowed to grow to the exponential growth phase (70–80 Klett units). This culture (5 mL) was inoculated into a 2000-mL Erlenmeyer flask containing 500 mL of the same medium, grown to the exponential growth phase and inoculated to the fermentor. The flasks were shaken at 240 rpm (28°C) in a Gallenkamp oribital shaker/incubator (model INR 200 010V).

The fermentation experiments were carried out in a 14-L laboratory bioreactor (Biostat E; B. Braun Melsungen, Melsungen, Germany) with a working volume of 10 L. The agitation speed was controlled at 500 rpm, and aeration was maintained at 0.5 vvm. Temperature and pH values of the medium were followed by a control unit. During the cultivations, pH was kept at 5.5 by means of 6 M NaOH and 3 M H_2SO_4. The three-stage process was divided into: (1) microbial growth, (ii) lipid accumulation and (iii) postaccumulation phases; and the temperatures of the phases were altered (Table 1). The temperature shift between phases was determined from the glucose consumption. The microbial growth was stopped, and the lipid accumulation was begun when about 1% (wt/vol) of the initial glucose was consumed. The postaccumulation temperature was chosen after the consumption of the initial (3%, wt/vol) and resupplemented (2% glucose in 1 L, wt/vol) glucose. Samples were collected for the determination of the cellular dry weight, optical density, glucose concentration and the cellular fatty acid composition.

Analytical methods. The cellular dry weight was determined after washing the cells (20 mL) with tap water and drying at 105°C for 24 h. The optical density of the culture medium was measured with a Klett-Summerson colorimeter (filter no. 66). Glucose concentration was determined by the dinitrosalicylic acid method (13). The cellular fatty acid composition and content were determined after washing the cells with tap water. The yeast cells were lyophilized and weighed, an internal standard (heptadecanoic

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TABLE 1

Summary of Fermentation Strategy and Temperatures Used

Fermentation	1	2	3	4
Temperature profiles of the fermentations				
Growth temperature (°C)	28	16–18	28	18
Accumulation temperature (°C)	28	16–18	15	28
Postaccumulation temperature (°C)	10	16–18, 28	15	18
Growth phase				
Duration of the growth phase (h)	37	110	40	200
Growth rate (h^{-1})	0.158	0.036	0.131	0.056
Lipids at the end of the growth phase (g/L)	0.5	0.8	0.7	1.0
State of fermentations at the end of the accumulation phase				
Duration of the accumulation phase (h)	40	114	71	40
Cellular dry weight (g/L)	15.2	14.9	15.2	15.2
Lipids of dry weight (%)	55	40	53	44
Total lipids (g/L)	8.4	6.0	8.1	6.7
Lipids accumulated (g/L)	8.2	5.2	7.4	5.7
Biomass yield (%)	30.4	29.8	30.4	30.4
Lipid yield (%)	16.8	12.0	16.2	13.4
Biomass production rate (g/L/h)	0.1776	0.0619	0.1362	0.0908
Overall lipid production rate (g/L/h)	0.0981	0.0249	0.0726	0.0400
Lipid production rate during accumulation phase (g/L/h)	0.3139	0.0549	0.1841	0.2820
Specific lipid production rate during accumulation phase (g/g glucose/h)	0.0051	0.0011	0.0026	0.0036

acid methyl ester, Sigma Chemical Co., St. Louis, MO) was added, and the fatty acid esters were saponified and extracted as methyl esters, as described previously (8).

Calculations. The relative fatty acid composition was estimated as a percentage of the total peak area. The total lipid content of the cells was defined as the sum of cellular fatty acid methyl esters. The degree of fatty acid unsaturation (DUS) in the lipid fraction was calculated as DUS (Δmol^{-1}) = [1.0 (% monoene) + 2.0 (% diene) + 3.0 (% triene)]/100. The biomass yield (%, w/w) was defined as the cellular dry weight per glucose consumed. The lipid yield (%, w/w) was calculated as the cellular lipid content per glucose used. The biomass production rate (g/L/h) was defined as the biomass (g/L) per growth time (h), and the lipid production rate (g/L/h) as the cellular lipid content in one liter per growth time (h). The specific rate of lipid production (g/g glucose/h) was estimated as the lipid produced per glucose consumed (g/g glucose) during the accumulation time (h).

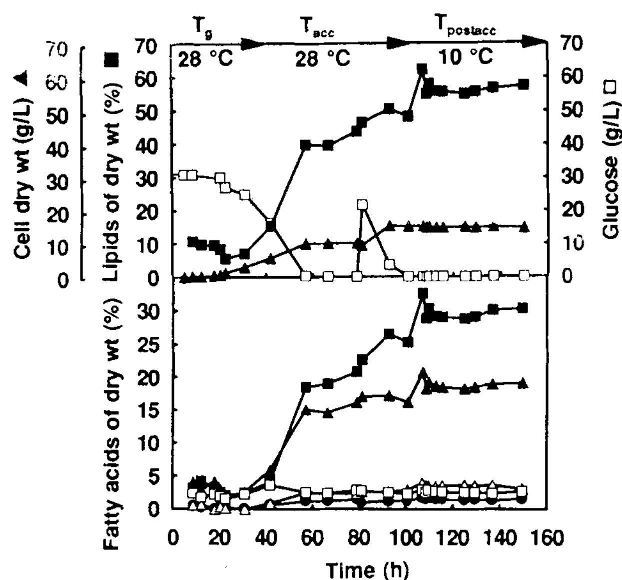
Chemicals. Components of the growth media and chemicals were obtained from Merck (Darmstadt, Germany) unless otherwise stated.

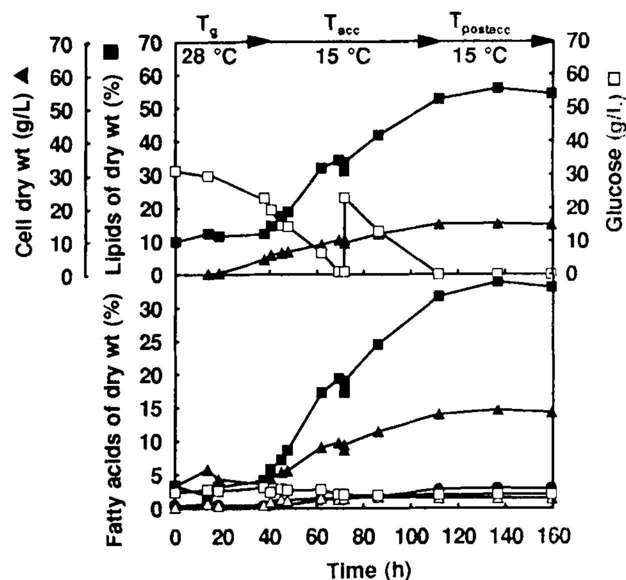
RESULTS AND DISCUSSION

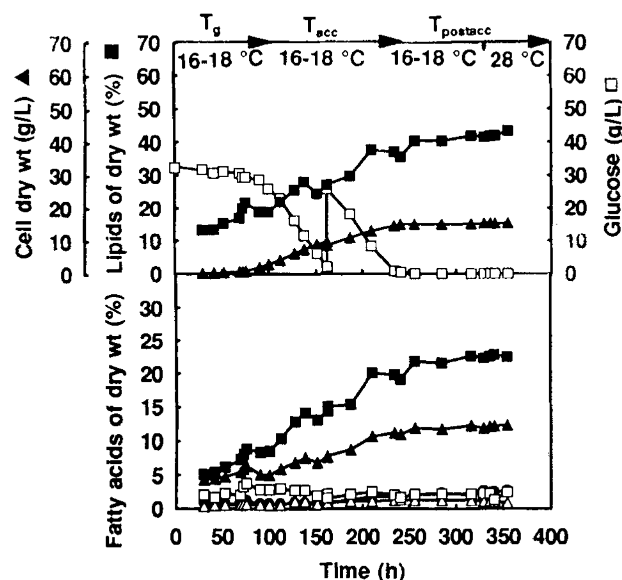
The effects of the growth phase temperature. The growth phase temperature affected the cellular fatty acid content during the phase, as well as the duration of the phase (Table 1 and Fig. 1). Total fatty acids accounted for as much as 21% of the cell dry weight at the end of microbial growth at 16–18°C, in contrast to less than 12% at 28°C. The yeast seemed to accumulate lipids even during the growth phase at 16–18°C because the fatty acid content increased up to 21% of the cellular dry weight, mainly in the form of palmitic and oleic acids, *i.e.*, the acids the yeast is known to accumulate in triglycerides (14). Reduction in the growth temperature from 28 to 16–18°C decreased the growth rate. Consequently, the duration of the growth phase increased from 37–40 h at 28°C to 100–110 h at 16–18°C.

Effects of the accumulation and postaccumulation phase temperatures. The accumulation phase temperature affected the quality of fatty acids in cellular lipids and the duration of the lipid accumulation, but had little effect on the cellular lipid content (Tables 1 and 2, Fig. 1). During lipid accumulation at 28°C, palmitic acid content increased from 2–5% of the cellular dry weight to 18%, and oleic acid content increased from 2–7 to 28%, while the other acids, *i.e*, palmitoleic, linoleic and linolenic acids, changed little with temperature. Consequently, the degree of fatty acid unsaturation decreased from 0.88 to 0.65–0.69 Δmol^{-1}. Instead, when the accumulation phase temperature was 15–18°C, palmitic acid content increased from 4–5 to 11–13% of the cellular dry weight and oleic acid from 5–9 to 20–28%, but the other acids were nearly unaffected by temperature. The degree of fatty acid unsaturation decreased from 0.89 to 0.73–0.75 Δmol^{-1}. Hence, the final fatty acid unsaturation was higher at the accumulation phase temperature of 15–18°C than at 28°C. The postaccumulation phase temperature affected neither the composition nor the content of the cellular lipids.

Effect of temperature on the state of fermentation at the end of lipid accumulation. The growth phase temperature seemed to affect the amount of lipids at the end of the accumulation phase (Table 1 and Fig. 1). The cellular lipid content varied between 40–55% of dry weight and was the highest (53–55% of dry weight) when the growth phase temperature was 28°C, in contrast to the significantly lower lipid content (40–44% of dry wt) at 16–18°C. Consequently, the amounts of total and accumulated lipids, lipid yield and the overall lipid production rate also were the highest at the growth phase temperature of 28°C. Although temperature considerably affected the cellular lipid content, the final cell dry weight and biomass yield were nearly the same in all the fermentations. Therefore, the amount of carbon used for cellular maintenance, due to the longer fermentation times at 16–18 than at 28°C,







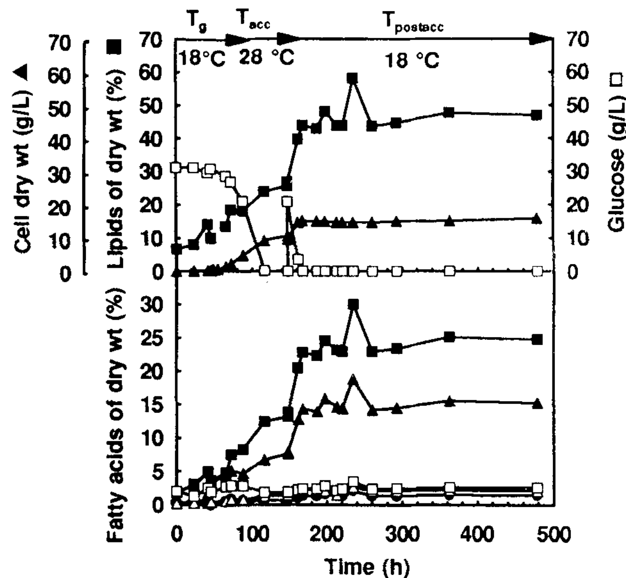


FIG. 1. The effects of growth (T_g), accumulation (T_{acc}) and postaccumulation ($T_{postacc}$) phase temperatures on the cellular dry weight (▲), lipid content (■), glucose consumption (□) and fatty acid profile of the cellular lipids: ▲, palmitic acid; ●, palmitoleic acid; △, stearic acid; ■, oleic acid; and □, linoleic acid.

can be regarded as insignificant. However, the same amount of nutrients in the fermentations might enable the use of the same amount of carbon for the growth of yeast cells, whereas significantly less carbon was used for the production of the cellular lipids at 16–18 than at 28°C. Hence, surplus carbon exists at 16–18°C, which was not used for neither cellular growth nor lipid production. The concomitant accumulation of another store material might be included at 16–18°C; for example, glycogen accumulation has been found to occur in yeasts temperature-dependently (2,15).

The accumulation phase temperature influenced lipid quality (Table 2 and Fig. 1). The degree of fatty acid unsaturation was the highest (0.75 Δmol^{-1}), and the average

TABLE 2

Proportions of Fatty Acids at the End of the Accumulation Phases

Fermentation	Growth temperature	Accumulation temperature	Relative fatty acid composition (%) 16:0	16:1	18:0	18:1	18:2	18:3	DUS[a] (Δmol^{-1})	C_{16}/C_{18}
1	28	28	33.7	2.6	6.3	52.7	4.8	—	0.65	0.569
2	16–18	16–18	29.4	5.2	3.2	53.9	5.3	2.0	0.73	0.537
3	28	15	26.5	5.4	2.9	60.5	3.8	0.5	0.75	0.471
4	18	28	32.7	2.9	5.5	51.8	5.4	0.9	0.69	0.560

[a]The degree of fatty acid unsaturation.

chainlength the longest (C_{16}/C_{18}), when the accumulation phase temperature was the lowest (15°C). Mainly, the proportions of palmitic and oleic acids could be altered by temperature. The oleic acid proportion increased from about 52 to over 60% when the accumulation temperature decreased from 28 to 15°C. Concomitantly, palmitic acid decreased from about 33 to 26%.

The duration of the lipid accumulation phase was the shortest (40 h) when the temperature was the highest (28°C) (Fig. 1 and Table 1). However, when the accumulation phase temperature was 15–18°C, lipid accumulation (71 h) occurred significantly faster with a growth phase temperature of 28°C than with a temperature of 16–18°C (114 h). The biomass production rate, as well as lipid production and specific lipid production rates, were the highest when the process time was the shortest, and *vice versa.* The growth of *L. starkeyi* at 28°C significantly shortened the final fermentation time and increased the lipid yield, while the composition of the accumulated lipids could be regulated by the accumulation phase temperature. In conclusion, the temperature profile for the highest lipid yield with desired composition would be: (i) the growth phase temperature that gives the maximum growth rate and (ii) the accumulation phase temperature that produces the desired ratio of $C_{16:0}$ to $C_{18:1}$.

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