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Accumulation of a Cocoa-Butter-Like Lipid by *Yarrowia lipolytica* Cultivated on Agro-Industrial Residues

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Abstract. *Yarrowia lipolytica* was cultivated on mixtures of saturated free fatty acids (an industrial derivative of animal fat called stearin), technical glycerol (the main by-product of bio-diesel production facilities), and glucose. The utilization of technical glycerol and stearin as co-substrates resulted in higher lipid synthesis and increased citric acid production than the combination of glucose and stearin. The lipids produced contained significant amounts of stearic acid (50–70%, wt/wt) and lower ones of palmitic (15–20%, wt/wt), oleic (7–20%, wt/wt), and linoleic (2–7%, wt/wt) acid. Single-cell oil having a composition similar to cocoa-butter up to 3.4 g/L was produced, whereas in some cases relatively increased citric acid quantities (up to 14 g/L) were excreted into the growth medium. The microorganism presented a high specificity for lauric, myristic, and palmitic acid, while a discrimination for the stearic acid was observed. As a conclusion, microbial metabolism could be directed by using mixtures of inexpensive saturated fats, glycerol, and glucose as co-substrates, in order to accumulate lipids with predetermined composition, e.g., cocoa-butter equivalents.

Oleaginous microorganisms are able to accumulate lipid up to 70% (wt/wt) of their dry matter [13, 14] when cultivated on media containing hydrophilic [8, 15, 16] or hydrophobic [1, 2, 4, 10, 11] carbon substrates. One of the most common applications of the oleaginous yeasts is to the production of lipids presenting composition similarities with various exotic fats (e.g., the cocoa butter) [13]. Since oleaginous yeasts accumulate lipids rich in unsaturated fatty acids and cocoa butter contains more than 60% (wt/wt) of saturated fats (palmitic and stearic acid) [13], several approaches have been used to increase the level of these acids in the yeast lipid, such as the cultivation of yeasts in stearic acid-rich media [5], the use of Δ^9 and Δ^{12} desaturase inhibitors [8], low oxygenation of the growth medium [3], and the genetic manipulation of strains [15, 16].

Recently it has been demonstrated that media containing saturated free fatty acids (C16:0, C18:0) favored growth and accumulation of storage lipid by *Yarrowia lipolytica*, while the lipids produced contained significant amounts of stearic acid [11]. In contrast, the same strain cultivated on glucose or technical glycerol in nitrogen-limited batch cultures produced a cellular lipid rich in oleic and linoleic acid. However, on glucose or glycerol, lipid accumulation was not favored, since under these culture conditions nitrogen limitation led to a significant production of citric acid in the growth medium [12].

In the present work, the biochemical behavior of *Y. lipolytica* cultivated on several agro-industrial residues (saturated free fatty acids, glucose, technical glycerol) was studied. The possibility of the utilization of these substrates to direct the microbial metabolism towards the synthesis of a cocoa-butter analog was also considered and discussed.

Materials and Methods

Microorganism and culture conditions. *Yarrowia lipolytica* ACA-DC 50109 (registered as LGAM S(7)1 in Papanikolaou et al. [10]) was used. The mineral composition of the medium was described previously [12]. The carbon sources used were: glucose monohydrate (purity 98%); technical glycerol co-product of the bio-diesel production process [French Agro-chemical industry EPILOR-R&D, glycerol con-

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tent 65%, wt/wt - impurities composed of potassium and sodium salts (4– 5%, wt/wt), methanol (1%, wt/wt), heavy metals-lignin (1%, wt/wt), non-glycerol organic materials (0.5%, wt/wt) and water (28%, wt/wt)]; stearin [an industrial derivative of animal fat composed of 100% free fatty acids (Hellenic Chemical Industry PAPOUTSANIS A S) containing (%, wt/wt): C12:0 10; C14:0 10; C16:0 25; C18:0 52; $^{\Delta 9}$C18:1 2; $^{\Delta 9,12}$C18:2 <0.5)]. Experiments were performed in 250-ml conical flasks, containing 50 ml of medium, inoculated with 10^8 cells, and incubated in an orbital shaker (agitation rate = 185 rpm) at 28°C. Cultures were conducted in oil-in-water emulsions, in which Tween 80 and PEG 20000 were used as emulsifiers, at 10% wt/wt of each, against the fat content. In preliminary trials, the influence of emulsifiers on cell growth and lipid accumulation was studied in fat-, glucose- and glycerol-free media, and it was found that these carbon components in the amounts used in the present study had negligible effect on cell growth and lipid accumulation.

Measurement of cell growth and chemical analyses. Determination of microbial growth, substrates (glucose, glycerol, stearin), products (storage lipids, citric acid), as well as thin layer chromatography, gas chromatography, and gas chromatography—mass spectrometry analyses of lipids, were performed as described elsewhere [10, 12].

Statistical analysis. Data from several cultures (carried out in triplicate) were statistically treated by the method of the maximum likelihood.Polynomial smoothing regressions were used, giving for biomass a correlation coefficient R = 0.95 and a variance of the measurement error 0.93 (confidence interval of 5% at ± 1.7 g/L). The regression of lipids presented an R = 0.64 with a variance of measurement error 0.28 (confidence interval of 5% at ± 0.7 g/L).

Results

Growth of *Yarrowia lipolytica* on mixtures of technical glycerol, glucose, and stearin. Substrate fat and hydrophilic carbon compounds (glucose or glycerol) were simultaneously consumed by *Y. lipolytica*, and storage lipid accumulation and citric acid excretion were observed (Figure 1A, B). In all cultures, a cessation of the substrate fat uptake occurred around 70 h after inoculation. Furthermore, in the late growth stages, the glucose uptake rate was decreased (Fig. 1B) while glycerol assimilation was uninterrupted (Fig. 1A), and in the latter case higher citric acid amounts were produced in the medium. The growth of *Y. lipolytica* on mixtures of all carbon substrates, glucose, technical glycerol, and stearin resulted in a sequential consumption of hydrophilic carbon components; glycerol was primarly assimilated and then, when the glycerol concentration became low, glucose consumption occurred (data not shown).

Several kinetic studies were conducted in media having different C/N molar ratios and various initial glucose, technical glycerol, and stearin concentrations (Table 1). In all cases, substantial biomass production and glucose or glycerol assimilation were observed, but a remarkable substrate fat amount remained unconsumed in the medium regardless of its initial concentration. Significant accumulation of storage lipid was observed

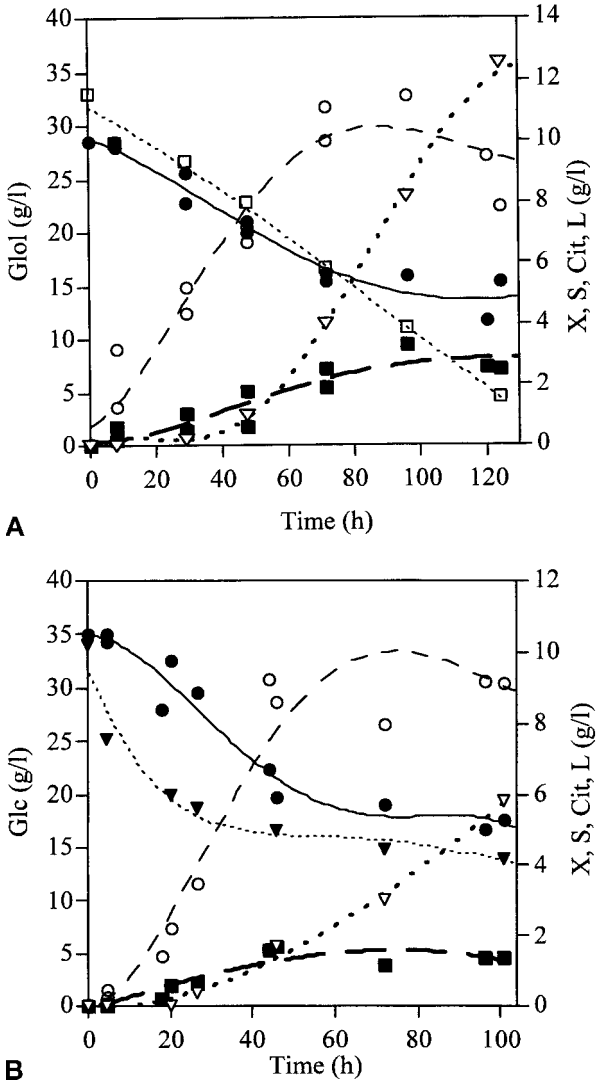

Fig. 1. Biomass (X, g/L, ○), cellular lipids (L, g/L, ■) and citric acid (Cit, g/L, □) production by *Y. lipolytica* growing at initial pH 6.5 and T 28°C, on a mixture of: (A) technical glycerol (Glol, g/L, □) and stearin (S, g/L, ●) (initial concentrations: glycerol 34 g/L, stearin 10 g/L, molar ratio C/N 175); (B) glucose (Glc, g/L, ▼) and stearin (S, g/L, ●) (initial concentrations: glucose 34 g/L, stearin 10 g/L, molar ratio C/N 180).

($Y_{L/X}$ = 0.27–0.30 g/g) only in stearin-rich media ($S_0 \geq$ 10 g/L), having glycerol as a fat co-substrate. Actually, in glycerol/stearin cultures, the substrate fat was deposited as storage material in significantly higher amounts compared with the glucose/stearin cultures ($Y_{L/S}$ = 0.41 g/g with glycerol against $Y_{L/S}$ = 0.23 g/g with glucose used as co-substrate) (Fig. 2A, B). In contrast, in experiments conducted in media with high glycerol or glucose and low stearin initial concentration (S_0 = 7 g/L), in spite of the significant growth observed, lipid production was low ($Y_{L/X}$ = 0.05–0.10 g/g) (Table 1). A production

Table 1. Biomass, single-cell oil and citric acid production by *Y. lipolytica* cultivated on mixtures of stearin, glucose, and technical glycerol. Culture conditions: growth in flasks, initial pH = 6.5, T = 28°C. Maximum values were obtained at incubation time 100–130 h

C/N	S_0[a] (g/L)	S_f[b] (g/L)	$Glol_0$[c] (g/L)	$Glol_f$[d] (g/L)	Glc_0[e] (g/L)	Glc_f[f] (g/L)	X_{max}[g] (g/L)	L_{max}[h] (g/L)	$Y_{L/X}$[i] (g/L)	Cit_{max}[i] (g/L)
55	10.0	5.5	10.0	0.0	–	–	9.7	2.6	0.27	0.9
110	13.0	8.6	10.0	0.0	–	–	9.0	2.0	0.22	2.8
100	10.0	4.4	11.0	0.0	–	–	9.2	1.9	0.21	2.5
140	10.0	4.4	23.0	0.5	–	–	10.1	2.7	0.27	9.5
175	10.0	4.2	34.0	4.5	–	–	11.4	3.4	0.30	12.5
140	7.0	4.0	28.0	0.0	–	–	10.5	1.0	0.10	14.1
100	10.0	4.1	–	–	10.0	0.0	7.7	0.5	0.07	1.7
145	10.0	4.9	–	–	21.0	5.9	9.3	2.0	0.22	3.9
180	10.0	5.3	–	–	34.0	13.9	8.7	1.7	0.20	5.9
130	7.0	2.5	–	–	24.0	9.5	11.3	0.8	0.07	6.0
130	7.0	3.2	8.0	0.0	7.0	0.3	9.6	0.8	0.08	7.2
110	7.0	3.7	11.0	0.0	7.0	0.1	10.3	1.6	0.15	9.5

[a] S_0 initial stearin concentration; [b] S_f final stearin concentration; [c] $Glol_0$ initial glycerol concentration; [d] $Glol_f$ final glycerol concentration; [e] Glc_0 initial glucose concentration; [f] Glc_f final glucose concentration; [g] X_{max} maximum concentration of biomass produced; [h] L_{max} maximum concentration of storage lipid produced; [i] $Y_{L/X}$ storage lipid in biomass; [j] Cit_{max} maximum concentration of citric acid produced.

of citric acid, which was notable (up to 14 g/L) especially in high C/N and rich in glycerol media, was observed. Other organic acids produced in low amounts were acetate, α-ketoglutarate, and iso-citrate. Given that substrate fat catabolism did not lead to a significant acidification of the culture medium [11], it may be assumed that citric acid biosynthesis was due mainly to hydrophilic carbon compound catabolism. Furthermore, the representation of citric acid produced as a function of glycerol or glucose consumed demonstrates that glycerol catabolism was preferentially directed towards the biosynthesis of citric acid ($Y_{Cit/Glol}$ = 0.49 g/g against $Y_{Cit/Glc}$ = 0.23 g/g) (Fig. 3A, B).

Analysis of the substrate fat showed that, regardless of the initial glucose or glycerol concentrations, the microorganism presented a high specificity for the shorter aliphatic chain fatty acids (C12:0 and C14:0), which rapidly disappeared from the culture medium, as well as for C16:0 (see, for example, Fig. 4). On the contrary, a metabolic discrimination against stearic acid, whose percentage increased from 52% to 72% wt/wt as a function of the cultivation time, occurred. A cessation of the substrate fat uptake was observed when C18:0 was the principal fatty acid remaining unconsumed in the culture medium.

Storage lipid composition. In the early growth stages, the concentration of unsaturated fatty acids (oleic and linoleic) was noteworthy, but it decreased afterwards in the cellular lipids. It also seems that when glucose was used as fat co-substrate, lipid produced in the early growth steps was more unsaturated as compared with glycerol (for similar runs) (Table 2).

In all of the cultures, the cellular lipids produced contained significant amounts of stearic, oleic, and linoleic acid, which were usually found in the storage lipids in higher concentrations than in stearin. The cellular lipid content, hence, was enriched with unsaturated fatty acids (Table 3). Indeed, when accumulation of storage lipid was low ($Y_{L/X}$ = 0.07–0.15 g/g), the stearic acid concentration was only 42–51% (wt/wt) in the reserve lipids, whereas the concentration of the unsaturated fatty acids was multiplied by two or even three times when compared with the cases in which remarkable fat accumulation occurred.

In several cases when the accumulation of lipid was favored ($Y_{L/X}$ = 0.22–0.30 g/g), cellular lipid analysis was carried out in order to quantify the main lipid fractions. The triacylglycerols (TAGs) were the principal lipid fraction of the accumulated lipid (around 55%, wt/wt, of total lipids); the free fatty acids (FFAs) were equally represented in significant amounts (35–40%, wt/wt, of total lipids).

Discussion

The aim of the present study was to investigate the growth of *Yarrowia lipolytica* ACA-DC 50109 on mixtures of stearin, technical glycerol, and glucose, in order to direct the cellular metabolism towards the accumulation of lipids presenting a composition similar to that of cocoa butter. During growth, the consumption of glucose (or glycerol) and fats was simultaneous (in agreement with results reported from Kim et al. [6] for *Torulopsis bombicola* growing on mixtures of soybean oil and glu-

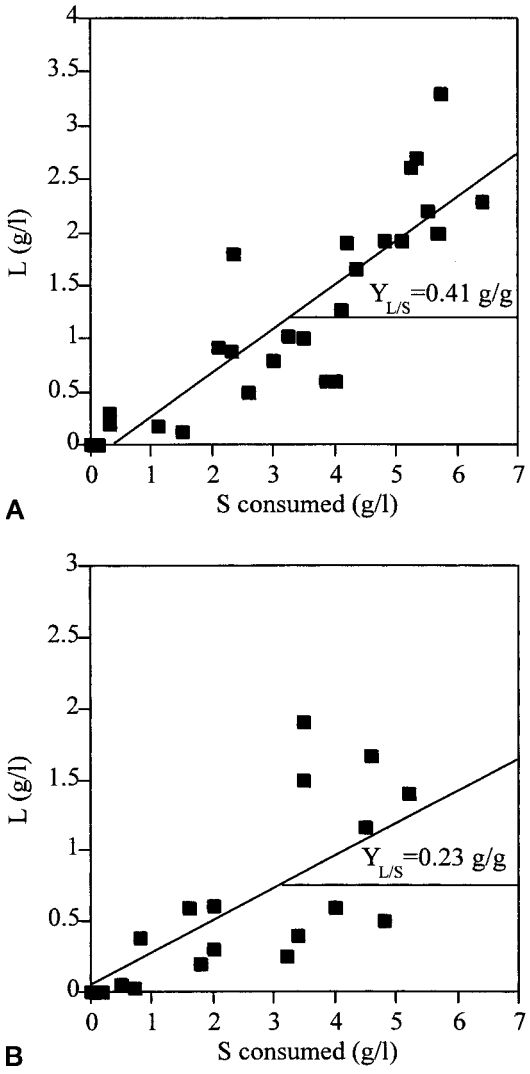

Fig. 2. Production of storage lipid (L, g/L, ■) as a function of substrate fat consumed in cultures with glycerol (A) or glucose (B) used as stearin co-substrate. Slope represents storage lipid yield on stearin consumed ($Y_{L/S}$, g/g). Culture conditions: initial pH = 6.5, T = 28°C.

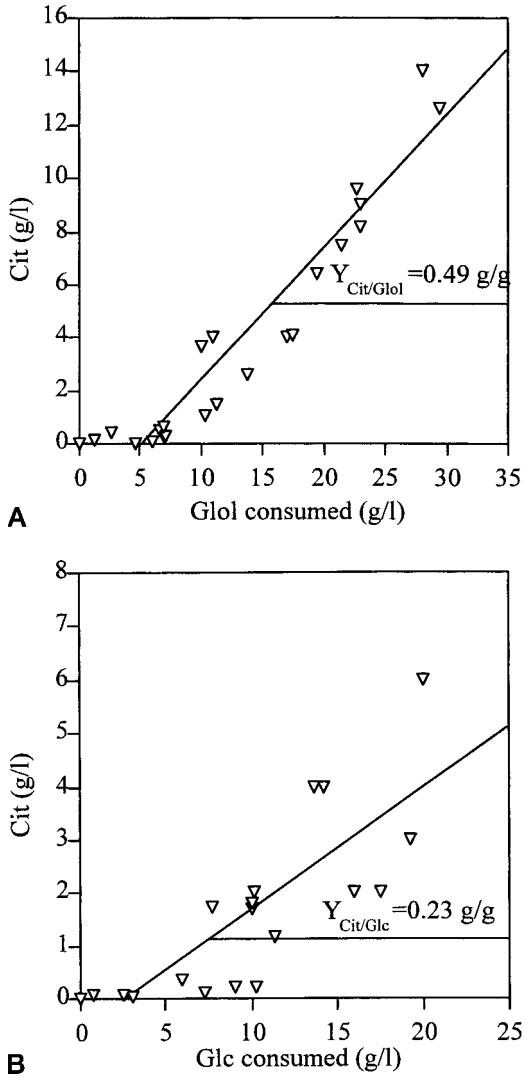

Fig. 3. Production of citric acid (Cit, g/L, □) as a function of glycerol (A) and glucose (B) consumed by *Y. lipolytica* growing on mixtures of stearin/technical glycerol and stearin/glucose respectively. Slope represents citric acid yield on substrate consumed ($Y_{Cit/Glol}$ or $Y_{Cit/Glc}$, g/g). Culture conditions: initial pH = 6.5, T = 28°C.

cose). Moreover, the catabolism of hydrophilic carbon compounds leading to citric acid biosynthesis was significantly altered by the presence of fat as a co-substrate in the culture medium. It was demonstrated that in the presence of stearin, higher glycerol uptake and increased citric acid biosynthesis occurred compared with the cultures with glucose as co-substrate. However, Papanikolaou et al. [12] have reported that *Y. lipolytica* growing on glucose or technical glycerol used as individual substrates consumed the carbon sources equally, and equivalent citric acid production occurred in both instances. It may be considered, therefore, that the presence of lipids could affect the carrier of glucose, but not that of glycerol, since it is known that the transporters of these compounds are different in various yeast strains (e.g., in *Saccharomyces ceravisiae* [9]). Furthermore, since the flow of glucose towards citric acid (via the EMP glycolysis) was decreased, it may be assumed that the catabolism of the fatty substrate resulting in high intracellular energy excess directed glucose towards the pentose-phosphate pathway (production of CO_2 and $NADPH+H^+$). This assumption is fortified by the fact that a higher unsaturated fatty acid content in the cellular lipids at early growth stages was observed when glucose was used as a fat co-substrate compared with the growth with glycerol as co-substrate (see Table 2). It is known that glucose catabolism in the respiratory-sufficient

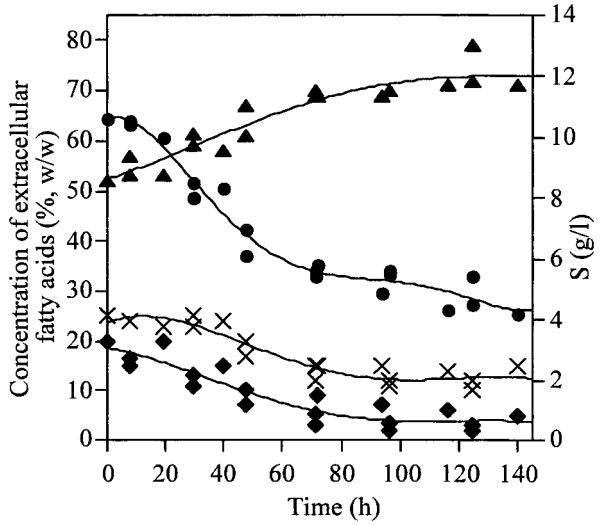

Fig. 4. Fatty acid composition of the substrate fat during growth of *Y. lipolytica* on a mixture of stearin and technical glycerol. Culture conditions: initial pH 6.5, T = 28°C, stearin = 10 g/L, initial glycerol concentrations = 10, 22, and 34 g/L. Total substrate fat (●) in g/L, stearic (▲), lauric & myristic (◆) and palmitic (✕) acid concentration (%, wt/wt) in the substrate fat.

yeasts occurs both via the pentose-phosphate and the EMP pathway while that of glycerol is conducted only via the EMP glycolysis.

In the present investigation, storage lipid production was lower than that reported when stearin has been used as sole carbon and energy substrate (maximum $Y_{L/X}$ obtained in this work was 0.30 g/g against 0.54 g/g on stearin fermentation [11]. However, an increase of the unsaturated fatty acid concentration has been observed in the lipid produced in the present experiments. The enrichment of storage lipid with unsaturated fatty acids indicates that de novo fatty acid biosynthesis occurred, in spite of the presence of exogenous fatty acids found in the culture medium. In similar experiments, though, Meyer and Schweizer [7] have reported that extracellular pentadecanoic acid even at low amounts (e.g., 0.3%, wt/wt) was capable of completely repressing the fatty acid synthetase activity of another *Y.* (*Candida*) *lipolytica* strain.

Y. lipolytica presented a specificity for C12:0, C14:0, and C16:0, which were preferentially incorporated, while a discrimination against C18:0 was observed. In addition, substrate fat uptake ceased when stearic acid was the principal fatty acid remaining unconsumed in the medium. Similar kinetic profiles have been observed during growth of *Y. lipolytica* on mixtures of saturated and unsaturated fatty acids [10] or *Pseudomonas oleovorans* on fatty and 3-hydroxy fatty acids [4].

The cellular lipid of *Y. lipolytica* contained TAGs

Table 2. Cellular lipid composition of *Y. lipolytica* growing on a mixture of technical glycerol or glucose and stearin. Culture conditions: initial pH 6.5, T = 28°C, S_0 = 10 g/L, Glc_0 = 34 g/L or $Glol_0$ = 34 g/L (for symbols, see Table 1)

	Fatty acid composition of stearin[a]			
	C16:0	C18:0	$^{\Delta 9}$C18:1	$^{\Delta 9,12}$C18:2
	25	52	2	<0.5
Fatty acid composition of microbial lipid; growth on a glycerol/stearin mixture				
Time (h)	$Y_{L/X}$ (g/g)	C16:0	C18:0	$^{\Delta 9}$C18:1	$^{\Delta 9,12}$C18:2
8	0.14	13	50	23	6
30	0.19	15	57	17	4
71	0.19	19	64	9	2
96	0.30	15	65	11	3
124	0.33	15	69	8	3
Fatty acid composition of microbial lipid; growth on a glucose/stearin mixture					
Time (h)	$Y_{L/X}$ (g/g)	C16:0	C18:0	$^{\Delta 9}$C18:1	$^{\Delta 9,12}$C18:2
5	0.11	15	44	27	8
20	0.26	8	34	34	13
26.5	0.17	10	31	38	13
46	0.19	15	68	8	3
72	0.20	13	76	6	3
100	0.19	16	67	8	3

[a] Other non-presented fatty acids were mainly C12:0 (10%, wt/wt) and C14:0 (10%, wt/wt).

(55% wt/wt, of total lipids), but also considerable amounts of FFAs (35%, wt/wt). Growth of oleaginous microorganisms on aliphatic compounds showed that TAGs were produced in relatively higher amounts in the cellular lipid compared with the present study (60–80%, wt/wt) [1, 2]. Lipids with composition similar to the cocoa butter were produced (Fig. 5). When the yeast accumulated large quantities of lipid, this lipid was rich in stearic acid (>60%, wt/wt), whereas the total saturated fatty acid content was over 70% (wt/wt) of the accumulated lipid [cocoa butter contains 62–70% (wt/wt) of saturated fatty acids]. In contrast, when lipid synthesis was not favored, the composition of cellular lipids was closer to that of the cocoa butter.

The strategy followed in order to synthesize a cocoa butter analog was different from those reported in the literature, as the strain used in the present study accumulated significant amounts of storage lipids with high stearic acid content (up to 80%, wt/wt) when it was cultivated on stearin as the sole substrate [11]. Thus, the main problem to solve was how to decrease the amount of C18:0 in the lipids produced by *Y. lipolytica* growing on stearin. Glycerol (or glucose) could be used as fat co-substrate in order to increase C18:1 and C18:2

Table 3. Composition of single-cell oil produced by *Y. lipolytica* cultivated on mixtures of technical glycerol, glucose, and stearin. Culture conditions: initial pH 6.5, T = 28°C, incubation time about 120 h (for symbols, see Table 1)

				Fatty acid composition of stearin (%, w/w)[a]				
				C16:0 25.0	C18:0 52.0	$^{\Delta 9}$C18:1 2.0	$^{\Delta 9,12}$C18:2 <0.5	U.f.a[b] 2.0
				Fatty acid composition of microbial lipid produced (%, w/w)				
S_0 (g/L)	$Glol_0$ (g/L)	Glc_0 (g/L)	$Y_{L/X}$	C16:0	C18:0	$^{\Delta 9}$C18:1	$^{\Delta 9,12}$C18:2	U.f.a.
10.0	10.0	–	0.27	15.0 ± 1.5	76.0 ± 2.0	5.0 ± 1.5	2.0 ± 0.5	7.0 ± 2.0
13.0	10.0	–	0.22	15.0 ± 1.0	70.0 ± 1.0	6.0 ± 1.5	2.0 ± 0.5	8.0 ± 2.0
10.0	11.0	–	0.21	15.5 ± 1.5	72.5 ± 2.5	5.0 ± 1.0	1.5 ± 0.5	6.5 ± 1.5
10.0	23.0	–	0.27	16.0 ± 1.0	67.5 ± 1.5	6.5 ± 1.5	2.0 ± 0.5	8.5 ± 2.0
10.0	34.0	–	0.30	14.0 ± 1.0	67.0 ± 2.0	9.5 ± 1.5	2.5 ± 0.5	12.0 ± 2.0
7.0	28.0	–	0.10	20.0 ± 1.5	50.0 ± 2.0	16.5 ± 1.0	6.5 ± 1.5	23.0 ± 2.5
10.0	–	10.0	0.06	20.0 ± 3.0	47.5 ± 2.5	18.5 ± 1.5	6.5 ± 1.5	25.0 ± 3.0
10.0	–	21.0	0.22	16.5 ± 1.5	66.0 ± 3.0	8.5 ± 1.5	3.0 ± 1.0	11.5 ± 2.5
10.0	–	34.0	0.20	14.0 ± 1.0	67.0 ± 2.0	8.5 ± 2.0	2.5 ± 0.5	11.0 ± 2.5
7.0	–	24.0	0.07	22.0 ± 2.0	45.0 ± 2.5	17.0 ± 2.5	6.5 ± 2.5	23.5 ± 5.0
7.0	8.0	7.0	0.08	22.0 ± 2.0	42.5 ± 2.5	17.0 ± 3.0	6.0 ± 2.0	23.0 ± 5.0
7.0	11.0	7.0	0.15	19.0 ± 1.5	48.5 ± 2.0	15.5 ± 2.0	5.5 ± 1.5	21.0 ± 3.5

[a] Other non-presented fatty acids were mainly C12:0 (10% wt/wt) and C14:0 (10% wt/wt).
[b] U.f.a.: Unsaturated fatty acids.

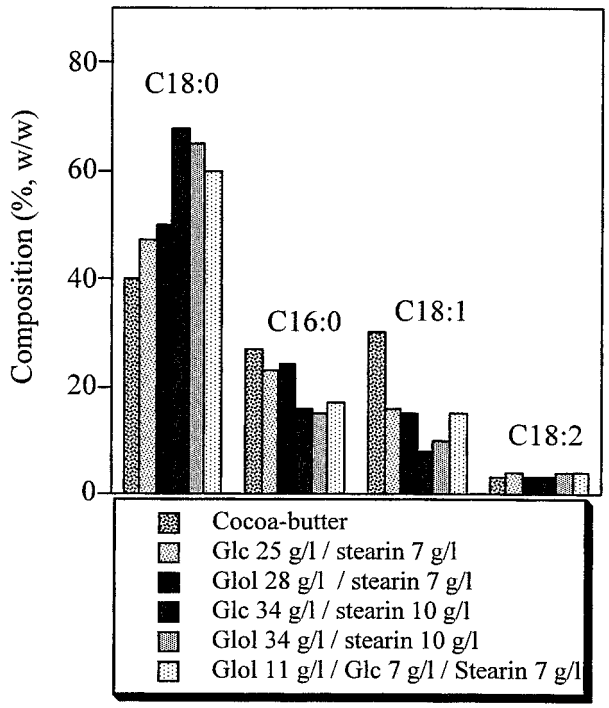

Fig. 5. Fatty acid composition (%, wt/wt) of a cocoa butter and cellular lipids of *Y. lipolytica* growing on different stearin (S, g/L), glycerol (Glol, g/L), and glucose (Glc, g/L) mixtures (data shown when maximum values of $Y_{L/X}$ were achieved).

amounts in the storage lipid. In contrast, research groups dealing with the production of cocoa butter substitutes by fermentative means confront exactly the opposite problem, which is how to increase the cellular C18:0 content, normally found in low concentrations in the oleaginous yeasts, and numerous approaches have been followed to obtain this result [3, 5, 8, 15, 16].

In conclusion, *Y. lipolytica* showed remarkable growth on mixtures of saturated free fatty acids and glycerol (and/or glucose) used as co-substrates. Accumulation and composition of storage lipid, as well as production of organic acids, were critically influenced by the mixtures used, and the cellular metabolism could be directed by using these inexpensive substrates in order to accumulate reserve lipids with a predetermined composition such as that of the cocoa butter.

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Literature Cited

1. Alvarez HM, Kalscheuer R, Steinbüchel A (1997) Accumulation of storage lipids in species of *Rhodococcus* and *Nocardia* and effect of inhibitors and polyethylene glycol. Fett-Lipid 99:239–246
2. Bati N, Hammond EG, Glatz BA (1984) Biomodification of fats and oils: trials with *Candida lipolytica*. J Am Oil Chem Soc 61:1743–1746
3. Davies RJ, Holdswoth JE, Reader SL (1990) The effect of low oxygen uptake rate on the fatty acid profile of the oleaginous yeast *Apiotrichum curvatum*. Appl Microbiol Biotechnol 33:569–573
4. Füchtenbusch B, Wullbrandt D, Steinbüchel A (2000) Production of polyhydroxyalkanoic acids by *Ralstonia eutropha* and *Pseudo-*

monas oleovorans from an oil remaining from biotechnological rhamnose production. Appl Microbiol Biotechnol 53:167–172
5. Gierhart DI (1982) Multistage process for the preparation of fats and oils. UK Patent 2091:285A
6. Kim ST, Oh DK, Lee KH, Kim JH (1997) Effect of soybean oil and glucose on sophorose lipid fermentation by *Torulopsis bombicola* in continuous culture. Appl Microbiol Biotechnol 48:23–26
7. Meyer KH, Schweizer E (1976) Control of fatty-acid synthetase levels by exogenous long-chain fatty acids in the yeasts *Candida lipolytica* and *Saccharomyces cerevisiae*. Eur J Biochem 65:317–324
8. Moreton RS (1985) Modification of fatty acid composition of lipid accumulating yeasts with cyclopropene fatty acid desaturase inhibitors. Appl Microbiol Biotechnol 22:41–45
9. Nevoigt E, Stahl U (1997) Osmoregulation and glycerol metabolism in the yeast *Saccharomyces cerevisiae*. FEMS Microbiol Rev 21:231–241
10. Papanikolaou S, Chevalot I, Komaitis M, Aggelis G, Marc I (2001) Kinetic profile of the cellular lipid composition in an oleaginous *Yarrowia lipolytica* capable of producing a cocoa-butter substitute from industrial fats. Antonie van Leeuwenhoek 80:215–224
11. Papanikolaou S, Chevalot I, Komaitis M, Marc I, Aggelis G (2002a) Single Cell Oil (SCO) production by *Yarrowia lipolytica* growing on an industrial derivative of animal fat in batch cultures (short contribution). Appl Microbiol Biotechnol 58:308–312
12. Papanikolaou S, Muniglia L, Chevalot I, Aggelis G, Marc I (2002b) *Yarrowia lipolytica* as a potential producer of citric acid from raw glycerol. J Appl Microbiol, 92:737–744
13. Ratledge C (1994) Yeasts, moulds, algae and bacteria as sources of lipids. In: Kamel BS, Kakuda Y (ed) Technological advances in improved and alternative sources of lipids. London: Blackie Academic and Professional, pp 235–291
14. Steinbüchel A (1991) Polyhydroxyalkanoic acids. In: Byrom D (ed) Biomaterials. London: McMillan, pp 123–213
15. Ykema A, Verbree EC, Nijkamp HJJ, Smit H (1989) Isolation and characterization of fatty acid auxotrophs from the oleaginous yeast *Apiotrichum curvatum*. Appl Microbiol Biotechnol 32:76–84
16. Ykema A, Verbree EC, Verwoert IIGS, Van der Linden KH, Nijkamp HJJ, Smit H (1990) Lipid production of revertants of Ufa mutants from the oleaginous yeast *Apiotrichum curvatum*. Appl Microbiol Biotechnol 33:176–182