Studies on Physicochemical Characteristics and Fatty Acid Composition of Lipids Produced by a Strain of *Rhodotorula Gracilis* CFR-1

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Rhodotorula gracilis **CFR-1 has been evaluated for its potential to produce lipids. The yeast lipids closely resembled palmolein, a liquid fraction of palm oil. It contained 2.3–3% free fatty acids, 64.4% tri-, 23.1% di-, and 6.1% mono-acylglycerols, 94.2% neutral and 5.8% polar lipids. Most abundant fatty acids were C18:1, C16:0, C18:2 and C18:0 (43.8, 28.5, 13.5 and 4.5%). All fatty acids, irrespective of the levels, followed definite patterns of increase or decrease during the advancement of fermentation. A pincers-shaped curve was obtained when the total saturation and unsaturation were plotted. Use of different glucose and molasses-based media did not show any significant overall effect on saturation (34.4–39.5%) and unsaturation (60.4–65.3%). Desaturation of fatty acids was found to be a metabolic function occurring in the process of cell maturation.**

KEYWORDS: Desaturation, growth associated, oleaginous, palmolein, pincers shaped curve, physicochemical, *Rhodotorula*, saturation, unsaturation.

Rhodotorula species produce lipids of composition closely similar to vegetable oils in which the 2-position of the triacylglycerol is occupied by unsaturated fatty acid. Fermentative synthesis of such microbial lipids as substitutes to vegetable oils can be considered as an attractive proposition in the context of increasing demand and short supplies of edible oils and fats (1). But most of these oleaginous yeasts have slow growth rates, and their adoption and consequent success depend on maximizing the exponential growth phase so as to yield higher quantities of biomass in short periods of fermentation. While studying different *Rhodotorula* strains, it was found that a strain of *Rhodotorula gracilis* CFR-1 had minimum lag phase of growth when grown in a selective medium. It displayed a high rate of cell multiplication (μ=0.16, hr^{-1}) during the initial stages (0–6 hr) of fermentation (2). Also, this strain was reported to produce more than 65% lipids intracellularly (3). Because of the potential of this strain we have studied the physicochemical characteristics and fatty acid composition of its lipids.

MATERIALS AND METHODS

The yeast *R. gracilis* CFR-1 was maintained on potato dextrose agar slopes. Unless otherwise specified, the fermentations were carried out by using a selective medium on a rotary shaker (250 rpm) (3). In order to find out the effect of two carbon sources (synthetic glucose and cane molasses) on the fatty acid profile of the yeast lipid, the yeast was grown in Enebo's medium (I) (4), Enebo's medium in which glucose was substituted with molasses (II), selective medium (III) (3) and selective medium in which glucose was substituted with molasses (IV). The methods of harvesting the cells and the lipid extraction were the same as reported elsewhere (3). The physical and chemical properties of the lipids were determined according to AOCS methods (5). Methyl esters of the lipids were analyzed by gas chromatography (6) in a Shimadzu GC (Shimadzu Scientific Instruments Inc., Columbia, MD) with a super Cowax-10 bonded phase super co-capillary column-294 99A of 30 m × 0.25 mm under the following conditions: phase, DEGS, film thickness 0.25 microns; sample size 0.4 μl; coating efficiency 69%; effective injector temperature, 220°C; column temperature 165°C; range 10 E; and chloroform retention time 7 min. Lipids were analyzed for tri-, di-, and mono-acylglycerides and free fatty acids (FFA) by column chromatography (7). The lipid class composition was determined by the hexane-ethanol partition method (8).

RESULTS AND DISCUSSION

The determined physical characteristics, such as specific gravity and refractive index (Butyrorefractometer reading), and the chemical characteristics, such as saponification value, percent free fatty acids, iodine value and unsaponifiable matter content are presented in Table 1. The lipids resemble palmolein, a liquid fraction of palm oil obtained from the fruits of *Elaeis guineensis*. The color of the lipids is also similar to palm oil, due to carotenoid pigments.

The yeast lipids contained 3% FFA, 64.41% tri-, 23.13% di- and 6.11% mono-glycerides. The lipid class composition consisted of 94.2% neutral lipids and 5.8% polar lipids (Table 2).

Instances of high FFA, ranging from 10–20%, have been reported in extracted microbial lipids, which causes serious concern when considering the utility of such lipids for edible purposes (1,10,11). Reasons for the presence of high FFA are due to activation of lipases and phospholipases and to treatment with acid or alkali during the initial steps of cell breakage for the extraction of intracellular lipids (1). However, in the present studies FFA ranged from 2.3–3% mainly due to milder conditions (0.1 N HCL hydrolysis and complete removal of the acid adhering to post hydrolyzed cells by careful washing) employed in the extraction procedure. In the event of large-scale extractions, adaptability of such treatments cannot be considered as specific to *Rhodotorula* yeasts, but depends on critical evaluation of cost factor and quality of end product.

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TABLE 1

Comparison of Physical and Chemical Properties of Lipids of *R. gracilis* CFR-1 with Oils of Ground Nut, Palm, Coconut and Soybean

Properties	*R. gracilis* CFR-1	Lipids of[a] palm (palmolein)[b]	Ground nut	Coconut	Soybean
Refractive index at 40°C	1.4566	1.4491–1.4552 (1.4450–1.4610)	1.4620–1.4640	1.4649–1.4710	1.4481–1.4491
Butyrorefratometer reading	46	35.5644 (43.72–52.5)	54–57.1	58.5–68	34.2–35.1
Free fatty acid (as % oleic acid)	2.3	<3 (<3)	<3	<1.25	<3
Saponification value	182.4	195–205 (195–205)	188–196	189–195	<250
Unsaponifiable matter (%)	1.3	<1.2	<1	<1.5	—
Iodine value	63.4	45–66 (55–62)	85–99	120–141	7.5–10

[a]Reference 9.
[b]Liquid fraction of palm oil.
All values for *R. gracilis* oil are mean determinations on three samples.

TABLE 2

Lipid Class Composition of *R. gracilis* CFR-1 Lipids

Lipid class	Weight (%)[a]
1. Neutral lipids	94.2
a) Free fatty acids	3.0
b) Triacylglycerols	67.4
c) Diacylglycerols	23.1
d) Mono-acylglycerols	6.5
2. Polar lipids	5.8

[a]Values are mean determinations of three samples.

Like any other *Rhodotorula* species, *Rhodotorula gracilis* CFR-1 produced only ordinary lipids. Barring slight differences, the fatty acid profile of *R. gracilis* CFR-1 was found to be almost similar to others reported in the literature (Table 3). In *R. gracilis* CFR-1 the most abundantly seen fatty acids were C18:1, C16:0, C18:2 and C18:0, in decreasing order (43.8, 28.5, 13.5 and 4.5%, Table 4).

The biomass, lipid content and the fatty acid profiles of the yeast lipids at regular intervals of growth (0–120 hr) are presented in Figure 1. The yeast displayed a growth-associated accumulation pattern, a feature quite unusual with oleaginous *Rhodotorula* yeasts (2). Also, the period between 0–20 hr of growth was reported to be critical, mainly because of active cell multiplication, lipid synthesis and storage. Fatty acids, irrespective of the levels, followed definite patterns of increase or decrease with time of growth. Figure 1 also shows that the individual fatty acids, except for C18:2, maintained levels that were attained at 24 hr until the end of fermentation. The initial levels of C8:0, C10:0 and C18:3 were low (2.31, 1.71 and 0.75%), and by 36 hr they were either less than 0.5% or were present only in traces. In the case of C18:2, the initial amount was 13.21% and increased to 17.91% by 12 hr. This was followed by a marked decrease to 8.32% by 36 hr. Presence of fatty acids like C16:1 was also observed in traces (less than 0.5%) after 24 hr of fermentation. It is interesting to note that the fatty acid composition of the initial inoculum (obtained by sterile distilled water washing of 48 hr potato agar culture slopes) did not show any resemblance to growing cells either at exponential or stationary phase. This suggests that yeast cells grown under solid and liquid conditions have different fatty acid compositions and syntheses.

The pattern of total saturation and unsaturation followed a pincers-shaped curve (derived from Fig. 1, but not presented as a separate figure). The initial

TABLE 3

Comparison of Fatty Acid Profiles of Three *Rhodotorula* Species

Species	% Saturated fatty acids					% Unsaturated fatty acids				
	C10:0	C12:0	C14:0	C16:0	C18:0	C16:1	C18:1	C18:2	C18:3	C20:0
R. graminis SN54[a]	nm	nm	1.0	24.0	6.0	1.0	56.0	10.0	1.0	—
R. graminis NCYC502[a]	nm	nm	1.0	27.0	12.0	1.0	48.0	8.0	2.0	—
R. glutinis AHU 3942[a]	nm	nm	2.0	29.0	8.0	2.0	47.0	9.0	3.0	1.5
R. glutinis OUT 6151[a]	nm	—	2.0	30.0	9.0	tr	40.0	16.0	3.0	tr
R. gracilis NRRL Y-1091[b]	nm	—	—	1.7	27.6	—	66.5	1.6	1.5	—
R. gracilis CFR-1[c]	4.6	0.6	1.3	28.5	4.5	—	43.9	13.5	3.1	—

[a]Reference 1.
[b]Reference 12.
[c]Data from Table 4, medium III; nm, not mentioned; tr, traces.

TABLE 4

Comparison of Fatty Acid Profiles of the Lipids of *R. gracilis* CFR-1 Grown in Different Media and to Some Vegetable Oils

	% Saturated fatty acids					% Unsaturated fatty acids				Total	
Lipid source	C10:0	C12:0	C14:0	C16:0	C18:0	C16:1	C18:1	C18:2	C18:3	Saturation	Unsaturation
Medium I	1.4	0.1	1.4	28.7	5.9	—	44.7	14.4	3.1	37.4	62.2
Medium II	4.9	0.4	1.2	26.1	2.6	—	52.8	11.2	0.9	35.1	64.9
Medium III	4.6	0.6	1.3	28.5	4.5	—	43.9	13.5	3.1	39.5	60.5
Medium IV	1.0	0.1	1.2	28.0	4.2	—	55.0	9.8	0.5	34.5	65.3
Palm[a]	—	—	1.0	43.0	5.0	1.0	40.0	10.0	—	45.0	51.0
Coconut[b]	6.4	50.3	19.1	7.5	1.6	—	4.7	1.8	—	84.9	6.5
Ground nut[c]	—	—	<0.1	6—15.5	1.3-6.5	<1.0	36-72	13-45	<1.0	<10.9	<51
Soybean[c]	—	—	<0.5	7—12	2.5-5	<0.5	29-30	48-58	4-10	< 2.9	<72

[a]Reference 1.
[b]Reference 13.
[c]Reference 14. Ground nut (d) and soybean (e) also contain C20:0, (d-2.5 and e<1.0); C20:1 (d 0.5-2.1 and e<1.0); C22:0 (d 1.5-4.8 and e<0.5); C22:1 (d<0.1) and C24:0 (d 1-2.5). Values for media I to IV are mean of three determinations.

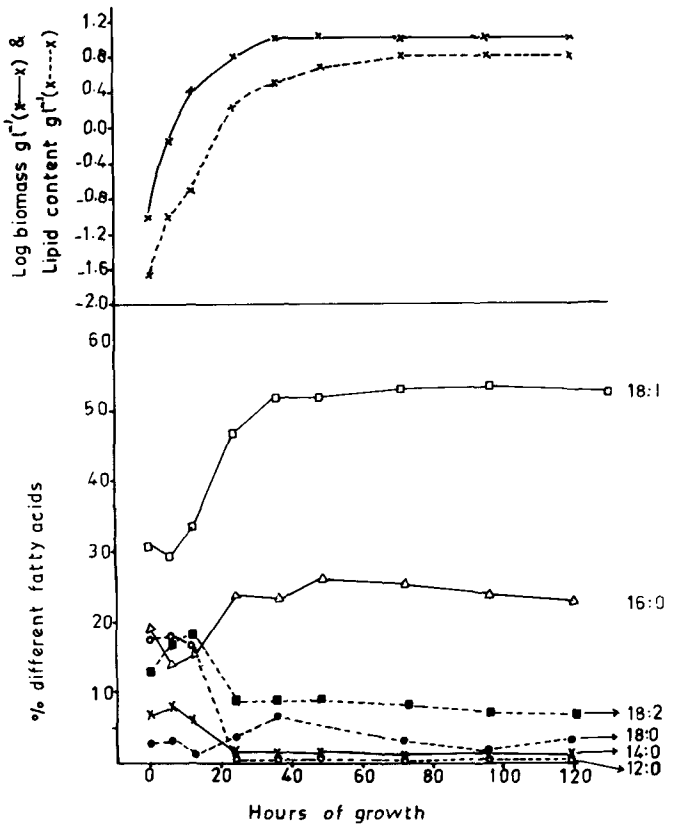

FIG. 1. Biomass, lipid content and changes in fatty acid profile of *R. gracilis* CFR-1 during growth.

unsaturation was 45% and this increased slowly with growth till 36 hr; the pattern was reversed for total saturation. The levels of both attained at 36 hr were maintained throughout the further course of fermentation. The intersection of the lipid curves occurred at 6 hr when the cells were under an active state of multiplication and protein and lipid syntheses. The changes in individual fatty acids and cumulative degrees of saturation and unsaturation provide some of the basic information about the synthesis and lengthening of the fatty acid chain by the yeast. Use of different media compositions did not show any significant effect on overall saturation (34.4-39.5%) and unsaturation (60.4-65.34%), despite some variations noted in the amounts of C18, C18:2 and C18:3 of cells grown in molasses media.

In general, data obtained from the late exponential or stationary growth phase of oleaginous microorganisms are reported as their total lipid content. In such cases, the changes in individual fatty acid levels during the earlier phases of growth are either ignored or not mentioned. The final degrees of saturation, unsaturation and chain length of fatty acids are obtained as the result of certain transitional changes that are governed by type and concentration of carbon and nitrogen sources and cultural conditions (15). Similar behavior is noticed in the present strain under investigation. Choi *et al.* (12) also reported that desaturation and fatty acid chain lengthening are found to be dependent on functions of oxygen uptake and growth rate in a strain of *R. gracilis* NRRL Y-1091 when grown in a continuous culture system (15). Desaturation can be considered as an inherent physiological phenomenon of oleaginous yeasts occurring in the process of cell maturation. Possible biochemical explanations of these events in some growing bacteria, yeasts and animal and plant cells already have been reported (16,17).

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