SYNTHESIS OF LIPIDS BY CERTAIN YEAST STRAINS GROWN ON WHEY PERMEATE

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Received for Publication May 29, 1998
Accepted for Publication August 28, 1998

ABSTRACT

Four yeast strains, Apiotrichum curvatum *ATCC 10567,* Cryptococcus albidus *ATCC 56297,* Lipomyces starkeyi *ATCC 12659, and* Rhodosporidium toruloides *ATCC 10788, were grown on whey permeate in order to synthesize lipid.* Lipomyces starkeyii *ATCC 12659 was identified as a high producer of lipid. It synthesized 36.9% lipids based on dry cell mass at a carbon:nitrogen ratio of 30:1 in shake-flask experiments. The significant ($p<0.05$) effect of both carbon:nitrogen ratio and yeast strain was observed on dry cell mass yield and lipid biosynthesis. All yeast strains except* Rhodosporidium toruloides *ATCC 10788 produced more triacylglycerols than phospholipids.*

INTRODUCTION

The capability of microorganisms to synthesize substantial amounts of lipids has been known for many years. Production of lipids by unconventional routes such as plant cell culture and fermentation has received much attention (Ratledge 1982; Ratledge and Boulton 1985; Moreton 1988; Ratledge 1988,1993,1994). Reasons for this interest in the production of lipids through microbial sources include the development of improved methods for large scale cultivation of microorganisms and some new practical applications of microbial lipids. In addition, use of cheap substrates such as inedible plant waste hydrolyzates and industrial waste materials as sources of more valuable products is a focal point of renewed research activities.

The economic feasibility of yeast lipid production would be more favorable if

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Journal of Food Lipids 5 (1998) 283-297.

high priced lipids resembling cocoa butter could be produced. It has been demonstrated that some mutants of the yeast *Apiotrichum curvatum* ATCC 20509, which lacks the Δ^9-desaturase responsible for converting stearic acid to oleic acid *in vivo*, produced high amounts of lipids consisting of more than 90% triacylglycerols. These triacylglycerols contained 60-64% saturated fatty acids and resembled the composition of cocoa butter when grown in the presence of oleic acid and Tween 80 in a semi-defined growth medium containing glucose as the carbon source (Ykema *et al.* 1989). Lyapkov and Kiseleva (1993) found the triacylglycerol profile of *Candida guilliermondii* to be similar to that of olive oil. Thus, the use of microbial lipids as substitutes for vegetable oils could have some merit.

Many studies have been carried out to identify yeast strains which can efficiently synthesize lipids by utilizing different carbon and nitrogen sources under various experimental conditions (Yoon and Rhee 1983a; Evans and Ratledge 1984a; Glatz *et al.* 1985; Hansson and Dostàlek 1986; Ykema *et al.* 1989; Hsiao *et al.* 1994). The objectives of this study were to investigate whey permeate as a sole carbon source and to determine the efficiency of synthesizing lipids by several yeast strains.

MATERIAL AND METHOD

Material

Yeast strains, *Apiotrichum curvatum* ATCC 10567, *Cryptococcus albidus* ATCC 56297, *Lipomyces starkeyi* ATCC 12659, and *Rhodosporidium toruloides* ATCC 10788 were obtained from the American type culture collection (Washington, DC). Agar sabouraud dextrose broth and YM broth were purchased from Difco Laboratories (Detroit, MI). Sweet whey permeate was obtained from the Dairy Plant at Michigan State University to be used as a substrate for biosynthesis of lipids. All chemicals were purchased from either Mallinckrodt Speciality Chemical Co. (Paris, KY) or EM Science (Cherry Hill, NY).

Storage and Analysis of Whey Permeate

Whey permeate was packed in twenty 10 L polyethylene bags (Cryovac Division, WR Grace and Co., Duncan, SC) and stored at −15C in walk-in freezer until used. The whey permeate was thawed at room temperature before using as the substrate for the production of lipids by yeast. The composition of the whey permeate was determined as: 3.64% lactose, 0.08% protein, 0.003% fat, 0.34% ash and 4.96% total solids. The pH value was 6.29.

Preparation of Culture Medium

Whey permeate was blended with asparagine (as a nitrogen source) and other nutrients as described by Ykema *et al.* (1988) and Vega *et al.* (1988). The nutrients included yeast extract 0.65 g/L (final concentration) and the following salts (all final concentrations given as milligrams per liter): calcium chloride, 50; cupric sulfate, 0.1; ferric chloride, 5; manganese sulfate, 20; megnesium sulfate, 750; potassium phosphate, 3,500; sodium phosphate, 1,000 and zinc sulfate, 5. Four culture media with carbon:nitrogen ratios of 30:1, 45:1, 60:1 and 75:1 were prepared by varying the amount of added asparagine, assuming 10.5% nitrogen and 26% carbon in the yeast extract (Ykema *et al.* 1988).

Fermentation Conditions

Yeast strains *Apiotrichum curvatum* ATCC 10567, *Cryptococcus albidus* ATCC 56297, and *Rhodosporidium toruloides* ATCC 10788 were grown in YM broth for 48 h before inoculating the culture medium. YM broth contained yeast extract 3 g/L, malt extract 3 g/L, peptone 5 g/L and dextrose 10 g/L. *Lipomyces starkeyi* ATCC 12659 was grown in sabouraud dextrose broth for 48 h. Sabouraud dextrose broth containing neopeptone (10 g/L) and dextrose (20 g/L) was used for subculturing *Lipomyces starkeyi* ATCC 12659. Twenty-five milliliters of the prepared culture medium (pH 5.5) to be used as a seed culture were transferred to 100 mL Erlenmeyer flasks with baffles at the bottom (Bellco Glass Inc., Vineland, NJ) and sterilized at 121C for 15 min. The seed culture was inoculated with 1.0 mL 48 h-old yeast cells and incubated at 30C in a water bath shaking at 200 rpm for 24 h. The seed culture was then aseptically transferred to 1 L Erlenmeyer flasks containing 250 mL sterilized culture medium. All yeast strains were incubated again at 30C in a water bath shaking at 200 rpm till the exhaustion of carbon and nitrogen source from the culture medium. The carbon and nitrogen levels in the culture medium during fermentation were determined at 24 h intervals. Optical density was also monitored at these times.

Analysis of Fermentation Medium

Growth of the cells in the fermentation medium was monitored by recording the optical density of an aliquot of the culture at 610 nm (Adler *et al.* 1985) using a double beam spectrophotometer (Bausch and Lomb Inc., Rochester, NY).

Lactose was determined by the method described by Teles *et al.* (1978). Quantitation was achieved by reading the absorbance at 520 nm against a similarly treated reagent blank. Color development was based on the combined action of phenol, sodium hydroxide, picric acid, and sodium bisulfite with lactose. Results were compared with a standard solution of lactose after reacting with reagents as described for the test samples.

Protein content was determined by the method of Waddel (1956). This method is based on differential measurements at 215 nm and 225 nm. The reading obtained at 225 nm was substracted from that at 215 nm and the difference multiplied by 144 which gave the protein concentration in the sample in μg/mL.

Harvesting of Yeast Cells

After the completion of fermentation, cells were harvested by centrifugation and removing the supernatant. Cells were washed twice with 0.1 M phosphate buffer, centrifuged, and the supernatant removed each time.

Determination of Dry Cell Mass

Dry cell mass was determined after harvesting the cells by the method of Vega *et al.* (1988), except that drying was carried out at 100C in an oven for 24 h instead of drying in a vacuum oven at 65C.

Lipid Extraction from Yeast Cells

Lipids from yeast cells were extracted by the method of Bligh and Dyer (1959). Extracted lipids were transferred to tared vials, weighed, dissolved in chloroform and flushed with nitrogen. Samples were stored at −20C for subsequent analyses.

Separation of Lipid Classes

Thin-layer chromatography was used to separate triacylglycerols and phospholipids as described by Engeseth (1990). A small amount of oil dissolved in chloroform for each yeast strain grown at different carbon:nitrogen ratios was dried under a stream of nitrogen gas. A volume of 400 μL of a mixture of dichloromethane and methanol (9:1, v/v) was added to each of the samples, and a 200 μL aliquot was streaked onto thin-layer chromatographic 20 cm × 20 cm glass plates coated with silica gel (particle size 10-40 μ) type G (Sigma Chemical Co., St. Louis, MO) at a thickness of 250 nm. The plates were activated in an oven at 105C for 2 h and then cooled in a desiccator before using. The plates were developed in a closed glass chamber using a mixture of petroleum ether, diethyl ether and glacial acetic acid (80:20:1, v/v/v). The developed plates were removed from the tanks, air-dried and sprayed with rhodamine 6G (0.5% in 95% ethanol) prior to viewing under ultraviolet light. The bands representing the triacylglycerol and phospholipid fractions were identified using phosphatidylethanolamine (Sigma Chemical Co., St. Louis, MO) and triacylglycerol standards on the same plate. The bands were scraped, collected, and the lipid fractions eluted with dichloromethane/methanol solvent (9:1, v/v) into tared vials. The vials were flushed with nitrogen and subsequently stored at −20C until further use.

Quantitation of Lipid Fractions

Triacylglycerol and phospholipid fractions from each lipid source were separated on silica gel G plates as described above. The lipid classes on the developed plates were visualized after spraying with a mixture of sulfuric acid and chromic acid (95:5, v/v) and subsequently charring at 150C for 20 min. Each fraction was quantified using a Shimadzu densitometer (Shimadzu Dual-Wavelength Thin Layer Chromato Scanner Model CS-930; Shimadzu Scientific Instruments, Columbia, MD). Individual triacylglycerol and phospholipid contents were expressed as a percentage of the total lipid content.

Statistical Analysis

The experimental designs used for this study were a split block design and a replicated two factor design. The data were analyzed statistically by using the procedures outlined by Gill (1988). Analysis of variance (ANOVA) for data was calculated using the MSTATC microcomputer program (Michigan State University 1991). Tukey's test was applied to separate the mean values.

RESULTS AND DISCUSSION

Fermentation Characteristics of Yeast Strains

Four yeast strains were grown on culture media containing whey permeate as the carbon source. The initial high carbon:nitrogen ratio of whey permeate makes it a good substrate for lipid production (Moon *et al.* 1978). As the carbon to nitrogen ratio of the growth medium seems to be critical for the production of yeast lipids (Evans and Ratledge 1984b; Turcotte and Kosaric 1989; Granger *et al.* 1993), four different carbon:nitrogen ratios were employed in the culture medium by incorporating yeast extract and asparagine as the nitrogen source. Vega *et al.* (1988) reported that the addition of asparagine boosted cell numbers and cell dry weight, which was the result of providing an easily used nitrogen source. It has been observed that a high nitrogen concentration relative to carbon in the culture medium enhances biomass production, but results in the synthesis of very low amounts of lipid (Moreton 1988). During the fermentation process, different parameters such as lactose utilization (results not shown), protein consumption (results not shown) and growth rate were investigated to monitor the point at which the fermentation process could be terminated for lipid extraction. The fermentation process was ended when the carbon source in the culture medium was almost completely exhausted and the cells were in the stationary phase.

Growth Rate

Growth rate was monitored by recording the optical density of the culture media at 610 nm. All the yeast strains under study behaved differently with regard to their growth on whey permeate (Tables 1-4). Statistical analysis of the data revealed that carbon:nitrogen ratios and cultivation time both have a significant ($p<0.01$) effect on the growth rate of *Apiotrichum curvatum* ATCC 10567 and *Cryptococcus albidus* ATCC 56297. The significant first-order interaction ($p<0.01$) between carbon:nitrogen ratios and cultivation time suggests that the effects of these variables are highly dependent upon each other. The carbon:nitrogen ratio and cultivation time also showed a significant ($p<0.01$) effect on the growth rate of *Rhodosporidium toruloides* ATCC 10788, but the nonsignificant first-order interaction suggests that the effects of these variables are independent of each other in the case of this strain. Only cultivation time had a significant ($p<0.01$) influence on the growth rate of *Lipomyces starkeyi* ATCC 12659.

Apiotrichum curvatum ATCC 10567 continued growing exponentially up to 48 h, whereafter its growth started declining gradually. However, maximum optical density of the culture medium with a carbon:nitrogen ratio of 30:1 was recorded at the end of 96 h of fermentation.

TABLE 1.
THE GROWTH RATE OF APIOTRICHUM CURVATUM ATCC 10567 AT DIFFERENT CARBON TO NITROGEN RATIOS AS MONITORED BY RECORDING THE OPTICAL DENSITY OF THE CULTURE MEDIUM AT 610 nm[1]

Cultivation	Optical Density at C:N Ratios[2]			
Time (h)	30:1	45:1	60:1	75:1
0	1.70±0.10[a]	1.68±0.08[a]	1.49±0.29[a]	1.79±0.09[a]
24	24.50±0.10[a]	26.30±1.00[a]	32.55±2.65[a]	30.65±0.15[a]
48	57.50±2.50[ab]	59.20±2.50[a]	54.35±1.85[ab]	46.40±0.50[b]
72	82.45±1.75[a]	74.25±0.05[a]	62.70±1.90[b]	54.95±2.85[b]
96	90.63±0.38[a]	81.25±2.35[a]	69.05±2.05[b]	59.35±4.25[b]
120	86.90±8.20[a]	80.50±5.00[a]	68.35±5.55[b]	60.30±6.30[b]

[1] All values represent the average of two replicates along with the standard deviation.
[2] C:N ratio, carbon to nitrogen ratio.
[a,b] Values in the same row bearing the same superscript are not significantly different from each other at $p<0.05$.

TABLE 2.
THE GROWTH RATE OF *CRYPTOCOCCUS ALBIDUS* ATCC 56297 AT DIFFERENT CARBON TO NITROGEN RATIOS AS MONITORED BY RECORDING THE OPTICAL DENSITY OF THE CULTURE MEDIUM AT 610 nm[1]

Cultivation	Optical Density at C:N Ratios[2]			
Time (h)	30:1	45:1	60:1	75:1
0	1.39±0.06[a]	1.43±0.08[a]	1.47±0.01[a]	1.50±0.02[a]
24	11.80±0.20[a]	10.55±0.45[a]	9.75±0.45[a]	9.60±0.20[a]
48	18.20±0.20[a]	15.80±0.20[a]	15.15±0.15[a]	14.00±0.30[a]
72	23.75±2.35[a]	20.60±2.10[ab]	20.20±2.00[ab]	18.85±2.45[b]
96	31.25±3.95[a]	28.70±3.60[a]	27.05±2.35[ab]	23.90±2.50[b]
120	39.25±2.75[a]	35.15±1.95[a]	30.60±1.80[b]	26.85±1.85[b]
144	45.45±1.75[a]	38.00±1.10[b]	32.50±1.80[c]	27.97±0.55[d]
168	49.30±1.00[a]	38.75±1.15[b]	34.05±0.85[c]	29.70±0.00[c]
192	51.15±0.35[a]	41.80±0.60[b]	35.45±0.35[c]	29.15±0.35[d]

[1] All values represent the average of two replicates along with the standard deviation.
[2] C:N ratio, carbon to nitrogen ratio.
[a,b,c,d] Values in the same row bearing the same superscript are not significantly different from each other at $p<0.05$.

TABLE 3.
THE GROWTH RATE OF *LIPOMYCES STARKEYI* ATCC 12659 AT DIFFERENT CARBON TO NITROGEN RATIOS AS MONITORED BY RECORDING THE OPTICAL DENSITY OF THE CULTURE MEDIUM AT 610 nm[1]

Cultivation	Optical Density at C:N Ratios[2]			
Time (h)	30:1	45:1	60:1	75:1
0	1.18±0.10[a]	1.20±0.08[a]	1.24±0.05[a]	1.18±0.06[a]
24	1.14±0.04[a]	1.17±0.05[a]	1.19±0.02[a]	1.21±0.01[a]
48	1.10±0.30[a]	2.10±0.50[a]	1.10±0.30[a]	1.15±0.35[a]
72	3.20±0.30[a]	3.90±0.30[a]	3.05±0.55[a]	3.10±0.40[a]
96	4.20±0.00[a]	4.40±0.40[a]	3.70±0.30[a]	3.65±0.85[a]
120	4.75±0.15[a]	5.60±0.60[a]	5.15±0.05[a]	5.80±0.00[a]
144	6.90±0.50[a]	7.20±0.90[a]	6.15±0.05[a]	6.50±0.90[a]
168	8.70±1.20[a]	8.00±1.20[a]	8.85±0.85[a]	11.75±1.45[a]
192	11.65±3.35[a]	11.10±1.70[a]	10.70±1.80[a]	13.70±0.00[a]
216	14.10±2.80[a]	12.75±0.35[a]	12.95±0.35[a]	15.80±0.00[a]
240	19.00±4.40[a]	16.30±0.10[b]	20.10±0.00[ab]	23.70±0.00[a]

[1] All values represent the average of two replicates along with the standard deviation.
[2] C:N ratio, carbon to nitrogen ratio.
[a,b] Values in the same row bearing the same superscript are not significantly different from each other at $p<0.05$.

TABLE 4.
THE GROWTH RATE OF *RHODOSPORIDIUM TORULOIDES* ATCC 10788 AT DIFFERENT CARBON TO NITROGEN RATIOS AS MONITORED BY RECORDING THE OPTICAL DENSITY OF THE CULTURE MEDIUM AT 610 nm[1]

Cultivation	Optical Density at C:N Ratios[2]			
Time (h)	30:1	45:1	60:1	75:1
0	1.73±0.07[a]	1.55±0.05[a]	1.49±0.09[a]	1.61±0.01[a]
24	7.70±0.40[a]	6.80±0.50[ab]	6.25±0.55[b]	6.10±0.50[b]
48	7.20±0.40[a]	6.65±0.45[a]	6.40±0.30[a]	6.40±0.70[a]

[1] All values represent the average of two replicates along with the standard deviation.
[2] C:N ratio, carbon to nitrogen ratio.
[a,b] Values in the same row bearing the same superscript are not significantly different from each other at $p<0.05$.

TABLE 5.
QUANTITATION OF TRIACYLGLYCEROL AND PHOSPHOLIPID FRACTIONS OF LIPIDS OBTAINED FROM DIFFERENT YEAST STRAINS GROWN ON CULTURE MEDIA HAVING DIFFERENT CARBON TO NITROGEN RATIOS

Yeast	Fraction	Contents as Percentage of Total Lipids at Carbon:Nitrogen Ratios			
		30:1	45:1	60:1	75:1
A.curvatum	TAG	58.5	72.0	67.3	76.8
	PL	7.6	11.0	6.9	10.4
C.albidus	TAG	43.0	34.4	38.4	46.8
	PL	23.6	20.9	19.4	19.5
L.starkeyi	TAG	50.8	40.5	46.5	66.8
	PL	5.3	6.5	4.5	7.7
R.toruloides	TAG	11.8	14.9	8.5	7.7
	PL	35.1	33.0	31.7	38.7

[1] All values represent the average of two replicates.
[2] TAG, triacylglycerol.
[3] PL, Phospholipid.

On the other hand, *Cryptococcus albidus* ATCC 56297 and *Rhodosporidium toruloides* ATCC 10788 reached their maximum exponential growth in about 24 and 192 h, respectively. However, *Lipomyces starkeyi* ATCC 12659 showed no signs of growth during the first 48 h of the fermentation period. This time was possibly used by this yeast strain to adapt its metabolic pathways to the available conditions of the fermentation. This yeast strain reached the plateau of its exponential growth phase after 240 h. Turcotte and Kosaric (1989) observed depression in growth rate around 20 h, although it took 70 h for the nitrogen source to be reduced to a zero level while growing *Rhodosporidium toruloides* ATCC 10788 in a culture medium containing glucose as the carbon source.

Dry Cell Mass of Yeast Strains

The weights of dry cell mass for all the yeast strains are presented in Fig. 1. *Apiotrichum curvatum* ATCC 10567 produced the greatest dry cell mass out of the four yeast strains evaluated. A yield of 18.6 g/L dry cell mass was obtained at the carbon:nitrogen ratio of 45:1. On the other hand, *Rhodosporidium toruloides* ATCC 10788 produced the smallest dry cell mass and this may be due to its inability to utilize lactose efficiently. The maximum dry cell mass yield (0.9 g/L) by this strain was achieved at carbon:nitrogen ratio of 60:1. *Cryptococcus albidus* ATCC 56297 produced its maximum dry cell mass (13.8 g/L) at the carbon:nitrogen ratio of 30:1, while *Lipomyces starkeyi* ATCC 12659 yielded its greatest amount (9.9 g/L) at a carbon:nitrogen ratio of 60:1. It is evident from these results that dry cell mass yield was highly dependent upon yeast strain and carbon:nitrogen ratio.

Satistical analysis of the data also showed that both yeast strains and carbon:nitrogen ratios had a significant ($p<0.05$) effect on the dry cell mass yield. However, the significant ($p<0.01$) first-order interaction indicates that the effects of both these variables were not independent of each other.

The dry cell mass yield was in close agreement to those reported in a number of other studies. Evans and Ratledge (1984a) reported 9.9 g/L and 11.2 g/L dry cell mass yield while growing *Lipomyces starkeyi* CBS 1807 and *Rhodosporidium toruloides* CBS 6016 in a culture medium containing glucose as carbon source and asparagine as nitrogen source, respectively. Vega *et al.* (1988) obtained dry cell mass yield of 11.85 g/L while growing *Apiotrichum curvatum* ATCC 20509 on 20% banana juice, supplemented with asparagine and mineral salts after 96 h of fermentation.

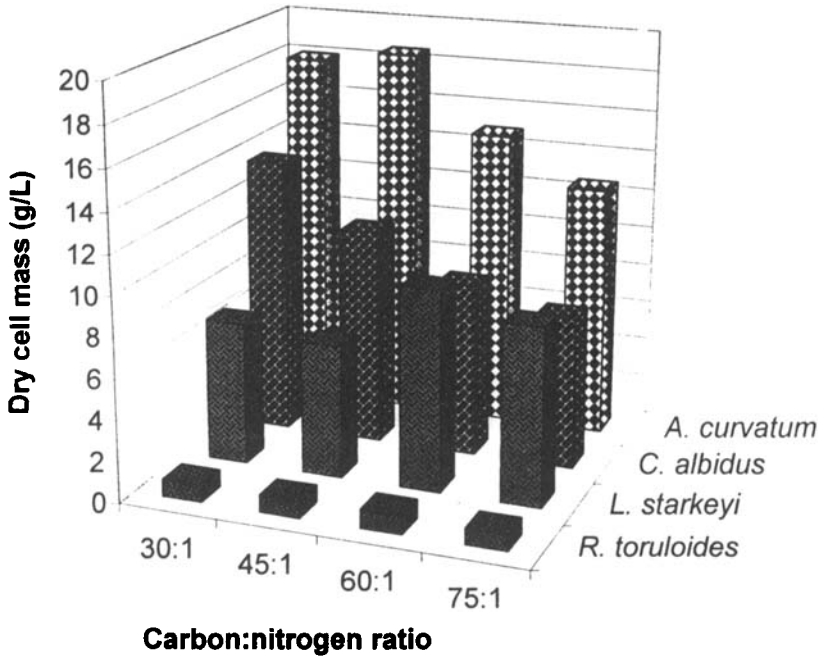

FIG. 1. THE YIELD OF DRY CELL MASS OF YEAST STRAINS GROWN ON CULTURE MEDIA HAVING DIFFERENT CARBON TO NITROGEN RATIOS

Lipid Yield of Yeast Strains

Lipid accumulation in yeast calls can be viewed as a two-stage process. In the first stage, the multiplication of cells takes place in the presence of an abundance of the nitrogen source. In the second stage, growth limitation results from the exhaustion of the nitrogen source, while excess carbon is converted into lipid. Lipid yields by the yeast strains are presented in Fig. 2 as a percentage of dry cell mass. Of all the strains investigated, *Lipomyces starkeyi* ATCC 12659 synthesized the greatest quantity of lipids (36.9% lipid) at the carbon:nitrogen ratio of 30:1. *Apiotrichum curvatum* ATCC 10567 was the second highest producer of lipid (16.6% at carbon:nitrogen ratio of 60:1). In contrast, *Rhodosporidium toruloides* ATCC 10788 synthesized 9.1% lipid at a carbon:nitrogen ratio of 45:1, while *Cryptococcus albidus* ATCC 56297 produced only 3.52% lipid at a carbon:nitrogen ratio of 75:1. Ykema *et al.* (1986) cultivated *Apiotrichum curvatum* ATCC 20509

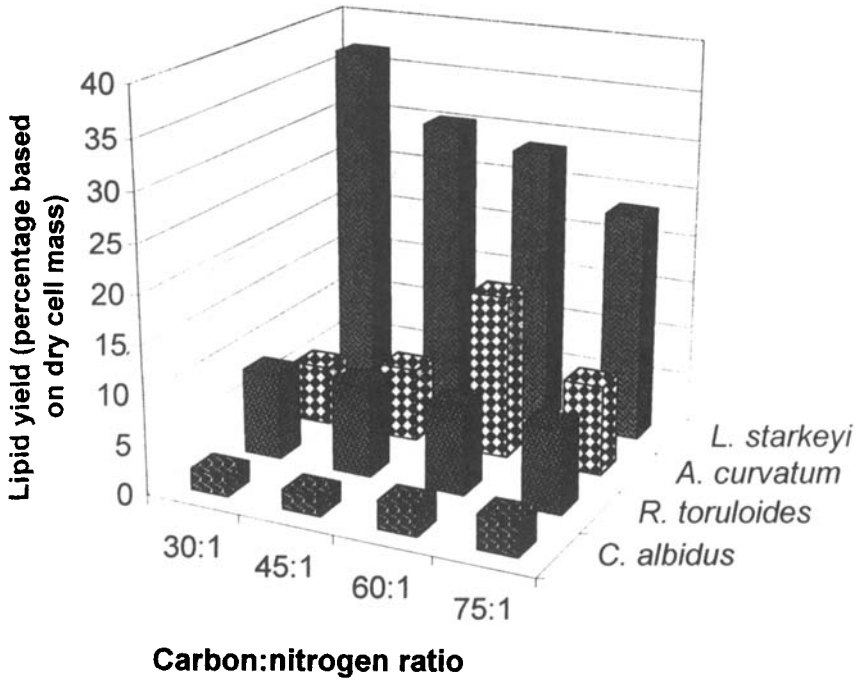

FIG. 2. THE PERCENTAGE OF LIPID YIELD OBTAINED FROM DIFFERENT YEAST STRAINS GROWN ON CULTURE MEDIA HAVING DIFFERENT CARBON TO NITROGEN RATIOS

in a semi-defined growth medium and reported 30% intracellular lipids on the basis of dry cell mass.

It is apparent from these results that yeast strain and carbon:nitrogen ratio influence the synthesis of lipids. Statistical analysis of the data showed that yeast strain had a major effect on lipid yield (significant at $P<0.01$). However, the significant ($P<0.05$) first-order interaction indicates that effect of yeast strain is dependent on the carbon:nitrogen ratios. A number of studies performed on other species of yeast also showed that the carbon:nitrogen ratio influenced lipid yield. Yoon *et al.* (1982) reported that the carbon:nitrogen ratio of approximately 41 affected the lipid content of *Rhodotorula gracilis* NRRL Y-1091 grown on rice straw. Ykema *et al.* (1988) studied lipid production by the oleaginous yeast strain, *Apiotrichum curvatum* ATCC 20509, in whey permeate and reported that

maximum lipid production rates were obtained at carbon:nitrogen ratios of 30-35:1. Turcotte and Kosaric (1989) found that an initial carbon:nitrogen ratio of about 77 was appropriate for maximum lipid production when using glucose or fructose as the carbon source, and either ammonium sulfate, ammonium nitrate, or urea as the nitrogen source.

Quantitation of Lipids Synthesized by Yeast Strains

Major components of yeast lipids include triacylglycerols, phospholipids, free fatty acids, steryl esters and sterols (Uzuka *et al.* 1975; Yoon and Rhee 1983b). Phospholipids are generally the second largest lipid fraction and mainly consist of phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol (Kaneko *et al.*1976). The major sterol component is ergosterol, with small amounts of zymosterol, lanosterol, fecosterol and episterol also present (Aries and Kirsop 1978).

The concentrations of the major classes of lipids synthesized by the four yeast strains are presented in Table 5. All yeast strains except *Rhodosporidium toruloides* ATCC 10788 produced greater amounts of triacylglycerols than phospholipids. Lipids from *Apiotrichum curvatum* ATCC 10567 contained 58.5-76.8% triacyglycerols and 6.9-11.0% phospholipids depending upon the carbon:nitrogen ratio. *Cryptococcus albidus* ATCC 56297 synthesized 34.4-46.8% triacylglycerols and 19.4-23.6% phospholipids. *Lipomyces starkeyi* ATCC 12659 accumulated 40.5-66.8% triacylglycerols and 4.5-7.7% phospholipids. In contrast, *Rhodosporidium toruloides* ATCC 10788, produced phospholipids in the range of 31.7-38.7% and triacylglycerols varied from 7.7-14.9% depending upon the carbon:nitrogen ratios. The rest of the components in the case of *Apiotrichum curvatum* ATCC 10567, *Cryptococcus albidus* ATCC 56297, *Lipomyces starkeyi* ATCC 12659 and *Rhodosporidium toruloides* ATCC 10788 (12.8-33.9%, 33.4-33.7%, 25.5-53.0% and 9.8-52.1%, respectively) may contain monoacylglycerols, diacylglycerols, free fatty acids, sterol esters and sterols.

Kessel (1968) reported 14% triacylglycerols and 26% phospholipids in *Rhodotorula gracilis* NCYC 59. Thorpe and Ratledge (1972) quantitated the lipid classes of the lipids isolated from *Candida* sp. no. 107 and reported 72-83% triacylglycerols and 5% phospholipids. The limits observed for triacylglycerols and phospholipids were slightly lower and higher, respectively, compared to the limits reported in a review by Ratledge (1982).

Microbial lipids have the potential to be vegetable oil substitutes in the context of increasing demand and short supplies of edible oils and fats. However, there is a need to develop techniques to maximize the exponential growth phase in order to obtain higher quantities of biomass in short periods of fermentation.

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