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Microbiology

COMPARISON OF LIPID PRODUCTION BY UNICELLULAR AND FILAMENTOUS FUNGI

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Eight different microorganisms including four unicellular fungi viz. *Rhodorula glutinis, Lipomyces starkeyi, Candida utilis* and *Saccharomyces cerevisiae* and four filamentous fungi viz., *Oospora sp., Fusarium oxysporum, Aspergillus luchuensis* and *A. niger* were tried to compare their capacity to produce cell biomass and lipids under varying cultural conditions. Lipid content of the unicellular fungi ranged from 6·3 to 53·6 % and of filamentous from 8·9 to 27·5 % with highest by *R. glutinis* and *F. oxysporum* respectively. Sucrose @ 50 g/litre medium and potassium nitrate as nitrogen source and incubation period of 12 days were found to be the best for lipid production from *R. glutinis* and *F. oxysporium*. Chemical analysis of lipids indicated more than 50 % of total lipids were non-polar fraction, comprised of mainly triglycerides and free fatty acids.

Although India is third major oilseed producing country in the world, yet the per capita consumption of lipid is only 25 % of the man's nutritional requirement, and moreover, these are deficient in unsaturated fatty acids. The shortage of lipids is becoming more acute with the expanding population. This can partially be solved by exploiting the microorganisms which have the advantage of their higher rate of metabolism, variety of metabolism and can convert the cheap crude forms of carbohydrates, e.g. molasses into fats (Lundin, 1949; Woodbine *et al.*, 1951; and Murray *et al.*, 1953). Though both unicellular and filamentous fungi are known to produce lipids yet no work seems to have been carried out to compare lipids of the two groups. The present study was, therefore, undertaken to compare lipid-producing capacity of unicellular and filamentous fungi and to standardize the cultural conditions of the organisms to maximize lipid productivity.

METHODS AND MATERIALS

Four unicellular and four filamentous fungi supplied by Dr C. P. Kurtzman** and Dr D. S. Chahal, respectively were used in this study.

The yeasts (Unicellular fungi) *Rhodotorula glutinis* (old name—*Rhodotorular gracillis* Harrison NRRm L-Y-1091), *Lipomyces starkeyi* Lodder et Kregar-van-Rij (1952) NRRL-Y-1389, *Candidi utilis* (Hennerberg) Lodder et Kregar-van-Rij NRRL-Y-900, *Saccharomyces cerevisiae* Meyer NRRL-Y-100.

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The moulds (filamentous fungi) Oospora sp-isolated from rotten spota fruit, *Fusarium oxysporum* Schlect, *Aspergillus luchuensis* Inni, *Aspergillus niger* van Tiegham.

The yeast cultures were maintained on yeast mannitol agar (Wickerham *et al.*, 1951) and filamentous fungi on Czapek's agar medium by fortnightly sub-culturing.

Modified Richard's medium was used as basal medium for lipid production.

Composition :

Sucrose, 50.0 g; Urea, 0.860 g; Potassium dihydrogen phosphate, 5.0 g; Magnesium sulphate, 2.5 g; Ferric chloride (Soln. 1.92 g/litre), 1 ml; Zinc sulphate (Soln. 44 g/litre), 1 ml; Distilled water, 1000 ml; *p*H, 5.5.

Basal medium was dispensed in equal volumes (50 ml) in Erlenmeyer flasks of 250 ml capacity in quadruplicate. After sterilization, flasks were inoculated with 1 ml cell/spore suspension (9×10^4 cells or spores/ml) and incubated at 25° C on rotary shaker at a speed of 130 strokes per minute and amplitude of 3.2 cm for ten days or otherwise as required in the experiment.

The biomass of unicellular fungi was harvested by centrifugation at 3800 rpm and of filamentous fungi by filtering through Whatman No. 42 filter paper followed by 2-3 washings with sterilized distilled water in both the cases. The biomass was dried at 60° C till constant weight in an oven.

Total lipids were extracted by the methods of Bhatia *et al.* (1972). Polar and non-polar fractions were separated out by solvent partition method of Nichols (1964). The different non-polar lipid classes were separated by thin layer chromatographic technique.

Results and Discussion

Eight different species including unicellular and filamentous fungi were taken to find one best from each group and then to study the cultural conditions to maximize lipid productivity.

The lipid content from 4 unicellular and 4 filamentous fungi was found to range from 6.4 to 43.0 % and 7.0 to 24.3 % respectively (Table I).

Although some unicellular fungi (*C. utilis* and *S. cerevisiae*) were as poor in lipid production as filamentous fungi, in general the filamentous fungi were less efficient to convert the substrate into lipid. On the other hand biomass co-efficient of filamentous fungi was as high as that of unicellular fungi indicating that lipid producing ability of certain unicellular fungi is based on the genetic make up. *R. glutinis* and *F. oxysporum* gave highest lipid per cent, total lipid and lipid co-efficient from each group and were selected for further studies to find optimum cultural condition to maximize their lipid producing capacity.

Effect of Different Nitrogen Sources

Rhodotorula glutinis—Out of 5 different nitrogen sources tried (Table II), potassium nitrate was found to be the best for lipid production, yielding 11.2 g lipid per litre (53.6 % lipid) with lipid coefficient of 22.4. The dry weight of biomass (20.860 g/l) and biomass coefficient (36.2) was also highest. Urea was the

next biomass source for the biomass (17.540 g/l) and lipid production (7.54 g/l). Urea has also been reported as best nitrogen source for lipid production by *R. glutinis* by Lubjana *et al.* (1953) and Chicago *et al.* (1952). Of course the present study indicated that potassium nitrate was the best nitrogen source but this was not tried by Lubjana *et al.* (1953).

TABLE I

Screening of unicellular and multicellular fungi for lipid production[a]

Organism	Dry weight of biomass/l (g)	Lipid (%)	Total lipid/l	Biomass* coefficient	Lipid** coefficient
Rhodotorula glutinis	17·540	43·0	7·540	35·1	15·0
Lipomyces starkeyi	15·420	21·4	3·300	30·9	6·6
Candida utilis	2·580	11·0	0·280	5·2	0·6
Saccharomyces cerevisiae	6·360	6·4	0·400	12·7	0·8
Oospora sp.	0·920	7·0	0·060	1·9	0·1
Fusarium oxysporum	9·740	24·3	2·400	19·5	4·7
Aspergillus Luchuensis	17·960	12·0	2·160	35·9	4·3
Aspergillus niger	19·840	9·8	1·960	39·7	3·9

*Biomass coefficient $= \frac{\text{Weight of dry biomass (mg)}}{\text{Weight of sugar supplied}} \times 100$

**Lipid coefficient $= \frac{\text{Weight of lipids (mg}}{\text{Weight of sugar supplied}} \times 100$

[a]Using sucrose + urea @ 50·0 g and 0·86 g per litre medium.

TABLE II

*Effect of different sources of nitrogen on biomass and lipid production**

Organism	N-source	Dry weight of biomass	Lipid %	Total Lipids/l	Biomass coefficient	Lipid coefficient
Rhodotorula glutinis	NH_4NO_3	6·860	14·0	0·960	13·7	2·0
	NH_4Cl	4·980	10·0	0·500	10·0	1·0
	KNO_3	20·860	53·6	11·180	36·2	22·4
	$(NH_4)_2SO_4$	3·460	13·4	0·460	7·0	1·0
	$(NH_2)_2CO$	17·540	43·0	7·540	35·1	15·0
Fusarium oxysporum						
	NH_4NO_3	10·020	17·4	1·740	20·0	3·5
	NH_4Cl	4·020	24·5	0·980	8·0	2·0
	KNO_3	11·160	25·8	0·280	22·3	5·8
	$(NH_4)_2SO_3$	3·940	16·3	0·640	19·9	1·3
	$(NH_2)_2CO$	9·740	24·3	0·240	19·5	4·7

*Using sucrose and nitrogen @ 50·0 and 0·4 g per litre medium.

Effect of Different Concentrations of Source

Rhodotorula glutinis—The increase in sucrose concentration from 10.00 to 70 g/l increased the biomass from 3.5 to 21.480 g/l and lipid percentage from 28.5 to 53.2 % (Table III). The maximum lipids obtained were 11.420 g/l on sucrose concentration of 70 g/per litre but the maximum lipid coefficient to 21.9 was on 50 g/l. But considering the biomass and lipids coefficient of 50 g/l can be suggested as the optimum. At this concentration C : N ratio was 73.5.

TABLE III

*Effect of different concentrations of sucrose on biomass and lipid production**

Organism	Conc. of g/litre	Dry weight of biomass/1 (g)	Lipid %	Total lipids/1 (g)	Biomass coefficient	Lipid coefficient
Rhodotorula	1·5	8·660	36·4	3·160	28·9	10·5
glutinis	2·0	14·940	41·5	6·200	37·4	15·5
	2·5	20·720	52·8	10·940	41·5	21·9
	3·0	20·800	53·0	11·020	34·7	18·4
	3·5	21·480	53·2	11·420	30·7	16·3
	4·0	21·080	53·0	11·180	26·4	14·0
Fusarium	1·5	6·760	19·4	1·320	22·6	4·4
oxysporum	2·0	8·440	20·0	1·700	21·1	4·2
	2·5	11·300	26·4	2·880	22·6	5·7
	3·0	9·080	29·5	2·220	15·1	3·7
	3·5	8·680	23·4	2·040	12·4	2·9
	4·0	5·940	21·6	1·280	7·4	1·6

* Using KNO_3 @ 2·88 g per litre medium.

The lipid percentage of 50-60% with lipid coefficient of 16 to 18 had been reported by Enebo *et al.* (1946) for *R. glutinis* using glucose and by Pan *et al.* (1949) using molasses.

Fusarium oxysporum

There was a continuous increase in the biomass with increase in sucrose up to 50 g/l but the biomass coefficient was highest (36.4) at 10 g/l (Table III). This concentration cannot be considered optimum because at this concentration, lipid coefficient was only 3.6 against 5.7 at 50 g/l at which the biomass coefficient was also fairly good (22.6). At this concentration C/N ratio comes to be 52.5. In a similar study Bhatia and Arneja (1970) reported that the growth and lipid production by this organism can be increased by growing the organism first on media with narrow C/N ratio (30) and then transferring it to a medium of wide C/N ratio (300) after 8 days growth on the previous media.

Effect of Different Carbon Sources

Rhodotorula glutinis—The total biomass of 42.100, 21.160 and 20.500 g/l with their respective biomass coefficients of 84.2, 42.3 and 41.1 were obtained on molasses,

sucrose and glucose respectively indicating that all these three carbon sources were good for growth that molasses giving the maximum (Table IV). From the percentage lipids as well as total lipids per litre point of view, sucrose proved to be the best yielding 11.160 g lipid per 1 (52.7 %) with the lipid coefficient of 22.3. The values for lipid per 1 and lipid coefficient obtained from the molasses were less than those obtained from the sucrose and the value for glucose (5.220 g lipid/1 and lipid coefficient 10.4) were lowest amongst these three carbon sources. The high biomass coefficient might be due to the presence of some inert carbon or other compounds which were not utilized and stayed there as much in the final weight.

TABLE IV

*Effect of different carbon sources on biomass and lipid production**

Organism	C-source	Dry weight of biomass (mg)	Lipid (%)	Total lipids/1 (g)	Biomass coefficient	Lipid coefficient
Rhodotorula glutinis	Sucrose	21·160	52·7	11·160	42·3	22·3
	Glucose	20·560	25·4	5·220	41·1	10·4
	Molasses	42·100	14·6	6·140	84·2	12·3
Fusarium oxysporum	Sucrose	11·200	26·5	2·960	22·4	5·9
	Glucose	10·020	24·8	2·480	21·1	5·0
	Molasses	9·900	15·9	1·580	19·8	3·1

* Using sugar and K NO_3 @ 50·0 g and 2·88 g per litre medium.

Fusarium oxysporum—Among the three carbon sources, sucrose was found to be the best for the biomass and lipid production yielding biomass coefficient and lipid coefficient of 22.4 and 5.9 respectively (Table IV). The same observations could be true for *F. oxysporum* on molasses giving almost as high biomass coefficient as that by sucrose and glucose but very low lipid coefficient.

Effect of Different Incubation Periods

Rhodutorula glutinis—A rapid increase in the biomass was observed with the increase in incubation period up to 12th day thereafter it remained more or less constant. The maximum biomass obtained was 20.680 g per 1 with the biomass coefficient of 41.4 and lipid coefficient of 21.8 after 12 days of incubation (Table VI). Though the percentage lipid (53.2) was highest after 15 days of incubation and hence incubation of 9-12 days should be considered optimum. Chicago *et al.* (1952) reported that *R. glutinis* when grown for 77 hr following 48 hr for incubation resulted only in 35 % lipid. Had they incubated further, more lipid might have been produced.

Fusarium oxysporum—There was continuous increase in biomass coefficient, percentage lipid, total lipid and lipid coefficient of this organism up to 12 days and thereafter it remained almost constant. Maximum biomass coefficient (22.4) and the

lipid coefficient (6.1) were obtained after 12 days of incubation and so this period was considered to be optimum (Table V).

TABLE V

*Effect of incubation period on biomass and lipid production**

Organism	Incubation period (days)	Dry weight of biomass/l (g)	Lipid %	Lipid/ litre (g)	Biomass coefficient	Lipid coefficient
Rhodotorula	9	15·920	43·2	6·880	31·8	13·8
glutinis	12	20·680	52·7	10·900	41·4	21·8
	15	20·460	53·2	10·880	40·9	21·8
	18	20·480	52·9	10·840	40·9	21·6
Fusarium	9	9·120	24·3	2·220	18·2	4·4
oxysporum	12	11· 20	27·5	3·080	22·4	6·1
	15	11·160	26·4	2·940	22·3	5·9
	18	9·520	21·5	2·040	19·0	4·1

* 2·88 g/litre, 50 g.

The best incubation period reported by Bhatia and Arneja (1970) is 10 days if the organism was grown on medium with C/N ratio of 30 under still conditions but when 8 days grown fungus was transferred to a first medium with wider C/N ratio (300), further incubation period of 8 days was required to obtain the maximum per cent of lipid (29.3) as well as the maximum lipid during the second phase of growth.

Chemical Analysis of Lipids

The chemical analysis of lipids indicated that the non-polar lipids were present up to the extent of more than 70 % in most of the test organisms except in case of *R. glutinis* and *A. niger* in which the values for this were only 52.7 and 66.8 % respectively. In other words lipids of *R. glutinis* were richer in polar lipids (47.3 %) followed by *A. niger* (33.2 %) as compared to that of others.

Thin layer chromatogram (Fig. 1) indicated the relative occurrence of the different non-polar lipid classes in the test organism. Their identity is based on the relative Rf values, with those of the standard compounds.

In all, six non-polar lipid classes were identified. Among these, monoglycerides (Spot 1) were found to be present only in three organisms viz. *C. utilis.*, *F. oxysporum* and *A. niger* while diglycerides (Spot 2) were present in all the test organisms except in case of *S. cerevisae.* Spot of free strol, free fatty acids and triglycerides (Spot 3, 4 and 5 respectively) were found to be present in the lipids of all the test organisms, while sterol esters and hydrocarbons (Spot 6) were present in lipids of *R. glutinis*, *C. utilis*, *F. oxysporum*, *A. luchuensis* and *A. niger* and were absent in others.

Ruinen (1963) reported that the lipids of *Lipomyces starkeyi* and *R. glutinis* to be mostly glycerides. In our studies it was not so with the latter, where, about equal

TABLE VI

Chemical analysis of lipids of different organisms

Organism	Lipid classes		Occurrence of different non-polar classes					
	Polar (%)	Non-polar (%)	Mono-glycerides	Di-glycerides	Tri-glycerides	Free fatty acids	Tri-glycerides	Sterol Esters, Hydrocarbons
Rhodotorula glutinis	47·3	52·7	—	+	+	+	+	+
Lipomyces starkeyi	10·4	89·6	—	+	+	+	+	+
Candida utilis	15·3	84·7	+	+	+	+	+	+
Saccharomyces cerevisae	21·6	78·4	—	—	+	+	+	—
Oospora sp.	26·6	73·4	—	+	+	+	+	—
Fusarium oxysporum	10·3	89·7	+	+	+	+	+	+
Aspergillus luchuensis	9·5	90·5	—	+	+	+	+	+
Aspergillus niger	33·2	66·8	+	+	+	+	+	+

amounts of polar and non-polar lipids were present. Bhatia *et al.* (1973) also reported the presence of monoglycerides, diglycerides, free sterols, free fatty acids and triglycerides in seven moulds.

Chemical analysis of lipids indicated that *R. glutinis*, *S. cerevisiae*, *Oospora* sp. and *A. niger* were quite rich in polar lipids containing more than 20 per cent of the total lipids as compared to others. Thin layer chromatography of the non-polar lipid fraction indicated the presence of the triglycerides and free fatty acids as the major classes in all the test organisms, but free fatty acids were comparatively much higher in *R. glutinis*.

From the comparative study on lipid production from different organisms, it can be concluded that although it is largely determined by the genetic capability, yet it can be enhanced to a great extent by providing the optimum cultural conditions to the organisms. In general, it was noticed that lipid production does not depend upon the unicellular or filamentous nature of the organism but on the genetic behaviour as *R. glutinis* a unicellular fungus gave the highest lipid production but

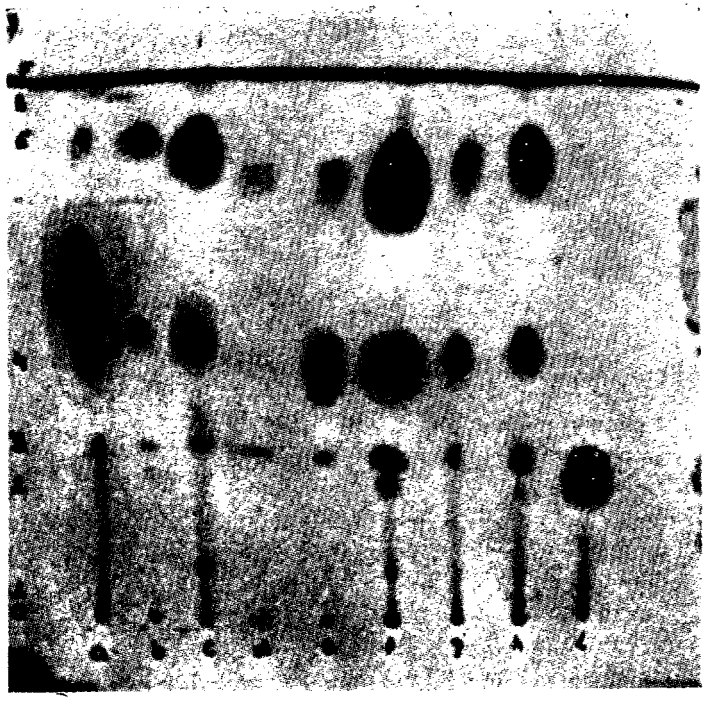

FIG. 1. Thin layer chromatogram of non-polar lipids of *different organisms. (a) R. glutinis; (b) L. starkeyi; (c) C. utilis; (d) S. cerevisiae; (e) Oospora sp.; (f) F. oxysporum; (g) A. luchuensis; (h) A. niger; (i) Standard cholesterol.*

[*Solvent system* :—Petroleum ether : Ethylene : Acetic acid = 80 : 20 : 1 V/v]

[Visulation : Spraying with cupric acetate phosphoric acid reagent (Fewer *et al.*, 1969)]

Identity components : — 0, origin; 1, Monoglycerides; 2, Diglycerides; 3, Free sterol; 4, Free Fatty acid ; 5, Triglyceride; 6, Sterol esters & hydrocarbons; 7, Solvent Front.

there were other filamentous fungi, *F. oxysporum*, *A. luchuensis* and *A. niger* gave comparatively more lipids than that of *L. starkeyi*, *C. utilis* and *S. cerevisiae*, unicellular fungi.

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