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Short Note

Effect of Carbon Sources on Lipid and Biomass Production by Oleaginous Yeast Cultures

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This study was undertaken to investigate the influence of carbon source on lipid production by oleaginous yeast cultures viz., *Rhodotorula glutinis, Rhodosporidium toruloides* and *Lipomyces starkeyi*. Among the carbon sources evaluated glucose exhibited higher biomass and lipid content. Among the yeast cultures, *Rhodotorula glutinis* exhibited higher lipid 2.43 g l^{-1} (23.78 per cent) and biomass 10.21 g l^{-1} in glucose, followed by *Rhodosporidium toruloides* with 2.29 g l^{-1} (22.54 per cent) and 10.15 gl^{-1} and *Lipomyces starkeyi* with 2.23 g l^{-1} (22.20 per cent) and 10.12 g l^{-1} of lipid and biomass respectively in glucose broth. Least amount of lipids was produced in sucrose containing broth. This study reveals the faster utilization of glucose for biomass production, which leads to the accumulation of lipids.

Key words: Lipids, Biomass, Oleaginous yeast, *Rhodotorula glutinis*, Carbon source

In biological systems, fatty acids are mostly encountered as components of lipids. Lipids are indispensable for growth and survival of all organisms. A fat is lipid material that is solid at room temperature whereas oil is a similar material that is liquid at room temperature. Natural oils and fats consist mainly of triacylglycerols. For this reason, the terms oil and fat are often used to denote triacylglycerols. The most common forms of sterol ester *viz.,* triacylglycerols and sterol esters as the main components of functional lipids in yeast are ergosterol esters.

Lipid production through microbial fermentation has long been an interesting research subject. Many molds, yeasts and algae exhibit the capacity to accumulate intracellular lipids in excess of 70 per cent of their biomass during metabolic stress periods. Among all heterotrophic microorganism, yeast showed its own advantages in terms of its fast growth rate and high oil content. The major component of oleaginous yeast and fungi is TAG (Triacyl glycerides), composed of C_{16} and C_{18} series long chain fatty acids, which are quite similar to those of vegetable oils, such as rapeseed oil and soybean oil. There fore microbial lipids should be useful for biodiesel production (Ratledge, 1991). However little information available on the use of microbial sources for lipid production as well as to exploit the microbial lipids for biodiesel production. Hence this present study was undertaken to optimize the carbon source for higher lipid production in through yeast cultures

Materials and Methods

Oleaginous yeast cultures *viz., Rhodotorula glutinis* (MTCC 247), *Rhodosporidium toruloides* (MTCC 1400) and *Lipomyces starkeyi* (MTCC 2974) were collected from MTCC, Chandigarh, India.

A lab experiment was conducted in the Dept. of Agricultural Microbiology, TNAU, to assess the lipid and biomass production by the above cultures in order to screen and to standardize the conditions for enhanced lipid production. This experiment was mainly aimed to find out the suitable carbon source for increased production of lipids.

Preparation of broth and inoculation

Screening broth (Dai et al. 2007) containing Yeast extract -15.0g,/l,Peptone - 5.0g/l was prepared with out carbon source, in which different carbon sources *viz.,* glucose, fructose and sucrose were added separately (0.22 M). The pH of the broth was adjusted to 6.0 and three replications were maintained for each carbon source. The 100 ml broth was inoculated with 24 hrs old cultures grown in YEPG broth at 10 per cent level containing $28x10^4$ cfu/ml in the broth. (Saxena *et al*., 1998).

Cultural conditions

Inoculated flasks were incubated at 30°C for 7 days in an incubator shaker at 200 rpm (Innova 4320, New Brunswick, USA) for the growth of culture. After seven days of growth, cultures were harvested by centrifugation and the cell pellets were obtained. Lipid content and biomass production of dried yeast cells were estimated by adopting the standard methods.

Cell biomass determination

For dry weight determination, culture sample was centrifuged at 6000 rpm for 10 min at room temperature and cell pellet was washed with 0.1 M

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phosphate buffer at pH 7.0. The supernatant was discarded and the cell pellet was dried at 65°C for 48 h. After cooling, the cell dry weight was estimated (Esther *et al*., 1988).

Identification of lipid bodies in oleaginous yeast cells

The harvested cell pellets were dissolved in small amount of sterile water to prepare the cell suspension. A known volume of the cell suspension was mixed with the same volume of 0.02 per cent Nile blue A solution and the same volume of 0.1 M glycine buffer (pH 10.0) and immediately the microscopic slides were prepared and observed under oil immersion objective for the presence of lipid bodies (Murphy and Vance, 1999).

Extraction of yeast lipid

Yeast lipid was extracted from the dried cells as described by Bligh and Dyer, (1959). Ten gram of dried cells were homogenized with 15 ml of methanol chloroform mixture 2:1 (v/v). Ten ml of chloroform was added again and homogenization was continued further for one min. To the homogenized contents, 10 ml of sterile distilled water was added and homogenized further for one min. Then the homogenized contents were filtered on a Buchner funnel through Whatman No.1 filter paper. While filtering, water in the contents was removed by adding one per cent anhydrous Na_2SO_4 to the filter paper. The filtrate was transferred to a graduated glass cylinder. Small quantities of yeast cells remaining in the blender and in the filter paper were washed with 10 ml of chloroform and the contents were transferred to the graduated cylinder and allowed for few min for the phases to separate. The lower volume of chloroform layer (20 ml added above) was recorded as 'x' ml. Upper methanol - water layer is removed by pipetting out along with layer of chloroform to ensure complete removal of methanol-water layer. Again the remaining lower volume of chloroform layer was recorded as 'y' ml. This volume was transferred quantitatively into a pre-weighted conical flask denoted as 'a' g. The contents in the conical flasks were evaporated at 40-50°C in water bath under stream of nitrogen gas. Remaining residues were cooled and dried over phosphoric anhydride in a vaccum desiccator. Weight of the conical flask was taken again and denoted as 'b' g.

Five ml of chloroform was added three times to dissolve the evaporated lipid. Again flasks were evaporated and dried as above. Weight of the flask was taken at third time and denoted as 'c' g. Amount of lipid present in the yeast cells was calculated by using the following formula.

Weight of lipid (g) = (b-a) - (c-a) ='d' g.

Where a - is the weight of empty flask.

$$\text{Total lipid (g)} = \text{Weight of lipid (d) x } \frac{\text{Total volume of chloroform layer (x ml)}}{\text{Volume of chloroform layer evaporated (y ml)}}$$

$$\text{Total lipid (per cent)} = \frac{\text{Total lipid (g)}}{\text{Weight of sample (g)}} \times 100$$

Table 1. Effect of carbon sources on lipid production by three oleaginous yeast cultures

Carbon source	Biomass (g l^{-1})				Lipid yield (g l^{-1})				Lipid in biomass (%)			
	C1	C2	C3	Mean	C1	C2	C3	Mean	C1	C2	C3	Mean
Glucose	10.21	10.15	10.12	10.16	2.43	2.29	2.23	2.34	23.80	22.54	22.23	16.19
Fructose	7.64	7.85	7.82	7.77	1.57	1.55	1.53	1.29	20.61	19.73	19.39	19.91
Sucrose	6.20	5.97	5.86	6.01	0.92	0.77	0.63	0.77	14.85	12.90	10.75	12.75
Mean	8.01	7.99	7.93		1.64	1.53	1.46		19.75	18.39	17.45	

	SE (d)	CD (0.05)	SE (d)	CD (0.05)	SE (d)	CD (0.05)
C	0.62	NS	0.21	NS	1.32	NS
T	0.62	1.31	0.21	0.44	1.32	2.77
C X T	1.08	NS	0.37	NS	2.28	NS

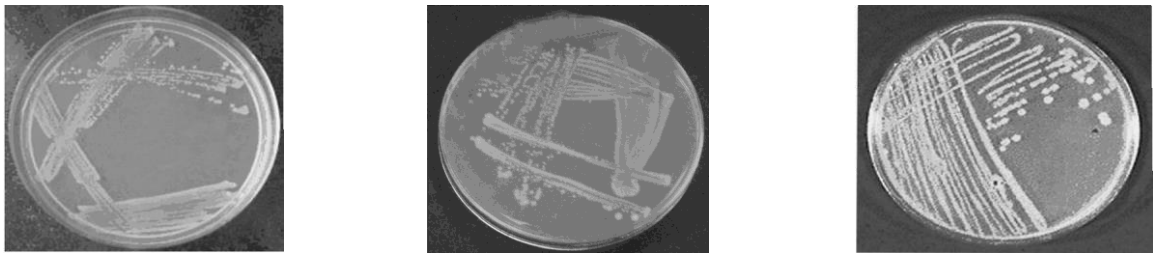
C1-*Rhodotorula glutinis* **C2-** *Rhodosporidium toruloides* **C3-** *Lipomyces starkeyi* **C-** Cultures **T-** Carbon source

Results and Discussion

Effect of different carbon sources *viz*., glucose, fructose and sucrose were used for the growth of yeast strains. Nile blue staining of the yeast cultures revealed the presence of lipids in all the three cultures evaluated.

Among the carbon sources evaluated glucose exhibited higher biomass and lipid content. Among the yeast cultures, *Rhodotorula glutinis* exhibited higher lipid 2.43 g l^{-1} (23.78 per cent) and biomass 10.21 g l^{-1} in glucose, followed by *Rhodosporidium toruloides* with 2.29 g l^{-1} (22.54 per cent) and 10.15 gl^{-1} and *Lipomyces starkeyi* with 2.23 g l^{-1} (22.20 per cent) and 10.12 g l^{-1} of lipid and biomass respectively in glucose broth. In fructose broth the cultures performed similarly and the maximum biomass of 7.64 gl^{-1} and lipid 1.57 g l^{-1} was observed in *Rhodotorula glutinis*. Least amount of lipids was produced in sucrose containing broth. (Table 1 & Fig.1)

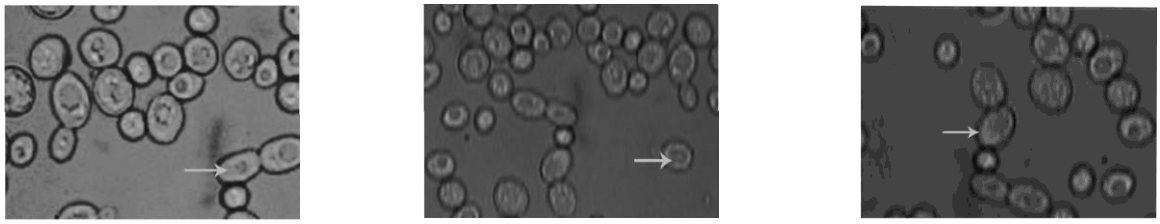
No significant difference was noticed on both lipid and biomass production among the cultures



A – *Rhodotorula glutinis* **B – *Rhodosporidium toruloides*** **C – *Lipomyces starkeyi***

Plate 1. Growth of oleaginous yeast cultures for lipid production

tested with the utilization of various carbon sources. Among the carbon sources evaluated, glucose stands first in biomass and lipid production of all the cultures. The results indicated the superiority of glucose over other sugars tested here. All these strains grew at a maximum in glucose and yielded more of lipids in biomass as reported by Jacob and Krishnamoorthy (1990) and Suutari *et al*. (1993).



A – *Rhodotorula glutinis* **B – *Rhodosporidium toruloides*** **C – *Lipomyces starkeyi***

Plate 2. Microphotograph showing the lipid particles in oleaginous yeast cultures

Similarly maximum lipid and biomass yield were reported in *Candida curvata* (Evens and Ratletge, 1984) and *Apiotricum* curvatum (Hassen *et al.,* 1993)

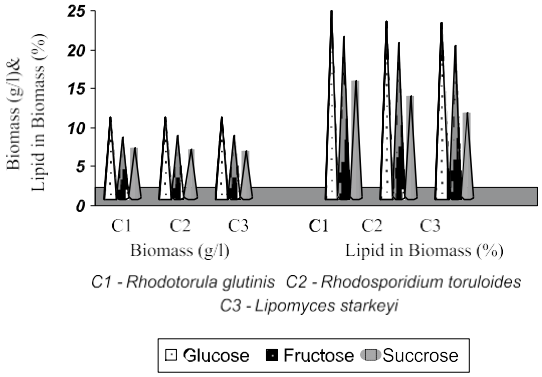


Fig. 1 Effect of carbon sources on lipid production by three oleaginous yeast cultures

in continuous culture system using glucose. Lipid accumulation of *Rhodotorula minuta* IIP-33 was maximum on glucose (0.48 w/w %) at the end of fed batch fermentation, compared to other carbohydrates such as sucrose, fructose and galactose as carbon source (Saxena *et al*., 1998) and this may be attributed to the simplicity of sugar (hexose) rather than the complex form of sugars.

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