PRODUCTION OF MICROBIAL LIPIDS FROM BEET MOLASSES

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SUMMARY: A variety of yeasts were cultivated on beet molasses (BM)-containing medium. Rhodotorula glutinis was superior to the other yeasts in lipid production (14% on dry weight basis), as well as in the efficient conversion of BM sugars to lipids (7%). Repeatedly centrifuged BM solution offered the best molasses sample which allowed the production of relatively high yeast yields. Maximum lipid yield (24.1%) was achieved with 8 days old cultures of Rhodotorula glutinis grown on a medium composed of (g/L): NH_4NO_3, 3; NaH_2PO_4, 0.7; K_2SO_4, 0.1, $ZnSO_4.7H_2O$, 0.05; $FeCl_3$, 0.05; centrifuged BM, 60. pH 7.0.

Key Words: Yeast, beet molasses, rhodotorula glutinis, microbial lipids.

INTRODUCTION

The greatest attention in microbial lipid production has been paid to yeasts because of their ability to accumulate high amounts of lipids, high lipid production rate, their relatively high growth rates and the resemblance of their triglycerides fraction to plant oils (2, 8). Many carbon sources proposed for the economic conversion to lipids have been described (13) of which molasses became a prime feedstock for the cultivation of lipid producing microorganisms (4, 9).

In Egypt, sugar industry by-products are not being utilized to their fullest potential. It is the objective of the present article to evaluate the suitability of this available cheap carbon source for the microbial production of lipid materials by yeasts.

MATERIALS AND METHODS

Microorganisms and cultivation

The identities as well as the sources of the different yeasts used are presented in Table 1. Stock cultures were maintained on glucose peptone agar slopes at 30°C. The beet molasses (BM) used as a sole carbon source was kindly provided by the Delta Sugar Company Egypt. The basal medium has the following composition (g/L) BM, 60 (equivalent to 3346 mg total reducing substances); $(NH_4)_2HPO_4$, 5; K_2HPO_4, 1; $MgSO_4.7H_20$, 1. The initial pH of the medium was adjusted to 6.0. Media were sterilized by autoclaving at 121°C.

The organisms were allowed to grow in 100 ml portions of the basal medium dispensed in 250 ml Erlenmeyer flasks. A 5% (v/v) inoculum was used and the inoculated media were incubated at 30°C±2 on a rotary shaker (200 shaked/min; amplitude 7 cm) for 8 days.

The BM treatments were carried on according to the method of El-Refai, *et al.* (5).

Media composition (g/L) I: Basal medium containing centrifuged BM as sole carbon source. II: $(NH_4)_2HPO_4$, 5; $MgSO_4.7H_2O$, 1; yeast extract, 1; $MnSO_4.4H_2O$, 0.2; $FeSO_4.7H_2O$, 0.005; $ZnSO_4.7H_2$, 0.005; centrifuged BM, 60. III: $NaNO_3$, 2; KH_2PO_4, 1; KCl, 0.5; $MgSO_4.7H_2O$, 0.5; $FeSO_4.7H_2O$, 0.01; centrifuged BM, 60. IV: peptone, 5; KH_2PO_4, 1; $MgSO_4.7H_2O$, 0.5; centrifuged BM, 60. V: NH_4NO_3, 2; KH_2PO_4, 0.5; $MgSO_4.7H_2O$, 0.5; $FeCl_3$, 0.05; centrifuged BM, 60. VI: NH_4NO_3, 3; NaH_2PO_4, 0.7; K_2SO_4, 0.1; $ZnSO_4.7H_2O$, 0.05; $FeCl_3$, 0.05; centrifuged BM, 60. VII: Urea, 1; KH_2PO_4, 1; $MgSO_4.7H_2O$, 1; $FeCl_3$, 0.05; $ZnSO_4.7H_2O$, 0.05; centrifuged BM, 60.

Analyses

The yeast growth was separated by centrifugation, washed and dried at 60°C to constant weight. The yeast dry weight was then estimated and analyzed for its content of crude protein by the micro-Kjeldahl method, while the crude lipids were extracted

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Table 1: Growth and lipid contents (mg/100 ml medium) of the tested yeasts.

Yeast	Source	Consumed sugar (%)	Dry wt. (mg)	Lipid		LCC
				mg	% of Dry wt.	
Candida tropicalis Y-21	NRRL	62.0	1008	44	4.4	2.1
C. utilis Y-30	NRRL	46.9	747	102	13.7	6.1
C. utilis Y-900	NRRL	50.2	840	55	6.5	3.3
Geotrichum tropicum	MIRCEN	69.9	1134	57	5.0	2.5
Hansenula polymorpa Y-7	NRRL	17.5	213	39	18.3	6.7
Pichia polymorpha	MIRCEN	40.6	644	90	14.0	6.6
Rhodotorula glutinis	NRRL	84.8	1420	199	14.0	7.0
Saccharomyces cerevisiae Y-2235	NRRL	40.1	672	62	9.2	4.6
S. Cerevisiae VIII	MIRCEN	32.8	534	38	7.1	3.5
S. cerevisiae IXV (SB)	MIRCEN	28.1	464	54	11.6	5.7
S. uvarum Y-1347	NRRL	52.6	871	59	6.8	3.4

MIRCEN, Microbiological Resource Centre, Ain Shams, University of Cairo, Cairo, Egypt.
NRRL, Northern Regional Research Laboratory, Peoria, Illinois, USA.

(12), purified (7) and the lipid content was calculated. The unassimilated sugars were determined in the culture filtrate (3).

The lipid conversion coefficient (LCC) = (mg lipid / mg sugar consumed) X 100

Economic coefficient (EC) = (Yeast dry wt. / wt. of consumed sugar) X 100

RESULTS AND DISCUSSION

Lipid production from crude BM

The results given in Table 1 clearly show that the tested yeasts manifested large variations in growth values and in their capacity to produce lipids. No consistent relationships were observed between the cell biomass yield and accumulation of lipids. As the efficiency of microbial lipid synthesis is usually estimated by the achievement of high lipid coefficient (6), thus *Rhodotorula glutinis* was considered to be most promising organism. For its high content of lipids, elevated lipid production rate and the relatively strong concentrations of both saturated and unsaturated fatty acids and of sterols and carotenoids, the genus *Rhodotorula* was previously reported (2,11,14).

Utilization of treated BM

The data illustrated in Table 2 indicate that good fermentation yields were achieved upon using the mud-free BM. The deleterious effect of the potassium ferrocyanide, decatonized and H_2SO_4-treated molasses on yeast activities were previously reported (5).

Suitability of the fermentation medium

The mud-free molasses solutions were separately supplemented with the ingredients necessary for yeast growth in an attempt to select a basal medium most favorable for lipid production. As illustrated in Table 3, the formulation of medium VI proved to be most suitable from the standpoint of the bioconversion of sugars into lipid, since the highest lipid conversion coefficient (8.8%) was recorded.

Table 2: *Rhodotorula glutinis* growth, lipid content and lipid conversion coefficient (LCC) on the basal medium supplemented one of a time with the differently treated BM samples.

Molasses type	Consumed sugar (%)	Dry wt. (mg)	Lipid		LCC
			mg	% of Dry wt.	
Crude (untreated)	84.8	1240	199	14.0	7.0
Centrifuged (mud-free)	87.7	1467	221	15.1	7.5
Potassim ferrocyanide-treated	30.7	466	54	11.6	5.3
H_2SO_4-treated	57.1	723	111	15.4	5.8
Calcium phosphate-treated	60.2	953	141	14.8	7.0
Decatonized	45.4	703	102	14.5	6.7

Table 3: *Rhodotorula glutinis* growth, lipid content and lipid conversion coefficient (LCC) as influenced with the composition of the fermentation medium.

Medium	Final pH	Consumed sugar (%)	Dry wt. (mg)	Lipid		LCC
				mg	% of Dry wt.	
I	7.5	87.9	1466	221	15.1	7.5
I	7.0	87.0	1456	206	14.2	7.1
III	7.5	82.2	1343	168	12.5	6.1
IV	7.5	91.4	1529	168	11.0	5.5
V	6.5	86.3	1444	169	11.7	5.9
VI	6.5	72.8	1218	214	17.6	8.8
VII	7.5	88.9	1486	124	8.3	4.2

Table 4: *Rhodotorula glutinis* nitrogen and sugar uptakes, dry weight, lipid content, protein percentage, economic coefficient (EC) and lipid conversion coefficient (LCC) during 10 day growth period.

Days	Final pH	N_2 uptake (%)	Consumed sugar (%)	Dry wt. (mg)	Protein % of Dry wt.	Lipid			
						mg	% of Dry wt.	EC	LCC
1	6.0	17.1	28.8	332	27.1	40	12.0	34.5	4.2
2	6.0	31.4	58.3	739	29.8	92	12.5	37.9	4.7
3	6.0	34.3	60.5	926	34.5	118	12.7	45.7	5.8
4	6.0	38.1	71.3	1118	35.4	148	13.2	46.8	6.2
5	6.0	40.0	71.3	1166	36.2	164	14.1	48.9	6.9
6	6.0	40.0	72.6	1202	36.8	177	14.7	49.5	7.3
7	6.5	42.9	73.3	1216	37.5	196	16.1	49.6	8.0
8	6.5	46.7	73.5	1233	36.4	218	17.7	50.1	8.9
9	6.5	46.7	74.3	1176	35.8	180	15.3	47.3	7.2
10	6.5	47.6	74.2	1159	35.3	156	13.5	46.7	6.3

Table 5: *Rhodotorula glutinis* growth and lipid content as influenced with the initial pH value of the medium.

pH value		Consumed sugar (%)	Dry wt. (mg)	Lipid		LCC
Initial	Final			mg	% of Dry wt.	
4	4.0	no growth	no growth	no growth	no growth	no growth
5	5.0	33.5	353	71	20.2	6.3
6	6.5	73.6	1233	218	17.7	8.9
7	7.0	74.1	1197	288	24.1	11.6
8	7.0	73.3	1191	208	17.5	8.5
9	8.0	73.7	1182	182	15.4	7.4
10	8.0	34.1	362	53	14.6	4.7

Growth-phase relations

The activities of the tested yeast were estimated during the different phases of growth using the formulation of the selected medium. The data presented in Table 4 indicate that maximal lipid content as well as the highest lipid percentage and conversion coefficient were recorded at the end of the stationary phase of growth (8 days). Similar results were recorded by many workers (10, 15).

pH relations

The effect of different starting pH values on the tested yeast activities was tested using aliquots of the basal medium which were initially adjusted either with 1N of HCl or NaOH to achieve pH values ranging from 4 to 10. Data represented in Table 5 show that pH 7.0 was found optimal for lipid biosynthesis where about 74% of the BM sugars were assimilated with the achievement of the highest lipid conversion coefficient (11.6%) and lipid

production (24.1%). Many workers have reported that no correlation had been shown between pH and lipid production within pH range optimum for growth (1).

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