SOME PHYSIOLOGICAL FACTORS INFLUENCING LIPID PRODUCTION *BY RHODOTORULA GLUTINIS* FROM EGYPTIAN BEET MOLASSES

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SUMMARY: The growth and lipid production by Rhodotorula glutinis cultivated on beet molasses (BM) containing medium were optimal at 8% BM level added at the beginning of the fermentation. Growth yields and lipid production were accelerated in presence of $(NH_4)_2SO_4$ at 5 g/L. and NaH_2PO_4 (0.5 g/L.). Varying levels of K_2SO_4, $ZnSO_4$, $7H_2O$ and $FeCI_3$ (one at a time) in the cultivation medium have almost no effect on the fermentation activities. Similarly, an exogenous supply of different concentrations of $MgSO_4.7H_2O$ exerts non-significant effect. On the other hand, the supplementation of corn-steep liquor (5g/L.) and cotton seed oil (1%) to the medium permitted maximal yeast activities.

Key Word: Rhodotorula glutinis.

INTRODUCTION

Lipid-producing (oleaginous) organisms have been known for many years, and their potential as alternative sources of animal or plant oils has been periodically assessed (15-17). The current status of lipid biotechnology has been recently reviewed (18, 19).

In previous communications (20) had selected *Rhodotorula glutinis* as the best lipid-producing organism. In the present work, the effect of some nutritional factors allowing better conversion of the beet molesses sugar into lipids was investigated.

MATERIALS AND METHODS

Micro-organisms and cultivation

Rhodotorula glutinis was kindly provided by NRRL, USA. Stock cultures were maintained on glucose-peptone agar slopes at 30°C. The beet molasses (BM) was kindly supplied by the Delta Sugar Company, Egypt. The BM used as a sole carbon source, was prepared by dilution with water to a concentration of 6% sugar. The diluted molasses was repeatedly centrifuged (3 times) at 4000 rpm for 20 min each. The muddy precipitate was then discarded. The basal medium has the following composition (g/L): BM, 60 (= 3346 mg total reducing sugars as glucose); NH_4NO_3, 3; NaH_2PO_4, 0.7; K_2SO_4, 0.1; $ZnSO_2.7H_2O$, 0.05; $FeCl_3$, 0.05. The initial pH of the medium was adjusted to 7.0. Media were sterilized by autoclaving at 121°C.

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

Analyses

The yeast growth was separated by centrifugation, washed and dried at 60°C to constant weight. The total lipids of the dried yeast were extracted (14), purified (4) and weighed. The original, as well as the unassimilated total reducing sugars (TRS) of the beet molasses were determined in the medium as glucose (2).

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

RESULTS AND DISCUSSION

Effect of BM level

Variation of the BM level between 2-10% revealed that 8% concentration supported maximal sugar bioconversions for cell biomass as well as lipid production (Table 1). Lower and higher BM concentrations proved to be in conductive for both growth and lipid formation of the

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Table 1: *Rhodotorula glutinis* growth and lipid content as influenced with the beet molasses (BM) concentration.

BM levels		Consumed Sugar (%)	Dry weight (mg)	Lipid		LCC
8%	TRS (g)			Dry wt. (mg)	% of Dry wt.	
2	1.115	73.0	409	84	20.5	10.3
4	2.230	75.7	843	197	23.4	11.7
6	3.346	74.1	1197	288	24.1	11.6
8	4.461	58.3	1344	446	33.2	17.1
10	5.577	58.1	1303	408	31.3	12.6

TRS = Total reducing sugars, D. wt. = Dry weight, LCC = Lipid conversion coefficient

experimental yeast. Similar results were reported by other investigators (5, 6, 9). Therefore, 8% BM (= 446 lmg / 100 ml reducing sugar) was applied in further experiments.

The growth and lipid biosynthesis activities were compared when the optimal BM level (80 g/L) was initially added at the beginning of the experiment as well as when being added in equal halves at different incubation periods. The data presented in Table 2 show that the addition of BM at the beginning of the fermentation process induced more lipid biosynthesis by the experimental yeast than the intermittent addition of the same BM level.

Nitrogen requirements

The metabolic pattern of the tested yeast proved to be greatly affected by the type of the nitrogen source employed (Table 3). The utilization of nitrogen in the form of NH_4 radicle stimulated accumulation to the largest extent. Maximal cellular lipid contents and sugar bioconversion estimates were achieved with $(NH_4)_2SO_4$.

The amounts of lipid produced were markedly affected by the nitrogen level in the medium, reaching its maximum at 5 g/L $(NH_4)_2SO_4$ concentration (Table 4). On the other hand, lipid productivity was decreased with increase of the nitrogen level of the medium. Similar metabolic activities were reported in different molds and yeasts (1,11,13). Several yeasts could accumulate large amounts of lipids when grown with an excess of carbon and a deficiency of nitrogen (16,17).

Table 2: *Rhodotorula glutinis* growth and lipid content as influenced with intermittent addition of BM.

Treatment	BM portions (g/L)	Time of BM addition (Days)	Consumed Sugar (%)	Dry wt (mg)	Lipid		LCC
					Dry wt (mg)	% of Dry wt	
Control	80	0	58.3	1344	446	33.2	17.2
1	40,40	0 and 2	76.3	1308	358	27.4	10.5
2	40,40	0 and 4	82.8	1432	423	29.5	11.5
3	40,40	0 and 6	81.1	1683	522	31.0	14.4

Table 3: *Rhodotorula glutinis* growth and lipid content as influenced with variation of nitrogen source in the medium.

Nitrogen source	Final ph	Comsumed sugar (%)	Dry wt (mg)	Lipid		LCC
				Dry wt (mg)	% of Dry wt	
$NaNO_3$	7.0	77.0	1419	410	28.9	11.9
KNO_3	7.5	70.9	1389	408	29.4	12.9
$(NH_4)NO_3$	7.0	58.3	1344	446	33.2	17.2
$(NH_4)Cl$	4.0	55.2	947	174	18.4	7.1
$(NH_4)_2SO_4$	5.5	41.9	1004	370	36.9	19.8
$(NH_4)_2HPO_4$	7.0	63.2	1517	502	33.1	17.8
Urea	8.5	58.4	1204	392	32.6	15.0
Asparagine	7.0	81.3	1756	416	23.7	11.5
L-cystine	6.5	86.6	2125	312	14.7	8.1

Table 4: *Rhodotorula glutinis* growth and lipid content as influenced with $(NH_4)_2SO_4$ concentration.

$(NH_4)_2SO_4$ level (g/L)	Consumed sugar (%)	Dry wt. (mg)	Lipid		LCC
			mg	% of D. wt.	
1	35.1	843	181	21.5	11.5
2	38.6	935	215	23.0	12.5
3	40.0	960	251	26.2	14.1
4	40.6	960	282	29.4	15.6
5	41.9	1004	370	36.9	19.8
6	42.0	906	306	33.8	16.3
7	42.3	847	186	22.0	9.9
8	40.5	818	177	21.6	9.8

Table 5: *Rhodotorula glutinis* growth and lipid content as influenced by some salt levels.

Salt used (g/L)	Consumed sugar (%)	Dry wt. (mg)	Lipid		LCC
			mg	% of Dry wt.	
NaH_2PO_4					
0.0	14.7	352	42	11.9	6.4
0.1	40.0	899	272	30.3	15.3
0.3	41.4	994	318	32.0	17.2
0.5	41.8	1004	374	37.3	20.0
0.7	42.0	1006	370	36.8	19.8
1.0	44.2	1053	328	31.2	16.7
2.0	45.1	1072	313	29.2	15.5
K_2SO_4					
0.00	41.9	1014	375	37.0	20.0
0.05	41.6	1007	371	36.8	20.0
0.10	41.8	1004	374	37.3	20.0
0.15	41.6	998	368	36.9	19.8
0.20	41.5	990	366	37.0	19.8
$ZnSO_4.7H_2O$					
0.000	42.0	1020	380	37.3	20.3
0.025	42.0	1017	377	37.1	20.1
0.050	41.9	1014	375	37.0	20.0
0.100	41.9	988	364	36.8	19.5
0.150	41.7	985	355	36.0	19.1
$FeCl_3$					
0.000	42.4	1001	384	38.4	20.3
0.025	42.3	1015	382	37.6	20.2
0.050	42.0	1020	380	37.3	20.3
0.100	41.6	1025	370	36.1	20.0
0.150	41.5	1031	369	35.8	19.9
$MgSO_4$ $7H_2O$					
0.0	42.3	1006	375	37.3	19.9
0.1	42.8	1018	378	37.1	19.8
0.5	42.9	1020	380	37.3	19.9
0.8	43.4	1031	387	37.5	20.0
1.0	43.5	1034	389	37.6	20.2
1.5	43.7	1038	390	37.6	20.0
2.0	43.9	1043	392	37.6	20.0

Table 6: *Rhodotorula glutinis* growth and lipid content as influenced with separate natural and oil additivies to the growth medium.

Supplement	Consumed sugar (%)	Dry wt. (mg)	Lipid		LCC
			mg	% of Dry wt.	
No additives*	41.8	1004	374	37.3	20.0
Natural additives (2 g/L)					
Corn step solid	35.1	663	414	62.4	26.5
Malt extract	36.6	671	311	46.4	19.0
Peptone	53.5	1285	399	31.1	16.7
Yeast extract	25.1	601	192	32.0	17.2
Beef extract	41.8	272	92	33.8	4.9
Oil additives 1(%)					
Cotton seed oil	36.7	1054	542	51.4	33.1
Corn oil	54.3	1087	447	41.1	18.5
Olive oil	57.9	1247	476	38.2	18.4
Castor oil	51.3	1125	301	26.8	13.2

*Composition of the medium (g/L): centrifuged BM, 80; $(NH_4)_2SO_4$, 5.0; NaH_2PO_4, 0.5

Initial sugar content = 4461 mg/100 ml medium.

Table 7: *Rhodotorula glutinis* growth and lipid content after 8 days of incubation as influenced with corn steep solid (CSS) and cotton seed oil (CSO) levels.

Level	Consumed sugar (%)	Dry wt. (mg)	Lipid		LCC
			mg	% of D. wt.	
0.00	41.8	1004	374	37.3	20.0
CSS (g/L)					
1.0	35.0	645	402	62.3	25.8
2.0	35.1	663	414	62.4	26.5
3.0	21.4	414	263	63.5	27.6
5.0	18.2	390	250	64.1	30.8
7.0	16.6	375	222	59.2	29.9
10.0	15.6	355	207	58.3	29.7
CSO (%)					
0.25	35.9	777	295	38.0	18.4
0.50	36.7	827	361	43.7	22.1
1.00	36.7	1054	542	51.4	33.1
1.50	36.0	564	269	47.7	16.7
2.00	35.9	528	263	49.8	16.4

Role of some salts

Weak growth and low lipid yields were maintained upon using medium formulation free of NaH_2PO_4. The addition of 0.5 g/L of the salt allowed the best fermentation yields (Table 5). On the other hand, varying levels of the following salts (one at a time): K_2SO_4, $ZnSO_4$ $7H_2O$ and $FeCl_3$ in the cultivation medium of the experimental yeast have almost no effect on the fermentation activities.

The supplementation of the culture medium with exogenous different concentrations of $MgSO_4$ $7H_2O$ exerts non-significant effect on the yeast activities (Table 5). This could be explained on the account that the used BM still had supplied adequate amounts of the constituents of these salts which enabled the tested yeast to maintain its normal metabolic activities and thus was not in need of exogenous supply of these elements. Therefore the modified medium used in further experiments composed of (g/L): centrifuged BM, 80; $(NH_4)_2SO_2$, 5.0; NaH_2PO_4, 0.5.

Effect of some natural and oil additives

The response of the experimental yeast to some natural additives has been examined. Corn steep solid (CSS) was superior in lipid accumulation by the tested

yeast (62.4% lipid on dry weight basis) as compared with the lipid content of the cells grown in absence of additives (37%) (Table 6). Similar results were reported (3, 8, 10). The optimal CSS level at which maximal values of lipid content as well as of lipid conversion coefficient (64.1; 30.8%) proved to be 5.0 g/L of the culture medium (Table 7).

Analogously, among the tested oils nothing was superior to cotton seed oil (CSO) which exhibited the optimal sugar bioconvertibility into lipid (LCC = 33.1%), maximal lipid output (54.2 mg/culture) and a relatively high lipid percentage (51.4%) (Table 6). The supplementation of the fermentation medium with CSO at a level of 10 g/L supported maximal yeast activities. The stimulation of lipid production in several fungi due to the addition of some lipid materials (including oils and fatty acids) has been recorded (7, 12).

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