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OPTIMIZATION OF LIPASE BIOSYNTHESIS BY STREPTOMYCES PARVULUS UNDER VARIOUS GROWTH CONDITIONS

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ABSTRACT

Among 60 streptomycete isolates, 16 exhibited lipolytic activities, S. parvulus proved to be the best lipase producer. The optimal culture conditions were 6 days of incubation at 30 °C under static culture conditions. The nutritional factors which affect lipase productivity were studied. 0.3% corn oil was the most suitable carbon source for the enzyme production (40.6 ug/ml). However, yeast extract stimulated lipase production (50.2 ug/ml) more than potassium nitrate (40.6 ug/ml) among the tested organic and inorganic nitrogen sources. Addition of amino acids as nitrogen sources to the basal medium showed that L-cystine (77 ug/ml), glycine (72 ug/ml), DL-alanine (42 ug/ml) and DL- valine (42 ug/ml) have stimulatory effect on lipase production, but glutamic acid (1.6 ug/ml), DL-serine (1.0 ug/ml) and DL-tryptophane (0.5 ug/ml) exerted strong inhibitory effect on lipase biosynthesis by S. parvulus.

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INTRODUCTION

Lipase enzyme production by microorganisms was studied by several investigators (Nelson, 1970; Arnold et al., 1975; Elwan et al., 1978 and 1986; Mohawed et al., 1985; and El-gamal and El-Sheikh, 1989). The composition of the culture medium and the concentration of its ingredients greatly affect the growth and enzyme production of the microorganism (Chopra and Chander, 1983). The presence of butter oil, vegetable oil and synthetic triglycerides as carbon sources caused a reduction in lipase activity, while 2% peptone and 0.5% yeast extract as nitrogen sources produced the highest value of the enzyme by Geotrichum candidum (Chander et al., 1983). Mohawed et al., (1985) stated that substituting tributyrin and sodium nitrate, originally present in the basal medium, by 13 carbon and 20 nitrogen sources added individually revealed that olive oil (0,3% W/V) and L-arginine or Dl-serine were the best substrates for lipase production by Aspergillus anthiceus IMI.

The present study is an endeavor to investigate the lipolytic activities of the tested streptomycetes and optimization of lipase production by S. parvulus.

MATERIALS AND METHODS

Lipolytic streptomycetes

Various streptomycetes isolated by El-Shribiny (1990) were tested in the present investigation for their lypolytic

activities. As a result of this study Streptomyces parvulus was found to be the most active lipase producer and it was, therefore selected for subsequent investigation.

Media used

a- Growth medium

Starch-nitrate agar medium (Waksman, 1959) was used for culture growing and mainterance of S. parvulus. This medium was composed of (g/1):

Starch	20.00
KNO3	2.00
MgSO4	0.50
NaCl	0.50
CaCO3	3.00
FeSO4	0.01
Agar	20.00
Distilled water	1000 ml

b- Production medium

The above liquid medium was used for the production of lipase by S. parvulus. The emulsified oils and fats (0.2% V/V) were prepared according to Elwan et al.,(1977). The prepared emulsions were added individually in place of starch to the growth medium.

Enzyme assay

The activity of lipase was determined by the pH meter assay method. The method was adopted by Wahlefeld (1974) and

modified by Mohawed (1983). The volume of enzyme activity was derived from the following equation.

$$\text{Volume activity (ug/ml sample)} = \frac{\text{Consumed NaOH}}{0.1 \text{ ml}}$$

where as, the specific activity of lipase enzyme was calculated from the following equation:

$$\text{Specific activity (ug/mg protein)} = \frac{\text{Volume activity}}{\text{protein content (mg/ml)}}$$

Determination of Protein

Total protein was estimated by the method of Lowry et al. (1951). Using bovine serum albumen as a standard protein. The optical denisties of the samples were measured at wave length 750 nm using Perkin-ELmar, Lambda I Spectrophotometer.

RESULTS AND DISCUSSION

Screening of the streptomycete isolates

The screening of the streptomycete isolates was carried on the bases of their lipolytic activities using the pH meter assay method. Among the 60 isolates, 16 exhibited lipolytic activities.

The data represented in table 1 showed that S. parvulus surpass all the tested isolates in its lipolytic activity (17.8 ug/ml sample) after 6 days of incubation at 30°C under static culture conditions, followed by S. caesius, S. autotrophicus, s. corchorusi and S. calvus. Streptomyces cepuensis, S. badius, S. galtieri and S. albovinaceus supported

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moderate lipolytic activities. Other isolates were found to be poor in their lipase activity. Thus, S. parvulus was selected for further study.

Effect of incubation periods

An experiment was conducted in order to follow the lipase production by the experimental isolate during the fermentation process. Samples were taken periodically every day. This was done to secure the proper time for maximum production of the enzyme.

The data given in table 2 revealed that the lipase formation was started after the second day of incubation period. Maximum lipase production, maximum protein concentration and the lighest biomass yield were attained on the 6th day of incubation. Afterwards, the lipase activity, protein content and biomass yield were decreased. The biomass growth seemed to be almost stable throughout the last four days. Our results were agreed with the results of Johnson and Sungg, 1974; Aisaka and Osame,(1979)and Chander et al.,(1980).

Effect of different lipids

Nine lipid sources namely, tributyrin, corn oil, cotton seed oil, caster oil, cocoanut oil, olive oil, sesame oil, lineseed oil, animal fat were tested for their efficiency to support the lighest production of lipase, protein and biomass.

Each lipid source was supplemented at a concentration of 0.2% (V/V) in replace of starch to the production meduim.

The data presented in table 3 showed that corn oil was the best lipid supported the highest yield of lipase (37.5 ug/ml), protein (0.25 mg/ml) and biomass (154 mg/50 ml culture) as compared with the other lipids used. This was followed by olive oil (25.3 ug/ml), tributyrin (17.8 ug/ml) and cotton seed oil (15.5 ug/ml). Other lipids used not suitable for lipase biosynthesis by S. parvulus.

Different concentration of corn oil (0.1 - 0.9% V/V) were tested. Data recorded in table 4 indicated that, 0.3% of corn oil was the optimum concentration for growth and lipase activity. Lower and higher levels of corn oil induced a decline in lipase production, protein content and biomass yield. In this connection Yashida et al. (1968) found that 0.2-0.6% olive oil gave maximal lipase production by Torulopsis ernoba. Mohawed et al. (1985) attained that 0.3% (W/V) olive oil was the best substrate for maximal lipase production by Aspergillus anthiceus. Similar results were obtained by Elwan et al. (1986); El-gamal and El-Sheikh (1989).

Effect of different nitrogen sources

In this experiment, nine different nitrogen sources were tested for their suitability in lipase production and

biomass yield by S. parvulus. Each nitrogen source was added to the basal medium in a concentration of 28 mg N/100 ml medium. The results in table 5 indicated that the production of lipase enzyme by S. parvulus was greatly affected by the type of the nitrogen source used, yeast extract (50.2 ug/ml), potassium nitrate (40.6 ug/ml) and peptone (40.1 ug/ml) supported the higher levels of lipase biosynthesis. Moderate levels of lipase production was obtained by casein, sodium nitrate and ammonium sulphate. Urea and ammonium chloride failed to support the production of lipase enzyme. In general, the tested organic nitrogen sources favoured lipase production more than inorgnic ones. These results coincide with the results of Akhtar et al. (1980), Chander et al. (1980) and El-gamal and El-sheikh (1989).

Effect of amino acids

The supplementation of different amino acids to the basal medium - free of nitrogen source in equimolecular nitrogen weights equivalent to the nitrogen content of 2 g. KNO3 (280 mg N/L) were tested.

The data presented in table 6 showed that the presence of L-cystine, glycine, DL-alanine and DL-valine enhanced lipase and biomass yields as compared with the control treatment containing KNO3 as a sole soruce of nitrogen. Glutamic acid, DL-serine and DL-tryptophane were inhibitors of lipase production by the experimental organism. The highest value of lipase

and biomass were obtained when L.-cystine was the only source of nitrogen. The requirment for amino acids seams to be versatile among the different producing organisms, Nashif and Nelson (1953) found that leucine, isoleucine and valine increased lipase production by Pseudomonas fragi and maximal value was obtained in the presence of L-lysine used as the sole nitrogen source. Mohawed et al. (1985) reported that the additoin of L-arginine or DL- serine (0.33% nitrogen) were the best sources of nitrogen for lipase production by Aspergillus anthiceus IMI.

Table 1 . The lipolytic activity of streptomycete isolates

Streptomycete isolate	Lipase activity (ug/ml sample)
Streptomyces albovinaceus	11.1
Streptomyces galtieri	12.2
Streptomyces albolongus	6.8
Streptomyces autotrophicus	16.0
Streptomyces badius	13.0
Streptomyces reseogriseus	4.9
Streptomyces corchorusi	15.6
Streptomyces diastaticus	9.3
Streptomyces macrosporeus	8.8
Streptomyces atroolivaceus	5.0
Streptomyces parvulus	17.8
Streptomyces caesius	16.5
Streptomyces capuensis	13.5
Streptomyces calvus	15.0
Streptomyces polychromogenes	5.6
Streptomyces roseosporus	6.4

Table 2 . Effect of incubation period on lipase production by Streptomyces parvulus.

Incubation period (in days)	Lipase activity (ug/ml sample)	Protein content (mg/ml sample)	Specific activity (ug% protein)	Biomass yield (mg/50 ml culture)
1	0.0	0.000	0.00	000.0
2	2.0	0.120	16.66	040.0
3	3.5	0.150	23.33	75.0
4	5.5	0.180	30.56	92.5
5	11.5	0.189	60.85	122.5
6	17.8	0.198	89.89	140.2
7	13.1	0.182	71.98	138.3
8	12.8	0.178	71.91	123.5
9	7.5	0.169	44.38	103.7
10	6.5	0.167	38.92	103.7
11	2.2	0.167	13.17	103.0
12	1.9	0.160	11.88	103.0

Table 3 . Effect of different lipids (0.2% V/V) on Lipase production by Streptomyces parvulus

Different lipids	Lipase activity (ug/ml sample)	Protein content (mg/ml sample)	Specific activity (ug/mg protein)	Biomass yield (mg/50 ml culture)
Tributyrin	17.8	0.198	89.89	140.2
Corn oil	37.5	0.250	150.00	154.3
Cotton seed oil	15.5	0.188	82.45	140.0
Caster oil	02.4	0.165	14.55	057.0
Cocoa-nut oil	05.2	0.169	30.77	106.0
Olive oil	25.3	0.240	105.42	148.0
Sesame oil	00.5	0.040	12.50	030.0
Lineseed oil	02.4	0.160	15.00	060.0
Animal fat	00.9	0.09	10.00	030.0

Table 4 . Effect of different concentrations of corn oil on lipase production by Streptomyces parvulus.

Corn oil concentration (V/V)	Lipase activity (ug/ml sample)	Protein content (mg/ml sample)	Specific activity (ug/mg protein)	Biomass yield (mg/50 ml culture)
0.1	20.9	0.22	95.00	145.7
0.2	37.5	0.25	150.00	154.3
0.3	40.6	0.26	156.15	156.9
0.4	37.0	0.26	142.30	154.0
0.5	20.0	0.23	86.96	145.0
0.6	20.0	0.23	86.96	145.0
0.7	08.2	0.16	51.25	093.5
0.8	04.6	0.11	41.82	076.7
0.9	03.2	0.10	32.00	75.0

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Table 5 . Effect of different nitrogen sources on lipase production by Streptomyces parvulus

Nitrogen Sources	Lipase activity (ug/ml sample)	Protein content (mg/ml sample)	Specific activity (ug/mg protein)	Biomass yield (mg/50 ml culture)
KNO_3	40.6	0.26	156.15	156.9
$NaNO_3$	20.3	0.22	92.27	125.5
Amm. sulphate	10.2	0.17	60.00	102.6
Amm nitrate	19.5	0.19	102.63	117.5
Amm. chloride	01.5	0.08	18.75	25.0
Urea	03.0	0.12	25.00	40.0
Peptone	40.1	0.28	143.21	161.0
Yeast extract	50.2	0.33	152.12	170.0
Casein	30.5	0.21	145.24	150.4

Table 6 . Effect of different amino acids on lipase production by Streptomyces parvulus

Amino acids	Lipase activity (ug/ml sample)	Protein content (mg/ml sample)	Specific activity (ug/mg protein)	Biomass yield (mg/50 ml culture)
KNO_3 (control)	40.6	0.26	156.15	156.9
Glycine	72.0	0.29	248.28	186.0
Glutamic acid	01.6	0.07	22.86	045.0
L.Asparagine	40.4	0.27	149.63	178.0
L. Cystine	77.0	0.29	265.52	230.0
DL.Alanine	42.0	0.26	161.54	200.0
DL.Valine	42.0	0.26	161.54	200.0
DL.Tryptophane	00.5	0.06	8.33	030.0
DL.Serine	01.0	0.1	10.00	040.0

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الظروف الانمائية المثلـــــى لانتاج أنزيـــــم الليبيز بواسطة استربتوميسيس بارفيولس

السيد على الشربينى ـ محمد فاروق غالـــــى
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من بين ٦٠ عزلة من الأسـ ـتربتوميسيتات وجد أن ١٦ عزلة لها القـــــدرة على انتاج أنزيم الليبيز . وقد أثبت استربتوميسيس بارفيولس أنها أفضل هـــــذه المعزولات فى انتاج أنزيم الليبيز بعد ٦ أيام من التحضين عند ٣٠ م تحت ظروف مزرعية ثابتة. ولقد درست العوامل الغذائية التى تؤثر على نشاط الكائن ووجد أن تركيز ٣و٠٪ من زيت الذره هو أنسب مصدر كربونى لانتاج أنزيم الليبيز وكذلك وجد أن مستخلص الخميرة يحفز الكائن لانتاج الأنزيم أكثر من نترات البوتاسيوم من بين المصادر النيتروجينية العضوية وغير العضوية المختبرة. وباضافة الأحماض الأمينية المختلفة إلى الوسط الغذائى وجد أن الحمض الأمينى سيستين ، الانين والفالين لهم تأثير منشط لانتاج الأنزيم بينما الحمض الأمينى جليوتاميك ، تربتوفان والسيرين لهم تأثير مثبط قوى على انتاج أنزيم الليبيز بواسطة استربتوميسيس بارفيولـــــس.