OPTIMIZATION OF LIPASE PRODUCTION IN SOME MICROBES

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Abstract

Bacterial strains showed maximum lipase production in Sugiura *et al.* (1977) basal mineral salt solution, while, Chander *et al.* (1980) basal medium gave the highest amount of fungal lipase. Enzyme production was maximized in third day of bacterial growth and after five days for fungal strains. Out of carbon source tested, glucose, gave the highest enzyme yield in the culture fluids for all bacterial and fungal organisms used except of *Aspergillus oryzae*, sucrose was found in the first order. Maximum lipase secretion was obtained in the presence of peptone and casin for *Bacillus subtilis* and *Pseudomonas aeruginosa*, respectively. However, wheat bran showed maximal amount of enzyme yield for fungal strains. Olive oil (1%) induced bacterial lipase biosynthesis, while, the addition of oil (1%) inhibited fungal lipase secretion. Addition of fatty acids to growth medium was inhibitory for lipase production.

The optimal pH for *B. subtilis* (12), *P. aeruginosa* and *A. oryzae* lipase production ranged from 5 to 7, while optimal pH for *S. cerevisiae* (4) and *A. flavus* (2) lipase production ranged from 6 to 8. The highest enzyme activity was reached at 30°C for both bacterial and fungal strains. When, the organisms were cultivated on a shaker, the enzyme production was increased.

INTRODUCTION

Microorganisms have been known to produce lipases and the nature of enzymes is known to vary in different species (Benzonana, 1974; Jensen, 1974 and Lobyreva & Marchenkova, 1979). Microbial lipases are responsible for degradation of fat by hydrolysis and the formed products contribute to the development of desirable flavors in food products. Microbial lipases are also used in different chemical industries (Linfield *et al.*, 1984 & 1985; Fujii *et al.*, 1986 and Khor *et al.*, 1986).

Although the extent to which lipase is formed varies considerably even between strains of the same species of organism, yet it is well known that the environmental and nutritional factors might affect the microbial lipase production. Thus, the aim of the present study is optimizing culture conditions and improve enzyme production using selected organisms, i.e., *Pseudomonas aeruginosa* and *Bacillus subtilis* (12), *Saccharomyces cerevisiae* (4), *Aspergillus oryzae*, and *Aspergillus flavus* (2) (Hauka *et al.*, 1997)

MATERIALS AND METHODS

Organisms:

Pseudomonas aeruginosa was obtained from Microbioloy Dept., Fac. of Pharmacy, Mansoura Univ., Mansoura, Egypt.

Bacillus subtilis (12) and *Aspergillus oryzae* were obtained from Microbiology Dept., Fac. of Agric., Mansoura Univ., Mansoura, Egypt.

Saccharomyces cerevisiae was obtained from National Research Center, Cairo, Egypt.

Aspergillus flavus was obtained from Dept. of Food Science, Fac. of Agric., Mansoura Univ., Mansoura, Egypt.

Culture media:

1. Sugiura *et al.* (1977) basal medium.

It contains: meat extract 0.3%, polypeptone 1.5%, glucose, 1.0%, urea 0.6%, KH_2PO_4 0.2%, KCl 0.05%, $MgSO_4$ 0.05%, and distilled water. pH is 6.0 This medium was used for propagation and maintenance of bacterial organisms by supplementing with 1.5% agar. The basal mineral salts solution supplemented with 1.0% olive oil was used for enzyme production.

2. Kennedy and Lennarz (1979) basal medium.

It contains: bacto-peptone 1.0%, Yeast extract 1.0%, NaCl 0.05%, NaH_2PO_4 0.04%, and distilled water. pH is 7.0. This medium was used for bacterial enzyme production.

3. Chander *et al.* (1980) basal medium.

It contains: peptone 2.0%, yeast extract 0.5%, NaCl 0.5%, dextrose 1.0%,

and distilled water. pH is 6.0. This medium was used for yeasts and fungal organisms for growth and lipase production.

4. Potato dextrose agar medium (PDA):

PDA medium was used to prepare the inocula of fungi. It is of the following constitution: 200.0 g infusion from white potatoes or peeled potato, 20.0 g dextrose (glucose), 15.0 agar, and 1000 ml distilled water. The pH was adjusted at 6.5. This is favourable medium for sporulation and maintenance of fungal organisms.

5. Concalves and Castello (1981) basal medium.

It contains: meat extract 0.3%, yeast extract 0.3%, peptone 0.5%, sucrose 2.0%, agar, 2.0%, and distilled water. pH is 6.0. This media was used for maintenace of yeast organisms.

6. Fukumoto *et al.* (1964) basal medium.

It consists of: peptone 7.0%, glucose 2.0%, KH_2PO_4 0.1%, $NaNO_3$, 0.1%, $MgSO_4$ 0.05%, and distilled water. pH is 6.0. This media was used for fungal lipase production.

7. El-Makhazangi (1989) basal medium.

It consists of (g/L): $NaNO_3$ 2, $K_2HPO4.7H_2O$ 0.5, KCl 0.5, $FeSO_4.7H_2O$ 0.01, Yeast extract 1, sucrose, 20, tributyrin emulsion 1 and reached up to 1000 ml with distilled water. The medium was used for fungal lipase production. Tributyrin emulsion was prepared according to El-Makhazangi (1989).

Inoculation procedure and microbial propagation:

Inoculation procedures have been described elsewhere (Hauka *et al.*, 1997).

Enzyme assay methods:

Lipase activity was assayed according to the method of Oi *et al.* (1969).

RESULTS AND DISCUSSION

Effect of medium composition on lipase production:

One of the most important factors in optimization the fermentation process is the design of the growth and production medium. Data in Table (1a) present the effect of two different media on the yield of bacterial lipase and data in Table (1b)

show the effect of three media on the secretion of yeast and fungal enzymes. The results obtained show that the production of lipase was greatly affected by the composition of the media used. The results also show that Sugiura *et al.* (1977) basal medium was the most favourable medium for bacterial lipase production, and gave the highest yield of enzyme (4.8 and 4.6 µmole FFA/ml/min) for *Pseudomonas aeruginosa* and *Bacillus subtilis* (12), respectively. Also, the ingredients of Chander *et al.* (1980) basal medium enhanced the secretion of yeast and fungal lipases. This indicates that the major components in all tested media varied greatly in their enhancing influence towards enzyme secretion.

Table 1. Effect of different media composition on lipase production (Enzyme activity is expressed as µmole FFA/ml/ min).

a. Bacterial strains

Media	Lipase activity	
	Pseudomonas aeruginosa	*Bacillus subtilis* (12)
Sugiura *et al.* medium (19970	4.8	4.6
Kennedy & Lennarz medium (1979)	0.8	0.6

b. Fungal strains

Media	Lipase activity		
	Saccharomyces cerevisiae	*Aspergillus oryzae*	*Aspergillus flavus*
Chander *et al.* medium (1980)	3.6	3.3	3.6
El-Makhazangi medium (1989)	1.2	0.6	1.0
Fukumoto *et al.* medium (1964)	0.8	1.2	0.9

Effect of time-course on lipase production:

The results achieved by the test strains are summarized in Table (2). The results achieved show that lipase occurred directly upon growing the microorganisms. The release of enzyme increased during fermentation time as such time proceeded and maximized on the third day of incubation for bacterial strains, and on the fifth day for yeast and fungal strians. The decline of lipase activity after three and five days could be explained on the basis that such enzyme may be attacked by proteinases in the culture fluids. Vadhera and Harmon (1969) found that the maximum production of lipase was attained after 5 days of incubation for *Staphylococcus aureus*. Hauka *et al.* (1991) also found that *Aspergillus fumigatus* lipase was at maximum after 5 days incubation.

Table 2. Time-course of lipase production for various organisms (Enzyme activity is expressed as µmole FFA/ml/min).

Organism / Time (days)	Lipase activity				
	P. aeruginosa	*B. subtilis* (12)	*S. cerevisiae*	*A. oryzae*	*A. flavus*
1	3.2	3.8	0.0	2.8	2.0
2	3.6	4.2	2.0	3.0	2.2
3	5.1	4.8	2.4	3.2	2.4
4	5.0	4.4	2.6	3.4	2.8
5	4.9	4.3	3.8	3.6	3.4
6	4.8	3.6	3.6	3.4	3.0
7	4.4	3.5	2.3	2.6	2.0
8	4.0	3.0	2.2	2.4	1.6
9	3.6	2.0	2.0	2.0	1.5
10	3.0	1.5	1..5	1.8	1.0

Effect of different carbon sources on lipase production:

The carbon-energy source is critical for optimal growth and product formation. Data on the effect of different carbon sources (1.0% concentration) on the production of lipase are presented in Table (3). Glucose promoted the highest enzyme activity with bacterial strains tested, *Saccharomyces cerevisiae* (4) and *Aspergillus flavus* (2), but for *Aspergillus oryzae*, sucrose stimulated lipase production. On the other hand, other carbohydrates showed reduction in lipase activity for these organisms. These findings are in accordance with those of Chander *et al.*, 1977 & 1981 and Hauka *et al.* (1991).

Effect of nitrogen sources on lipase production:

Peptone and urea were replaced by different nitrogen sources at equivalent percentages of elemental nitrogen in the bacterial medium. In the fungal medium only, peptone was replaced by other nitrogen sources. The results are presented in Table (4). It is obvious from the data that casein and peptone allowed a high production of lipase and the maximum activity was 5.6 and 6.4 µmole FFA/ml/min. by *Pseudomonas aeruginosa* and *Bacillus subtilis* (12), respectively. Wheat bran gave maximum yield of lipase of yeast and fungal organisms. It can be inferred from the results that lipase activity is dependent on the nitrogen source in the medium. These results are in harmony with those obtained by Hosono & Tokita (1970) and Chander *et al.* (1980).

Table 3. Biosynthesis of lipase tested organisms after growth on various carbon sources (Enzyme activity is expressed as µmole FFA/ml/min).

Organism / Carbon sources	Lipase activity				
	P. aeruginosa	*B. subtilis* (12)	*S. cerevisiae*	*A. oryzae*	*A. flavus*
Glucose	5.2	4.8	4.8	3.6	3.4
Galactose	3.2	3.0	3.2	3.2	2.4
Fructose	4.8	2.8	4.0	3.0	2.0
Mannitol	4.6	2.8	4.0	3.5	2.0
Glycerol	4.0	3.0	2.8	3.4	1.6
Maltose	3.9	2.4	3.2	3.4	1.4
Sucrose	4.0	2.7	4.4	4.4	2.0
Lactose	2.6	2.4	4.0	3.2	2.2
Molasses	3.6	2.0	3.6	2.8	1.8
Gum Arabic	3.8	2.0	3.8	2.8	2.4
Pectin	4.0	2.0	3.2	3.6	2.2

Table 4. Biosynthesis of lipase by different organisms after growth on various nitrogen sources (Enzyme activity is expressed as µmole FFA/ml/min).

Organism / nitrogen sources	Lipase activity				
	P. aeruginosa	*B. subtilis* (12)	*S. cerevisiae*	*A. oryzae*	*A. flavus*
Control	5.2	4.8	4.8	4.4	3.4
Casein	5.6	5.6	4.0	4.4	3.6
Wheat bran	4.8	6.0	4.8	4.5	4.0
Peptone	5.2	6.4	3.6	3.2	3.4
Soybean meal	3.0	5.6	3.9	3.0	3.2
Urea	2.0	4.8	3.6	3.0	3.0
Gelatin	2.4	3.2	3.8	3.3	2.8
Sodium nitrate	4.8	5.4	3.4	2.8	3.0
Ammonium sulphate	2.0	2.4	3.7	3.6	2.4
Potassium nitrate	1.8	1.8	3.8	3.2	2.4

Effect of different lipids on lipase production:

Oils were added in the growth medium at 1.0 & 0.1% concentrations for bacteria and fungi, respectively. In general, Table (5) shows that plant oils increased lipase secretion for bacterial strains. On the other hand, synthesized triglycerides and animal lipids showed inhibitory effect on the lipase production. In Table (5) different lipid sources repressed lipase production with fungal organsims. Finally, these results suggest that lipase of bacterial organisms appeared to be inducible with plant oils, but fungal lipases are constitutive enzymes.

Our results are in agreement with those obtained by Khan *et al.* (1967). They found that *Achromobacter lipolyticum* produces lipase and the yield of lipase can be increased three folds by the addition of olive oil, coconut oil, or butter fat to the medium. Hauka *et al.* (1991) in their work on *Aspergillus fumigatus* found that lipids represeed lipase production.

Table 5. Effect of different oils and fats on the production of lipase (Enzyme activity is expressed as µmole FFA/ml/min).

Organism / Oil and fat	Lipase activity				
	P. aeruginosa	*B. subtilis* (12)	*S. cerevisiae*	*A. oryzae*	*A. flavus*
Olive oil	5.6	6.4	3.1	3.4	3.4
Soybean oil	4.0	4.8	4.5	4.4	3.9
Coconut oil	4.0	3.6	2.4	2.8	2.6
Sunflower oil	4.4	4.0	3.0	3.0	2.8
Corn oil	4.0	4.0	3.4	3.6	3.4
Cotton seed oil	3.2	3.2	2.6	3.0	2.7
Stearin	2.4	2.8	2.2	2.6	2.4
Olein	2.0	1.2	2.8	2.8	2.8
Butter oil	2.8	2.0	2.6	2.6	2.8
Tallow fat	2.6	2.0	3.0	3.4	3.0
Caster oil	2.4	3.2	3.0	4.0	3.2
Control	2.8	2.6	4.8	4.5	4.0

Effect of different fatty acids on lipase production:

The stimulation or inhibition of lipase production by fatty acids are shown in Table (6). Fatty acids were added with 0.5% concentration to the growth medium. Butyric acid showed slight inhibition for the enzyme formation by all tested organisms. Other fatty acids were inhibitory for lipase production by the tested strains. These findings are in agreement with those obtained by Chander *et al.* (1979).

Table 6. Effect of addition of different fatty acids to the defined medium on the production of lipase (Enzyme activity is expressed as µmole FFA/ml/min).

Organism / Fatty acids	Lipase activity				
	P. aeruginosa	*B. subtilis* (12)	*S. cerevisiae*	*A. oryzae*	*A. flavus*
Control	5.6	6.4	4.8	4.5	4.0
Butyric acid	5.6	5.2	4.4	3.6	3.8
Palmitic acid	4.8	4.0	2.8	1.8	2.4
Stearic acid	4.0	4.4	3.2	1.8	2.0
Oleic acid	4.4	3.2	2.8	1.6	2.8

Effect of lipid concentration:

This experiment was designed to investigate the influence of olive oil and soybean oil concentration with and without sugar (carbohydrates) on the production of bacterial and fungal lipases. Synthesis in absence of lipid sources indicate that lipase is constitutive enzyme. The results achieved show also that the absence of carbohydrates, from the growth medium, greatly inhibited lipase production in the presence of olive oil and soybean oil concentrations for bacterial and fungal strains, respectively. Olive oil (1.0%) slightly enhanced bacterial lipase production (Table 7). Soybean oil (0.2%) slightly enhanced fungal enzyme synthesis. Finally, the results obtained indicated that lipase was slightly inducible. Also, the poor synthesis of enzyme in absence of essential energy source (carbohydrates) by all test organisms indicate that lipase is constitutive enzyme. This result is in agreement with those obtained by Iwai and Tsujisake (1974).

Table 7. Effect of oil concentration on the biosynthesis of lipase (Enzyme activity is expressed as µmole FFA/ml/min).

Organism / Oil concentration %	Lipase activity									
	Olive oil				Soybean oil					
	P. aeruginosa		*B. subtilis* (12)		*S. cerevisiae*		*A. oryzae*		*A. flavus*	
	a	b	a	b	a	b	a	b	a	b
0.1	5.2	2.4	6.2	1.6	4.5	1.9	4.3	1.8	3.9	2.0
0.2	5.2	2.6	6.2	2.2	5.0	1.8	4.6	1.7	4.1	1.8
0.4	5.2	2.8	6.2	2.4	4.0	1.6	3.5	1.6	3.2	1.6
0.6	5.4	3.2	6.2	2.5	2.0	0.8	3.2	0.6	2.7	1.4
0.8	5.4	3.6	6.4	2.4	1.5	0.6	2.5	0.5	2.4	1.3
1.0	5.6	3.6	6.4	2.4	0.0	0.0	1.6	0.5	1.4	1.2
Oilless	5.3	2.4	6.0	1.0	4.8	1.6	4.5	0.8	4.0	1.6

a = With sugar source in media. b = Without sugar source in media.

Effect of pH on lipase production:

In industrial fermentation the maintenance and control of pH is important for optimal growth and/or product formation. The results of the effects of different pH values on lipase production are shown in Table (8). It is seen that the pH greatly affected the enzyme activity of culture fluids of the organisms. A pH range of 5.0-7.0 seemed to be optimal for lipase production from bacteria and *Aspergillus oryzae* and a pH range of 6.0-8.0 seemed to be optimal for *Saccharomyces cerevisiae* (4) and *Aspergillus flavus* (2) lipase production. Outside these ranges, the enzyme was inhibited. The maximum lipase productivity by the procedures strains was noted at pH 6.0. Our results are in close agreement with the findings of Chander *et al.* (1980).

Table 8. Effect of initial pH value of the culture medium on the biosynthesis of lipase (Enzyme activity is expressed as μmole FFA/ml/min).

Organism / pH	Lipase activity				
	P. aeruginosa	*B. subtilis* (12)	*S. cerevisiae*	*A. oryzae*	*A. flavus*
4	1.2	3.0	2.4	3.2	1.6
5	4.2	5.2	4.0	4.2	2.4
6	5.6	6.4	5.0	4.6	4.1
7	3.6	5.0	4.6	4.0	4.0
8	3.0	4.0	4.4	3.0	3.2
9	2.4	3.5	4.0	2.7	2.0

Effect of incubation temperature:

Temperature affects the efficiency of carbon-energy conversion to cell mass. Thus, in process of optimization, it is important to realize that temperature may affected growth rate and product synthesis rate differently. From Table (9), it can be observed that the incubation temperature greatly affected lipase production. The highest enzyme activity was reached at 30°C for the tested strains. No difference in lipase productivity by *Bacillus subtilis* (12) at 25 and 30°C. Enzyme production was decreased at other temperature degrees. These results are in conformity with the findings of Chander *et al.* (1981).

Effect of aeration:

Highest enzyme activity was observed in shake cultures in comparison to stationary cultures. The results in Table (10) show that organisms require large quantities of dissolved oxygen for high lipase production. These results are in line to those obtained by Chander *et al.* (1980).

Table 9. Effect of incubation temperature on the biosynthesis of lipase (Enzyme activity is expressed as µmole FFA/ml/min).

Organism / Temperature °C	Lipase activity				
	P. aeruginosa	*B. subtilis* (12)	*S. cerevisiae*	*A. oryzae*	*A. flavus*
20	0.4	4.0	2.5	3.2	2.4
25	4.7	6.4	3.5	3.6	4.0
30	5.6	6.4	5.0	4.6	4.1
35	4.9	3.6	2.5	2.5	4.0
40	4.0	1.6	1.5	2.2	2.4
45	2.4	1.2	1.0	2.0	1.8

Table 10. Effect of shaking on lipase production by the test organisms (Enzyme activity is expressed as µmole FFA/ml/min).

Organism / aeration	Lipase activity				
	P. aeruginosa	*B. subtilis* (12)	*S. cerevisiae*	*A. oryzae*	*A. flavus*
Without shaking	5.6	6.4	5.0	4.6	4.1
With Shaking	7.6	9.0	5.6	6.0	5.5

REFERENCES

1. Benzonana, G. 1974. Some properties of an extracellular lipase from *Rhizopus arrhizus*. Lipids, 9: 166-172.

2. Chander, H., V.K. Batish, S.S. Sannabhadti, and R.A. Srinivasan. 1980. Factors affecting lipase production in *Aspergillus wentii*. J. Food Sci., 45: 598 - 600.

3. Chander, H., V.K. Batish, and D.R. Ghodekar. 1981. Factors affecting lipase production in *Rhizopus nigricans*. J. Dairy Sci., 64: 193-196.

4. Chander, H., B. Ranganathan, and J.Singh. 1979. Role of some fatty acids on the growth and lipase production by *Streptococcus faecalis*. J. Food Sci., 44:1566-1567.

5. Chander, H., S.S. Sannabhadt, J. Elias, and B.Ranganathan. 1977. Factors affecting lipase production by *Penicillum chrysogenum*. J. Food Sci., 42: 1482.

6. Concalves, J.A., and F.J. Castillo. 1981. Partial purification and chrysralization of B-galactosidase from *Kluyveromyces marxianus*. J. Dairy Sci., 56:2085.

7. El-Makhazangi, A.M.A. 1989. Studies on microbial lipases. Ph.D. Thesis, Food Sci., Fac. of Agric., Zagazig Univ., Zagazig, Egypt.

8. Fujii, T., T. Tatara, and M. Miagawa. 1986. Studies on application of lipolytic enzyme in detergency. 1. Effect of lipase from *Candida cylindracea* on removal of olive oil from cotton fabric. JAOCS, 63:796-799.

9. Fukumoto, J., M. Iwai, and Y. Tsujisaka. 1964. Studies on lipase, purification and properties of a lipase secreted by *Rhizopus delemar*. J. Gen. Appl. Microbiol., 10 (3): 257.

10. Hauka, F.I.A., M.M.A. El-Sawah, and I.I. Ismail. 1991. Factors influencing lipase activity and production in *Aspergillus fumigatus*. J. Agric. Sci. Mansoura Univ., 16 (1): 74-82.

11. Hauka, F.I.A., I.I. Ismail, R.A. Hassan, and T.S.M. Shady. 1997. Factors controlling lipase activity in certain microorgansims (Under publication).

12. Hosono, A., and F.Tokita. 1970. The lipolytic properties of *Candida mycoderma* and *Debaryomyces klockeri* isolated from limurg cheese and some poperties of lipase produced by these yeasts. Jap. J. Zootech. Sci., 41:519.

13. Iwai, M., and Y. Tsujiska. 1974. The purification and properties three kinds of lipase from *Rhisopus delemar*. Agr. Biol. Chem., 38:1241.

14. Jensen, R.G. 1974. Characterization of lipase from the mold *Geotrichum candidum*. Lipids, 9:149-157.

15. Kennedy, M.B., and W.J. Lennarz. 1979. Characterization of the extracellular lipase of *Bacillus subtilis* and its relationship to a membrane-bound lipase found in a mutant strain. J. Biol. Chem., 354:1080-1089.

16. Khan, I.M., C.W. Dill, and R.C. Chander. 1967. Production and properties of extracellular lipase of *Chromobacter lipolyticum*. Biochim. Biophys. Acta, 132:68-77.

17. Khor, H.T., N.H. Tan, and C.L. Chua. 1986. Lipase-catalyzed hydrolysis of palm oil. JAOCS, 63:538-540.

18. Lifield, W.M., R.A. Baraukas, L. Sivieri, S. Serota, and R.W. Stevensonsr. 1984. Enzymatic fat hydrolysis and synthesis, JAOCS, 61:191-195.

19. Linfield, W.M., S. Serota, and L.Sivieri. 1985. Lipid-lipase interactions. 2. A new method for the assay of lipase activity. JAOCS, 62:1152-1154.

20. Lobyreva, L.B., and A.I. Marchenkova. 1979. Lipolytic activity of the spore-forming strains of *Bacillus subtilis*. Mikrobiologiya, 48:208-211.

21. Oi, S., A. Sawada, and Y. Satomura. 1969. Purification and some properties of fungal lipase preparation used for milk flavouring. Agric. Biol. Chem., 33:729.

22. Sugiura, M., T. Oilkawa, K. Hirano, and T. Inukai. 1977. Purification, crystallization and properties of triacylglycerol lipase from *Pseudomonas fluorescens*. Biochim. Biophys. Acta, 488:353-358.

23. Vadhera, D.V., and L.G. Harmon. 1969. Factors affecting production of Staphylococcal Lipase. J. Appl. Bacteriol., 32:293-304.

الظروف المثلى لإنتاج الليبيز من بعض الكائنات الحية الدقيقة

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بعد أن إحتلت الميكروبات المرتبة الأولى فى إنتاج الليبيز فى أيامنا الحالية إتجهت الأبحاث الحديثة نحو توفير ظروف مثلى لإنتاج الإنزيم من الميكروبات المختلفة حيث أنه من المعروف تباين الإنتاج بإختلاف الميكروب وكذلك بإختلاف ظروف الإنتاج سواء الغذائية منها أو البيئية ولذلك إستهدفت الدراسة فى هذا البحث توفير ظروف مثلى لإنتاج أعلى كمية من الإنزيم من الميكروبات وقد وجد أن بيئة Sugiura *et al.*, (1977) هى الأفضل لإنتاج الليبيز من البكتريا وأن بيئة Chander *et al.* (1980) هى الأفضل لإنتاج الإنزيم من الفطريات، وقد وصل الإنتاج أقصاه بعد ٣ أيام للبكتريا و ٥ أيام للفطريات. وقد وجد أن الجلوكوز هو أفضل مصدر كربون لإنتاج الإنزيم بإستثناء السكروز الذى أعطى أعلى إنتاج من فطر *Aspergillus oryzae* وقد أعطى الكازين والببتون أعلى نشاط للإنزيم من بين مصادر النتروجين المستعملة مع كل من *Bacillus subtilis, Pseudomonas aeruginosa* على الترتيب فى حين أن ردة القمح أعطت أعلى إنتاج للإنزيم من الفطريات. وقد وجد أن زيت الزيتون هو أفضل الزيوت والدهون لإنتاج الإنزيم بالنسبة للبكتريا فى حين أدت الليبيدات المستخدمة الى تقليل إنتاج الإنزيم من الفطريات.

أدت إضافة الأحماض الدهنية لبيئة النمو إلي تثبيط إنتاج الإنزيم. وعند غياب مصدر الطاقة الأساسى من بيئة النمو (الكربوهيدرات) قل بدرجة كبيرة إنتاج الإنزيم. وقد أظهر الجلوكوز (١٪) وزيت الزيتون (١٪) أعلى نشاط للإنزيم البكتيرى، بينما كان الجلوكوز ١٪ وزيت فول الصويا ٠,٢٪ أفضل للإنتاج الإنزيمى من الفطريات بإستثناء فطر *A. oryzae* الذى أعطى أعلى إنتاج مع السكروز ١٪ وزيت فول الصويا ٠,٢٪ ووجدت درجة pH ٦ ودرجة الحرارة ٣٠ ٥م هى المثلى لإنتاج الإنزيم سواء من البكتريا أو الفطريات علماً بأن الرج أدى إلى زيادة إنتاجية الإنزيم.