

## **Role of some fermentation parameters affecting lipase production by *Fusarium solani***

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### **Abstract**

The lipase production by *F. Solani* was investigated on using shaking flask and laboratory stirred fermenter. The results indicated that the best yield 380 U/ml (specific enzyme activity 9.04 U/mg protein) was obtained by using Erlenmeyer flask 250 ml containing 50 ml of culture with a medium composition g%: Sucrose 2, corn oil 0.2 ml, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.05, KCl 0.05, KH<sub>2</sub>PO<sub>4</sub> 0.1, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.23, at pH 6.2 using citrate phosphate buffer and inoculum size 6 ml/50 ml medium. However, on using a bench top fermenter 5 L, the yield was increased by about one and half fold (520U/ml) under optimized fermentation conditions.

**Keyword:** Lipase production, *F. Solani*

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### **Introduction**

Lipases (triglycerol ester hydrolase) are enzymes which catalyze the hydrolysis of fats to produce monoglycerides, diglycerides, free fatty acid and glycerol. With the rapid development of enzyme technology a considerable attention has been focused on the biotechnological application of lipases to fats and oil industry (Macrae and Hammond, 1985).

Lipolytic enzymes have an increasing use in food & detergent industries. Recently, their role in paper & pulp manufacture has been emphasized (Fujita *et al.*, 1992; Hate *et al.*, 1996). Furthermore, lipases are used in the manufacturing of monoacyl-glycerols which are widely used as emulsifiers in food of dairy industry, pharmaceuticals, cosmetic industries as well as hair care additives (Bornscheure, 1995).

Although, lipases occur in animals, plants and microorganisms, microbial lipases have a broad spectrum of industrial application, since they are more stable compared with plant and animal lipases and they can be obtained cheaply.

The lipases from *Candida rugosa* (Kong and Rhee, 1989, *Georrichun candidum* (Hedrich *et al.*, 1991). *Aspergillus niger* (Ellaiah *et al.*, 2004) and *Mucor sp.* (Abbas *et al.*, 2002) have been used successfully in industrial applications and lipase from *Candida rugosa* is commercially available (Sonnet and Gazzillo, 1994). However the industrial demand for highly active preparations of lipolytic enzymes continues to stimulate the search for new enzyme sources (Lee and Rhee, 1993).

The lipases production are dependent on several environmental factors such as cultivation temperature (Maliszewska and Mastalerz, 1992; Berto *et al.*, 1997; Chattopadhyay *et al.*, 1999) pH of the growth medium (Chopra and Chander, 1983; Chen *et al.*, 1998) incubation period (Beruil and Kushner, 1975; Chattopadhyay *et al.*, 1999) as well as inoculum size (Elwan *et al.*, 1985).

The goal of the present work is to optimize the lipase production by *Fusarium solani* as well as studying the production process under controlled fermentation condition using 5 liter laboratory fermenter.

## Materials and Methods

### Materials

#### Microorganism

The strain of *Fusarium solani* was kindly obtained from Natural and Microbial Products Chemistry Dept., National Research Center, Dokki, Cairo, Egypt.

#### Chemicals

All the chemicals used in the current work were laboratory reagents purchased from Merck and all the organic solvents were HPLC grade.

### Methods

#### Maintenance of the microorganism

The culture used in the current work was a descendant from a single slant of the pure culture. The stock culture was maintained on agar slops of the following, medium (w/v) (Oxoid, 1982) glucose 2, NaNO<sub>3</sub> 0.2, KCL 0.05, KH<sub>2</sub>PO<sub>4</sub> 0.1, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.05, FeSO<sub>4</sub>.7H<sub>2</sub>O 0.005 and agar 2. The pH was initially adjusted to 6.8. The slant culture was stored in a refrigerator with regular transfer every month.

## Inoculum preparation

The spore suspension of the experimental organism was prepared by adding 6 ml sterile distilled water into 6 days old slant culture and scratched with a sterile inoculated needle. Erlenmeyer flasks 250 ml containing sterile 100 ml of cultivation medium was inoculated by 5 ml of previously prepared spore suspension ( $8.2 \times 10^7$  spore/ml.). According to Elwan *et al.* (1986) the cultivation medium has the following composition g/l: sucrose 2, corn oil 0.2, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.05, KCL 0.05, KH<sub>2</sub>PO<sub>4</sub> 0.1, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.23. The ratio of gum arabic to corn oil is 1:1 and the pH was adjusted to 5.4 using citrate phosphate buffer (0.1 M). Growth was continued at 200 rpm  $30 \pm 1^\circ\text{C}$  for 72 h.

## Preparation of triglyceride

According to the method described by Starr (1941) 10 % (v/v) of the triglycerides (olive oil) emulsified in 10% (w/v) gum arabic in hot water and homogenized in a top drive homogenizer for 10 min to form an emulsion of triglyceride used.

## Determination of the lipolytic activity (Parry *et al.*, 1966)

A suitable volume (1ml) of the culture filtrate was mixed with 2.5 ml of deionized water and the pH was adjusted to 7.7 1 ml of 0.1 Tris-HCl buffer (pH 7.7) and 3 ml of 10% (v/v) triglyceride emulsion prepared in 10% (w/v) gum arabic by homogenizing in top drive homogenizer for 10 min were then added. The mixture was incubated for 2 h at 37°C in shaking water bath after which 10 ml of 99% acetone (absolute) was added. The resulting mixture was then titrated against 0.05 N NaOH using thymolphthalein indicator. It should be noted that NaOH is previously standardized against standard 0.05 N HCl using phenolphthaleine indicator. Blanks, obtained on using-boiled enzyme samples and the activities were obtained in terms of U/ml of enzyme solution. The lipase unit was defined as that produces 1  $\mu$ mol of free fatty acids under assayed conditions.

## Protein estimation

The protein content of the enzyme preparations was determined according to the method described by Lowry *et al.* (1951).

## Biomass estimation

The mycelial biomass obtained after growth, was separated by filtration washed by distilled water and dried. It was dried in an oven 90°C and reweighed till constant weight.

## Bioreactor studies

The fermentation process was achieved by using 5 L laboratory fermenter (B. Braun, Biotech international micro DUC-200). The production process carried out as follow, sterile 3 L of the growth medium (Elwan *et al.*, 1986) medium, was inoculated by the tested microorganism as 6 ml/50 ml medium. The growth was continued at temperature 30°C, dissolved oxygen 1 v/v/min and the stirred speed was controlled at 600 rpm to maintain DOT above 20%.

## Results and Discussion

Since, the constitution of the fermentation medium clearly affected on the lipase production, the study started by investigating the effect of four different nutritive media on lipase production. The results in Table 1 represent the different media used. The best lipase activity 200 U/ml was obtained on using the fermentation medium reported by Elwan *et al.* (1986a), where this medium was characterized by containing 0.2 corn oil. However, the medium of Tamerler and Keshavarz (2000), which contains rapeseed oil 1.25, showed a considerable lipase activity 150 U/ml.

It is clearly obvious that the media which contained oils showed greater lipase production. This may be attributed to that corn oil and rapeseed oil were used as inducer in the fermentation process (Elwan *et al.*, 1986; Elibol and Ozer, 2000). Also, Kanwar *et al.* (2002) reported that the addition of tributyrin at concentration 0.05 % (v/v) to the culture medium enhanced the lipase production by 2.4 folds.

The results presented in Fig 1 showed the time course of lipase production by the experimental microorganism. It is clearly indicated that the best lipase activity (250 U/ml) was obtained at 72 h of incubation. These results are in accordance with the concept that the production of enzymes is advantageous to the microorganism only when nutrients become limiting i.e. during the late logarithmic phase or early stationary phase (Lee and Rhee, 1993).

On the other hand, Maia *et al.* (2001) found that the maximum specific lipase activity was obtained at 96 h cultivation using *F.solani*. Similarly, Ellaiah *et al.* (2004) found that the 96 h cultivation period was the best for lipase production using *Aspergillus niger*.

The effect of different inoculum size of *F.solani* on lipase production was studied, and the results are shown in Fig 2. The lipase production by the experimental microorganism increased on increasing the inoculum size until it reached 290 U/ml at 6 ml inoculum size ( $8.2 \times 10^7$  spore/ml). As the inoculum increased above 6 ml a sharp decrease in the activity is recorded (180 U/ml at 10 ml). These results are in accordance with that obtained by Elwan *et al.* (1986); Mohawed, (1990). Also Chattopadhyay *et al.* (1999) found that the best concentration of spore suspension was  $8 \times 10^4$  spores/ml.

With regard to the effect of different buffers values of the fermentation medium on lipase production (Table 2), two buffers were used to adjust the fermentation medium for lipase production to the specific pH values, Citrate phosphate (pH 3.5-7.0) and phosphate buffer (pH 6.0-8.0). It is clear that the optimum pH for lipase production by *F.solani* was 6.2 using citrate phosphate buffer (0.1M). Lipase production was found to be sensitive to narrow changes in pH value. Higher acidity or alkalinity resulted in decrease in lipase productivity by nearly 55% and 25% respectively. Similar results were found by El-Negar and Mohawed (1990) where the optimum pH was 5.6 using *A.sydowi*.

Mecki *et al.* (1994) found on determining the effect of pH the production medium, both lipase productivities and mycelial dry weight by four tested fungus were pH 5.5 for *Thermascus lonuginosous*, and pH 6.0 for *Sportichum thermophilum* and pH 6.5 for *Aspergillus fumigatus*. Bertro *et al.* (1997) found also that best pH was 5.5 for *Alternaria brassicicola*. Generally the optimum pH for microbial lipases in the range of 6.0-9.0 (Fox and Stephaniak, 1983).

The effect of addition of oils and fat compounds to oil-free medium was studied. In the course of study, results in Table 3 indicated that *F.solani* lipases were constitutive. The results showed that maximum lipase activity 350 U/ml (specific enzyme activity 8.50 U/ml protein) as well as the best growth of the microorganism was also exhibited on adding corn oil 0.2 % to the production medium. Corn oil was followed by olive oil and tween 80 where lipase activity and specific enzyme activity were 270 U/ml, 255 U/ml and 7.48 U/mg protein, 6.82 U/mg protein respectively. On the other hand, reduced levels have been obtained on using Tween 20, Tween 40 (150% 175 U/ml). The discrepancy of being constitutive or inducive is in favour of the claim and that a fixed property for lipases is unlikely, the property is somewhat arbitrary, depending on growth conditions (Davies, 1964; Elwan *et al.*, 1986). Similar results were obtained by Elwan *et al.* (1986b) on using *A.sydowi* grown on corn oil medium, Elibol and Ozer (2000) on

using *Rhizopus arrhizus* grown also on corn oil medium (Mohawed, 1990) found that the presence of corn oil was the best oil lipase production from *Pencillium aurantiogriseumm* and *Alternaria alternate*. Olive oil shown to be the best additive by Mahadik *et al.* (2002) and Ellaiah *et al.* (2004) when *Aspergillus niger* was studied for lipase production.

In addition, the strength of tested microorganism lipase to hydrolyze different oil were tested. From Table 4, it is evident that degree of hydrolysis was proportional to the number of carbon atoms and the presence of double bonds. Thus, *F.solani* lipase hydrolyzed corn oil showing lipase activity 380 U/ml followed by olive oil of lipase activity 350 U/ml. This may be attributed to the ratio of unsaturated fatty acids in these oils is greater than that of saturated fatty acid. Corn oil contains approximately 86% unsaturated fatty acids while olive oil 89% unsaturated fatty acids. On the other hand rapeseed oil contains 27% unsaturated fatty acids.

The study extended to investigate the lipase activity on using bench top fermenter 5 L under the optimum fermentation conditions. The results presented in Fig 3 showed that the maximum lipase activity 520 U/ml was obtained at 96 h growth period, 1 v/v/min aeration and 6 ml/50 ml medium inoculum size a temp 30°C. As the growth period increased a considerable decrease in lipase activity was noticed which may be due to nutrient limitation and the cells reached the decline phase.

The above results showed that, the laboratory fermentation is more suitable for fermentation process, since the growth conditions were controlled as well as the priority of using controlled fermentation condition by laboratory fermentor, than shaking flasks.

## Conclusion

The present study clearly indicated that the lipase productivity depended on same physiological and fermentation parameter such as, growth period, pH, inoculum size as well as oxygen requirement. The best yield was obtained on using fermentation medium containing corn oil 0.2 %, growth time 96 h, pH 7.5, inoculum size 6ml / 100ml medium.

Table 1: Effect of different medium on lipase production by *F.solani*

Fermentation Medium	Lipase activity U/ml	Cell dry wt g/100 ml	S.E.A. U/mg protein
1	200	3.45	5.55
2	75	2.88	2.08
3	100	3.01	2.77
4	150	3.25	4.16

S.E.A: specific enzyme activity

Medium 1: g % (Elwan *et al.*, 1986a)

Sucrose 2, corn oil 0.2, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.05, KCl 0.05, KH<sub>2</sub>PO<sub>4</sub> 0.1, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.232.

Medium 2: g % (Elwan *et al.*, 1986b)

Sucrose 2, NaNO<sub>3</sub> 0.2, KH<sub>2</sub>PO<sub>4</sub> 0.1, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.05, FeSO<sub>4</sub>.7H<sub>2</sub>O traces, KCl 0.05. pH 6.2

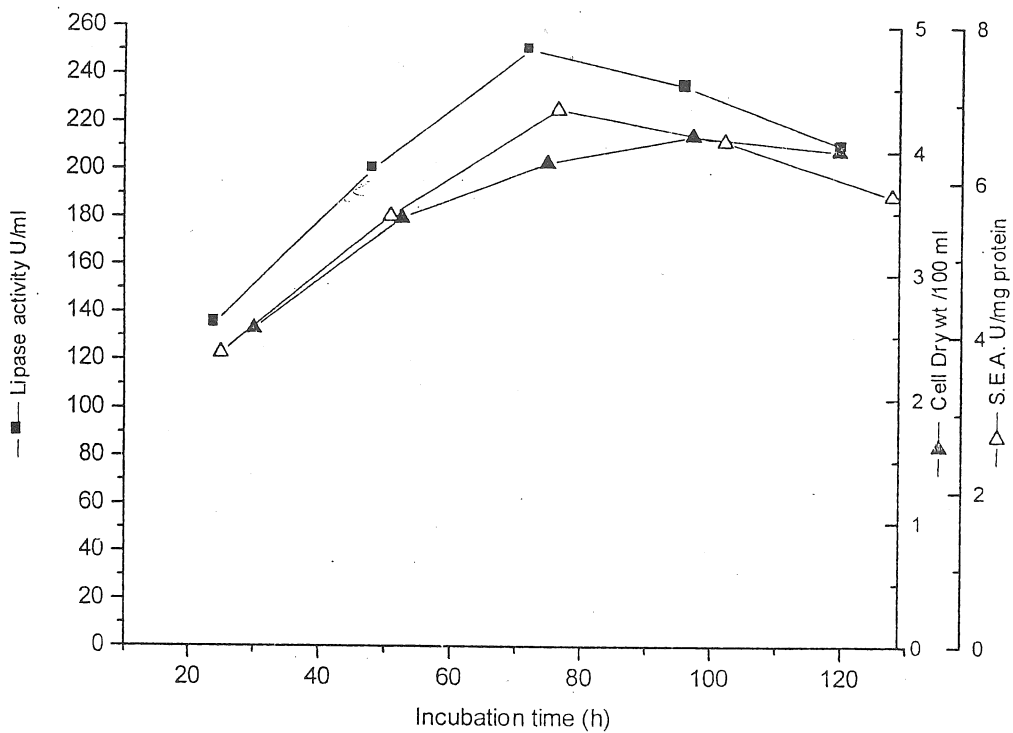
Medium 3: g % (Elwan *et al.*, 1986c)

Cotton seed oil 0.5, NaNO<sub>3</sub> 2, KH<sub>2</sub>PO<sub>4</sub> 0.1, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.05, KCl 0.05, FeSO<sub>4</sub>.7H<sub>2</sub>O, yeast extract 0.3.

Medium 4: g % (Tamerler and Keshavarz, 2000)

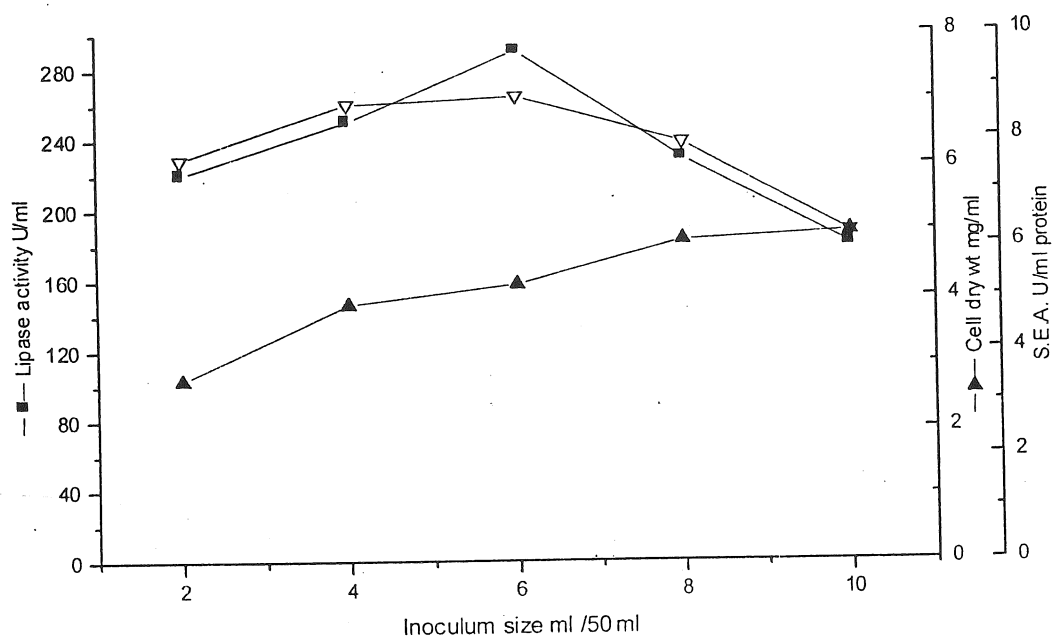
NaH<sub>2</sub>PO<sub>4</sub> 1.2, KH<sub>2</sub>PO<sub>4</sub> 0.2, CaCl<sub>2</sub>.2H<sub>2</sub>O 0.033, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.03, ZnSO<sub>4</sub>.7H<sub>2</sub>O 0.03, MnSO<sub>4</sub>.4H<sub>2</sub>O 0.0012, FeSO<sub>4</sub>.7H<sub>2</sub>O 500 µg, rape seed oil 1.25 cm<sup>3</sup> (w/v), yeast extract 2.0.

Fig1: Effect of different incubation periods on lipase production by *F. solani*



Medium (Elwan *et al.*, 1986a) g %  
 Sucrose 2, corn oil 0.2, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.05, KCl 0.05, KH<sub>2</sub>PO<sub>4</sub> 0.1,  
 (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.2) pH 5.4 using citrate-phosphate buffer 0.1M.

Fig 2: Effect of different inoculum size lipase production by *F. solani*



Medium (Elwan *et al.*, 1986a) pH 5.4 using citrate-phosphate buffer 0.1 M at 72 h incubation



Table 2: Effect of different pH values of fermentation medium on lipase production using *F.solani*

Buffer	Initial pH	Final pH	Lipase activity U/ml	Cell dry wt mg/ml	S.E.A. U/mg protein
Citrate phosphate	3.5	5.38	160	3.65	4.50
	5.4	6.60	290	4.20	7.04
	6.2	6.80	350	4.68	8.50
	7.0	7.50	320	4.45	7.85
Phosphate	6.0	6.65	300	4.55	7.36
	6.5	7.20	330	4.81	8.00
	7.5	8.10	285	4.23	5.50
	8.0	8.30	240	3.95	5.00

Citrate phosphate (0.1M) 3.5-7.0

Phosphate (0.1M) buffer 6.0-8.0

Table 3: Effect of different oils and dispersed agents on the lipase production using *F.solani*.

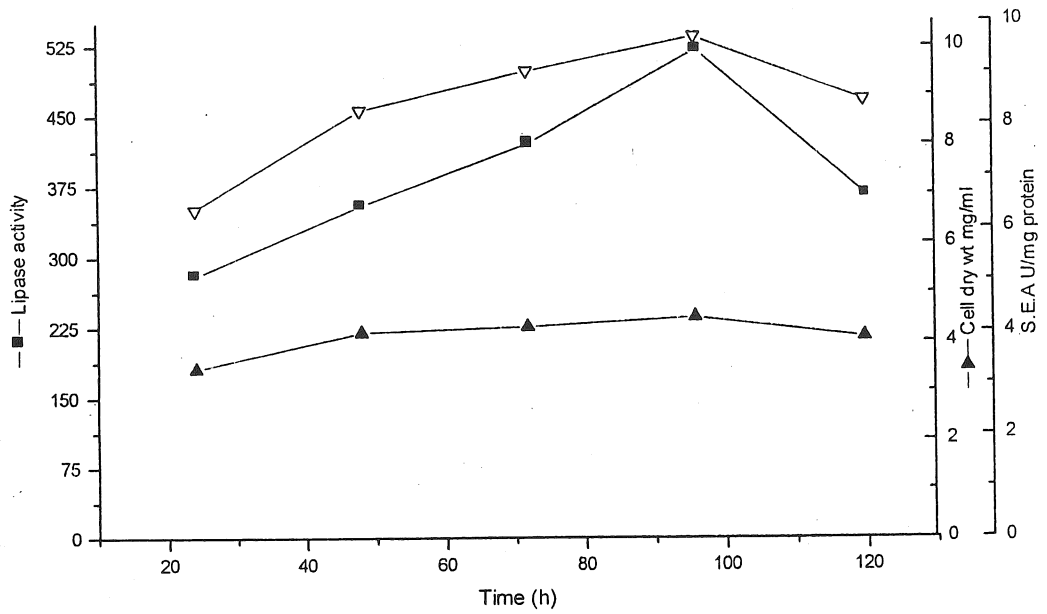
Different oils & dispersal agent	Lipase activity	Cell dry wt	S.E.A
	U/ml	Mg/ml	U/mg protein
Control	95	2.94	2.08
corn oil	250	3.90	6.93
olive oil	210	3.10	4.6
sun flower oil	175	2.98	3.83
cotton seed oil	190	3.00	4.16
Tween 20	150	2.82	3.28
Tween 40	240	3.77	5.26
Tween 80	200	3.04	4.38

Using 0.1 M Citrate phosphate buffer pH 6.

Table 4: Effect of lipase activity produced by *F. solani* on hydrolysis of different oils

Different oils	Final pH	Lipase activity U/ml	Cell dry wt mg/ml	S.E.A. U/mg protein
Olive	7.55	350	4.68	8.50
Cotton	7.59	340	4.60	8.13
Corn	7.59	380	4.65	9.04
Sesame	7.61	250	4.50	5.40
Rape seed	7.63	218	4.55	4.15

Fig (3) Lipase production by *F. solani* using laboratory fermentor



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