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EFFECT OF SOME PHYSICAL FACTORS ON LIPASE PRODUCTION BY SOME SELECTED FUNGI

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ABSTRACT:

Seven fungi have been experimentally screened to get the most active two lipase producers. The experimental conditions were Czapek's yeast extract tributyrin emulsion (CYET) liquid medium, incubated at 30 °C &pH 5.0 for 8 days.

Classical kinetic technique of enzymatic analysis has been used to study the effect of physical factors (pH, temperature &incubation period). Optimal yield of lipase produced by Aspergillus niger after 8 days of incubation at 30 °C &pH 5.0, while optimal yield of lipase produced by Rhizopus nigricans after 6 days of incubation at 30 °C &pH 6.0 .

***Conclusively,** from this study, it could be concluded that the optimal yield of lipase was produced by Aspergillus niger at pH 5 and 30 °C for 8 days incubation, while the optimal yield of lipase produced by Rhizopus nigricans at pH 6 and 30 °C for 6 days incubation.*

Keywords: Some physical factors, lipase production, some selected fungi

INTRODUCTION:

The importance of lipases in industrial processes has been throughly discussed in review articles (Rattary, 1984 and Yamane, 1987). Use of these enzymes in production of food flavors has been well documented (Nelson, 1972; Dwivedi, 1973; Arnold *et al.,* 1975 and Shahani *et al*., 1976).

With the advent of biotechnology, fermentation processes have been employed to produce a wide variety of lipases from microbial sources (Kamimura *et al.,* 2001; Elibol and Ozer, 2000). Molds have produced lipase, and the nature of the enzymes varies among species (Fukumoto *et al.,* 1963; Iwai *et al.,* 1964; Jensen, 1974; Kinsella and Hwang, 1976; Chander *et al*., 1980 &

1981; Fodiloglu and Erkmen, 1999 and Kader *et al*., 2007). Enzyme- producing microorganisms include bacteria (Kulkarni and Gadre, 2002; Babu *et al*., 2006), yeast (Corzo and Revah, 1999) and actinomycetes (Sommer *et al.*, 1997).

Lipases from microorganisms have drawn much attention especially for their potential use in biotechnology, mainly due to their availability and stability (Ghosh *et al.*, 1996 and Wang *et al.*, 1995).

Microbial enzymes that have been used to generate cheese flavors from milkfat have also been discussed by Jolly& Kosikowski (1973); Huang & Dooly (1976); Sood & Kosikowski (1979); Arbige *et al*. (1986) and Omar *et al.* (1986).

The objective of the present work was to potentially characterize the effect of some physical factors on lipase production by some selected fungi, and to use these organisms as natural and economical source of the enzyme.

MATERIALS AND METHODS

Organisms:

Seven fungi (*Aspergillus niger, Aspergillus oryzae, Fusarium oxysporium, Penicillium italicum, Rhizopus nigricans, Rhizoctonia solani & Trichoderma viridae*) obtained from National Collection of Yeast Cultures (NCYC), Agric. Res. Counsel, food Res. Inst., Colney lane Norwich. These cultures were maintained with periodic transfer on Czapek's yeast extract – tributyrin agar slant.

Growth medium:

Fungal spores were grown in a sterilized (121°C/15 min) growth medium composed of (%): 3 sucrose; 0.3 $NaNO_3$; 0.1 KH_2PO_4; 0.05 $MgSO_4$. $7H_2O$; 0.001 $FeSO_4$. $7H_2O$; 0.1 yeast extract, and 1 ml of tributyrin emulsion.

Production of lipase:

Fungal organisms were inoculated into 40 ml of the growth liquid medium in 250 ml Erlenmyer flasks at the rate of 3 ml spore suspension. Then incubated at 30 °C for 8 days for the production of lipase. Mycelium was separated by filtration then centrifugation at 5000 x g for 20 min.

The cell- free extract was the source of lipase enzyme. Lipase production was determined by the method of Fukumoto *et al*.(1963) with some modifications. The reaction mixture contained 3 ml of the substrate (olive oil for lipase production); 1 ml of 0.1 *M* acetate buffer (pH 5.6); 1 ml of 0.2 *M* $CaCl_2$, and 1 ml of the enzyme solution to be tested.

The mixture was incubated at 30 °C for 150 min. with constant shaking in calibrated water bath. At the end of incubation, the reaction mixture was mixed with 10 ml ethanol (90%) in a beaker to stop the reaction, and the free fatty acids formed by the enzyme reaction was titrated with 0.05 M KOH using a

potentiometer titrator. The activities of lipase enzyme were expressed in terms of units, one unit being defined as one ml of the difference between the volume of the alkali solution consumed for titrating the test solution and the blank containing boiled enzyme.

Measurement of growth:

The dry weight of mycelium was measured by filtration of the cell culture through Whatman Nº 1 filter paper, washing with distilled water, and drying in an oven at 90 °C for 24 hr.

RESULTS AND DISCUSSION

Screening of lipolytic activity of the fungi:

Among seven fungi, those were screened for lipase production under the same experimental conditions the most active two organisms which produce high amount of lipase were *Aspergillus niger* and *Rhizopus nigricans* followed by *Aspergillus oryzae* (Table 1), while the other four fungi produced small amounts of the enzyme under these conditions and medium composition.

Table 1. Screening of lipolytic microorganisms [a]

Organisms	Wt. of biomass mg mycelium/ml medium	Lipase activity [b] (µmoles FFA)
Aspergillus niger	120.30	1.000
Aspergillus oryzae	73.30	0.150
Fusarium oxysporium	9.45	0.125
Penicillium italicum	82.90	0.150
Rhizopus nigricans	22.13	0.350
Rhizoctonia solani	94.28	0.025
Trichoderma viridae	20.35	0.050

a. Average of four trials.

b. Lipase activity is expressed as µmoles of free fatty acids liberated by 1 ml of medium.

Influence of physical factors on the production of lipases:

Because of the higher lipase production *Aspergillus niger and Rhizopus nigricans* were selected for further physical studies.

Incubation period:

Out of five different periods, namely 2,4,6,8, and 10 days, the maximum amount of lipase was produced by *Aspergillus niger* and *Rhizopus nigricans* after 8,6 days respectively, (Table 2). Thus it is considered as optimum incubation period for these fungi were grown in Czapek's medium in stationary culture. The obtained optimum incubation period for *Aspergillus niger* was 8 days, while the

Table 2. Effect of incubation period on lipase production by *Aspergillus niger* and *Rhizopus nigricans* [a]

Incubation period (days)	*Aspergillus niger*		*Rhizopus nigricans*	
	Wt. of biomass mg mycelium/ ml medium	Lipase activity [b] (µmoles FFA)	Wt. of biomass mg mycelium/ ml medium	Lipase activity [b] (µmoles FFA)
2	114.07	0.150	19.88	0.175
4	174.23	0.200	117.58	0.250
6	127.95	0.250	105.38	0.350
8	104.96	0.900	67.73	0.300
10	101.13	0.450	63.60	0.125

a. Average of four trials.

b. Lipase activity is expressed as µmoles of free fatty acids liberated by 1 ml of medium.

one observed by Chander *et al*, (1980) was 5 days for *Aspergillus wentii*, while Vaidehi and Jagadamba (1984) found that it was 6 days for *Aspergillus flavus*. The difference between these incubation periods could be due to the type of Aspergillus species *(A. niger, A. wentii, A. flavus)* and the composition of growth media used by different mentioned researchers.

In studying *Fusarium oxysporium* and *Rhizoctonia solani* Vaidehi and Jagadamba (1984) found that it was 8 days.

The observed optimum incubation period for *Rhizopus nigricans* (6 days) was in good agreement with Chander *et al*. (1981) observations for the same organism grown on yeast dextrose medium. Ogendero (1980) showed that *Humicola grisea* var. *themoidea* and *Mucor pusillus* produced the maximum amount of lipase on 6 days, while the optimum incubation period for *Talaromyces thermophilus* and *Termoascus crustaceus* was 8 days.

Incubation temperature:

Five different incubation temperatures, 20, 25, 30, 35, and 40 °C were tested under the previously mentioned optimum incubation period for each fungus. The maximum production of lipase was found at 30 °C (Table 3). These results are in excellent agreement with those obtained by Fukumoto *et al.*(1963) and Chander *et al.*(1981) for *Aspergillus niger* and *Rhizopus nigricans*, respectively.

Confirmation can be obtained by Chander *et al.* (1980), Eitenmeller *et al.* (1970), and Chander *et al.*(1977) for *Aspergillus wentii*, *Penicillium roqueforti*, and *Penicillium chrysogenum* respectively, with different incubation periods. Nevertheless, maximum amount of lipase production at 30-45 °C were also

Table 3. Effect of incubation temperature on lipase production by *Aspergillus niger* and *Rhizopus nigricans* [a]

Incubation temperature (°C)	*Aspergillus niger*		*Rhizopus nigricans*	
	Wt. of biomass mg mycelium/ ml medium	Lipase activity [b] (µmoles FFA)	Wt. of biomass mg mycelium/ ml medium	Lipase activity [b] (µmoles FFA)
20	103.32	0.175	95.55	0.200
25	115.83	0.350	123.13	0.250
30	119.10	0.875	94.33	0.350
35	136.15	0.300	91.80	0.150
40	138.58	0.300	92.09	0.100

a. Average of four trials.
b. Lipase activity is expressed as µmoles of free fatty acids liberated by 1 ml of medium.

observed by Liu *et al.*(1972) and Somkuti & Bable (1969) for other fungi, *Humicola lanuginose* and *Mucor pusillus*, respectively.

Starting pH values:

The growth medium was adjusted to pH 4, 5, 6, 7 and 8. Maximum lipase was produced at pH 5.0 by *Aspergillus niger* and 6.0 by *Rhizopus nigricans* (Table 4).

Table 4. Effect of starting pH on lipase production by *Aspergillus niger* and *Rhizopus nigricans* [a]

Starting pH	*Aspergillus niger*		*Rhizopus nigricans*	
	Wt. of biomass mg mycelium/ ml medium	Lipase activity [b] (µmoles FFA)	Wt. of biomass mg mycelium/ ml medium	Lipase activity [b] (µmoles FFA)
4	118.78	0.225	78.00	0.300
5	119.10	0.875	94.33	0.350
6	118.23	0.375	141.95	0.550
7	109.38	0.175	118.23	0.450
8	105.10	0.150	114.93	0.150

a. Average of four trials.
b. Lipase activity is expressed as µmoles of free fatty acids liberated by 1 ml of medium.

These results agree with findings of Fukumoto *et al.* (1963) with *Aspergillus niger* and with findings of Chander *et al.*(1977, 1980 and 1981) and

Hosono *et al*.(1973)who demonstrated maximum yield of lipase by *Rhizopus nigricans, Aspergillus wentii*, *Penicillium chrysogenum* and *Candida muscorum*, respectively at pH 6.0 . On contrary, higher pH optima was noted in *Penicillium camemberti* and *Gleosporium olvarium* (Dolezalek and Minarik, 1969 and Grasas, 1973).

Conclusively, from this study, it could be concluded that the optimal yield of lipase was produced by *Aspergillus niger* at pH 5 and 30 °C for 8 days incubation, while the optimal yield of lipase produced by *Rhizopus nigricans* at pH 6 and 30 °C for 6 days incubation.

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تأثير بعض العوامل الفيزيائية على انتاج بعض الفطريات لانزيم الليبيز

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تم انتخاب سبعة فطريات مختلفة لتحديد افضلها انتاجا لإنزيم الليبيز باستخدام بيئة تشابك مع مستخلص الخميرة ومستحلب الترايبيوترين السائلة كبيئة لتنمية هذه الفطريات والتي حضنت عند ٣٠ °م ودرجة pH ٥.٠ ولمدة ٨ ايام.

وجد ان اعلى انتاج لانزيم الليبيز كان عن طريق فطر ***Aspergillus niger*** يليه فطر ***Rhizopus nigricans*** ثم فطر ***Aspergillus oryzae*** حيث كان اقلها انتاجا للانزيم. لذلك فقد استخدمت فطريات ***Aspergillus niger*** ، ***Rhizopus nigricans*** لدراسة العوامل الفيزيائية مثل درجة حرارة التحضين (٢٠ ، ٢٥ ، ٣٠ ، ٣٥ ، ٤٠ °م) و pH البيئة المبدئي عند درجات (٤ ، ٥ ، ٦ ، ٧ ، ٨) وكذلك مدة التحضين (٢ ، ٤ ، ٦ ، ٨ ، ١٠ ايام) بدون رج.

تم الحصول على افضل انتاج لانزيم الليبيز بواسطة فطر ***Aspergillus niger*** بعد ثمانية ايام تحضين عند درجة حرارة ٣٠ °م ودرجة pH مبدئي للبيئة عند ٥.

أما امثل إنتاج لإنزيم الليبيز من فطر ***Rhizopus nigricans*** فقد كان بعد ستة ايام من التحضين عند درجة حرارة ٣٠ °م وفي بيئة ضبطت درجة ال pH المبدئية لها عند ٦.