Assiut Vet. Med. J. Vol. 42 No. 84, January 2000

Dept. of Botany & Microbiology,
Fac. of Science, AL- Azhar University, Assiut, Egypt.

FACTORS AFFECTING LIPASE PRODUCTION BY *GEOTRICHUM CANDIDUM*

(With 1 Table and 4 Figures)

By
A.A. EL-SHANAWANY

(Received at 29/12/1999)

تأثير بعض العوامل على إفراز أنزيم الليبيز بواسطة فطرة جيوتريكم كانديديم

عبد الرحيم أحمد الشنوانى

يهدف هذا البحث إلى دراسة أنسب الظروف البيئية والغذائية لإفراز إنزيم الليبيز فى الوسط الغذائى وقد استخدمت فطرة جيوتريكم كانديديم والتى عُزلت بدرجات عالية من منتجات الألبان ولها القدرة العالية على إفراز هذا الانزيم. ولقد أظهرت النتائج أن أعلى انتاجية للانزيم بعد يومين من التحضين فى الوسط الغذائى الثابت والمحتوى على الزبدة وعباد الشمس كمصدر كربونى ذو رقم هيدروجينى ٦ والتحضين عند ٢٥°م .

SUMMARY

Geotrichum candidum was isolated at high frequency from milk products (butter and cheese) and achieved high lipase production among the screened fungi. The production of such extracellular lipase by *G. candidum* under various conditions was investigated. The fungus showed maximum lipase production after 2 days at 25°C with pH 6. Lipase activity was stimulated by adding 0.1% of both butter or sunflower oil to the growth medium, while corn, olive and palm oils reduced the production of lipase.

Key words: *Factors affecting lipase production by Geotrichum candidum.*

INTRODUCTION

Lipases are one of the important classes of industrial enzymes in terms of their versatility (Sidhu et al., 1998). They are used extensively in biotechnological field such as food technology, clinical and industrial chemistry (Brockman, 1984; Zaks and Klibanov 1985 and Garcia-Lepe et al., 1997).

Many industrially used lipases are prepared from fungi (Iwai and Tsujisaka, 1974; Wisdom et al., 1987; Derewenda et al., 1994). The lipolytic activity of fungal cultures depends on the species and individual isolate employed (Sviridenko et al., 1979; Baillargeon et al., 1989), culture conditions such as pH, incubation temperature, age of culture and the nature of the lipid substrate employed (Selezneva and Kazanina, 1986; Mohawed et al., 1988; Valero et al., 1991; Chen et al., 1992, Serra et al., Haas and Bailey, 1993).

The present work was aimed at the study of the ability of *Geotrichum candidum* to hydrolyse butter and vegetable oils. The conditions influencing the synthesis and activity of its extracellular lipases were also examined.

MATERIAL and METHODS

Organism:

Geotrichum candidum isolated from milk product samples (raw butter and cheese) and highly active in producing lipase enzyme was selected. The organism was maintained on malt extract agar slants at 25°C for 72 hr.

Growth medium :

Chander et al., (1980), medium with the following composition (g/L): Peptone, 20; yeast extract, 5; NaCL, 5; glucose, 10; pH =6 and sterlized at 121°C for 15 min has been used for the growth of the organism and lipase production.

Production and assay of lipase:

One ml spore suspension of *G. candidum* was inoculated into 25 ml of the growth medium dispensed in 100 ml Erylenmeyer flasks and incubated at different temperatures and periods. The medium was filtered through Whatman No 1 filter paper and the filtrate was centrifuged at 5000 r.p.m for l0 min. The supernatant was taken as the source of lipase enzymes. Using the method of Oi et al. (1969) with

some modificantions, the reaction mixture contained 5ml of 5% butter emulsified in 7% Acacia gum in distilled water, 5ml of 0.02 M Tris-Hcl-buffer (PH 7.5), 2ml of 0.2 M $CaCl_2$ solution, 1ml enzyme solution and 2ml distilled water were incubated in a shaker incubator at 37°C for 3 hr. The total amount of fatty acids liberated was titrated againest 0.01 mol/l NaOH. The blank was used by assaying the mixture containing the boiled enzyme.

Temperature and incubation periods:

The organism was cultivated in the basal growth medium and incubated at 15, 20, 25, 30, 35°C. The lipase activity of cell free extracts was determined at intervals of 1, 2, 3, 4, 5, and 6 days.

Effect of pH:

The initial pH of the basal medium was adjusted to different values ranging from 3 - 9 by the addition of 0.1 N HCl or 0.1 N NaOH. After inoculation, the cultures were incubated at 25°C for 48 hr. and the supernatant was assayed for lipase activity.

Effect of aeration :

To test the effect of shaking on the production of lipase by *G. candidum*, two sets of flasks with basal medium were prepared and inoculated with the organism. One set was incubated without shaking and the second set was kept continuously on a rotary shaker (150 rev / min). Both groups were incubated at 25°C for 48hr.

Effect of different lipid sources.

To study the effect of different lipid sources on lipase production sterilized raw butter, corn , olive, palm or sunflower oils was added to the basal medium at 0.1% concentration and lipase enzyme activity was then assayed.

RESULTS

Results are obtained at Table 1 and Fig. 1,2,3 & 4.

Table 1: Effect of aeration on growth and lipase production by *G. candidum*.

	Lipase activity per ml broth (μ moles FFA)	Weight of biomass mg myclium/ ml broth
Stationary	13.6	0.27
Shaked	9.4	0.20

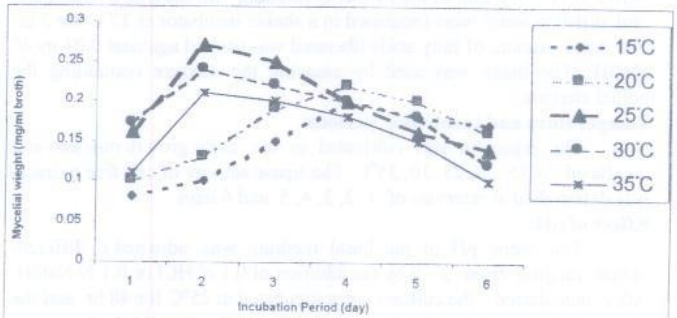

Fig. 1: Effect of incubation period and temperature on mycelia dry wieght by G.candidum

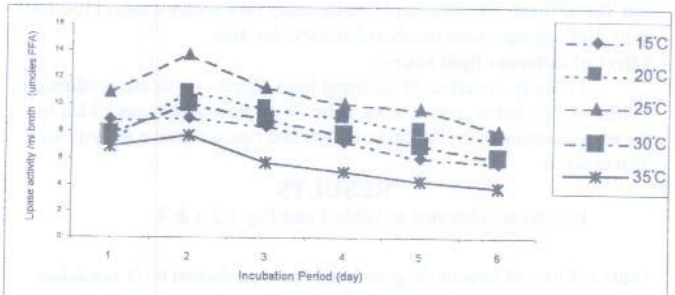

Fig. 2: Effect of incubation period and temperature on lipase production by *G.candidum*

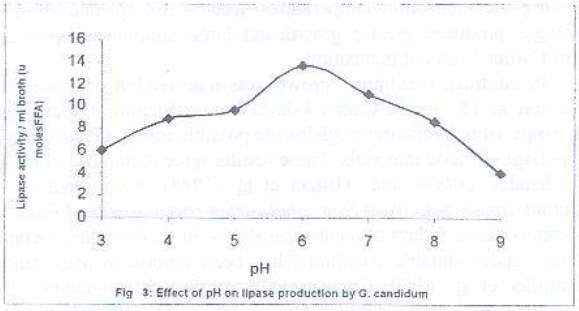

Fig 3: Effect of pH on lipase production by G. candidum

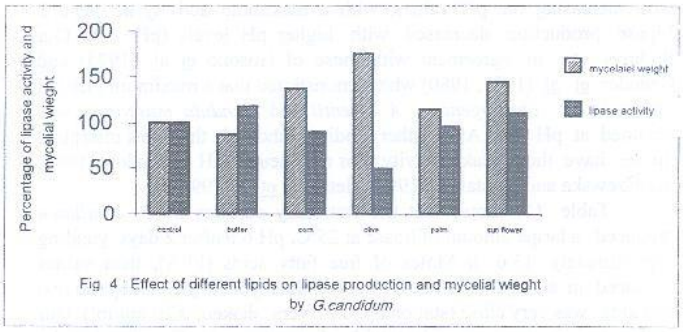

Fig 4 : Effect of different lipids on lipase production and mycelial wieght by *G.candidum*

DISCUSSION

Data in Fig. (1 and 2) reveal that *G. candidum* had a broad optimum temperature for lipase production ranging between 15- 35°C after 2days, while the peak (13.6 *u* Moles free fatty acids) was recorded at 25°C, but the amounts of lipase production was decreased with increasing the incubation temperatures tested. Also, the data show that the fungus produced greater growth and larger amounts of lipase at 25 and 35°C after 2 days of incubation.

In contrast, maximum growth was achieved but with less lipase production at 15 and 20°C after 4 days. The production of lipase over a wide range of temperatures explains the possible role of *G. candidum* in the spoilage of food materials. These results agree with those of Chopra and Chander (1983) and Osman et al. (1988). They reported that maximum lipase activity of *Syncephalastrum racemosum* and *Fusarium* was achieved after 2 days of incubation at 25 - 30°C. Also the production of lipase under suitable conditions has been repoted by many authors (Eitenmiller et al., 1970; Chander et al., 1977, 1980; Ogundero, 1982; Mohawed et al., 1988; Haas and Bailey, 1993; Saad, 1995; Lopes-Diaz et al., 1996; Yadav et al., 1998; Barakat and Abdel-Sater, 1999).

Lipase activity was examined under different pH levels ranging from 3.0 - 9.0. Results of Fig. (3) show a slow increase in lipase activity with increasing the pH values, with a maximum activity at pH 6.0. Lipase production decreased with higher pH levels (pH 7-9). Our findings are in agreement with those of Hosono et al. (1973) and Chander et al. (1977, 1980) who demonstrated that a maximum yield of lipase in *P. chrysogenum, A. wentii* and *Candida muscorum* was obtained at pH 6.0. Also, other findings showed that most microbial lipases have their peak activity at or near neutral pH (Ogundero, 1982; Maliszewska and Mastalevz, 1992; Metwally et al., 1996).

Table (1) shows that the stationary cultures of *G. candidum* produced a larger amount of lipase at 25°C, pH 6.0 after 2 days, yielding approximately 13.6 *u* Moles of free fatty acids (FFA), than values produced in shaked cultures. However, the mycelial growth by the two methods was very close (stationary, 0.27 vers. shaken, 0.20 mg/ml). Our results are in close agreement with observations in *G. candidum* by Alford and Smith (1965); *Aspergillus candidus* (Tipograf and Petina, 1966); *Syncephalastrum racemosum* (Sannabhadti, 1969) and *Penicillum*

citrinum (Abdel-Fattah et al., 1972) which produced more lipase under static conditions.

The effect of 0.1 % of different oils on lipase production and mycelial growth was studied (Fig.4) and, the butter and sunflower oils were the best substrates (as carbon source) for lipase production. They stimulated lipase production of *G. candidum* by 17.6 and 10.3%, respectively, while the mycelial growth was decreased in presence of butter and increased by adding sunflower oil to the medium. On the other hand corn, olive and palm oils caused decreases in lipase production by 11.8, 1.8 and 5.9 %, respectively, whereas promotion of the mycelial growth occurred. In this respect, strong lipase production by *G. candidum* was observed on rice, bran, olive oil, oleic acid and linoleic acid (Iwai et al., 1973) and according to other reports, exclusively oils or fatty acids (Jensen, 1974; Tsujisaka et al., 1973). Ogundero (1980) reported that, the presence of lipids in the growth medium of some thermophilic fungi enhanced the production of extracellular lipases, and the lack of lipids in the medium did not induce their synthesis. Chander et al. (1980) observed that butter and olive oils caused inhibition of lipase activity of *Aspergillus wentii* by 53 and 63 %, respectively. On the contrary, lipase production by *A. tamarii* was stimulated by the addition of 0.15 % olive oil (Saad 1995). Promotion of lipase production was observed by the addition of different lipid substrates (Eitenmiller et al., 1970; Ogundero, 1982; Chopra and Chander, 1983; Valero et al., 1991; Chen et al., 1992; Maliszewska and Mastalerz, 1992; and Haas and Bailey, 1993).

It was concluded that the optimum conditions for lipase production by *G. candidum* were achieved after 2 days of incubation at 25°C in stationary culture containing butter or sunflower oil at pH 6.0. Bekhtereva and Konova (1980) reported that, fungal mycelial possessed a capacity for intensive accumulation of lipids similar in their composition to natural vegetable oils. The replacement of food fats and oils to fulfil industrial needs by fats of microbial origin is a subject of considerable interest (Kazantsev et al., 1979; Solozhenkin et al., 1979; Olama and EL-Sabaeny, 1993).

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