

PRODUCTION OF LIPASE (S) BY SOME YEASTS

I. EFFECT OF TEMPERATURE, pH AND TRIGLYCERIDES

By

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ABSTRACT

An investigation was carried out to evaluate lipolytic activities of three anascosporogenous yeasts viz. Rhodotorula rubra, Candida utilis, C. guilliermondii and an ascosporogenous yeast Debaryomyces hansenii. Lipase (s) was synthesized under the influence of different incubation time, pH, temperature and triglycerides. R. rubra gave the highest level of enzyme after 6 days at 30°C, on sugar - free peptone yeast extract-corn oil medium, of pH 4.

Both C. utilis and C. guilliermondii exhibited optimum conditions for maximum lipase biosynthesis in presence of olive and coconut oil respectively. Corn oil induced enzyme biosynthesis in R. rubra, D. hansenii, C. utilis and C. guilliermondii in presence of 0.2%, 0.6%, 0.8% and 1% respectively.

However, the ascosporogenous yeast D. hansenii which was grown on the same medium showed optimum lipase biosynthesis at 20°C after 48 hours of growth.

INTRODUCTION

Recently, microbial lipase (s) witnessed a tremendous progress and development regarding their production for industrial and clinical application. Production of yeast extracellular lipase (s) is confined largely to

anascosporogenous species, particularly members of the genus candida (Werner, 1960).

Among the active producers of extracellular lipase (s) that have been studied are Candida lipolytica (Vickery, 1936), Mycotorula lipolytica (Peters and Nelson, 1948), C. cylindrica (Yamada and Machida, 1962), C. paralipolytica (Ota and Yamada, 1966), C. humicola (Bours and Mossel, 1969) and Torulopsis ernobii (Motai et al., 1966). It is well known that production of lipase (s) by different microorganisms is affected by many environmental and nutritional requirements.

The present work shows the effect of temperature, pH and different triglycerides on the biosynthesis of lipase by three anascosporogenous yeasts, Rhodotorula rubra, Candida utilis and C. guilliermondii and the ascosporogenous yeast Debaryomyces hansenii.

MATERIALS AND METHODS

Test organisms:

Candida utilis was kindly provided by the Department of Microbial Chemistry, National Research Centre, Cairo. However, Rhodotorula rubra (Domme), Lodder as well as Candida guilliermondii (Cast.), Iangeron et guerra had already been isolated from apricot and pears respectively and identified by Mahmoud et al., (1980). Also,

Debaryomyces hansenii was isolated from a sample of an Egyptian cheese brine called Mesh, and identified by Mahmoud (1978).

Growth medium:

The nutrient medium used throughout these investigations is constructed as follows (g/L): peptone, 5.0; yeast extract, 5.0, KH_2PO_4 , 2.0, MgSO_4 , 7 H_2O , 0.5, corn oil, 10 initial pH 6.5.

Lipase assay:

Lipase (s) in the growth filterates of the yeasts under study were assayed by the tributyrin cup plate clear zone technique (TCZ) as described by Elwan et al., (1977), devised from the technique of Lawrence et al., (1967). Standard curve showing linear relation between (Fulka AG, Buchs SG pancreatic lipase) concentrations and mean clearing zone diameters (mm) as determined by TCZ assay at pH 8 and after 24 hours incubation at 30°C was used for lipase assay in growth filtrates.

Factors affecting lipase biosynthesis:

The organisms were grown in 25 ml portions of the liquid growth nutrient medium in 100 ml capacity Erlenmeyer flasks. The liquid nutrient medium was inoculated with the yeasts under study.

Four levels of incubation temperatures were tested namely, 20, 25, 30 and 35°C. The initial pH of the nutrient medium was adjusted in the range from 4 to 8.

The effect of different triglycerides and corn oil concentration on lipase (s) biosynthesis was also investigated. Medium supplemented with 1% of each of corn oil, coconut oil, cotton seed oil and olive oil at pH 6.5 were inoculated, incubated at 30°C and lipase enzyme was assessed after 2, 4 and 6 days.

RESULTS AND DISCUSSION

Incubation temperature in relation to lipase biosynthesis

Table (1) represents the levels of lipase biosynthesis by the organisms under study as they were grown at 20, 25, 30 and 35°C. From the results it is obvious that 20°C is the optimum temperature for lipase biosynthesis by D. hansenii through the incubation periods, where the maximum lipase yield (6.310 ug/ml) was attained after 2 days incubation.

Optimum temperature for lipase production by R. rubra, C. utilis and C. guilliermondii was 30°C. No lipolytic activity was detected in case of C. guilliermondii after 4 or 6 days at 35°C. This difference in optimum temperature for lipase biosynthesis could be correlated with the optimum growth temperature for the different

yeasts. Phaff et al. (1966) reported that D. hansenii multiply at or close 0°C and have maximum growth temperature at 35°C, while C. utilis can grow only at minimum temperature of 5 - 10°C, Rose and Evison (1965). The production of enzymes are related to the internal biosynthetic processes inside the organism, these processes are quite complicated.

Previous investigations revealed that a fixed period of incubation could not be considered optimum for the production of an enzyme even by members of the same genus or species. Johnson and Snygg, (1974) claimed that lipase (s) production was attained after 2 - 7 days incubation by the four lipolytic microorganisms: Saccharomyces lipolytica, Micrococcus caseolyticus, Bacillus licheniformis and Staphylococcus sp. It is worthy to mention that the optimal temperature for the production of lipase (s) enzyme varied with different yeast strains. Thus, optimal incubation temperature of 33°C was recorded for Torulopsis ernobii (Yoshida et al. 1968), 30°C for Mycotorula lipolytica (Peters and Nelson, 1948), C. paralipolytica (Ota and Yamada (1966) and Saccharomyces lipolytica (Johnson and Snygg, 1974).

Effect of pH on lipase productivity:

Table (2) represents the levels of lipase activity at different pH ranges from 4 to 8 of the yeasts

under investigation. Maximum lipase (s) biosynthesis was attained at pH 4, followed by pH 7. Incubation time of 6 days at pH 8 was found inhibitory to lipase biosynthesis by all the yeasts under test though this pH was optimal for maximum enzyme activity (unpublished data). Lipolytic activity could not be detected also after 6 days incubation at pH 6 or 7 by both C. utilis and D. hansenii. Several reports indicate different optimum pH values for yeast lipase (s) optimum of pH 5 was recorded for Torulopsis ernobii (Yoshida et al. 1968), pH 5.8 for Torulopsis sp. (Dessouky, 1976), pH 9.5 for Saccharomycopsis lipolytica (Johnson and Snygg, 1974) and pH 4.5 for Mycotorula lipolytica (Peters and Nelson, 1948).

Effect of triglycerides on lipase (s) production:

The effect of different oils as inductive substrates on the biosynthesis of lipase (s) by the yeasts under study was investigated (Table 3). One noticed that lipase enzyme could be suggested to be constitutive in case of both ascosporogenous and anascosporogenous tested yeasts.

Corn oil induced lipolytic activity in all the tested yeasts. Olive oil retarded lipase productivity in case of C. guilliermondii and D. hansenii. Lipase productivity by D. hansenii was also retarded in presence of either coconut oil or cotton seed oil. Also C. guil

guilliermondii failed to produce lipase when grown on cotton seed oil throughout the tested period. Maximal induction of lipase (s) production by R. rubra and/or D. hansenii were obtained when cotton seed oil was used as a substrate. Both olive oil and cotton seed oil induced maximal lipase production in case of C. utilis, while C. guilliermondii produced maximum lipolytic activity only when grown on olive oil.

Table (4) represents lipase productivity by the different yeasts in presence of different concentrations of corn oil. Maximum lipase (s) activity was detected in presence of 0.2% corn oil by R. rubra, 0.6 - 1% by D. hansenii and C. guilliermondii and 0.6 - 0.8% by C. utilis. It has been already found that the preferential oil and its optimum concentration varied greatly with oil structure and type of microbe. Yoshida et al. (1968) reported that the maximum lipase production by Torulopsis ernobii was at 0.2 - 0.6% (w/v) olive oil while Dessouky, 1976 recorded 0.2 - 0.3% (w/v) corn oil for Torulopsis sp. The maximum production at 0.2 - 0.3% (w/v) of corn oil for Torulopsis sp.

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Table (1)

Effect of incubation temperature on lipase (s) Produced by C. utilis, S. roulliermondii, R. rubra and D. hansenii
 Data are expressed as Uf/ml (ug lipase/one ml cell free filtrates)

| Temp. (°C) | Organisms | | | | | | | | | | | |
|------------|------------------|-------|--------|--------------------------|-------|--------|-----------------|-------|--------|--------------------|-------|--------|
| | <u>C. utilis</u> | | | <u>S. roulliermondii</u> | | | <u>R. rubra</u> | | | <u>D. hansenii</u> | | |
| | 48 h. | 96 h. | 144 h. | 48 h. | 96 h. | 144 h. | 48 h. | 96 h. | 144 h. | 48 h. | 96 h. | 144 h. |
| 20 °C | 5.495 | 5.495 | 3.476 | 2.570 | 3.467 | 2.951 | 3.467 | 3.15 | 2.951 | 6.310 | 3.467 | 3.981 |
| 25 °C | 2.570 | 3.981 | 3.981 | 1.905 | 2.239 | 2.239 | 3.467 | 3.981 | 3.476 | 3.467 | 3.981 | 3.476 |
| 30 °C | 5.971 | 5.813 | 6.344 | 2.237 | 3.040 | 3.914 | 4.677 | 5.044 | 6.822 | 4.677 | 3.467 | 3.467 |
| 35 °C | 4.677 | 3.981 | 3.467 | --- | --- | --- | 4.677 | 3.467 | 2.951 | 3.467 | 2.570 | 2.239 |

Table (2)

Effect of pH on Lipase (a) produced by C. utilla, C. guilliermondii,
R. rubra and D. hansenii

Data are expressed as $\mu\text{p/ml}$ (μg Lipase/one ml cell free filtrates)

Organisms

| pH | <u>C. utilla</u> | | | <u>C. guilliermondii</u> | | | <u>R. rubra</u> | | | <u>D. hansenii</u> | | |
|----|------------------|-------|--------|--------------------------|-------|--------|-----------------|-------|--------|--------------------|--------|--------|
| | 48 h. | 96 h. | 144 h. | 48 h. | 96 h. | 144 h. | 48 h. | 96 h. | 144 h. | 48 h. | 96 h. | 144 h. |
| 4 | 7.586 | 7.586 | 7.586 | 6.310 | 7.586 | 7.586 | 6.310 | 7.730 | 6.310 | 7.586 | 10.990 | 6.310 |
| 5 | 2.951 | 3.467 | 3.467 | 7.604 | 3.467 | 4.677 | 2.570 | 3.467 | 4.677 | 5.495 | 3.467 | 3.467 |
| 6 | 5.495 | 3.981 | --- | 3.467 | 2.570 | --- | 3.981 | 6.310 | 2.951 | 3.981 | 4.677 | --- |
| 7 | 5.495 | 5.495 | --- | 7.586 | 4.677 | 2.570 | 7.604 | 7.604 | 7.604 | 3.981 | 4.677 | --- |
| 8 | 4.677 | 3.981 | --- | 6.310 | 3.467 | --- | 4.677 | 4.677 | --- | 4.677 | 3.981 | --- |

Table (3)

Effect of different triglycerides on Lipase (a) produced by C. utilis, G. stipitiformis, R. rubra and D. hansenii when the expressed as mg/ml (as lipase / one ml cell free filtrates)

| Triglyceride | C. utilis | | | G. stipitiformis | | | R. rubra | | | D. hansenii | | |
|-----------------|-----------|-------|--------|------------------|-------|--------|----------|-------|--------|-------------|-------|-------|
| | 48 h. | 96 h. | 144 h. | 48 h. | 96 h. | 144 h. | 48 h. | 96 h. | 144 h. | 48 h. | 96 h. | |
| Corn Oil | 5.971 | 5.813 | 5.344 | 2.237 | 3.040 | 3.167 | 4.677 | 5.044 | 6.882 | 4.877 | 3.467 | 3.981 |
| Coconut Oil | 5.495 | 7.604 | 7.604 | 7.506 | 7.586 | 3.901 | 2.570 | 3.467 | 4.677 | --- | 3.981 | 3.981 |
| Cotton seed Oil | 7.604 | 7.604 | 7.604 | --- | --- | --- | 4.677 | 5.495 | 5.129 | --- | 2.570 | 7.604 |
| olive Oil | 7.586 | 7.604 | 7.962 | --- | --- | 3.981 | 3.467 | 3.981 | 4.677 | --- | 2.951 | 3.467 |
| NO | 7.604 | 7.962 | 8.730 | 2.239 | 2.570 | 2.570 | 3.981 | 6.110 | 6.310 | 2.570 | 5.495 | 5.495 |

Organisms

Table (4)

Effect of corn oil concentration on lipase (a) produced by S. utilla, C. zwittermora, R. rubra and D. hansenii
 Data are expressed as μ/ml (40 ml/one ml cell free filtrates)

| Oil concn % | Organisms | | | | | | | |
|-------------|------------------|--------|-----------------------|--------|-----------------|--------|--------------------|--------|
| | <u>S. utilla</u> | | <u>C. zwittermora</u> | | <u>R. rubra</u> | | <u>D. hansenii</u> | |
| | 96 h. | 144 h. | 96 h. | 144 h. | 96 h. | 144 h. | 96 h. | 144 h. |
| 0.2 | 4.677 | 3.981 | 3.467 | 3.981 | 5.495 | 7.604 | 2.570 | 3.467 |
| 0.4 | 4.677 | 3.981 | 2.570 | 3.981 | 3.467 | 7.604 | 2.951 | 3.981 |
| 0.6 | 7.566 | 3.981 | 3.981 | 3.981 | 3.467 | 3.981 | 3.467 | 3.981 |
| 0.8 | 7.604 | 3.981 | 3.981 | 3.981 | 3.467 | 3.981 | 3.467 | 3.981 |
| 1.0 | 5.003 | 6.344 | 3.040 | 3.981 | 5.044 | 6.822 | 3.467 | 3.981 |

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

انتاج انزيم الليبوز بعوض الخافض
١- تأثير درجة الحرارة - الرقم الايدروجيني والجلسيدات

سومى محمد أحمد الجبال و منى اسحق ريف
قسم النبات - كلية النبات - جامعة عين شمس

هدت محاولة لتقدير النفاط الليبوزى لى ثلاثة خمائر غير متكيفة وهى
رودوثورولا زهرا ، كانديدا يوتلس و كانديدا جويليروموندى واحسيد
انواع الخمائر المتكيفة وهى دياروسيس هاتسنى .

وقد انتج انزيم الليبوز تحت ثوات مختلفة عنها فترات التحضين
الرقم الايدروجينى ، ودرجات الحرارة وكذلك انواع مختلفة
الجلسيدات .

وقد اهدت رودوثورولا زهرا اهدى نفاط انزيم بعد لفترة تحضين
مئة ايام عند ٢٠م على بيئة خالية من السكر وتحتوى على زيت المينوره
عدد رقم ايدروجينى ٠٤ . وقد اهدت كل من كانديدا يوتلس و كانديدا
جويليروموندى اهدى نفاط انزيم عند نفس الظروف السابقة ولكن لى
وزن زيت الذره بالنسبة للكائن الاول وزيت جوز الهند للثانى . زيت
الذره احسن على انتاج الانزيم لكل من رودوثورولا زهرا ، دياروسيس
هاتسنى ، كانديدا يوتلس و كانديدا جويليروموندى عند تركيبات
٢ ، ٦ ، ٨ ، ١٠ % بالترتيب .

ولكن بالنسبة للخيمرة المتكيفة التى نهدت على نفس البيئة اهدت
احسن نفاط انزيم عند ٢٠م بعد ٤٨ ساعة نمو .