SOME FACTORS CONTROLLING LIPASE ACTIVITY IN CERTAIN MICROORGANISMS

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Abstract

Screening of twenty four bacterial organisms for their lipase activity revealed that they have different enzymatic potentialities. *Bacillus subtilis* (12) was the most active strain. Fifteen fungal strains and seven yeasts were also tested for lipase activity. *Aspergillus oryzae* and *Saccharomyces cervisiae* (4) were found in the greatest order for fungal and yeast strains. A 9.0%, 0.1% and 0.2 M of acacia gum, sodium desoxychlorate and Ca^{+2}, respectively seemed to be optimal activators for lipase activity. The highest levels of *Pseudomonas aeruginosa, Cellulomonas* sp. (9), *Saccharomyces cerevisiae* (4) and *Aspergillus oryzae* were achieved at pH 7.5, while pH 8.5 exhibited similar effects on *Bacillus subtilis* (12) and *Aspergillus flavus* (2) as regards to lipase activity. The highest enzyme activity was recorded at 40°C for *Pseudomonas aerugionsa, Cellulomonas sp.* (9), *Saccharomyces cerevisiae* (4) and *Aspergillus oryzae*. Whilst, 35°C showed similar activity for *Bacillus subtilis* (12) and *Aspergillus flavus* (2). Olive oil was the favourable substrate among oils studied for lipase activity in all microorganisms.

INTRODUCTION

A number of microorganisms were found to be lipase producers. Lipases produced by the majority of them are extracellular. Microbial lipases have been studied much less than other lipases, despite their usefulness as reagents or food additives (flavor-modifying enzymes). Lipase production is known to vary among different species (Sugiura *et al.*, 1977; Watanabe *et al.*, 1977; Horiuti & Imamura, 1978; Chander *et al.*, 1979a and Kennedy & Lennarz, 1979). Microbial lipases are responsible for degrading fat by hydrolysis and the products thus formed contributed to the development of desirable flavors in food products. Therefore, attention to the more

general aspects of lipase production and activity by microorganisms have been devoted. The present work describes the results of comparative study among many microbial lipase potentialities. Also the study deals with the effect of various factors on lipase activity of selected organisms.

MATERIALS AND METHODS

Microorganisms:

Bacillus subtilis (1), *Bacillus polymyxa, Aspergillus niger, Trichoderma viride* (1), *Fusarium monipiforme* and *Rhizoctonia solani* were obtaied from Microbiological Resource Center Cairo Mircen (CAIM), University of Ain Shams, Cairo. *Arthrobacter simplex, Bacillus circulans, Bacillus licheniformis, Cellulomonas* sp. (5), *Cellulomonas sp.* (9), *Bacillus megaterium* (66, 10, 13, 14, 15 and 19), *bacillus subtilis* (8, 9, 11, 12, 16, 17, 18 and 20), *Streptococcus tchermophilus, Penicillium digitatum, Aspergillus terreus, Aspergillus oryzae, Kluyveromyces lactis, K.fragilis* and *Candida tropicalis* were obtained from Microbiology Department, Fac. of Agric., Mansoura Univ., *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* were obtained from Microbiology Dept., Fac. of Pharmacy, Mansoura Univ., *Aspergillus fumigatus* (2), *Trichoderma viride* (2), *Aspergillus flavus* (3), Myceliophthora ferqusii, *Aspergillus flavus* (1) and *Aspergillus fumigatus* (1) were obtained from Botany Dept., Fac. of Science, Mansoura Univ. *Aspergillus flavus* (2) and *Hermodenderum clodesporiodes* were obtained from Food Science Dept., Fac. of Agric., Mansoura Univ. *Saccharomyces cerevisiae* (1, 2, 3 and 4) were obtained from National Research Center, Dokki, Cairo.

Media used:

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%, pH 6.0, distilled water, olive oil 1.0%. This medium was used for growing bacterial organisms and lipase production.

Chander *et al.* (1980) Basal medium:

It contains, pepton 2.0%, yeast extract 0.5%, Nacl 0.5%, dextrose 1.0%, pH 6.0 and distilled water and this medium was used for growing yeasts, fungi and lipase production.

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 daxtrose (glucose), 15.0 agar, 1000 ml distilled water. The pH was adjusted at 6.5. This is a favourable medium for sporulation and maintenance of fungal organisms.

Concalves and Castillo (1981) Basal medium:

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

Preparation of fungal spore suspension:

Spores appeared on PDA slants were scrapped by using 5 ml sterilized distilled water and dispensed in 50 ml sterilized distilled water containing 8.0g NaCl/ liter (Gewaily, 1977).

Production of lipase:

Cells of bacteria and yeasts as well as spores of fungi (molds) were grown on the appropriate media. After incubation time, depending on the testing factor examined, the contents of the flasks were filtrated and centrifuged at 8000 rpm for 15 min. Culture fluids were used as a source of lipase enzyme.

Culture procedure:
Bacterial experiments:

Agar slants were inoculated with organisms, each in duplicate, and after 72 hr. incubation at 30°C, fairly good growths were obtained. The salnts were stored in refrigerator at 5°C or used directly as inocula (or working cultures) for inoculation batch fermentation flasks. 50 ml portions of the fermentation medium, i.e. basal mineral salt solution of Sugiura *et al.* (1977), were dispensed in 250 ml conical flasks, sterilized at 121°C for 15 min. 5 ml inoculum was added to each flask. For preparing inocula, the growth on agar slants was scraped using 5 ml sterile tap water and dispensed in a flask containing 50 ml of the above medium. These cell suspensions were considered as suitable inocula. After incubation at 30°C for 5 days. The flasks were centrifngeded at 8000 rpm for 15 min. The lipase activity was estimated in culture fluids in three replicates.

Yeasts screening:

According to the previous technique on the screening of bacteria, different strains of yeasts were grown on Chander *et al.* (1980) basal medium solution for 5 days at 30°C for both growth and lipase production. After the incubation period, the flasks were centrifuged at 8000 rpm for 15 min. The culture fluid was used as the

source of enzyme. Lipase activity was determined by the method of Oi *et al.* (1969).

Fungal screening:

50 ml portions of the fermentation medium, i.e. basal mineral salt solution of Chander *et al.* (1980) were dispensed in 250 ml conical flasks, sterilized, then inoculated with 5 ml spore suspension per flask. After incubation at 30°C for 5 days, the medium was filtered then centrifuged at 8000 rpm for 15 min. Culture fluid was used as the source of lipase.

Lipase assay:

Lipase activity was determined by the method of Oi *et al.* (1969) with some modifications. The reaction mixture contained 5.0 ml of 5% olive oil emulsion in 7.0% acacia gum, 5.0 ml of 0.2 M tris-HCl buffer (pH 7.5), 2.0 ml 0.2 M $CaCl_2$, 1.0 ml enzyme solution and 2.0 ml of distilled water. After the incubation period under the assay condition, the total amount of liberated fatty acids was titrated against N/100 NaOH. The blank used was an assay mixture containing boiled enzyme.

RESULTS AND DISCUSSION

Screening of organisms for their lipase activity:

Data on the comparative lipase activity through a standard experiment given in Table (1). The screening of the twenty four bacterial strains for their lipase potentiality revealed that *Bacillus subtilis* (11and 12) are the most active lipase procedures. Their lipase activity was of the same level (3.8 µmol free fatty acids (FFA) / ml/min). *Bacillus polymyxa* and *Bacillus megaterium* (14 and 15) were in the second order of lipase production.

With respect to yeasts and fungal organisms, *Saccharomyces cervisiae* (4) and *Aspergillus oryzae* showed the highest lipase activity. The enzyme activities recorded was 3.2 and 2.6 µmol FFA/ml / min. for the above organisms, respectively.

It is also to be concluded from the data presented in Table (1) that bacteria, in general, proved to be active as lipase producing organisms.

In second experiment, *Bacillus subtilis* (12), *Pseudomonas aeruginosa* and *Cellulomonas sp.* (9) were used as bacterial strains for studying factors controlling lipase activity. Also, *Saccharomyces cerevisiae* (4), *Aspergillus oryzae* and *Aspergillus flavus* (2) were selected as yeast and fungal organisms in further experiment.

Table 1. Lipase activities by various microorganisms (enzyme activity expressed as µmole FFA/ml/min.).

Serial No.	Organisms	Lipase activity
1	*Arthrobacter simple*	2.4
2	*Cellulomonas sp.* (5)	2.5
3	*Cellulomonas sp.* (9)	2.6
4	*Pseudomonas fluorescens*	2.0
5	*Pseudomonas aeruginosa*	2.6
6	*Bacillus subtilis* (1)	2.0
7	*Bacillus subtilis* (8)	2.0
8	*Bacillus subtilis* (9)	2.0
9	*Bacillus subtilis* (11)	3.8
10	*Bacillus subtilis* (12)	3.8
11	*Bacillus subtilis* (16)	2.6
12	*Bacillus subtilis* (17)	2.6
13	*Bacillus subtilis* (18)	2.4
14	*Bacillus subtilis* (20)	2.4
15	*Bacillus licheniformes*	2.9
16	*Bacillus circulans*	2.0
17	*Bacillus polymyxa*	3.6
18	*Bacillus megaterium* (66)	2.0
19	*Bacillus megaterium* (10)	2.0
20	*Bacillus megaterium* (13)	2.8
21	*Bacillus megaterium* (14)	3.0
22	*Bacillus megaterium* (15)	3.0
23	*Streptococcus thermophilus*	2.8
24	*Bacillus megaterium* (19)	2.4
25	*Candida tropicalis*	2.0
26	*Saccharomyces cerevisiae* (1)	2.2
27	*Saccharomyces cerevisiae* (2)	1.8
28	*Saccharomyces cerevisiae* (3)	1.8
29	*Saccharomyces cerevisiae* (4)	3.2
30	*Kluyveromyces lactis*	2.0
31	*Kluyveromyces fragilis*	2.0
32	*Trichoderma viride* (1)	1.8
33	*Fusarium monipiforme*	2.0
34	*Rhizoctonia solani*	2.0
35	*Myceliophthora ferqusii*	2.0
36	*Penicillium digitatum*	1.4
37	*Trichoderma viride* (2)	1.2
38	*Aspergillus oryzae*	2.6
39	*Aspergillus terreus*	2.0
40	*Aspergillus flavus*	2.0
41	*Aspergillus fumigatus* (1)	2.0
42	*Aspergillus niger*	1.6
43	*Aspergillus fumigatus* (2)	1.5
44	*Aspergillus colodesporioides*	1.4
45	*Aspergillus flavus* (2)	2.1
46	*Aspergillus flavus* (3)	1.7

Effect of Acacia gum concentration:

The results on the effects of acacia gum concentrations are shown in Table (2). It is clear that acacia gum concentration greatly affected the lipase activity of culture fluids of the tested strains. The maximum lipase activity for all strains was reached at 9.0% acacia gum concentration. Better results are necessary for making the lipase substrates in the suitable interface system for lipase action. The results obtained are in accordance with those of Huka *et al.* (1991).

Table 2. Effect of acacua gum concentration on lipase activity. (enzyme activity expressed as µmole FFA/Ml/min.).

Organisms / Gum acacia conc.	*P. aerug-inosa*	*B. subtilis* (12)	*Cellulomonas sp.* (9)	*S. cerevisiae*	*A. Oryzae*	*A. flavus*
1%	0.0	0.4	1.1	0.4	0.5	0.8
3%	1.0	1.3	1.2	0.8	1.0	1.4
5%	1.8	1.8	1.6	2.0	1.6	1.6
7%	2.8	3.8	2.6	3.2	2.6	2.1
9%	4.4	4.0	3.0	3.2	3.0	2.2
11 %	3.0	2.0	1.5	1.8	0.9	0.7

Effect of bile salts and tweens:

Of the stabilizers tested, i.e. bile salts and tweens, only sodium desoycholate at 0.1% increased lipase activity for all tested organisms (Table 3). Other stabilizers decreased the lipase activity when added at varying concentrations. Also, enzyme activity was not increased when increasing of sodium desoxycholate concentration more than 0.1%. These findings are in agreement with those obtained by Adams and Brawley (1981) and Hauka *et al.* (1991).

Effect of different metal ions:

Among the metal ions tested (Table 4), Ca^{+2} was the best metal activator of the lipase activity for all tested strains. In general, all metal ions, i.e. Mg^{+2}, Na^{+}, K^{+}, Fe^{+3}, $Hg^{+2}$and Sn^{+2} were found as lipase activators for the tested organisms. The degree of activation was different according to the type of metal ion and the source of enzyme. Therefore, the lipase could be considered as a metalloenzyme or metal activated enzyme (Jensen and Pitas, 1976).

Table 3. Effect of stabilizers on lipase activity. (enzyme activity expressed as µmole FFA/ml/min.).

Organisms	Control	Bile salts (%)								Tweens										
		Sodium desoxycholate (%)					Sodium tauroglycholate (%)			Tween 80			Tween 60				Tween 40			
		0.05	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.5	1.0	2.0	0.5	1.0	1.5	2.0	0.5	1.0	1.5	1.5
Pseudomonas aeruginosa	4.4	4.5	4.8	4.0	3.8	2.0	2.0	1.2	1.2	3.4	3.2	1.8	2.9	3.2	4.0	2.4	1.2	2.6	3.8	3.0
Bacillus subtilis (12)	4.0	4.2	4.4	4.0	3.0	2.2	2.2	2.0	1.8	3.0	2.8	2.0	2.6	2.8	3.6	2.0	1.8	2.2	3.2	2.6
Cellulomonas sp. (9)	3.0	3.8	4.4	4.4	3.2	1.6	2.8	1.5	1.0	2.5	2.5	2.4	2.0	2.1	2.4	1.8	1.4	1.8	3.0	2.2
Saccharomyces cerevisiae	3.2	3.3	3.6	3.2	3.0	2.2	2.2	2.0	1.6	2.6	2.6	2.2	2.3	2.5	2.8	1.6	1.5	2.4	2.6	2.0
Aspergillus oryzae	3.0	3.1	3.3	3.0	2.0	1.8	2.2	1.4	1.2	3.0	1.9	1.6	1.8	2.4	2.6	1.4	1.6	2.0	2.6	2.0
Aspergillus flavus (2)	2.2	2.4	2.8	2.0	1.6	1.0	2.0	1.7	1.1	1.9	1.5	0.9	1.8	2.2	2.6	1.0	1.4	1.8	2.4	1.6

Effect of $CaCl_2$ concentration:

The enhancing of lipase activity by Ca^{+2} ions may be attributed to: 1) Ca^{+2} accelerates the hydrolysis of triglycerides by unpurified preparations of microbial lipases (Shipe, 1951). 2) Ca^{+2} inhibits the resynthesis of ester linkages, which would effectively shift the reaction in the direction of hydrolysis. 3) stabilization of the enzyme protein by Ca^{+2} ions, Wills (1960). and/or 4) removal of free fatty acids from the reaction system by the formation of Ca^{+2} salts and thereby acceleration of hydrolysis in accordance to the mass at the substrate-water interface by removing the free fatty acids or glycerides which cover the surface of the triglycerides emulsion (Liu *et al.*, 1973). The present results are also in line with those obtained by Chander *et al.* (1979b), Muderhwa *et al.* (1985) and Hauke *et al.* (1991).

The data in Table (5) show that the highest lipase activities (4.8, 4.4, and 4.4 µmol FAA/ml/min) were reached at 0.2 M $CaCl_2$ for *Pseudomonas aeruginosa, Bacillus subtillis* (12) and *Cellulomonas sp.* (9), respectively. Also, yeast strains and fungal strains show that similar high lipase activity at 0.2 M Ca^{+2}. In general, Ca^{+2} ions were shown as metallo-ion activators for lipase activity. 0.2M Ca^{+2} was proved to be favourable for lipase action for both bacteria and fungi. Increasing Ca^{+2} ion concentration showed inhibitory effect on lipolysis. Hauka *et al.* (1991) found that the best concentration for *Aspergillus fumigatus* lipase activity was 10^{-3} M of Ca^{+2}.

Optimum pH of lipase activity:

The influence of pH on lipase activity was studied and the results obtained are shown in Table (6). It could be noticed that the pH greatly affected the enzyme activity in all crude enzyme preparations. The optimum pH of the enzymes from *Pseudomonas aeruginosa, Cellulomonas sp.* (9), *Saccharomyces cerevisiae* (4) and *Aspergillus oryzae* through the hydrolysis of oil were found to be 7.5 But, the optimum pH for *Bacillus subtilis* (12) lipase and for *Aspergillus flavus* (2) lipase appeared to be 8.5. However, the range of pH 7.5-8.5 seens almost suitable for enzyme activity. At pH 5.5, for all test organisms, little activity or no lipase activity was found. Also, the results showed a good activity of the enzyme in alkaline conditions, but not in acid conditions. These results are in conformity with those obtained by Razak *et al.* (1983), Khor *et al.* (1986) and Hauka *et al.* (1991).

Optimum temperature of lipase activity:

Datermination of the optimum temperature for lipase activity is shown in

Table 4. Effect of different metal ions (at 10^2 M concentration) on lipase activity. (enzyme activity expressed as µmole FFA/ml/min.).

Organisms / Metal ions	*P. aerug-inosa*	*B. subtilis* (12)	*Cellulomonas sp.* (9)	*S. cerevisiae*	*A. Oryzae*	*A. flavus*
0.00	2.4	2.2	1.2	1.4	1.0	0.8
$CaCl_2$	4.8	4.4	4.4	3.6	3.3	2.8
$MgCl_2$	4.0	3.0	2.6	1.8	2.5	2.4
NaCl	2.4	3.0	2.0	2.2	1.8	1.4
KCl	3.0	2.0	1.4	1.2	2.0	2.0
$FeCl_3$	3.2	2.2	2.0	2.4	2.4	2.0
$HgCl_2$ (10^{-4})	3.0	3.8	1.2	3.6	3.0	1.8
HgCl	2.4	2.8	2.7	3.4	2.8	2.6
$SnCl_2$	3.0	3.0	3.4	3.5	3.0	2.0

Table 5. Effect of Ca^{+2} concentration on lipase activity. (enzyme activity expressed as µmole FFA/ml/min.).

Organisms / Ca^{+2} conc.	*P. aerug-inosa*	*B. subtilis* (12)	*Cellulomonas sp.* (9)	*S. cerevisiae*	*A. Oryzae*	*A. flavus*
0.0 M	2.4	2.2	1.2	1.4	1.0	0.8
0.1 M	3.8	4.0	4.0	3.4	3.0	1.8
0.2 M	4.8	4.4	4.4	3.6	3.3	2.8
0.3 M	3.7	3.0	3.6	3.4	2.4	2.3
0.4 M	3.0	2.5	1.8	2.6	2.4	2.1
0.5 M	1.8	1.9	1.5	1.5	1.5	1.35

Table 6. Effect of pH on lipase activity. (enzyme activity expressed as µmole FFA/ml/min.).

Organisms / pH	*P. aerug-inosa*	*B. subtilis* (12)	*Cellulomonas sp.* (9)	*S. cerevisiae*	*A. Oryzae*	*A. flavus*
5.5	0.0	0.4	0.0	0.2	0.2	0.4
6.5	0.3	0.7	0.4	1.0	1.4	0.7
7.5	4.8	4.0	4.4	3.6	3.3	2.8
8.5	4.0	4.2	4.0	3.5	3.2	3.4
9.5	2.0	1.0	1.4	2.0	1.4	1.8

Table 7. Effect of incubation temperature on lipase activity. (enzyme activity expressed as µmole FFA/ml/min.).

Organisms / °C	*P. aerug-inosa*	*B. subtilis* (12)	*Cellulomonas sp.* (9)	*S. cerevisiae*	*A. Oryzae*	*A. flavus*
25	0.5	0.8	0.6	0.3	0.4	0.5
30	0.8	1.2	1.4	1.0	1.2	1.0
35	4.2	4.6	3.8	3.3	2.8	3.6
40	4.8	4.2	4.4	3.6	3.3	3.4
45	4.4	4.0	4.2	3.1	2.9	2.8
50	3.6	3.6	2.8	1.8	2.2	1.6

Table (7). The results obtained show clearly that 40°C favoured the highest activity of enzyme prepared by *Pseudomonas aeruginosa, Cellulomonas sp.* (9), *Saccharomyces cerevisiae* (4), and *Aspergillus oryzae.* The optimum incubation temperature, required to get highest lipase activity for *Bacillus subtilis* (12) and *Aspergillus flavus* (2) was found to be 35°C. These results are in agreement with results obtained by Adams and Brawley (1981), Khor *et al.* (1986) and Hauka *et al.* (1991).

Substrate hydrolysis by microbial lipases:

The substrate specificity for lipases of different microorganisms is presented in Table (8). It could be seen that olive oil exhibited considerably greater lipase activity for all microbial strains than that by other oils. Moreover, lipases were active on all substrates and readily hydrolyzed ones, but, the degree of hydrolysis is different according to the type of substrate. These findings are in agreement to those obtained by Fukumoto *et al.* (1964).

Table 8. Effect of different oils as substrates on lipase activity. (enzyme activity expressed as μmole FFA/ml/min.).

Organisms / Oils	*P. aeruginosa*	*B. subtilis* (12)	*Cellulomonas sp.* (9)	*S. cerevisiae*	*A. Oryzae*	*A. flavus*
Olive oil	4.8	4.6	4.4	3.6	3.3	3.6
Cotton seed oil	2.4	2.4	2.4	2.8	3.1	2.0
Corn oil	2.4	2.5	3.6	3.6	3.2	1.8
Soybean oil	2.0	2.6	2.8	2.8	2.6	2.0
Caster oil	2.2	2.0	2.8	2.4	2.8	1.5
Coconut oil	2.6	3.6	2.0	2.0	3.0	2.4
Sunflower oil	2.6	1.6	1.5	2.0	2.6	1.6
Stearin	1.6	3.8	3.4	2.6	3.2	1.8
Olein	1.2	2.0	1.9	3.0	2.8	2.0
Butter oil	2.0	2.2	1.7	2.2	2.9	2.6

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الظروف المثلى التى تحكم نشاط الليبيز من بعض الكائنات الحية الدقيقة

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لاقت الكائنات الحية الدقيقة إهتماما كبيراً لإنتاج إنزيم الليبيز منها حديثاً نظراً لأهميتها فى مجالات متعددة ولذلك إتجهت الدراسة فى هذا البحث نحو إنتاج ودراسة خواص الليبيزات البكتيرية مقارنة بليبيزات الفطريات والخمائر وقد إستهدف هذا البحث التوصل إلى أفضل السلالات المنتجة لليبيز من بين ٢٤ سلالة بكتيرية و ١٥ سلالة فطر و ٧ خمائر وقد وجد أن بكتريا *Cellulomonas sp.* (9), *Pseudomonas aeruginosa, Bacillus subtilis* (12) هى أغضل البكتريا وان *Saccharomyces cerevisiae, A. flavus* (2), *A. oryzae* هى أفضل الفطريات والخمائر المختبرة إنتاجا للإنزيم. وقد إستخدمت هذه الكائنات العالية الإنتاج فى التجارب. وجد أن تركيز ٩٪ من صمغ الاكسيا يكون مستحلب جيد يعطى أعلى نشاط للإنزيم، وقد أظهر تركيز ٠,١٪ من ملح الصفراء (ديزوكسى كولات الصوديوم) زيادة ملحوظة فى نشاط الإنزيم ومن بين الأيونات المعدنية المختبرة وجد أن الكالسيوم أفضلها كمنشط للإنزيم عند تركيز ٠,٢ مولر ، وقد وجد أن درجة pH ٧,٥ ودرجة الحرارة ٤٠ ٥م تعطى أعلى نشاط للإنزيم من البكتريا والفطريات باستثناء *Aspergillus flavus*, *Bacillus subtilis* (12) الذى أعطى أعلى نشاط عند pH ٨,٥ وحرارة ٣٥ ٥ م ، وجد أن زيت الزيتون هو أفضل مادة تفاعل للإنزيم من بين مواد التفاعل المختلفة المستخدمة والذى إستطاع الإنزيم تحليلها جميعاً.