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STUDIES ON THE INDUCTION OF LIPASE(S) BY THERMOALKALOPHILIC *BACILLUS bREVIS* B_2

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Abstract

Bacillus brevis-B_2 was selected as the most patent lipase(s) producer among nine thermoalkalophilic bacterial isolates isolated from agro industrial wastes at 70ºC and pH 10.5. Of the tested nutrient medium, tap water containing 1% fish wastes and 1% yeast extract is the best inducer for lipase synthesis by *B. brevis* B_2. Maximum enzyme biosynthesis could be obtained after 24 hr of incubation. The optimal pH and temperature for enzyme formation are 11.5 and 70ºC. Enzyme yield increased in presence of sucrose or lactose in the nutrient medium, also the presence of 100 ppm zinc sulphate and 500 ppm folic acid supported high induction of lipase by *B. brevis* growing under shaking condition and inocula size of 2ml/100ml culture medium. A very slight increase in enzyme formation occur under exposure of bacterial isolate to 1 KGy of γ-irradiation.

Key words: Thermoalkalophilic bacteria, Lipase, Physiology.

Introduction

In a world of diminishing resources and increasing needs, every opportunity for recycling waste materials must be sought (Haddadin *et al*., 2009). Lipase(s) (triacylglycerol acylhydrolase, E.C. 3.1.1.3) constitutes a group of enzymes that catalyze the hydrolysis of triglycerides at the lipid-water interface. Lipase have become an integral part of the modern food industry and a large number of lipases are produced on an industrial scale for flavor development in dairy products, processing of foods such as meat, and in vegetables, fruits, baked goods and beer (Aravindan and Viruthagiri, 2007). Microbial lipases have received particular attention due to their diverse properties, relative ease of preparation and broad substrate specificity, including chain-length selectivity, position selectivity and stereoselectivity (Jantaporn and Chuenchit, 2007). In the last few years, there has been an increasing interest in the use of enzymes for the biosynthesis of secondary metabolites using organic media (Gargouri *et al*., 2002; Castillo *et al*., 2003).

Recently, a number of thermophilic bacteria producing thermoactive lipases and esterases have been purified and characterized (Lee *et al*., 1999; Dharmsthiti and Luchai, 1999; Markossian *et al*., 2000; Immamura and Kitaura 2000). Thermophilic lipases exhibit higher thermostability, higher activity at elevated temperatures and often more resistance to chemical denaturation, making them ideal tools in industrial and chemical processes where relatively high reaction temperatures and/or organic solvents are used. As each industrial application may require specific properties of the biocatalysts, there is still an interest in finding new lipases that could create novel applications.

The present study deals with isolation of thermoalkalophilic lipase forming bacterial isolates from agro-industrial wastes and to study the formation of lipase by *B. brevis* B_2 the most potent bacterial isolates under different physiological conditions.

Materials And Methods

1) Isolation media:

a. Isolation medium (Lima *et al*., 2003). It contains the following composition (g/l): Yeast extract 5 and olive oil 10. The mineral salt solution 10 ml contained (g/l): KNO_3 2.0; $MgSO_4.7H_2O$ 0.5, K_2HPO_4 1.0, $ZnSO_4$ 0.44, $FeSO_4.7H_2O$ 1.1 and $MnSO_4.7H_2O$ 0.2 and distilled water 1L, pH was adjusted at 10.5. This is a selective medium for isolation and also used for production and symbolized medium C.

b. Czapek-Dox's agar medium Dox (1910): Czapek-Dox's agar medium contains the following (g/l): $NaNO_3$ 3, K_2HPO_4 1, KCl 0.5, $MgSO_4.7H_2O$ 0.5, $FeSO_4.7H_2O$ 0.01, sucrose 30, agar 20, and distilled water 1L, pH was adjusted at 9.5.

c. Nutrient agar medium (Shiriling and Gottlieb, 1966): The medium contains peptone 5, beef extract 3, NaCl 5 and agar 20. pH was adjusted at 9.5. The nutrient medium used for lipase formation and symbolized medium N.

The previous media were supplemented with 10 ml tributyrin grinding with 10ml Arabic Gum to obtain a selective medium for isolating lipolytic microbial isolates.

d. Preparation of enviro-agar industrial wastes: Agar-industrial wastes (sun flower, cotton seeds, soyabean, olive seed and linseed wastes) and environmental

wastes (Fish and pulletery wastes) were dried at 70ºC for 48 hours, grinded and used as substrate for isolation of lipolytic microorganisms and lipase(s) formation.

2) Production medium:

a. Production medium according to (Aisaka and Terada 1980). It contains the following composition (g/100ml): Sun flower oil 1.5, Peptone 0.5, Glucose 1, KH_2PO_4 0.25, KCl 0.05 and $MgSO_4.7H_2O$ 0.05. pH was adjusted at 9.5. This medium symbolized medium A.

b. Production medium according to (Chen *et al*., 1998): It contains the following ingredients (g/l): Tryptone 10, yeast extract 5, NaCl 10 and NH_4Cl 1. pH was adjusted at 9.5. This medium symbolized medium D.

c. PY medium according to (Lee *et al*., 2003): PY medium has the following composition (g/100 ml): K_2HPO_4 0.2, KH_2PO_4 2, poly-peptone 4, beef extract 0.5, yeast extract 0.5, glucose 2, $MgSO_4.7H_2O$ 0.01, $ZnSO_4.7H_2O$ 0.0001, $MnSO_4.7H_2O$ 0.0001 and $FeSO_4.7H_2O$ 0.001. pH was adjusted at 9.5. This medium symbolized medium E.

3) Isolation of thermoalkalophilic bacterial isolates:

The thermoalkalophilic bacterial isolates were isolated from the seven wastes previously mentioned as follows: Ten grams of sandy and/or clay soil mixed with 10g of different wastes and incubated for 10 days at 70ºC and pH 10.5. Then 1 ml from each sample was dissolved in 50 ml nutrient liquid medium, incubated for 2 days at the same pH and temperature. Fifty µl from each sample were used to inoculate on medium C according to Lima *et al*., (2003), Czapeks Dox's agar medium and nutrient agar medium and incubated for 1, 2, 3, 4 and 5 days at pH 10.5 and 70ºC.

The purification of thermoalkalophilic bacterial isolates was carried out according to the agar streak technique (Collins and Lyne, 1985).

4) Identification of the most potent lipase producing bacterial isolates:

The bacterial isolates were subjected to screening in relation to lipase formation and screening again in relation to the formation of lipase with different wastes as a source of carbon. Then the most potant lipolytic bacterial isolates were subjected to

characterization using the keys of Hensyl (1994). All experimental study was carried out according to Collins and Lyne (1985). The morphological, physiological and biochemical characteristic of bacterial sample were carried out in Fermentation Biotechnology and Applied Microbiology Center (Al-Azhar University, Cairo, Egypt).

5) Enzyme assay:

1. **Clear zone method:** According to Cardenas *et al*., (2001), lipase activity on agar plate is frequently conducted by using tributyrin as substrate. Clear zone around the colonies indicate formation of lipase(s).

2. **Titrimetric method:** Lipase activity was assayed by alkali titration using using olive oil as substrate as described by Nahas (1988). The reaction mixture contained: 3.5 ml olive oil, 3.5 ml phosphate buffer at pH 8.0, 0.1M and 2 ml crude enzyme. One unit of enzyme activity is defined as the amount of enzyme which catalyze the release of 1 µ mol free fatty acids in 30 min at 60ºC.

3. **Colorimetric method:** Lipase activity was assayed quantitatively by using p-nitrophenyl palmitate (PNPP) as substrate according to Winkler and Stuckmann, (1979). The reaction mixture contained: 2.4 ml substrate solution freshly prepared and 0.1 ml enzyme solution.

   Substrate solution contained 30 mg of p-nitrophenyl palmitate in 10 ml isopropanol; 207 mg sodium deoxycholate and 100 mg Arabic gum in 90 ml phosphate buffer pH 8, 0.05M.

   One unit of enzyme activity is defined as the amount of enzyme which catalyze the release of one mol. of p-nitrophenol in 30 min at 60ºC.

6) Parameters controlling lipase activity by the most potent bacterial isolate:

The effect of different parameters on lipase(s) formation were studied as follows: incubation period, pH, incubation temperature, fish wastes concentrations, inoculum size, incubation condition, carbon source, nitrogen source, yeast extract concentrations, surfactants, metallic ions, vitamins requirement and irradiation doses.

Results And Discussion

Isolation of thermoalkalophilic bacteria from agar-industrial wastes:

Data cited in table (1) reveals that nine thermoalkalophilic bacterial isolates were isolated from different wastes; three isolates from clay soil with sunflower oil wastes, two isolates from clay soil, one isolate from each of clay soil with cotton seed oil wastes, sandy soil, clay soil with soyabean oil wastes and sandy soil with cotton seed oil wastes.

Table (1): Isolation of the thermoalkalophilic bacteria from agro-industrial wastes.

Bacterial isolates (code no.)	Types of wastes	Isolatation temperatures (^{o}C)
B_1	Fermented clay soil with sun flower oil wastes.	70
B_2	Clay soil.	70
B_3	Clay soil.	70
B_4	Fermented clay soil with cotton seed oil wastes.	70
B_5	Sandy soil.	70
B_6	Fermented clay soil with soyabean oil wastes.	70
B_7	Fermented sandy soil with cotton seed oil wastes.	70
B_8	Fermented clay soil with sun flower soil wastes.	50
B_9	Fermented clay soil with sun flower oil wastes.	50

Screening of thermoalkalophilic lipase forming bacterial isolates:

Maximum enzyme formation could be attained by bacterial isolates B_1, B_2 and B_3, followed by bacterial isolate B_4 while bacterial isolate B_5 could form weak level of enzyme (Table 2). No enzyme activity was recorded by the other bacterial isolates.

Table (2): Screening of thermoalkalophilic lipase forming bacterial isolates.

Bacterial isolates (code no.)	Lipase activity	Temperature	
		30°C	70°C
B_1	+ + + -		+
B_2	+ + + -		+
B_3	+ + + -		+
B_4	+ + -		+
B_5	+ -		+
B_6	- -		+
B_7	- +		+
B_8	- +		-
B_9	- +		-

(Enzyme activity is determined by clearing zoon technique CZT).

Influence of enviro-agro industrial wastes on lipase formation by thermoalkalophilic bacterial isolates:

As listed in table (3) bacterial isolates B_2 and B_3 synthesized remarkable amounts of lipase on all tested wastes than the other two bacterial isolates. These two bacterial isolates, the most potent bacterial isolates, were subjected to further studies.

Table (3): Screening of thermoalkalophilic, lipase forming bacterial isolates with different wastes.

Different wastes	Lipase activity (Unit/ml)			
	B_1	**B_2**	**B_3**	**B_4**
Cotton seed	2.50±0.2	4.14±0.2	3.33±0.2	2.88±0.3
Soyabean	1.80±0.1	2.25±0.3	3.15±0.3	1.65±0.1
Sunflower	**4.68±0.3**	**6.75±0.1**	**6.50±0.3**	5.04±0.2
Linseed	3.69±0.1	4.59±0.4	5.48±0.2	3.60±0.3
Olive seed	1.35±0.2	2.16±0.2	3.06±0.1	1.62±0.2
Fish	2.00±0.1	4.50±0.2	5.04±0.3	2.25±0.3
Poultery	2.50±0.2	3.96±0.3	4.50±0.2	2.25±0.3

(Thermoalkalo-stable lipase activity is determined by titration method).

Influence of different nutrient media on lipase formation by the most potent bacterial isolates:

Bacterial isolates B_2 and B_3 were allowed to grow on different nutrient media (Basal medium and medium with supplements of fish and poultery wastes) to investigate the effect of these media on lipase formation by the bacterial isolates. Results listed in table (4) demonstrate that the level of lipase increased in most tested media with added fish wastes or poultery wastes than the basal medium. Maximum induction of lipolytic enzyme by the two bacterial isolates could be attainted by using medium A with added fish wastes.

Table (4): Influence of different culture media on lipase formation by the most potent bacterial isolates.

Media	Media with added					
	Basal media		Fish wastes		poultery wastes	
	Lipase activity (Unit /ml).					
	B_2	B_3	B_2	B_3	B_2	B_3
A	3.96±0.2	4.14±0.1	5.4±0.2	5.94±0.1	6.3±0.2	4.86±0.2
C	2.34±0.1	2.7±0.3	2.88±0.1	2.7±0.2	2.7±0.2	3.6±0.1
D	3.6±0.2	3.6±0.3	6.3±0.3	3.6±0.1	2.7±0.1	4.68±0.3
E	2.7±0.3	2.52±0.1	3.42±0.3	3.6±0.1	5.4±0.2	3.42±0.2
N	3.24±0.3	3.78±0.2	5.22±0.3	3.96±0.2	4.86±0.3	4.5±0.3

(Lipase activity is determined by titration method).
A: Aisaka and Terada (1980). E : Pymedium C: Lima *et al.*, (2003).
N: Nutrient broth D: Chen *et al.*, (1998)

Selection of the most potent lipase forming bacterial isolate in relation to different substrates in media A :

In a preliminary experiment maximum lipolytic formation could be induced on medium A with fish wastes. It is interest to choose the most potent bacterial isolate from the two tested isolates with best substrate of medium A for maximum yield of enzyme.

Table (5) illustrates that the induction of lipolytic enzyme by bacterial isolate B_2 was higher than that of bacterial isolate B_3 in all cases, and medium A in present of fish is the most suitable medium.

Table (5): Selection of the most potent lipase forming bacterial isolate in relation to different substrates in media A.

Media A with	Lipase activity (Unit/ml).	
	B_2	B_3
Oil	10.36± 0.2	5.92 ±0.03
Oil & fish wastes	13.32±0.02	6.66+0.04
Oil & pulletery wastes	8.15±0.04	8.88±0.02
Fish wastes	**14.80±0.04**	**12.58±0.02**
pulletery wastes	8.88±0.01	7.40±0.01

(Enzyme activity determined by clolorimetric method)

Concerning the enhanced effect of fish wastes on enzyme formation by bacterial isolate B_2, it is important to study the effect of fish wastes as nutrient medium (1 g/100ml tap water) also investigate medium A with fish wastes (1 g/100ml) and nutrient medium contained fish wastes with yeast extract (1 g and 1/2 g respectively per 100 ml tap water) media were adjusted at pH 11.5.

Table (6) reveals that nearly equal enzyme induction could be attained by bacterial isolate B_2 grown on tap water containing fish wastes and medium A with fish wastes. However, the enzyme synthesis was more stimulated on fish wastes in presence of yeast extract.

El-Tayeb *et al*., (2001) stated that it is better to replace the usual nitrogen source in microbiological media with natural proteins as cheaper sources of nitrogen of the fermentation. Ghorbel *et al*., (2003) used fishing industry waste water for production of solvent stable protease of *Bacillus cereus* BGI.

Table (6): Selection media for lipase formation by the most potent bacterial isolate, B_2.

Media	**Lipase activity (Unit/ml).**
Fish wastes & tap water	24.54 ± 0.004
Fish wastes & yeast extract	29.64 ± 0.09
Fish wastes & media A	25.07±0.002

(Enzyme activity determined by clolorimetric method)

In the light of these results one may conclude that tap water containing fish wastes and yeast extract is the best inducer medium for lipase formation by bacterial isolate B_2.

Identification trails of bacterial isolate B_2 indicated that it is related to genus Bacillus and according to the key of Bergey's Manual of Systematic Bacteriology (1984) was identified as *Bacillus brevis* B_2.

Effect of environmental and nutritional conditions on lipase formation by *B. brevis* B_2 :

Studies on lipase formation by *B. brevis* B_2 during growth are shown in fig.(1), the level of lipase synthesis increased till reached its maximum on media A 70ºC at a culture age of 24 hr, after which enzyme formation decreased reaching about 61% of the maximum activity after 72 hr. Kashmiri *et al*., (2006) stated that the rate of lipase secretion by *trichoderma viride* into fermentation broth could be achieved between 18 to 24 h. the level of the extracellular lipase was the maximum till 48 h, then the level slightly decrease due to denaturation of the formed enzyme.

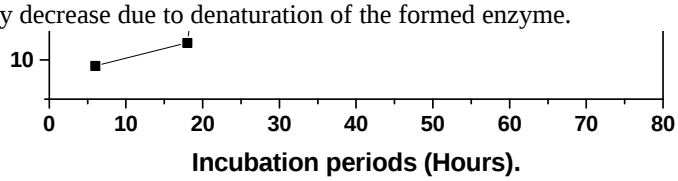

Fig.(1): Effect of different incubation periods on lipase formation by *B. brevis* B_2

It is clear from fig.(2) that increasing the initial pH of the nutrient medium from 7.5 to 11.5 a gradual increase in the formed enzyme could be detected and the highest yield was obtained at pH 11.5. Further increase in pH (12-14) resulted in a decrease in enzyme formation.

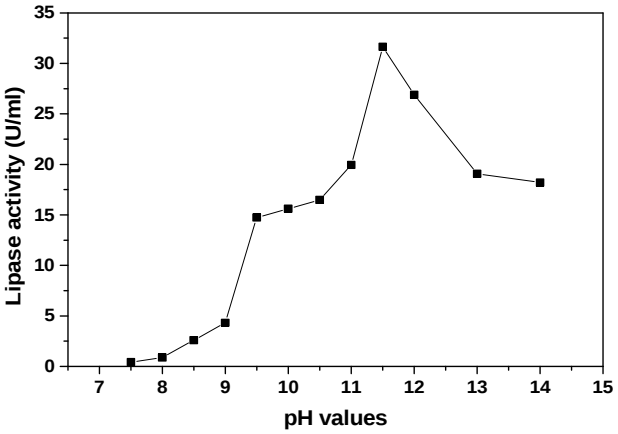

Fig.(2): Effect of different pH values on lipase formation by *B. brevis* B_2.

The pest temperature used for lipase formation takes place in range of 50°C – 85°C (Fig.3). At 70°C maximum amount of lipase units were formed, these results indicate the thermo-alkalophilic nature of the organism. These results were in accordance with Malhotra *et al*., (2000). Deive *et al*., (2009) attained that the final value of extracellular and intracellular lipolytic activities of *T. thermphilus* were obtained after growth at 70°C.

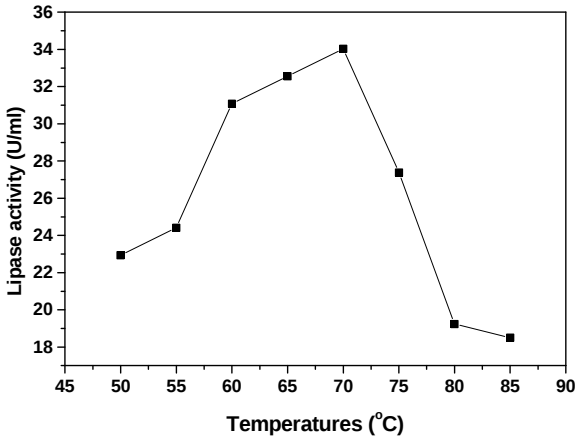

Fig.(3): Effect of different temperatures on lipase formation by *B. brevis* B_2

Effect of different fish wastes concentrations, used as a sole carbon source in fermentation medium, on lipase formation were tested. Fig.(4) indicate that maximum enzyme biosynthesis could be detected at fish wastes concentration of 1% (w/v), This means that the cheapest solid natural substrate fulfilled both energy and nutritional requirement for *B. brevis* B_2 to be able to produce the highest lipase formation.

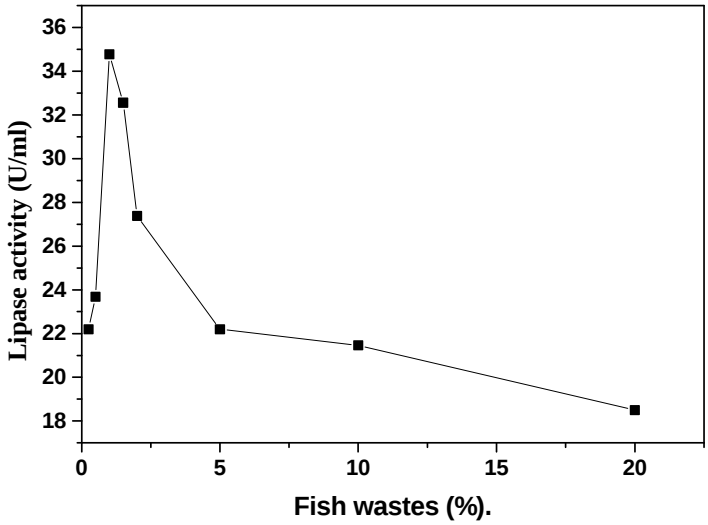

Fig.(4): Effect of different fish wastes concentrations on lipase formation by *B. brevis* B_2.

Regarding the effect of different inoculum sizes of *B. brevis* B_2 it is evident that maximum lipase formation was recorded in presence of 2 ml bacterial suspension (Fig.5). Lipase formation by *B. brevis* B_2 slightly increased under submerged condition. In a trial to study the effect of introducing some carbon sources on lipase formation, it was found that the tested disaccharide carbon sources (sucrose, lactose and maltose) are the best carbon for maximum lipase biosynthesis by *B. brevis* B_2 (Fig.6). Concerning the effect of supplementing different nitrogen sources on the biosynthesis of lipase by *B. brevis* B_2, it was found that all tested nitrogen sources failed to increase lipase formation (Fig.6). Kim *et al*., (1998) reported that biosynthesis of a highly alkaline thermostable lipase by *Bacillus stearothermophilus* L1 could be achieved in a medium contained beef and palm oil.

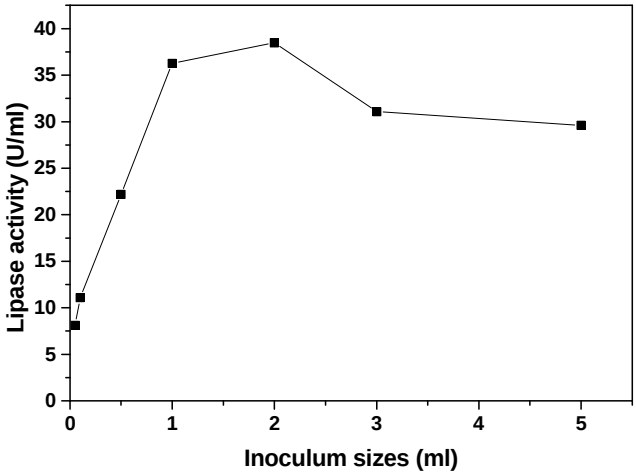

Fig.(5): Effect of different inoculum sizes on lipase formation by *B. brevis* B_2.

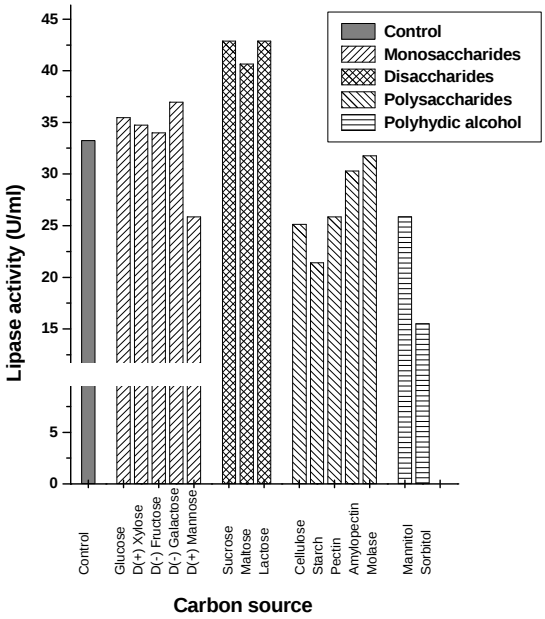

Fig.(6): Effect of different carbon sources on lipase formation by *B. brevis* B_2.

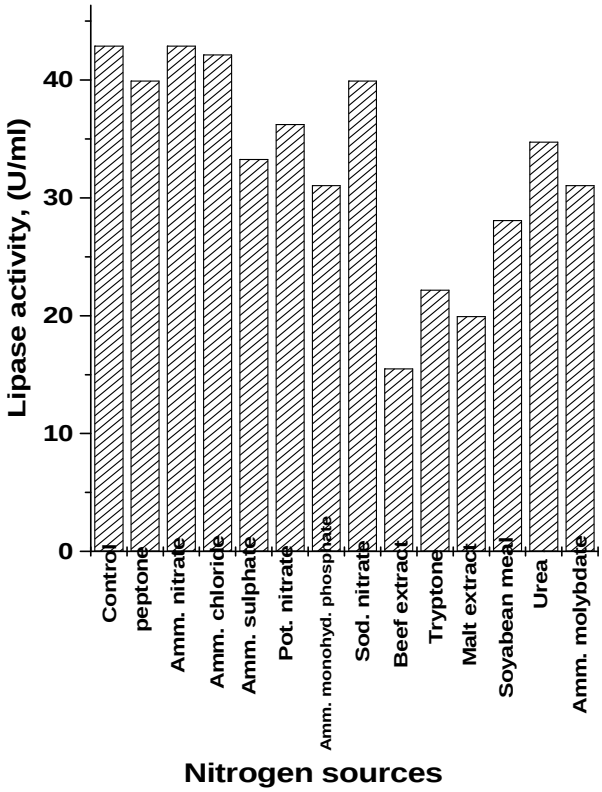

Fig.(7): Effect of different nitrogen sources on lipase formation by *B. brevis* B_2 .

The optimum concentration of yeast extract that induced maximum lipase formation is 1%. However, increasing yeast extract concentration up to 4.5% revealed a decreasing effect on enzyme level.

To check the effect of surfactant on lipase formation by *B. brevis* B_2 it was found that Tween 20 exhibited slight increase on enzyme biosynthesis while Triton X-100 revealed the synthesis of enzyme by about 16.37% (Table 7). Deive *et al*.,(2009) found that the level of extracellular lipase from *T. thermophila* HB 72 increased by supplementing of the nutrient medium by Tween 80, Tween 20 and Triton X-100.

Table (7): Effect of different surfactants on lipase formation by *B. brevis* B_2.

Surfactants	Lipase activity, (Unit/ml).
Control	50.32 ± 0.07
Triton X-100	42.00 ± 0.0014
Tween 20	**53.97± 0.05**
Tween 80	49.07 ± 0.06

Concerning the effect of some tested metal ions on lipase formation, it is clear from results in Fig.(8) that $ZnSO_4$, $MgSO_4$ and $Fe_2(SO_4)_3$ stimulated the enzyme synthesis by *B. brevis* B_2 by about 66, 55, 45% respectively. On the other hand, Cu^{2+}, Mn^{2+}, Cd^{+} and Na^{+} showed no appreciable effect on lipase formation while the other tested metal ions and EDTA repressed the enzyme biosynthesis by values ranging from 33% to 75%. Ammar *et al.*, (1985) revealed that Ni^{2+} and Co^{2+} at certain concentration exhibited a good yield of lipase by *B. stearothermophilus*. Lipase formation by a thermophilic *Bacillus* sp. is increased several folds when Mg^{2+}, F^{3+} and Co^{2+} were added to the nutrient medium (Janssen *et al*., 1994).

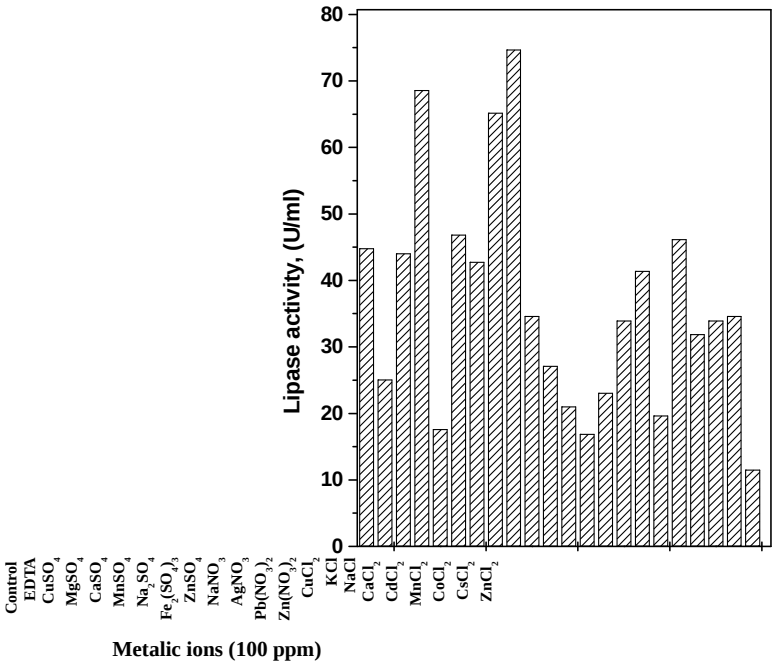

Fig.(8): Influence of some metal salts and EDTA on lipase formation by *B. brevis* B_2.

The addition of some vitamins to the basal nutrient medium is recoded in Fig.(9). The results obtained demonstrate that folic acid (500 ppm) has a stimulatory effect on lipase formation by *B. brevis* B_2. Alternatively histidine (500, 1000 ppm) repressed it by 18 and 22% respectively.

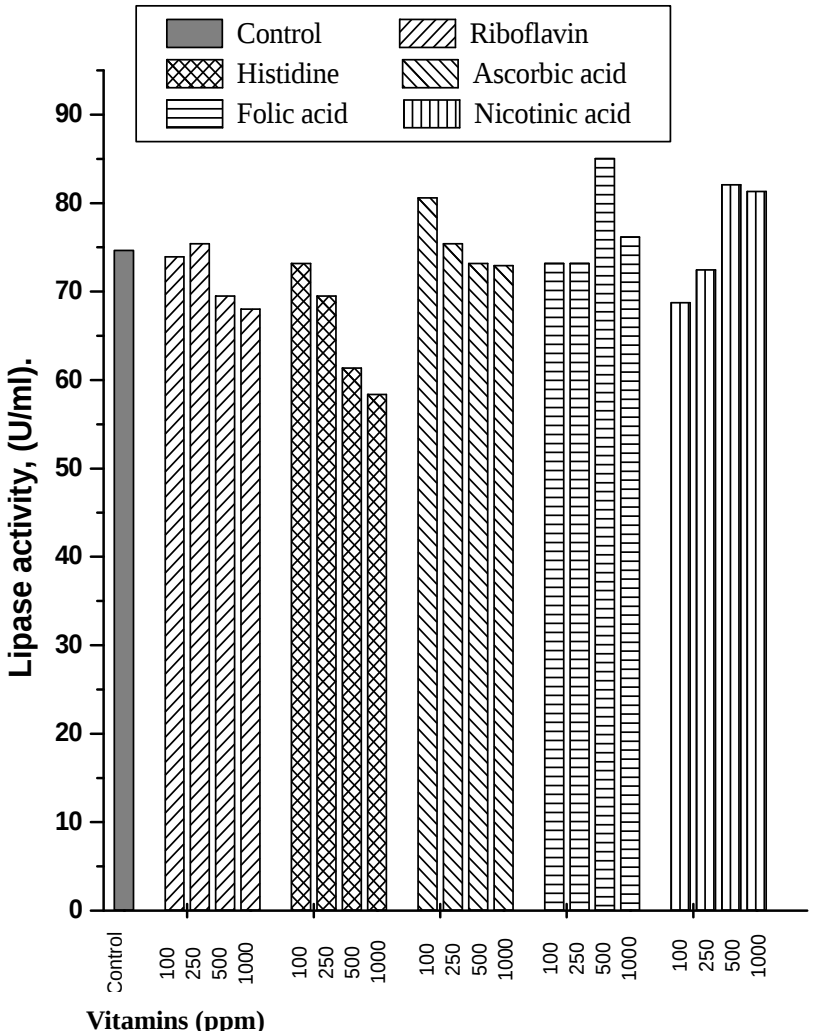

Fig.(9): Effect of some vitamins on lipase formation by *B. brevis* B_2.

Studies on the effect of gamma-irradiation on lipase formation by *B. brevis* B_2 reveled that γ-rays of 1 KGy exerted a slight increase on lipase biosynthesis. Ferdes and Ferdes (1992) reported that γ-radiation of microbial cells increases the activity of some enzymes and decrease the activity of others. Also, Rosenthal (1992) reported that, the enzymes are not very much affected by doses of ionizing radiation.

References

1. AISAKA, K. AND TERADA, O. (1980): Purification and properties of lipoprotein lipase from *Rhizopus japanicus.* Agric. Biol. Chem., 44: 799-805.
2. AMMAR, M.S.; MOSTAFA, S.A.; AND BARRADA, S.H. (1985): Production of thermostable lipase(s) from obligate thermophilic bacteria in relation to α-amylase biosynthesis. *Aspect.*Saudi Arabia, March, 12 -14.
3. ARAVINDAN, R. AND VIRUTHAGIRI, T. (2007): Optimization of medium composition for lipase production by *Candida rugosa* NCIM 3462 using response surface methodology. Canadian Journal of Microbiology, 53(5): 643-655.
4. Bergey's Manual of Systematic Bacteriology (1984): Vol. 1, Ed. (Novel. R. Kreig) William and Wilkins. Baltmore, London.
5. CARDENAS, J.; ALVARZ, E.; DE CASTRO-ALVAREZ, M.S.; SANCHEZ-MONTERO, M.; VALMASEDA, M.; ELSON, S.W.; SINISTERRA, J.V. (2001): Screening and catalytic activity in organic synthesis of novel fungal and yeast lipases. J. Mol. Catal. B: Enzyme, 14: 111-123.
6. CASTILLO, E.; PEZZOTTI, F.; NAVARRO, A.; LOPEZ-MUNGUIA, A. (2003): Lipase catalysed synthesis of xylitol monoesters: solvent engineering approach. J. Biotechnol., 102: 251-259.
7. CHEN, S.J.; CHENG, CHEN, C.Y. AND CHEN, T.L. (1998): Production of an alkaline lipase by *Acinetobacter radioresistens.* J. Fernent Bioeng., 86: 308-312.
8. COLLINS, C.H. AND LYNE, P.M. (1985): Microbiological Methods Fifth edition. Butter Worth and Co.(Publishers) Ltd.
9. DEIVE, F.J.; VARVALHO, E.; PASTRANA, L.; RUAB, M.L.; LONGO, M.A. AND SANROMAN, A.M. (2009): Strategies for improving extracellular lipolytic enzyme production by *Thermus thermophilus* HB27. Bioresour. Technol., 2-53.
10. DHARMSHITI, S. AND LUCHAI, S. (1999): Production, purification and characterization of thermophilic lipase from *Bacillus* sp. THL027. FEMS Microbiol. Lett., 179: 241-246.
11. DOX, A.W. (1910): The intracellular enzymes of penicillium and *Aspergillus* with special reference to those of *P. Camemberti.* U.S. Sept. Agr. Bur. Animal/ Ind. Bull., 120, 170.

12. El-TAYEB, O.; HASHEM, A.; MOHAMMAD, F.; AND ABOULWATA, M. (2001): Optimization of the industrial production of bacterial α-amylase in Egypt. III. Production in solid state fermentation and on natural protein sources by two strains of *Bacillus subtilis* and *Bacillus amyloliquefaciens. Egypt J. Biotechnol.* 10, 56-67.

13. FERDES, O.S. AND FERDES, M. (1992): Irradiation dose-effect relationship and phenomenological behavior to gamma-irradiation of some enzymes. Romanian Journal of Biophysics, 2(2): 113-120.

14. GARGOURI, M.; DROUET, P. AND LEGOY, M.D. (2002): Synthesis of a novel macrolactone by lipase-catalysed intra-esterification of hydroxyl-fatty acid in organic media. J. Biotechnol., 91: 259-266.

15. GHORBEL, B.; SELLAMI-KARNOUN, A. AND NASRI, M.(2003): Stability studies of protease From *Bacillus cereus* BGI. *Enzyme Micrab. Technol.* 32: 513-8.

16. HADDADIN, M.S.Y.; HADDADIN, T.; ARABIYAT, O.I. AND HATTAR, B. (2009): Biological conversion of olive pomace into compost by using *Trichoderma harzianum* and *Phanerochaete chrysosporium:* Bioresource. Technol., doi: 10. 1016/j. biortech. 40-47.

17. HENSYL, W.R. (Ed.) (1994): Bergy's Manual of Determinative Bacteriology 9th edition, Williams & Wilkins, Baltimore.

18. IMAMURA, S. AND KILAURA, S. (2000): Purification and characterization of a monoacylglycerol lipase from the moderately thermophilic *Bacillus* sp. H-257, J. Biochem., 127: 419-425.

19. JANSSEN, P.H.; MONK, C.R. AND MORGAN, H.W. (1994): A thermophilic lipolytic *Bacillus* sp., and continuous assay or its p-nitrophenyl palmitate esterase activity. *FEMS Microbiol Lett*. 120, 195-200.

20. JANTAPORN, T. AND CHUENCHIT, B. (2007): Molecular cloning and functional expression of a novel extracellular lipase from the thermotolerant yeast *Candida thermophila,* FEMS Yeast Research, 7: 232-243.

21. KASHMIRI, M.A.; ADNAN, A. AND BUTT, B.W. (2006): Production, Purification and partial characterization of lipase from *Trichoderma viride*. Afr. J. Biotechnol., 5(10), 878-882.

22. KIM, H.K.; PARK, S.Y.; LEE. J.K. AND OH, T.K. (1998): Gene cloning and characterization of thermostable lipase from *Bacillus stearothermophilus* L1. *Bioscienc., Biotechnol. Biochem.* 62, 66-71.

23. LEE, D.; KOK, Y.; KIM, K.; KIM, B.; CHOI, H.; KIM, D.; SUHARTONO, M.T. AND PYUN, Y. (1999): Isolation and characterization of a thermophilic lipase from *Bacillus thermoleovorans* ID-1. FEMS Microbiol. Lett., 179: 393-400.

24. LEE, M.H.; JAE, T.S.; YOON-HO, C.; SEUNG-PYO, H.; EUGENE, R.; HYUNG, K.; SEUNG-GOO, L.; HARYOUNG, P.; SANG, C.L.; YOUNG, B.S. AND MOON-LEE, S. (2003): High-level expression and secretion of *Bacillus pumilus* lipase B26 in *Bacillus subtilis* Chungkookjang. J. Microbiol. Biotechnol., 13(6): 892-896.

25. LIMA, N. MURALIDHAR; CHIRUMAMILA, R. AND MARCHANT, R.R. (2003): Production, regulation and some properties of lipase activity from *Fusarium oxysporum* f. sp. Vasinfectum. Enz. Microbiol. Technol., 18: 932-938.

26. MALHORTA, R.; NOORWEZ, S.M. AND SATYANARAYANA, T. (2000): Production and partial characterization of thermostable and calcium-independent α-amylase of an extreme thermophile *Bacillus thermooleovorans* NP54. Letters in Applied Microbiol., 31: 378-384.

27. MARKOSSIAN, S.; BECKER, P.; MARKL, H. AND ANTRANIKIAN, G. (2000): Isolation and characterization of lipid-degrading *Bacillus thermoleovorans* IHI-91 from a Icelandic hot spring. Extremophiles, 4: 365-371.

28. NAHAS, E. (1988): Control of lipase production by *Rhizopus oligosporus* under various growth conditios. J. Gen. Microbiol., 134: 227-233.

29. ROSENTHAL, I. (1992): Electromagnetic Radiations in food science. Spinger-Verlag, pp. 244.

30. SHIRILING, E.M. AND GOTTLIEB, D. (1966): Methods for characterization of *Streptomyces* species. Int. J. Syst. Bact., 16: 313-340.

31. WINKLER, U.K. AND STUCKMANN, M. (1979): Glycogen, hyaluronate and some other polysaccharides greatly enhance the formation of exolipase by serratia marcescens. J. Bacteriol., 138: 663-670.

دراسات على حث وتكوين انزيم الليباز بواسطة باسيلس بريفس بـ 2 المحب للحرارة والقلوية

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ملخص البحث

تم إختيار العزلة المعرفة باسليس بريفس بـ 2 من بين العزلات البكتيرية التسع المحبة للحرارة والقلوية والمعزولة من بعض المخلفات الصناعية كأقوى العزلات تكويناً لانزيم الليباز. ومن بين البيئات الغذائية المستخدمة تم إختيار البيئة الغذائية المحتوية على 1% من مخلفات الاسماك و 1% من مستخلص الخميرة المذابين بالماء العادى كأحسن بيئة محفزة للتخليق الأحيائى لإنزيم الليباز بواسطة باسليس بريفس بـ 2 تم الحصول على أعلى تكوين للإنزيم بعد 24 ساعة. وأثبتت الدراسة أن أنسب تركيز من أيون هيدروجينى لتحفيز تكوين الانزيم هو 11.5 وعند درجة حرارة 75°م. وقد أدى وجود سكروز أو لكتوز فى البيئة الغذائية للكائن إلى زيادة مستوى تكوين الانزيم كما كان لإضافة كبريتات زنك (100 جزء فى المليون) وحمض فوليك (500 جزء فى المليون) أكبر الأثر فى تدعيم تكوين الانزيم بواسطة باسليس بريفس تحت ظروف التحضين المغمورة وحجم 2مل معلق بكتيرى لكل 100مل بيئة غذائية. وقد حدثت زيادة طفيفة فى تكوين الانزيم عند تعريض العزلة البكتيرية لجرعة مقدارها 1 كيلو جراى من أشعة جاما.