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"نظرة بين الحاضر والمستقبل
30 - 31 ديسمبر –2019 - اسطنبول – تركيا
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Detection of Some Types of Oils That Induce Lipase Production in Some Bacterial Isolates.

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Abstract: Bacterial lipases are a highly versatile class of hydrolytic enzymes which catalyze the hydrolysis of triglycerol to glycerol and fatty acids. They play an important role in biotechnological and industrial processes, including oleo chemical, food, pharmaceutical detergent formulation, cosmetic, leather, textile, and paper industries applications. Tremendous interest among scientists and industrialists has been arisen in the last decade in microbial lipases due to their versatility and ease of mass production.

In the current work, natural oils such as Triglyceride, Tween 20, Tween 80, Olive oil, Sweet almond, Celery oil, Sesame, Rosemary oils were implemented to enhance lipase production by soil and pathogenic bacterial isolates. Results revealed that triglyceride and tween 80 had the best enhancing activity for lipase production in most isolates. It was also shown that Pseudomonas and Bacillus were the most potential lipase producing bacteria.

A novel modified tributary agar was prepared by the addition of Sudan blue dye and used for the detection of lipase producers. It was shown to give a very clear zone even in the case of weak producing strains as compared to the original medium.

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Specific lipase activity had been determined Staphylococcus aureus (in the present research. The highest lipase activity was demonstrated by Pseudomonas (4.5u/ml), while Staphylococcus aureus did not show any lipase activity.

Keywords: Modified medium, Sudan blue dye, inducing oils, bacteria

اكتشاف بعض أنواع الزيوت التي تدل على إنتاج اللايبيز في بعض العزلات البكتيرية

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INTRODUCTION

Lipases are water soluble enzymes that are capable of hydrolyzing triacylglycerol and release free fatty acids and glycerol. They belong to the serine hydrolase family that react with carboxylic ester bonds of mono-, di-, and tri- glycerides releasing glycerol and fatty acids [1]. Lipases are considered a major group of biocatalysts that have enormous biotechnology application and have been isolated and purified from fungi, yeast, bacteria, plant and animal source [2].Currently bacterial lipase are of great demand because of potential industrial application [3] , [4.[

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These are considered as nature's catalysts, but commercially, only microbial lipases are being used significantly. They have enormous application in the industries of detergents, pulp ,fat and oil processing, oleochemical industry, food industry, and paper industry, tea processing, biosensors and cosmetics and perfumery [5. [

Due to high yields, ease of genetic alteration, broad range of catalytic activities, tolerance to environmental conditions and fast growth rates ; microbial lipases are used in bioremediation which is the use of microorganism or microbial process to detoxify and degrade environmental contaminants [6] . The interest in microbial lipase production has increased in the last decades because of its large potential in industrial application as additive for foods flavor modification, waste water treatment,decomposition and removal of oil substances [7], cosmetic (removal of lipase), pharmaceutical digestion of oil and fat in food ,leather (removal of lipid from animal skin) [8] and medical blood triglyceride assay [9.[

Generally industrial enzymes are produced in the presence of inducers . Most of the well studied microbial lipases are inducible extracellular enzymes. As for lipases, it was found that triacyl glycerol vegetable oils, surfactants, oil industry wastes or their hydrolysis products in the culture medium have, an inducible effect on lipase production.

The main goal of this research was to find a cheaper and natural compound that induces lipase production from different bacterial soil and pathogenic isolates then determine the specific activity of this enzymes

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Material and Methods:

Bacterial Isolates: Bacterial isolates were obtained from Microbial Bank/ College of science / Biology Department .

Media:

Bacterial isolates were inoculated on tributary agar base according to [3] method with a slight modification. The modified media containing 0.05% peptone, 0.3% yeast extract, 2% agar, (PH:7) was used to screen lipase producing strains. Every 50 ml of media was mixed with 0.001 gm Sudan blue, 0.5ml of one of the oils tested, stirred for ½ hr. then poured into sterilized plate. Sudan Blue II is a synthetic dye, with a functional azo group and aromatic ring. It is widely used to stain alcohols, esters, hydrocarbon derivatives, oils, fats and waxes .

Bacterial isolates were inoculated on these plates with different oil substrates, incubated for 2 days and a zone of clearance was observed due to hydrolysis of different types of oils such as

Triglyceride , Tween 20 , Tween 80, Olive oil, Sweet almond ,Celery oil , Sesame, Rosemary oil. Determination of lipase activity was measured by titrimetric method according to [10].

Titrimetric lipase assay method:

Lipase activity was measured by titrimetric method using different types of oils as substrates at PH 7.0. the reaction cocktail was prepared by 5% (w/v) olive oil emulsified in 5% (w/v) gum acacia in 100mM sodium phosphate

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buffer, PH 7.0. 1ml of each crude lipase enzyme was added to the reaction cocktail of 10 ml separately at their respective culturing temperatures and incubated for 15 min. at 100 rpm in a shaker incubated. The reaction was quenched and fatty acids were extracted by adding 1 ml of acetone: ethanol solution (1:1) and swirling the contents swiftly. Phenolphthalein indicator of 2-3 drops was added to each of the reaction mixture with respect to different crude lipase enzymes of isolates and the control. The contents of each reaction mixture were titrated with 0.05M NaOH solution to an end point of pink colore at pH10.0 . Lipase activity was calculated as micromoles of free fatty acids formed from olive oil per ml of crude lipase enzyme as per equation .

Activity = ((Vs-VB).N.1000) /s

Where, Vs.: is the volume of 0.05M NaOH solution consumed by the enzyme-substrate cocktail (ml), VB: is the volume of 0.05M NaOH solution consumed in the titration by the substrate (control) cocktail (ml). N: is the molar strength of the NaOH solution used for titration (0.05 M). S: is the volume of substrate cocktail solution (10 ml). One unit (U) of lipase enzyme is defined as the amount of enzyme required to liberate 1µmol of fatty acids from triglycerides.

Result and Discussion :

The screening of lipase production by 6 bacterial isolates showed that all of them produce lipase with different extent but Pseudomonas aeruginosa was the most potent, followed by Bacillus and Actinomyces isolated from soil

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as shown in table (1) .The results also revealed that the best substrate for lipase production was triglyceride and tween 20 among all oil substrates utilized in this research (Triglyceride, tween 20 ,tween 80, Olive oil, Sweet almond ,Celery oil , Sesame, Rosemary oil) . These results agrees with [11] who concluded that high lipase production had been observed when organic nitrogen source as peptone and yeast extract were utilized as a good nitrogen supplement that gives 66.25% by 2acillus and Pseudomonas and tween 20 . Also [12] found that tween 80 is effective in inducing lipase production.

Table (**1**): lipase production by bacterial isolates from different sources.

Type of oil / Nam of bacteria	Triglyceride	Tween 20	Tween 80	Olive oil	Rosemary oil	Sesame oil	Celery oil	Sweet almonds oil
Bacillus (soil)	++	+++	++	–	+	–	–	–
Pseudomonas (soil)	+	++	+	–	+	+	–	–
Actinomyces (soil)	++	+	+	–	–	–	–	–
Staphylococcus aureus	+	–	+	–	–	–	–	–
Pseudomonas aeruginosa	+++	+++	++	–		–	–	––
Actinomyces	+	++	+++	–	–	–	–	–

*strong (+++), moderate (++) and weak (+).

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Results also demonstrated that lipase production from *Staphylococcus aureus was* very weak. Clear inhibition zones around the colonies inoculated in tributary agar with different oil substrates was used. Triglycerides and Tween 80 among all oils used proved to be the most suitable natural oils for lipase production. In addition to that, results demonstrated an overall increase in extracellular lipase production as compared with shake flask method. A study by [13] found that extracellular lipase secretion starts a 12-16 hours and hen reached maximum activity at 46-48 hours in pilot scale fermentation through an increase in cell mass. Another work by [14] confirmed that clear hydrolysis zone around bacterial colonies are considered as an important method for selecting lipase producing isolates which correlates with the present results obtained. Also, it was found that nutrient broth was more convenient for production which agrees with [1] who concluded that the utilization of nutrient broth gave maximum extracellular lipase production (almost 1.3 fold) higher than Luria broth.

Titrimetric lipase assay with triglycerides showed high lipolytic activity in tributyric agar. Pseudomonas soil isolates gave the highest activity (4.5u/ml) as shown in table (2), while *Staphylococcus aureus* had no activity. It is assumed that oily environment may provide better conditions for lipase producing microorganisms [15]. A study by [16] also confirmed that the maximum production of lipase enzyme from *Bacillus amyloligrefacieces* after 48 hr. incubation at pH 9 and highest activity had been achieved by utilizing fish oil as a substrate.

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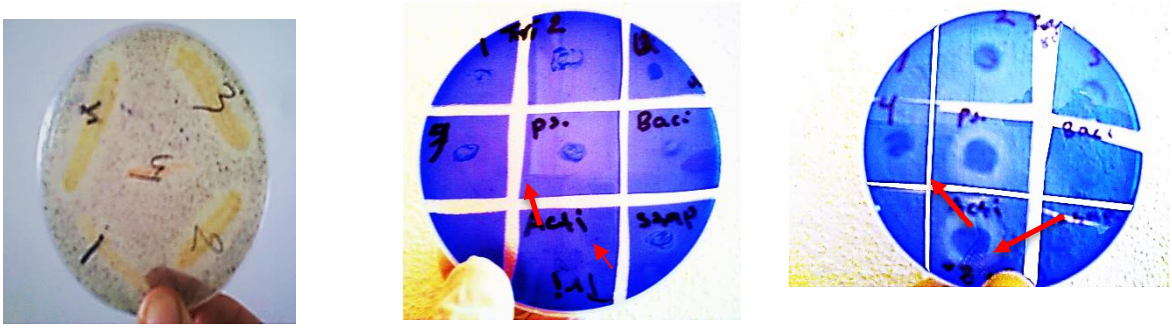
Table (**2**): Lipase activity by bacterial isolates.

Name of bacteria	Lipase Activity	Activity score
Bacillus (soil)	2 U/ml	+
Pseudomonas (soil)	4.5	+++
Actinomyces (soil)	0	–
Staphylococcus aureus	2	+
Pseudomonas aeruginosa	3.5	++
Actinomyces	2	++



(**A**) (**B**) (**C**)

Figure (**3**): Bacterial isolates on modified agar showing clear zones of lipolytic activities. A: Tributary agar. B: Modified Tributary agar with Triglyceride as lipase substrate and Sudan blue. C: Modified Tributary agar with Tween 80 as lipase substrate and Sudan blue.

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CONCLUSION:

Our results show that the modified medium presented in this study was suitable in giving a more clearly defined hydrolytic zone than the original Tributary agar. Sudan blue was chosen due to its solubility in oils as it is used to stain alcohols, esters, hydrocarbon derivatives, oils, fats and waxes. Triglycerides and tween 80 proved to enhance lipase production in Pseudomonas and Bacillus. This could help in reducing the media cost and enhancing lipase enzyme production.

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