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Research article

Effect of cultural conditions on lipase production *Pseudomonas aeruginosa* isolated from Iraqi soil

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ABSTRACT

Seventy *Pseudomonas* isolates were obtained from different Iraqi soils samples. Thirty five of these isolates have the ability to produce lipase. These isolates were screened on Rhan medium. The highest lipase activity was found with *P. aeruginosa*. ps12. The effect of media composition, pH and temperature on production of lipase were determined. Maximum lipase production appeared in the medium containing (0.5%) glucose as carbon source, (0.5%) urea as nitrogen source and 1% of olive oil with pH 8 and incubated at 30 ^{O}C for 48 h with 1 ml (O.D 600= 0.8) of 24 h of bacterial culture.

Keywords: *Pseudomonas*, **glucose, urea, lipase, crude oil, tween 20.**

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INTRODUCTION

Lipases (triacyglycerol ester hydrolases, E.C. 3.1.1.3) promote the hydrolysis of acylglycerides and in general a wide range of low and high molecular weight fatty acid esters, thiol esters, amides, polyol/polyacid esters, etc. Furthermore in suitable environmental conditions, lipases are capable of catalyzing the reverse reaction of synthesis just as efficiently [1].

Lipases have received great attention as industrial biocatalysts in areas like oils and fats processing, detergents, baking, cheese making, surface cleaning, or fine chemistry. They can catalyze reactions of insoluble substrates at the lipid-water interface, preserving their catalytic activity in organic solvents [2]. Although a wide variety of Gram-positive and Gram-negative bacteria species produce lipases, the most widely used enzymes originate from the genus *Pseudomonas* [3].

Pseudomonas species, ubiquitous in soil and water, are of considerable scientific and technological importance and comprise a tax on metabolically versatile organism capable of utilizing a wide range of simple and complex organic compounds [4].

Bacterial lipases are generally produced in the presence of oil or any other lipid substrate (viz. fatty

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acid esters, fatty acids, and glycerol) as carbon in the presence of any complex nitrogen source. This study aimed to obtain and screening of Pseudomonas isolates for lipase production and optimization of lipase production and use it for degradation of crude oil.

MATERIALS AND METHODS

Collection of samples for isolation of bacteria

Seventy samples of oil contaminated soils included 40 samples from Baghdad city, 20 samples from Kut, and 10 samples from Hilla, each sample was collected in sterile container then transported to the laboratory until using [5].

Media for lipase production Rhan agar medium

This medium was prepared as solid by adding agar 2% (for qualitative screening) and as liquid (use for lipase production) [6]. The composition of medium was mentioned in **table 1.**

Table 1. composition of Rhan medium

K_2HPO_4	5gm	$FeCl_3$.6H2	1gm
$(NH_4)_2PO_4$	5gm	Agar-Agar	15gm
$MgSO_4$.7H2O	5gm	Olive oil	10gm
$CaCl_2$.6H_2O	1gm	Tween 80	Few drops (2-5) as emulsifier

All components were dissolved in one liter of distilled water and pH was adjusted to 7. The mixture was sterilized by autoclave at 121 ^oC for 15 min. This medium was used to detect the ability of bacteria to produce lipase.

Isolation of bacteria

One gm of each soil samples was added to 9 ml of sterile D.W and shacked to homogenize and serial dilutions were prepared for each sample, then 0.1 ml of each dilution was spread upon Rhan agar medium and incubated at 37 ^oC for 2 days. The bacterial colonies were purified by sub culturing on nutrient agar medium until pure culture was obtained and maintained on nutrient agar slant.

Screening for lipase producing bacteria

A. Qualitative screening

The bacterial isolates were cultured on Rhan agar medium and incubated at 37 ^oC for 48 h. The growth of bacteria and precipitation, (opaque region) around colonies were suggested as indicator for lipolytic activity.

B. Quantitative screening

Bacterial isolates were inoculated in 100 ml of the production medium (Rhan broth medium with 1 ml (O.D 600= 0.8) of 24 h bacterial cultures. The Rhan medium was incubated at 37 ^oC for 48 h. The cultures were centrifuged at 6000 rpm for 30 min. The cells free supernatant was separated and lipase activity was assayed.

Assay of lipase

Lipase activity was assayed according to Van Tigerstrom, (1988). Enzyme solution (0.3 ml) was added to 3.7 of reaction solution and incubated at 37 ^OC for 30 min. The reaction was stopped by addition of 3 ml ethanol (95%) and O.D was measured at 540 nm [7]. Enzyme activity was expressed in units; one unit 1U is defined as the amount of enzyme that liberates 1 µg fatty acid/min. For turbidimetric assay, 1 unit is equivalent to 0.9 O.D 540/min.

Determination the effect of different conditions on lipase production

The following steps were followed in each experiment of enzyme production under different conditions. The production medium 50 ml of Rhan broth medium was inoculated with 1 ml (O.D 600 = 0.8) of 24 h bacterial cultures, and then the culture was centrifuged after, the end of the incubation time at 6000 rpm for 30 min. The supernatant was separated (crude enzyme) and the enzyme activity, protein and specific activity were assayed. The following media and conditions were used for this purpose.

1. Effect of carbon source

Rhan medium containing different carbon sources 0.5 % (glucose, sucrose, starch, castor oil, cod liver oil, sesame oil) was inoculated with bacteria and incubated at 30 ^oC for 48 h then the enzyme was extracted and the enzyme activity was assayed.

2. Effect of nitrogen source

Rhan medium containing different nitrogen sources 0.5 % (ammonium sulphate, urea, pepton, yeast extract) and glucose as a carbon source was inoculated with bacteria and incubated at 30 ^oC for 48 h then the enzyme was extracted and the enzyme activity was assayed.

3. Effect of crude oil concentration

Rhan medium containing different concentrations of crude oil (0.5, 1, 1.5, 4, and 5 %) and glucose as a carbon source. While urea used as a nitrogen source. This media was cultured with bacteria at 30 ^oC for 48 h and the enzyme was extracted and activity was assayed.

4. Effect of the olive oil concentration

Rhan medium containing different concentrations of olive oil (0.2, 0.4, 0.8, and 1 %) and composed of glucose and urea was inoculated with bacteria and incubated at 30 ^oC for 48 h then the enzyme was extracted and the enzyme activity was assayed.

5. Effect of incubation time

Rhan medium composed of glucose, urea and 1 % olive oil was inoculated with bacteria and incubated at different time intervals (5, 24, 48, 72, and 96 hrs) at 30 ^{o}C.

6. Effect of Temperature

Rhan medium composed of glucose, urea and olive oil was inoculated with bacteria and incubated at different temperatures (30, 40, and 50 ^{o}C) for 48 h.

RESULT AND DISCUSSION

Isolation and identification of *Pseudomonas*

In the present study, thirty five Pseudomonas isolates were obtained from seventy samples collected from three Iraqi cities. *Pseudomonas* genus was identified according to morphological feature of bacterial colonies on nutrient agar. Most of colonies were mucoid and produced pyocyanin pigment. *Pseudomonas* is a Gram-negative rod measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm. Almost all strains are motile by means of a single polar flagellum.

Many bacterial species are present in the soil normally and adapting to the soil conditions and almost the bacteria degrade the oil present in the soil that contaminated with oil, which help in clean the soil from oil products [8,9]. One of very dominant bacteria found in the oil contaminated soil is Pseudomonas [10,11].

Screening for lipase producing bacteria

The ability of *Pseudomonas* isolates to produce lipase was examined using qualitative and quantitative methods.

A. Qualitative screening

The maximum diameter of hydrolysis zone (2.3 mm) was found with ps 12 isolate followed by ps 24, while the lowest zone was seen in case of ps 21. The 5 isolates (ps 12, ps 24, ps 5, ps 21 and ps 6) produced the highest hydrolysis zones (2.3, 2.2, 2.1, 2.0 and 2.0 respectively) were selected for the quantitative screening.

Quantitative screening

The five isolates (ps 12, ps 24, ps 5, ps 6, and ps 21) were examined in quantitative method. The maximum production was found in the supernatant of *P. aeruginosa* ps 12 culture (specific activity 60 U/mg) (**Fig. 1**).Thus it was selected for the further steps and identified by VITEK2system.

The optimum conditions of lipase production from *P. aeruginosa* ps 12.

1. Carbon source

The effect of different carbon sources (glucose, sucrose, starch, castor oil, sesame oil and cod liver oil) on the production of lipase enzyme from *P. aeruginosa* ps 12 was studied.

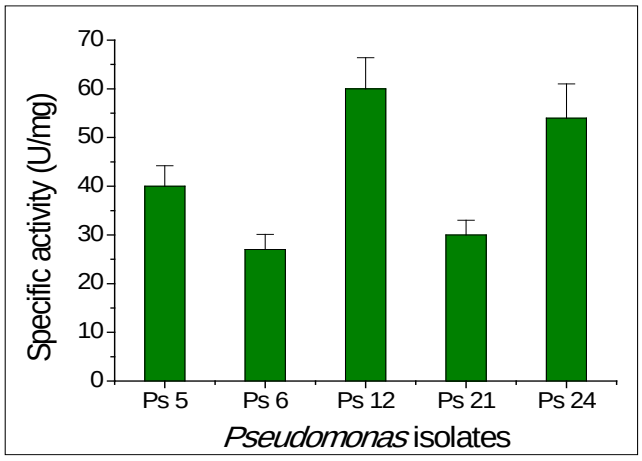

Fig. 1. Lipase production by *Pseudomonas* isolates in Rhan medium, pH 8 at 30 ^{o}C for 48 h.

Fig. 2 revealed that the highest lipase production (55 U/mg) was obtained when the medium contained 0.5% glucose.

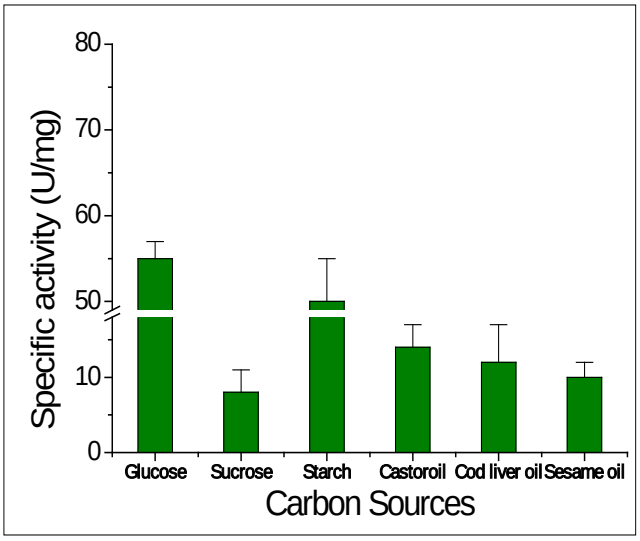

Fig. 2. Effect of different carbon sources on the lipase production from *P. aeruginosa* ps 12 in Rhan medium, pH 8, at 30 ^{0}C for 48 h.

The results indicate that *P. aeruginosa* has the ability to utilize various carbon sources as substrates for growth as well as for the concomitant production of lipase. *Pseudomonas* being heterotrophy, obtain their required nutrients from the organic matter in the environment through the presence of efficient and extensive systems of powerful enzymes. They are able to utilize complex carbon sources as energy source. Glucose (monosaccharide) may support a maximum biomass yield followed by starch (polysaccharide). Glucose supported the maximum productivity better than disaccharides (sucrose) this

may be due to that glucose is simple sugar and easy to utilize for glucose and metabolism. Prasad, (2013) reported that the media supported by glucose stimulate bacteria to produce lipase [12].

In another side oils gave lower specific activity than the carbohydrate because oils are considered as a complex substrate, therefore it required long time to utilize. The result obtained indicates that lipas production seems to be constitutive and independent of lipase substrate. Marie, (2012) showed that this enzyme (lipase) is produced in to presence of lipids such as oil or triglyceride [13].

2. Nitrogen sources

Four nitrogen sources were introduced in the production medium (ammonium sulphate, urea, peptone and yeast extract). It was found that urea gave production more than the other sources reached to (50 U/mg) (**Fig. 3**). Urea is a simple organic compound and can be an important source for the construction of cells and this may stimulate enzyme production such as lipase.

During growth and enzyme production, the bacterial cells probably hydrolyze the organic nitrogen releasing their mineral component and other growth factors.

Some reports found that the maximum activity of *Aspergillus*, *Bacillus*, *Pseudomonas*, and *Mucor* lipase in medium containing urea as nitrogen source. Prasad, (2013) found a good relationship between urea as nitrogen sources and lipase production [12].

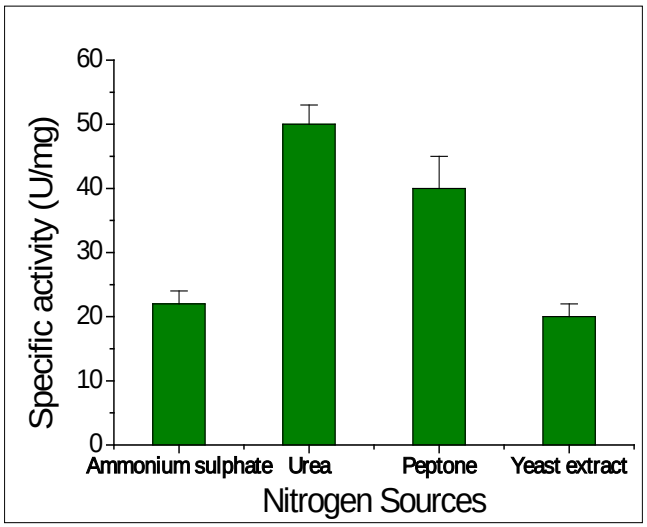

Fig. 3. Effect of different nitrogen sources on lipase production from *P. aeruginosa* ps 12 in Rhan medium containing glucose as carbon source, pH 8, at 30 ^{0}C for 48 h.

3. Incubation time

The effect of incubation time on the enzyme production is explained in **Fig. 4**. The production of enzyme increased dramatically with time up to 48 hr an which it reached its maximum value (50 U/mg)
This result may indiacate that lipase of *P. aeruginosa* ps 12 is produced in the early stages of growth and increased with time and reached to maximum level at stationary phase and then declined. Narasimha et al., (2010) found the maximum lipase production of *P. aeruginosa* (ps12) at 24 h [14]. While, Tembhurkar et al. (2012) reported that the optimum lipase production produced from *P. aeruginosa* (enzyme activity) was after 72 h [15].

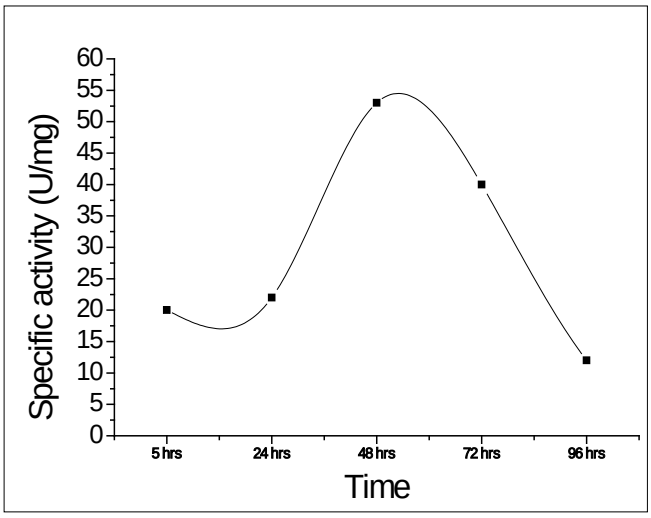

Fig. 4. Effect of incubation time on lipase production from *P. aeruginosa* ps 12 in Rhan medium containing glucose as carbon source and urea as nitrogen source, pH 8, at 30 ^{o}C for 48 h.

4. Temperature

The effect of temperature on lipase production produced from *P. aeruginosa* ps12 was determined. The maximum enzyme productivity was found at 30 ^{o}C (45 U/mg) (**Fig. 5**).

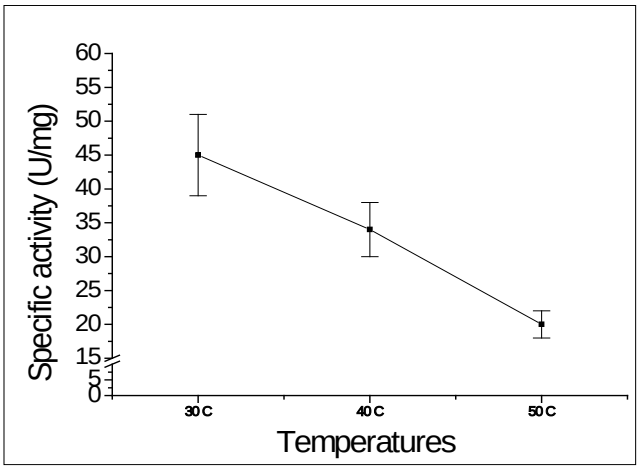

Fig. 5. Effect of different temperatures on lipase production from *P. aeruginosa* ps12 in Rhan medium containing glucose as carbon source and urea as nitrogen source, pH 8, at 30 ^{0}C for 48 h.

It can be concluded that *P. aeruginosa* ps 12 can produce lipase at a wide range of temperatures from 30 to 50 ^{o}C but it favored the moderate temperaturs and it can tolerant temperatures above 30 up to 50 ^{0}C. This character helps the bacteria to survive and produce their enzymes in the environment at different levels of temperature and help in degradation of lipids.

Narasimha et al., (2011) depicted that the maximum productivity of *P. aeruginosa* lipase isolated from soil was at 30 ^{o}C [14]. Tembhurkar et al. (2012) showed the optimum temperature for lipase produced from *P. aeruginosa* that isolated from soil was 50 ^{o}C [15].

5. Effect of crude oil concentration on lipase production

The production of lipase was estimated in the presence of different concentrations of oil (heavy oil 150). The maximum lipase production (35 U/mg) was observed at concentration 1% (**Fig. 6**). Results indicates that this kind of bacteria has good ability to degrade wide levels of oil concentrations and this is use for treatment of polluted soil.The presence of heave oil in growth medium may increase the viscosity of the medium especially at high concentration and this will reduce the transfer of nutrients and oxygen molecules and hence will reduce the microbial growth and metabolism.

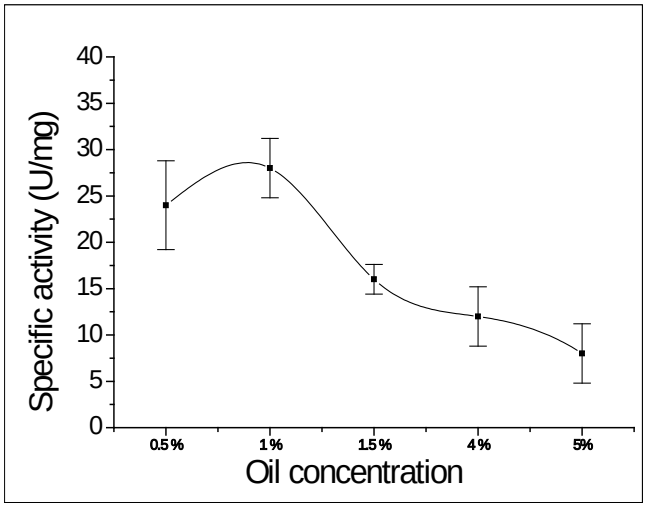

Fig. 6. Effect of different concentrations of crude oil on the lipase production by *P. aeruginosa* ps 12 in Rhan medium containing glucose as carbon source and urea as nitrogen source, pH 8, at 30 ^{0}C for 48 h.

Several study focused on the ability of Pseudomonas species to degradate different kinds of oil. Catallo et al, (1992) found that the lipase produced from *P. aeruginosa* isolated from soil can easily hydrolyze the salmon oil and also found that the concentration of crude oil play an important role in lipase production [16] and their results were going on with our study.

6. Effect of olive oil concentration on lipase production

In this study, the effect of olive oil concentration on lipase production by *P. aeruginosa* ps 12 was studied. The enzyme production increased positively with increase of olive oil concentration. The highest concentration of olive oil (1 %) used in this study gave the maximum activity of lipase (55 U/mg) (**Fig. 7**). The presence of oil in the medium stimulates bacteria to produce lipase to utilize the oil work as a nutrient source. Olive oil was employed as a carbon source for producing lipase from several bacteria such as *Acinetobacter radioresistens*, *Bacillus* sp., *Geobacillus* sp., *P. putida, P. mendocina* and *P. aeruginosa* [14,17].

Most of the bacterial lipases reported are constitutive and nonspecific in their substrate specificity [2]. Narasimha et al. (2011) used 1 % olive oil as the best concentration for lipase production [14].

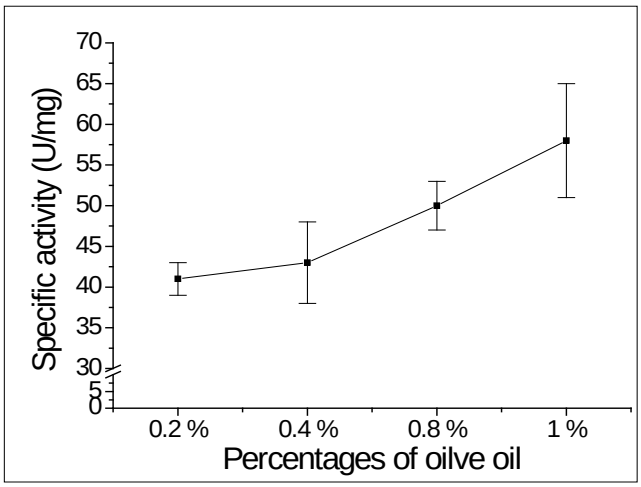

Fig. 7. Effect of different concentration of olive oil on lipase production by *P. aeruginosa* ps 12 in Rhan medium containing glucose as carbon source and urea as nitrogen source,, pH 8, at 30 ^{o}C for 48 h.

Conflict of interest

The authors declare that they have no conflict of interests.

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