Isolation of Pseudomonas aeruginosa and testing of lipase (EC 3.1.1.3) production conditions.

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Abstract
Pseudomonas aeruginosa isolates were obtained from soil contaminated with petroleum oils and cultured in different nutrient media. The appropriate isolate was chosen for the production of lipase enzyme and the change in production conditions was carried out as temperature, where the maximum activity of lipase enzyme reached (3U/ml) at a temperature of 37 degrees Celsius, while the pH was the best activity (3.2U/ml) at pH 7 and the change of carbon sources reached the maximum activity of the enzyme (2.9U/ml) at the use of glucose as a carbon source, while at Nitrogen sources, the maximum enzyme activity (3.5U/ml) was reached when using peptone as a source. The lipase produced from the soil will be useful in increasing industrial production.
Keywords: Lipase producing bacteria, pseudomonas aeruginosa, application.

1. Introduction

Lipase is an enzyme responsible for breaking down fats and converting them into fatty acids and glycerides (Mohammed, 2017). The enzyme lipase is present in the secretions of the pancreas and is responsible for the digestive process (Tanaka et al., 2021, Joshi and Kuila, 2018). The lipase enzyme belongs to the hydrolase family of the triglyceride ester (Ado et al., 2013). The lipase enzyme has many biotechnological applications (Cristian, 2005). One of the most popular types of lipase is the enzyme derived from microorganisms (bacteria and fungi). Microbial lipase is better than lipase derived from animals and plants, due to the rapid growth of the medium and the possibility of genetic manipulation (Sirisha, 2010; Veerapagu et al., 2013; Mongkolthanaruk and Boonmahome, 2013). Microbial lipase is glycoproteins but extracellular bacterial lipase is lipoproteins)Abdul-Hammid et al, 2017; Sagar et al., 2013(. The lipase enzyme is affected by many physical factors such as temperature, pH, and incubation period. Because lipase is a cofactor, the enzyme is produced in the presence of an oil, fat, or other catalyst. Nevertheless, the sources of nitrogen, carbon, and micronutrients are being considered for the growth and improvement of production
(Veerapagu et al., 2013). Microbial lipases constitute an important group of biotechnologically valuable enzymes, mainly because of versatility of their applied properties and ease of mass production (Godris H. L. et al 1989, Prita S., et al, 2009) Lipases catalyze the hydrolysis of triacylglycerol to glycerol and free fatty acids. In contrast to esterases lipases are activated only when adsorbed to an oil–water interface (Rohit Sharma et al., 2001) and do not hydrolyze dissolved substrates in the bulk fluid. An important characteristic of lipases is their ability not

only to hydrolyze the ester bonds, trans-esterify triglycerides and resolve racemic mixture, but also to synthesize ester bonds in non-aqueous media (Prita S., et al, 2009). Given the importance of the lipase enzyme, this current study aims to find the optimum conditions in the isolate bacteria from different sources of oil-contaminated soil and study the activity.

2. Materials and methods

2.1 Collection and Isolation of Lipase Producing Bacteria

Various soil samples were collected from oil refineries in Baghdad. At a depth of 5-10 cm. The serial dilution method was used to isolate bacterial strains (Sagar et al., 2013). Pooled samples are cultured in broth and incubated at 37 °C for 24 h. Each 100 µl of the dilution was then sprayed onto an agar plate and incubated at 37 °C for 48 h (Veerapagu et al., 2013; Sagar et al., 2013). Pseudomonas aeruginosa was examined for lipase production.

2.2 Screening Lipase Producing Bacteria

A test for the detection of lipase-producing bacteria using a nutrient agar medium with olive oil (Bharathi and Rajalakshmi, 2019;Veerapagu et al., 2014; Astuti et al.,2019). The strains producing lipase are inoculated and identified on the diffusion plates for 48 hours at 37 ° C (Alhamdani and Alkabbi, 2016).

2.3 Lipase assay

The activities of the enzyme lipase were measured by spectrophotometry by a method proposed by Hong et al. (2003). The reaction mixture consisting of 1 ml of fat emulsion, 1 ml of crude enzyme extract and 1 ml of ammonium chloride was incubated at 37 °C for half an hour then the absorbance was measured at 440 nm. The unit of activity (U) was defined as the amount of enzyme that releases micromoles of fatty acids under the conditions of estimation (Takaç and Şengel, 2009).

2.4 Protein determination

Bradford method was used to determine the amount of protein in the determination of a specific activity (Lafferty et al., 2013).

2.5 Determination of optimal culture media for P.aeruginosa

Tests were conducted to select the optimum conditions for lipase enzyme production which included selection of temperature (20-42°C) and optimum pH selection (4-9).

2.5.1 Effect Carbon source

Lactose and glucose were used in different proportions as a carbon source to study its effect on lipase enzyme activity.

2.5.2 Effect Nitrogen source

Peptone and Tryptone were used as a nitrogen source to study its effect on lipase activity.

3. Results and discussion

Fifteen strains obtained from soil samples were isolated by serial dilution according to their morphology and appearance on nutrient agar plates (Mobarak-Qamsari et al., 2011). The isolates with the greatest potential for lipase production are selected based on the clear area of the nutrient agar plate as shown in Figure 1. The five bacterial strains (SP1, SP2, SP3, SP4, SP5) are

shown in table 1. Figure 1 shows a high density of clarity, while the other isolates did not show any area around the colonies.

Figure 1: screening lipase production from P.aeruginosa on nutrient agar plates at 37°C for 24h. The highest species capable of producing lipase enzyme was selected.

Table 1: Bacterial sequences capable of producing lipase enzyme

Strain	Activity
SP1	4
SP2	4
SP3	3.3
SP4	3
SP5	1.8

Using curve, bovine serum albumin was prepared with different concentrations. As shown in the table 2.

Table 2: Preparation of Bovine serum albumin concentrations

Tube number	Volume of stock solution (BSA) (mL)	Volume of distilled water (mL)	Protein (BSA) concentration (mg/mL)
1	0.0	1	0
2	0.1	0.9	0.1

3	0.2	0.8	0.2
4	0.3	0.4	0.3
5	0.4	0.6	0.4
6	0.5	0.5	0.5
7	0.6	0.4	0.6
8	0.7	0.3	0.7
9	0.8	0.2	0.8
10	0.9	0.1	0.9
11	1	0.0	1

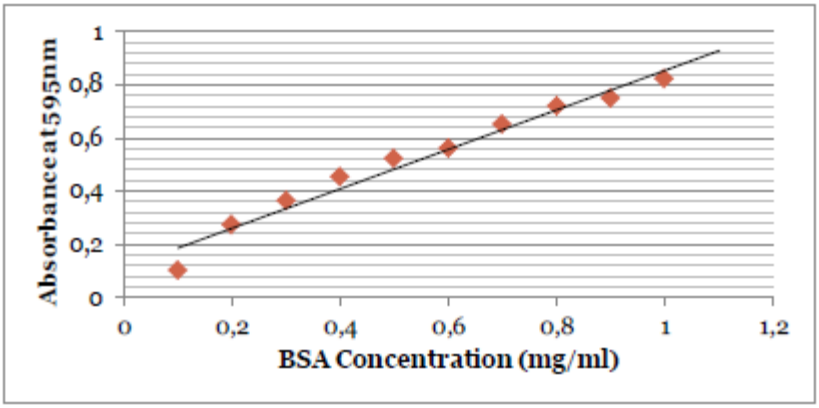

Figure2: Standard curve of BSA

3.1 pH Effect

Figure 3 shows the effect of pH on the lipase production at temperature of 37oC, and incubation period of 24 h. Different degrees of pH were selected to find out the optimum pH for lipase production, ranging from (4-9), where the lipase activity increases with increasing of pH from 4-7 then starts to decreasing with increasing of pH. The highest activity of lipase is obtained at pH 7. These results are agreement of results of Myers et al., 2018.

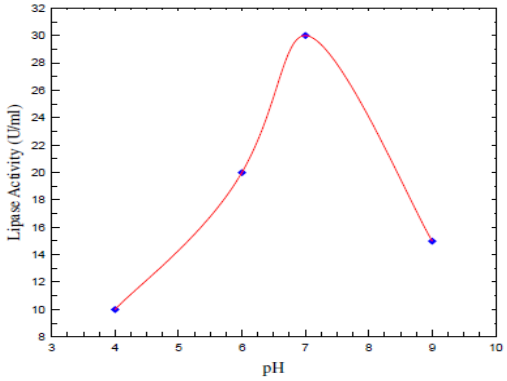

Figure 3: Effect of pH on the activity of lipase at temperature 37 °C and period incubation 24h.

3.2 Temperature Effect:

Figure 2 shows the effect of incubation temperature on the enzyme activity in culture medium, where the activity of enzyme increases with increasing of temperature up to 37oC where the highest activity of enzyme is obtained (30.1 U/ml), then the activity drops remarkably with temperature at 37oC and these results are agreement with results of Gswami and Sharma, (2017), and Baharum et al. (2003). This behaviour is due to that improving of temperature is lively for cell growth and production of enzyme. Higher temperature increases the rates of enzymatic reactions in cells until they reach an optimum level. Besides the optimum temperature, the enzyme is inactivated due to protein denaturation which slows down the metabolism of cells and affects cell growth and productivity (Abdel-Hamied et al.,2017)

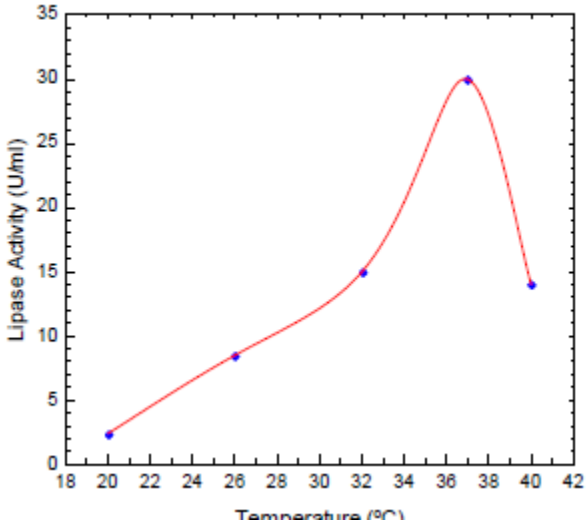

Figure4: Effect of temperature on the activity of lipase at pH7 and period incubation 24h.

3.2 Effect of Carbon Source:

Figure 5 shows the effect of carbon source on the lipase activity at temperature of 37oC, pH 7, and time incubation 24 h. From figure 5, it can be seen that the PM gives us the highest activity of lipase (30 U/ml) while the activity with glucose and lactose are 29 and 27 U/ml respectively.

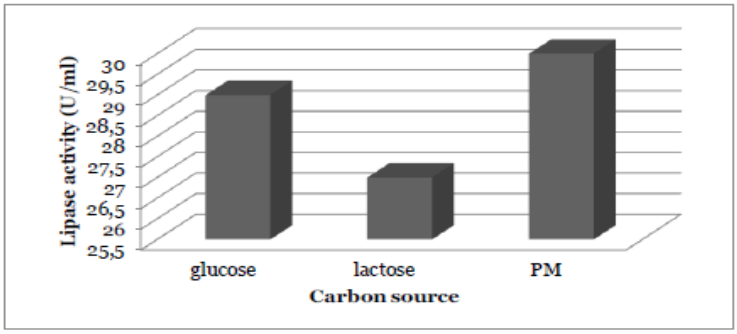

Figure 5: Effect Carbon source on lipase activity at temperature 37°C, PH 7 and time incubation 24h.

3.3 Effect of Nitrogen Source:

Figure 4 shows the effect of nitrogen source on the lipase activity at temperature of 37oC, pH 7, and time incubation 24 h. From figure 4, it can be seen that the peptone gives us the highest activity of lipase (37 U/ml) compare with tryptone and PM. This is agreement with that results of **Sztajer H, Maliszewska I.** (1989).

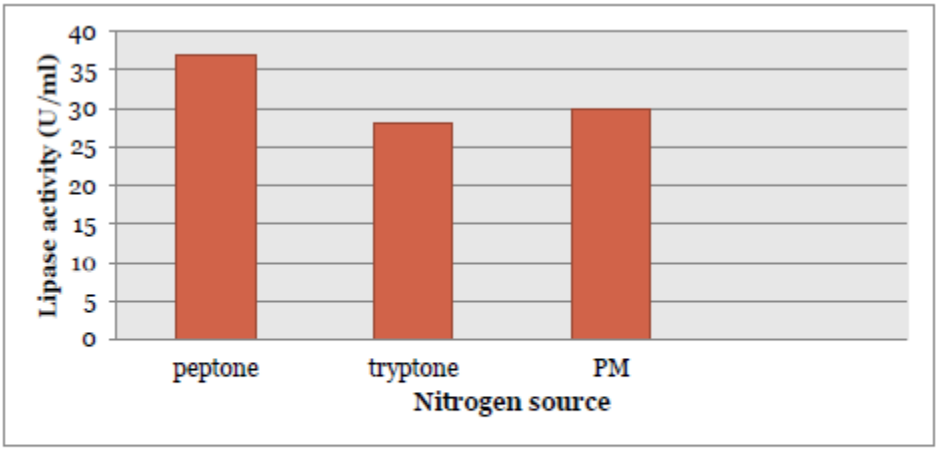

Figure 6: Effect Nitrogen source on lipase activity at temperature 37°C, PH 7 and time incubation 24h.

4. Conclusion

It can be concluded that the soil contaminated with petroleum oils is suitable for the growth of Pseudomonas aeruginosa bacteria. It is also possible to choose a better environment for the growth of bacteria. The maximum activity of the lipase enzyme produced from *P.aeruginosa* was shown after 48 hours of deliberate incubation at 37 °C. The current study provides benefit from improving culture conditions such as temperature and pH . This study also shows the spread of *P. aeruginosa* in polluted places.

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