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Optimization and Characterization Growth Conditions of Lipase-Producing Bacteria from Oil Contaminated Soil and Sewage

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Abstract: Ten samples of oil contaminated sewage and soil were collected from generators locations and car repair locations in Baghdad, Iraq for isolation of lipase- producing bacteria. Enriched selective media technique was used for isolation of lipase- producing bacteria in which different inducers (olive oil and Tween 80) used for enzyme production. The effect of different pH and temperatures were investigated. Fifteen lipase - producing bacteria had been isolated; *Pseudomonas aeruginosa* (6 isolates), *Escherichia coli* (5 isolates), *Klebsiella pneumonia* (3 isolates) and *Staphylococcus epidermidis* (1 isolate). Olive oil was found to be the more suitable source of carbon and lipid than Tween 80 for lipase-producing bacteria. All lipase- producing bacteria were capable to grow at different pH except of *E.coli* and *S. epidermidis* which could not grow at pH 9. All lipase- producing bacteria were capable to grow at different temperature except of *E.coli*, *K.pneumoniae* and *S. epidermidis* could not grow at 4^{o}C. *P. aeruginosa* was considered the best lipase- producing bacteria which could handle the different conditions, and the optimum conditions for growth and enzyme production was at 37 ^{o}C, pH 7 and incubation period 48hrs. or more.

Keywords: lipase- producing bacteria, oil contaminated sewage, oiled soil.

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Introduction

Lipases are glycerol ester hydrolases (EC: 3.1.1.3), which hydrolyzed ester bonds of glycerides at water-oil interface (1). During hydrolysis, lipases dredge acyl group from glycerides resulting in formation of lipase-acyl complex, and then transports its acyl group to OH group of water (2). Lipases (EC: 3.1.1.3) hydrolyze triglycerides to fatty acids and glycerol. They are greatly disseminated in nature. Although lipases have been got in many animals, plants, bacteria, yeasts and fungi, lipases from microorganisms are most important because of their potential uses in different industries like food, dairy, pharmaceutical detergents, textile, biodiesel and cosmetic industries, and in synthesis of fine chemicals, agrochemicals and polymeric materials (3,4).

Although many of bacterial lipases are obtainable, only a little are commercially developed as wild or recombinant strains (5). Lipase from *Pseudomonas* are greatly used for different biotechnological applications (5,6). *Pseudomonas mendocina* (lumafast), and *Pseudomonas glumae* are being available commercially in detergent combinations, but most of them have supreme-temperature optima (5).

Lipase from *Pseudomonas* is distributed into three groups according to their molecular mass and biochemical characteristics with the following typical enzymes (7). *Pseudomonas aeruginosa* lipase for group I, *Pseudomonas cepacia* and *P. glumae* which have been renamed *Burkholderia cepacia* and *Burkholderia glumae* for group II and *Pseudomonas fluorescens* lipase for group III. *P. aeruginosa* produces an extracellular lipase, which has been extracted from culture media of either industrial fermentation of wild type *P. aeruginosa* or PAC1R or an overexpressing *P. aeruginosa* strain holding a plasmid with the cloned lipase gene (8). This study aimed to: Isolation of lipase-producing bacteria from oil contaminated soil and sewage, optimization growth on selective media, and study the effect of pH and temperature on lipase-producing bacteria.

Materials and Methods

Sample Collection

Ten samples of oil contaminated sewage and soil were collected from generators locations and car repair locations in Baghdad, Iraq using sterile containers until samples transported to the laboratory (9).

Isolation of Lipase-Producing Bacteria

The isolation process was performed by serial dilution of samples on olive oil agar (modified) plates. The composition of olive oil agar medium is (per liter) 5g peptone, 3g yeast extract, 10ml olive oil and 15g agar. Culture plates were incubated at 30 ̊C for 48hrs. Colonies showing opaque zone around them were indicated for lipase –producing bacteria (10).

Identification of Lipase-Producing Bacteria

The isolates were identified depending on microscopical and colonial characteristics, biochemical properties and VITEK-2 system at AL-Numan Hospital in Baghdad.

Qualitative Screening for Lipase-Producing Bacteria

Two media were used for qualitative screening of lipase – producing bacteria in which two different substrates were used which acted as inducers in production of lipase. The composition of the first medium (Rhan agar medium) is (per liter) K_2HPO_4 5g, $(NH_4)PO_4$ 5g, $MgSO_4.7H_2O$ 5g, $CaCl_2.6H_2O$ 1g, $FeCl_3.6H_2O$ 1g, Agar-Agar 15g, Olive oil 10ml and Tween 80 (2-5) drops as emulsifier. All components were dissolved in one liter of distilled water and pH was adjusted to 7. The mixture was autoclaved at 121 ^{o}C for 15 min. This medium was used for the screening the capability of bacteria to produce lipase. Colonies showing opaque zone around them after 48hrs. at 37 ^{o}C were indicated for lipase –producing bacteria (9).

The composition of the second medium (Tween 80 medium) is (per liter) peptone 10g, NaCl 5g, $CaCl_2$ 0.1g, agar 20g and Tween 80 10ml.All components were dissolved in one liter of distilled water and pH was adjusted to 7.4. The mixture was autoclaved at 121^{o}C for 15 min. This medium was used for the screening the capability of bacteria to produce lipase. Colonies showing opaque zone around them after 24-48hrs. at 37 ^{o}C were indicated for lipase –producing bacteria (11).

Optimization Growth of Lipase-Producing Bacteria

Tween 80 medium was adjusted to different pH 4, 7 and 9 using 0.1 N HCl and 0.1 N NaOH and isolates were inoculated to check the pH range that they could grow in and incubated at 37 ^{o}C for 48hrs. To test effect of temperature; culture media were incubated at 4 ^{o}C, 37 ^{o}C and 42 ^{o}C at pH 7 for 48hrs.

Results and Discussion

Isolation and Identification of Lipase-Producing Bacteria

The selective medium technique enabled the isolation of bacteria from oil contaminated soil and sewage with lipolytic activity using olive oil agar medium (modified). The lipolytic bacteria were further studied and characterized by their microscopical and colonial morphology, biochemical and VITEK-2 system which indicated that the suspected bacteria were *P.aeruginosa* (6 isolates),*Escherichia coli*(5 isolates), *Klebsiella pneumoniae* (3 isolates) and *Staphylococcus epidermidis* (1 isolate).

Olive Agar Medium for Primary Isolation

The basic factor of lipase activity has been carbon, since lipases are inducible enzymes (12) and thus mostly secreted in the existence of a lipid provenience like oil or any other inducer, such as triacylglycerols, fatty acids, hydrolysable esters, tweens, bile salts and glycerol (10).

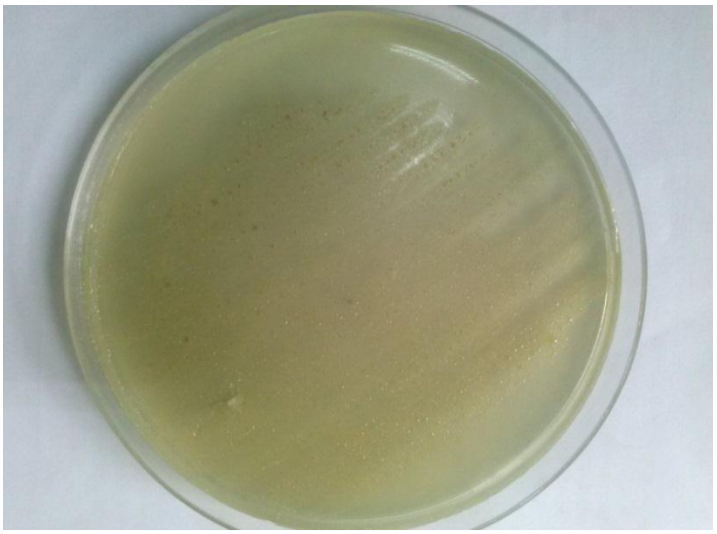
In this study, we used olive oil as a carbon and lipid source at a concentration 1% which made lipase-producing bacteria to grow successfully. The existence of oil in the culture medium promotes bacteria to produce lipase to consume the oil as a nutrient provenience. Olive oil was consumed as a carbon provenience by many lipase-producing bacteria like *Acinetobacter radioresistens*, *Bacillus* sp., *Geobacillus* sp., *Pseudomonas putida*, *P. mendocina* and *P. aeruginosa* (13,14). In addition to carbon provenience we used peptone and yeast extract as a nitrogen provenience, the type of nitrogen provenience in the culture medium also affected lipase secretion. In general, microorganisms produce high yields of lipase when organic nitrogen provenience are existed, like peptone and yeast extract, which utilized for lipase production by different thermophiles like Bacillus sp. and different Pseudomonads (15).

Qualitative Screening for Lipase-Producing Bacteria

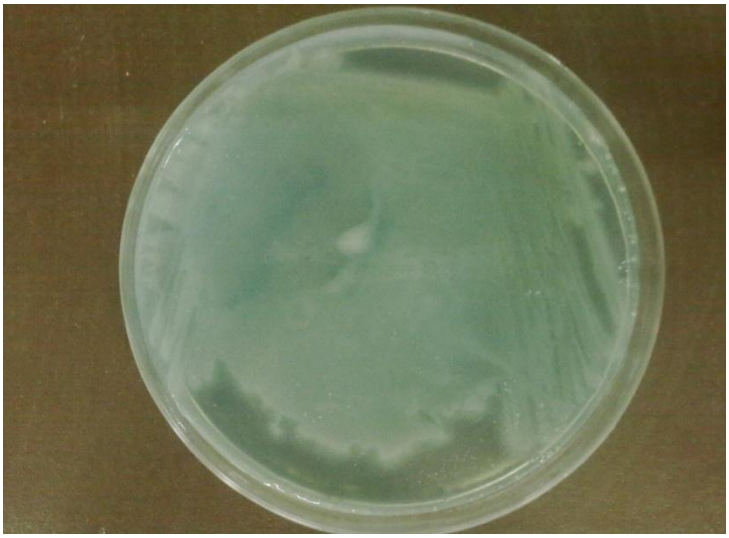
The fifteen isolates were grown in both selective media. Their growth indicated that they can use olive oil and Tween 80 as a carbon and lipid source and indicated their ability to produce lipase. Evaluation of lipase producing - bacteria based on existence of black

sediment in and around the colonies as shown in (Figure 1).

Olive oil was found to be the most suitable source, as shown in figure (1) the colonies on Rhan agar medium were more regular and there was black sediment after 48 hrs., while the growth was irregular but heavier and there was no black sediment observed on Tween 80 agar medium.



(a)



(b)

Figure (1) : *Pseudomonas aeruginosa* on (a) Rhan agar medium (b) Tween 80 medium

Lipase production study on Tween 80 agar medium observed a 75% decrease in the production of lipase based on the results obtained by Zouaoui and Bouziane (8) and they indicated that olive oil was better inducer than Tween 80 under the test conditions. Most published data had shown that lipid carbon sources (especially natural oils) promote lipase production (16).The highest concentration of olive oil (1 %) used by Ghafil and Hassan (9) gave the maximum activity of lipase (55 U/mg). The maximum activity of olive oil was illustrated at 2% (v/v) (0.46U/ml) (10).

Effect of pH and Temperature on Lipase-Producing Bacteria

Effect of pH and temperature on lipase-producing bacteria illustrated in table (1).

Table (1): Effect of pH and temperature on lipase-producing bacteria

Bacterial isolates		*Pseudomonas aeruginosa*	*Escherichia coli*	*Klebsiella pneumoniae*	*Staphylococcus epidermidis*
pH	4	+	+	+	+
	7	+	+	+	+
	9	+	-	+	-
Temperature	4°C	+	-	-	-
	37 °C	+	+	+	+
	42 °C	+	+	+	+

As shown in table (1), all lipase-producing bacteria were capable to grow at different pH except of *E.coli* and *S. epidermidis* which could not grow at pH 9. All lipase- producing bacteria were capable to grow at different temperature except of *E.coli*, *K.pneumoniae* and *S. epidermidis* could not grow at 4°C. *P. aeruginosa* was considered the best lipase- producing bacteria which could handle the different conditions.

The culture media pH is one of the greatest essential environmental parameters affecting both growth and lipase secretion. The results showed by Zouaoui and Bouziane (8) revealed that *P. aeruginosa* was able to grow at pH range from 6 to 8 and reached lipase maximum activity 38.5U/ml at pH 7. Another study revealed that the enzyme was most active at pH 6 and 9 (10). Growing activity at pH 7-9 can described as an alkalophilic enzyme but high lipase activity at pH 6 can be consider the enzyme compliant at acidic pH condition (10). Generally, bacterial lipases are stable in a wide range of pH from 4 to 11 (17).

P. aeruginosa can yield lipase at a broad range of temperature from 30 to 50 °C but it preferred moderate temperatures and can handle above 30 °C up to 50 °C. This characteristic helps *P. aeruginosa* to survive and yield their enzymes in variety environmental conditions and help in degradation of lipids (9).

Conclusions and Recommendations

Olive oil is a better inducer than Tween 80 for lipase -producing bacteria growth. *P.aeruginosa* is the best bacteria that grow at different environmental conditions and the optimum conditions for growth are at 37 ^{o}C, pH 7 and incubation period 48hrs. or more. So, our recommendations are extraction and purification of the enzyme and study the effects of different environmental conditions on its stability and activity. Since bacterial lipases especially from *P.aeruginosa*is widely used in industrial applications, we recommended to use it as a natural alternative material of chemical materials in variety of industries as detergents.

References

1- Garlapati, V. K.; Vundavilli, P.R. and Banerjee, R. (2010). Evaluation of lipase production by genetic algorithm and particle swarm optimization and their comparative study. Appl. Biochem. Biotechnol., 162(5):1350-61.

2- Ramani, K.; Kennedy, L.J.; Ramakrishnan, M. and Sekaran, G. (2010). Purification, characterization and application of acidic lipase from *Pseudomonas gessardii* using beef tallow as a substrate for fats and oil hydrolysis. *Process Biochem*., 45:1683-91.

3- Saxena, R.K.; Ghosh, P.K.; Gupta, R.; Davinson, W.S.; Bradoo, S. and Gulati, R. (1999). Potential biocatalysis and future industry. *Curr.Sci.*, 77:110-15.

4- Jaeger, K.E. and Eggert, T. (2002). Lipases for biotechnology. *Curr.Opin.Biotechnol*., 13:390-97.

5- Jaeger, K.E.; Ransac, S.; Dijkstra, B.W.; Colson, CH.; Heuvel,M. and Misset,D. (1994). Bacterial lipases. *FEMS Microbiol.Rev.*, 15:29-63.

6- Pandey, A.,; Benjamin, S.; Soccol, C. R., ; Nigam, P.; Krieger, N. and Soccol, V.T. (1999). The realm of microbial lipases in biotechnology. *Biotechnol. Appl. Biochem*., 29:119-31.

7- Bisht, D.; Yadav, S.K. and Darmwal, N.S. (2012). Enhanced production of extracellular alkaline lipase by an improved strain of *Pseudomonas aeruginosa* MTCC 10,055. *Am. J. Appl. Sc.*, 9:158-67.

8- Zouaoui, B. and Bouziane, A. (2012). Production, optimization and characterization of the lipase from *Pseudomonas aeruginosa .Rom. Biotech. Lett.*, 17(2):7187-93.

9- Ghafil, J.A. and Hassan, SH.S. (2014). Effect of cultural conditions on lipase production *Pseudomonas aeruginosa* isolated from Iraqi soil. World J. Experm. Biosci., 2(1):13-18.

10- Mobarak-Qamsari, E.; Kasra-Kermanshahi, R. and Moosavi-Nejad, Z. (2011). Isolation and identification of a novel, lipase-producing bacterium, *Pseudomonas aeruginosa*KM110. *Iran. J. Microbiol.*, 3(2):92-98.

11- EL-Sanousi, S.M.; El-Saraj, S.A. and Mohamed, S.A. (1987). Properties of *Serratia marscens* isolated from disease honeybee (*Apismelifera*) larvae. *J.Gener. Microbiol*., 133:215-19.

12- Lotti, M.; Monticelli, S.; Montesinos, J.L.; Brocca, S.; Valero, F. and Lafuente, J. (1998). Physiological control on the expression and secretion of *Candida rugosa*lipase. *Chem.PhysLipids*, 93:143-48.

13- Sugihara, A.; Tani, T. and Tominaga, Y. (1991). Purification and characterization of a novel thermostable lipase from *Bacillus sp*. *J. Biochem.*, 109: 211-15.

14- Narasimha, G.; Praveen, A. and Subramanyamm, D. (2011). Production and optimization of lipase enzyme by *Pseudomonas*sps. *Biotechnol.*, 5: 36-42.

15- Sharma, R.; Soni, S.K.; Vohra, R.M.; Jolly, R.S.; Gupta, L.K. and Gupta, J.K. (2002). Production of extracellular alkaline lipase from a *Bacillus sp*. RSJ1 and its application in ester hydrolysis. *Ind.J. Microbiol.* 42: 49-54.

16- He, Y.Q. and Tan, T.W. (2006). Use of response surface methodology to optimize culture medium for production of lipase with *Candida sp*. *J. Mol. Catal*. B-Enzym., 43:99-125.

17- Gupta, R.; Gupta, N. and Rathi, P. (2004). Bacterial lipases: an overview 4. of production, purification and biotechnological properties. *Appl. Microbiol. Biotechnol.*, 64:763-81.