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Optimization of the Cultural Conditions to Enhance Lipase Production by Environmental Bacteria

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Abstract

The production of lipase enzymes has played an essential role in biotechnology since they are used in various environmental applications, including oil pollution treatment. The present study aims to increase the production of lipase enzymes by optimizing nutritional and environmental factors from bacteria isolated from the soil and water from different sites in Basrah province. Seven bacterial isolates out of the fifty that showed positive results in the primary screening exhibited the highest activity in the secondary screening. These isolates belong to the species including *Bacillus subtilis* strain QD517, *Bacillus velezensis* strain Bac104, *Bacillus subtilis* strain PK9, *Bacillus cereus* strain RB1, *Enterobacter cloacae* strain YY-2, *Bacillus subtilis* strain SPA N1, and *Bacillus velezensis* strain FJ23. The values of lipase enzyme activities of the seven isolates under normal cultivation conditions were 49 U/ml, 28 U/ml, 24 U/ml, 23 U/ml, 23 U/ml, 18 U/ml, and 18 U/ml, respectively. Nutritional and environmental factors including carbon sources, nitrogen sources, phosphorus sources, temperature, pH, and incubation period were examined in this study to increase lipase enzyme activity. The optimal environmental and nutritional conditions were 37 °C, a pH of 7, sunflower oil as the best carbon source, peptone as the best nitrogen source, $NH_4H_2PO_4$ as the best phosphorus supply and four days as the optimum period of incubation. The maximum lipase activity values for the seven bacterial isolates under optimal conditions were 89 U/ml, 91 U/ml, 86 U/ml, 92 U/ml, 88 U/ml, 88 U/ml, and 89 U/ml, respectively for the bacterial isolates of *Bacillus velezensis* strain Bac104, *Bacillus subtilis* strain PK9, *Bacillus cereus* strain RB1, *Bacillus subtilis* strain QD517, *Bacillus subtilis* strain SPA N1, *Bacillus velezensis* strain FJ23, and *Enterobacter cloacae* strain YY-2. Optimizing culture conditions for the production of large quantities of lipase enzymes is important because lipase-producing bacteria have a wide range of applications.

Keywords: Lipase, Optimization, lipolytic bacteria, cultural conditions, lipase producing bacteria

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INTRODUCTION

Lipase is a member of the hydrolytic enzyme class that breaks down lipids into glycerol and fatty acids. In non-aqueous media, it can also reverse this reaction [1]. The utilization of plant and animal sources for lipase enzyme production is restricted by the presence of active or hazardous chemicals, such as phenolic compounds in plants and enzyme inhibitors in plant and animal tissues. Advanced biotechnological methods that utilize microorganisms producing enzymes like lipase enzyme are being sought as a replacement for conventional chemical procedures. This offers a financially feasible alternative that is also ecologically sustainable [2]. The lipase exhibits excellent pH stability and strong temperature resistance. Additionally, it is highly soluble in a wide range of metal ions and industrial organic solvents, and it demonstrates a high resistance to common commercial detergents and enzyme inhibitors [3]. After amylase and protease, lipase is the third most commonly utilized enzyme in biotechnology due to its wide range of catalytic capabilities and capacity to

function in diverse media [4]. Although lipase has been extracted and purified from bacterial, fungal, plant, and animal sources, but bacterial lipase has been shown to be a more stable and effective enzyme [5].

Lipase-producing bacteria can be isolated from various environments, including contaminated soil, dairy factories, vegetable oils, and industrial and agricultural waste [6]. The most significant bacterial genera known to produce the lipase enzyme include *Stutzerimonas stutzeri, Aeromonas caviae, Serratia marcescens, Bacillus sp., Halomonas sp.,* and *Stutzerimonas balerica* [7]. Typically, bacteria produce lipase enzyme in modest amounts under normal environmental conditions; however, this production can be enhanced by modifying environmental factors such as temperature, pH, sample size, and the availability of carbon and nitrogen sources [8, 9].

The potential metabolic activities of microorganisms are significantly influenced by the composition of the culture media. The optimization process typically involves varying one variable at a time. The factors influencing media composition include nutritional factors, such as carbon and nitrogen sources, as well as physicochemical factors, including incubation time, temperature, pH, and the presence of lipids as inducers, cofactors, and inhibitors different media exert varying stimulatory effects on lipase enzyme production [10]. A number of variables can impact lipase synthesis, including carbon source, nitrogen source, temperature and pH value. In all kinds of microbial sources, carbon supplies are crucial for lipase induction, while nitrogen is crucial for lipase synthesis. The increased synthesis of lipases from different microbial species has been significantly influenced by a variety of organic and inorganic nitrogen sources [11]. The ideal temperature is crucial for enzyme secretion. The higher concentration of lipase enzyme was affected by temperature, therefore, the production of high concentrations of the lipase enzyme are usually produced at temperatures ranging from 33 up to 38°C that boosts lipase synthesis. The lipase production is reduced at low temperatures, while enzyme function is similarly affected at higher temperatures. Bacterial lipases often have neutral or alkaline pH values, bacterial cells can produce more lipase if they grow in optimal pH [12]. The current study aims to evaluate several nutritional and environmental factors to enhance the activity of lipase-producing bacteria isolated from various contaminated soils and water in Basrah province, Iraq. These include carbon sources, nitrogen sources, phosphorus sources, temperature, and pH, all of which may improve lipase enzyme production by the isolated bacteria.

MATERIALS AND METHODS

Isolation and Characterization of Lipase Producing Bacteria

The bacteria that used in the present study were isolated and identified in a previous study [13]. The samples were collected from soil and water samples contaminated with oil from various areas in Basrah province. The 16S ribosomal DNA gene sequence analysis was used to identify the bacterial isolates. The 16S rDNA gene was amplified using universal primers 27F and 1492R. The PCR mixture was prepared in a total volume of 25 µL, comprising 12.5 µL of master mix (Promega, USA), 2 µL of DNA, 1 µL each of forward and reverse primers, and 9.5 µL of nuclease-free water. The PCR program included an initial denaturation step at 96 °C for 3 minutes, followed by 27 cycles of denaturation at 96 °C for 30 seconds, primer annealing at 56 °C for 25 seconds, and extension at 72 °C for 15 seconds. A final extension was performed at 72 °C for 10 minutes.

Screening and evaluation of lipase producing bacteria

The primary and secondary screening method to determine the ability of isolated bacteria to produce the lipase enzyme was conducted in a previous study [13] by streaking the bacteria on Tween 80 agar. Bacteria that produced a clear zone around their colonies were identified as positive isolates. The lipase-positive bacteria from the primary screening were subsequently purified on nutrient agar for secondary screening. Nutrient broth was used to activate the bacterial isolates. A 100 mL solution of modified mineral salt media (MSM) was prepared, containing NaCl (0.25 g/L), peptone (0.2 g/L), $NH_4H_2PO_4$ (0.1 g/L), $MgSO_4 \cdot 7H_2O$ (0.04 g/L), $CaCl_2 \cdot 2H_2O$ (0.04 g/L), Tween (1-2 drops), and olive oil (2 mL), and mixed with 2.5 mL of activated bacteria. This mixture was then incubated for three days [14]. Lipase activity was estimated using a mixture of 2 ml of phosphate buffer (pH 10), prepared by dissolving 1 g of phosphate buffer in 100 ml of distilled water. This was combined with 4 mL of olive oil, 2.5 mL of distilled water, 0.5 mL of $CaCl_2$ (2.19 g of $CaCl_2$ in 100 mL of distilled water), and 1 mL of diluted lipase enzyme. The mixture was incubated for fifteen minutes at 27 °C in a shaking incubator. Following incubation, two to three drops of phenolphthalein (0.5 g in 50 mL of ethanol) were added to 1 mL of 96% ethanol. NaOH (0.2 g in 100 mL of distilled water) was then added gradually until the mixture turned bright pink, indicating the endpoint of the titration. A control sample was prepared without the diluted lipase enzyme, and the reaction was halted by adding NaOH [15]. The amounts of fatty acids

liberated in each sample was calculated based on the volume of NaOH used to reach the titration endpoint by following equation:

$$\text{fatty acid/ subsample (U)} = \frac{(Vs - Vb) \times N \times 1000}{(5\ mL)} \quad (1)$$

while U is µmol of fatty acid released/ml; Vs is ml NaOH for sample; Vb ml NaOH for blank; and N is the normality of the NaOH titrant used.

Enhancing the Activity of the Lipase Enzyme

Experiments were conducted to determine how different cultural conditions such as sources of carbon, nitrogen, and phosphorus, as well as pH, temperature, and incubation period affect the growth and lipase production of the selected bacteria. The aim was to improve and enhance the activity of the lipase enzyme.

Effect of carbon sources

The optimal carbon source for enhancing lipase enzyme production was determined by testing various sources, including glucose, sunflower oil, and starch. Each carbon source was added to MSM individually at a mass of 2 g or 2 mL, and the cultures were incubated at 37°C and pH 7 for 72 hours [16].

Effect of nitrogen sources

The optimal nitrogen source for enhancing lipase enzyme production was determined by testing several sources, including yeast extract and $NaNO_3$. Each nitrogen source was individually added to MSM at a mass of 0.2 g and the cultures were incubated at 37°C and pH 7 for 72 hours [17].

Effect of phosphorus sources

To enhance the production of the lipase enzyme, two phosphorus sources including KH_2PO_4 and $Ca(H_2PO_4)_2$ were employed. They were separately added to MSM at a mass of 0.1% and incubated at 37°C and pH 7 for 72 hours.

Effect of temperature on lipase production

The culture media were incubated for 72 hours at three different temperatures (27, 37, and 40 °C) maintaining a pH of 7 to determine the optimal temperature for maximizing lipase enzyme synthesis [18].

Effect of pH on lipase production

The culture media were carried out at three pH values (5.5, 7, and 8.5) in order to determine the optimal pH for producing lipase enzyme. The pH was adjusted by using either 0.1 N HCl or 0.1 N NaOH. The cultures were then incubated at 37°C for 72 hours [19].

Effect of incubation period

To determine the optimal incubation period for lipase enzyme production, we evaluated several periods including 2, 3, 4, and 5 days. The cultures were incubated at 37°C and a pH of 7 [2].

RESULTS

Isolation and Characterization of Lipase Producing Bacteria

Fifty-two bacterial isolates have been isolated from soils and water containing oil collected from five sites in the Basrah province. The isolates were identified based on amplification by the PCR technique and sequence analysis of the 16S rDNA gene of the bacterial species. The most frequently recorded species in this study belong to the genera *Bacillus, Brevibacillus and Lysinibacillus*, while the most frequently distributed species in the habitats was *Bacillus subtilis*.

Screening and evaluation of lipase producing bacteria

The tween agar medium has been used for the primary screening of lipase enzyme production. Fifty bacterial isolates exhibited positive results in the primary screening, as they formed clear zones around their colonies. The isolates that showed positive results in the primary screening were subjected to secondary screening to investigate their ability to produce the lipase enzyme by using MSM, in which olive oil is utilized as the sole source of carbon and peptone is sole source of nitrogen as shown in **Figure 1**. The results of the secondary screening of lipase producing bacteria showed that the best seven bacterial isolates belong to *Bacillus subtilis strain QD517, Bacillus velezensis* strain Bac104, *Bacillus subtilis strain PK9, Bacillus cereus strain RB1, Enterobacter cloacae strain YY-2, Bacillus subtilis strain SPA N1 and Bacillus velezensis strain FJ23* with values of lipase activity of 49 U/ml, 28 U/ml, 24 U/ml, 23 U/ml ,23 U/ml ,18 U/ml and 18 U/ml respectively.

Figure 1. Flasks containing MSM with bacterial isolate to produce lipase enzyme.

Enhancing Activity of Lipase Enzyme

Effect of carbon sources

Different carbon sources have been investigated to produce the lipase enzyme. The results exhibited that sunflower oil was the best carbon source for lipase enzyme production by *Bacillus subtilis* strain QD517, *Enterobacter cloacae* strain YY-2, *Bacillus subtilis* strain SPA N1, *Bacillus subtilis* strain PK9, *Bacillus cereus* strain RB1, and *Bacillus velezensis* strain FJ23 and with lipase activities of 55, 32, 28, 26, 26, and 23 U/ml respectively as shown in **Table 1**. Olive oil and glucose were good sources for production of lipase enzyme by all seven selected bacterial strains. Starch was the poorest source of carbon for all bacteria except *Bacillus subtilis* strain QD517 and *Bacillus velezensis* strain FJ23 with lipase activities of 19 U/ml and 21 U/ml.

Table 1. The effect of different carbon sources on lipase enzyme activity

Isolates codes	The bacterial isolates	Lipase activity (U/ml)			
		olive oil	Sunflower oil	Glucose	Starch
A1	*Bacillus velezensis* strain Bac104	28	27	16	4
A3	*Bacillus subtilis strain PK9*	24	26	17	4
A4	*Bacillus cereus strain RB1*	23	26	18	8
A5	*Bacillus subtilis strain QD517*	49	55	18	19
C8	*Bacillus subtilis strain SPA N1*	18	28	17	4
D5	*Bacillus velezensis strain FJ23*	18	23	20	21
J3	*Enterobacter cloacae strain YY-2*	23	32	16	17

Effect of nitrogen sources

The results revealed that peptone was the best nitrogen source for lipase enzyme production with lipase activities of 49 U/ml, 28 U/ml, 24 U/ml, 23 U/ml ,23 U/ml ,18 U/ml and 18 U/ml for *Bacillus subtilis* strain QD517, *Bacillus velezensis* strain Bac104, *Bacillus subtilis* strain PK9, *Bacillus cereus* strain RB1, *Enterobacter cloacae* strain YY-2, *Bacillus subtilis* strain SPA N1 *and Bacillus velezensis* strain FJ23 respectively as shown in **Table 2**. In contrast, $NaNo_3$ was the poorest source of nitrogen for lipase production *Bacillus subtilis* strain PK9, *Bacillus velezensis* strain FJ23, *Enterobacter cloacae* strain YY-2, *Bacillus velezensis* strain Bac104 and *Bacillus cereus* strain RB1 and with value of activity 12, 10, 10, 8 and 7 U/ml respectively except by *Bacillus subtilis* strain QD517 and *Bacillus subtilis strain* SPA N1 were recorded the best lipase activity with $NaNO_3$ with 22 U/ml. Meanwhile, yeast extract was the poorest source of nitrogen for lipase enzyme production for *Bacillus subtilis* strain SPA N1with a value of 9 U/ml.

Table 2. The effect the different nitrogen sources on lipase activity

Isolates codes	The bacterial isolates	Lipase activity (U/ml)		
		Peptone	Yeast extract	$NaNO_3$
A1	*Bacillus velezensis* strain Bac104	28	20	8
A3	*Bacillus subtilis strain PK9*	24	16	12
A4	*Bacillus cereus strain RB1*	23	20	7
A5	*Bacillus subtilis strain QD517*	49	44	22
C8	*Bacillus subtilis strain SPA N1*	18	9	22
D5	*Bacillus velezensis strain FJ23*	18	16	10
J3	*Enterobacter cloacae strain YY-2*	23	18	10

Effect of phosphorus sources

Different phosphorus sources have been investigated to produce the lipase enzyme. The results exhibited that $NH_4H_2PO_4$ was the best phosphorus source for lipase enzyme production by *Bacillus subtilis* strain QD517, *Bacillus velezensis* strain Bac104, *Bacillus subtilis* strain PK9, *Bacillus cereus* strain RB1, *Enterobacter cloacae* strain YY-2, *Bacillus subtilis* strain SPA N1 and *Bacillus velezensis* strain FJ23 with values of lipase enzyme activities of 49, 28, 24, 23, 23, 18 and 18 U/ml respectively as shown in **Table 3**. KH_2PO_4 was the poorest source of phosphorus for all bacterial except *Bacillus subtilis strain QD517* and *Enterobacter cloacae* strain YY-2 with lipase enzyme activities of 40 and 16 U/ml.

Effect of temperature on lipase production

Various variables were evaluated in the current study to determine the best conditions for lipase

enzyme production by the seven best bacterial isolates. Different temperature values (27 ºC, 37 ºC and 40 ºC) were investigated in the production of lipase enzyme. The results showed that the optimum temperature for lipase production was 37 ºC with lipase activity values 86, 48, 44, 42, 36, 28 and 26 U/ml for *Bacillus subtilis* strain QD517, *Bacillus subtilis* strain PK9, *Bacillus velezensis* strain Bac104, *Bacillus cereus* strain RB1, *Enterobacter cloacae* strain YY-2, *Bacillus subtilis* strain SPA N1 and *Bacillus velezensis* strain FJ23 respectively as shown in **Table 4**.

Table 3. The effect of different Phosphorus sources on lipase enzyme activity

Isolates codes	The bacterial isolates	Lipase activity (U/ml)		
		$NH_4H_2PO_4$	KH_2PO_4	$Ca(H_2PO_4)_2$
A1	*Bacillus velezensis* strain Bac104	28	12	
A3	*Bacillus subtilis strain PK9*	24	9	16
A4	*Bacillus cereus strain RB1*	23	12	17
A5	*Bacillus subtilis strain QD517*	49	40	36
C8	*Bacillus subtilis strain SPA N1*	18	5	7
D5	*Bacillus velezensis strain FJ23*	18	13	14
J3	*Enterobacter cloacae strain YY-2*	23	16	10

Table 4. The effect of different temperatures on lipase enzyme activity

Isolates codes	The bacterial isolates	Lipase activity (U/ml)		
		27 ºC	37 ºC	40 ºC
A1	*Bacillus velezensis* strain Bac104	10	44	9
A3	*Bacillus subtilis strain PK9*	12	48	12
A4	*Bacillus cereus strain RB1*	8	42	13
A5	*Bacillus subtilis strain QD517*	8	86	11
C8	*Bacillus subtilis strain SPA N1*	9	28	11
D5	*Bacillus velezensis strain FJ23*	10	26	12
J3	*Enterobacter cloacae strain YY-2*	8	36	13

Effect of pH on lipase production

The values of pH including (5.5, 7 and 8) were investigated for lipase enzyme production. The best value of pH was 7 for bacterial *Bacillus subtilis* strain QD517, *Bacillus velezensis* strain Bac104, *Bacillus subtilis* strain PK9, *Bacillus cereus* strain RB1, *Enterobacter cloacae* strain YY-2, *Bacillus subtilis* strain SPA N1 *and Bacillus velezensis* strain FJ23 with lipase enzyme activities of 49, 28, 24, 23, 23, 18 and 18 U/ml respectively. The lowest value of pH was 8 for the seven selected bacterial isolates as shown in **Table 5.**

Effect of incubation period

The optimal lipase enzyme production was determined by measuring the seven-day incubation periods for each of the seven samples. The results exhibited that the samples responded best to an incubation period of four days by *Bacillus subtilis* strain QD517, *Bacillus subtilis* strain PK9, *Bacillus velezensis* strain Bac104, *Enterobacter cloacae* strain YY-2, *Bacillus subtilis* strain SPA *N1, Bacillus velezensis* strain FJ23 and *Bacillus cereus* strain RB1 with lipase enzyme activities of 92, 91, 89, 89, 88, 88 and 86 U/ml respectively as shown in **Table 6.** The worst incubation period was one day for the same seven bacteria with lipase enzyme production values of 7.5, 8, 8.5, 8.5, 8.5, 9.5 and 11 U/ml by *Enterobacter cloacae* strain YY-2, *Bacillus velezensis* strain Bac104, *Bacillus cereus* strain RB1, *Bacillus subtilis* strain QD517, *Bacillus subtilis* strain SPA N1, *Bacillus subtilis* strain PK9 and *Bacillus velezensis* strain FJ23 respectively.

The Optimization of Culture Media Under Optimal Conditions

The maximum lipase activity for the seven selected bacterial isolates was observed after the parameters of the media components and culture conditions were optimized by selecting the best variables that improved lipase enzyme activity, as shown in **Table 7**. These variables included sunflower oil as a carbon source, peptone as a nitrogen source, $NH_4H_2PO_4$ as a phosphorus source, a temperature of 37 °C, a pH of 7, and four days of incubation period.

Table 5. The effect of different pH values on lipase enzyme activity

Isolates codes	The bacterial isolates	Lipase activity (U/ml)		
		pH 5.5	pH 7.0	pH 8.0
A1	*Bacillus velezensis* strain Bac104	11	28	10
A3	*Bacillus subtilis strain PK9*	8	24	10
A4	*Bacillus cereus strain RB1*	7	23	7
A5	*Bacillus subtilis strain QD517*	9	49	7
C8	*Bacillus subtilis strain SPA N1*	7.5	18	7
D5	*Bacillus velezensis strain FJ23*	11	18	7.5
J3	*Enterobacter cloacae strain YY-2*	9	23	8.5

Table 6. Effect of incubation period on lipase enzyme activity

Isolates codes	The bacterial isolates	Lipase activity (U/ml)					
		1 days	2 days	3 days	4 days	6 days	7 days
A1	*Bacillus velezensis* strain Bac104	8	15.5	22	89	82	78
A3	*Bacillus subtilis strain PK9*	9.5	17	26	91	82	80
A4	*Bacillus cereus strain RB1*	8.5	21.5	21	86	83	82
A5	*Bacillus subtilis strain QD517*	8.5	26	25	92	87	79
C8	*Bacillus subtilis strain SPA N1*	8.5	17	20.5	88	84	82
D5	*Bacillus velezensis strain FJ23*	11	25	21.5	88	84	81
J3	*Enterobacter cloacae strain YY-2*	7.5	19.5	20	89	82	80

Table 7. lipase enzyme activity (U) before and after the optimization

Isolates codes	The bacterial isolates	lipase activity (U/ml) before the optimization	lipase activity (U/ml) after the optimization
A1	*Bacillus velezensis* strain Bac104	28	89
A3	*Bacillus subtilis strain PK9*	24	91
A4	*Bacillus cereus strain RB1*	23	86
A5	*Bacillus subtilis strain QD517*	49	92
C8	*Bacillus subtilis strain SPA N1*	18	88
D5	*Bacillus velezensis strain FJ23*	18	88
J3	*Enterobacter cloacae strain YY-2*	23	89

DISCUSSION

Microbial enzymes tend to be more stable than their plant and animal counterparts, and their production is more convenient and safer, enhancing their importance for commercial applications. The oily environments of vegetable oil-processing factories, industrial waste sites, soil contaminated with oil, and diesel-fuel-polluted soil provide suitable habitats for lipase-producing bacteria [20]. The lipase enzyme has unique properties that help it interact between liquid and non-liquid media. It has been proven that it can be used in a wide range of substrates and is very stable under harsh conditions of temperature, pH, and organic solvents [17]. Generally, bacteria produce their lipase enzymes under natural environmental conditions in small quantities that can be increased by changing nutritional sources such as carbon and nitrogen sources and cultivation conditions such as temperature, pH, and inoculum size to find the most suitable variables to increase the growth of bacteria and the production of enzymes. Bioremediation, typically occurs in both aerobic (with oxygen) and anaerobic (without oxygen) conditions, depending on the specific contaminants and the microorganisms involved. While many contaminants are more readily degraded in the presence of oxygen (aerobic bioremediation), some contaminants are more effectively broken down under anaerobic conditions [8, 9].

Thongpoo [21] improved lipase production using optimum conditions, which included 0.5% yeast extract as a nitrogen source and 0.5% soybean oil as a carbon source at 200 rpm with an incubation period of 54 hours. The results indicated that the bacteria have the potential to be used in treating wastewater contaminated with fats. The study by Pham *et al.* [17] showed that the bacteria *Lysinibacillus* PL33 and *Lysinibacillus* PL35 exhibited high activity in producing lipase enzyme at temperatures of 35-40°C and at a pH of 6-7, reaching the highest production values of 480 units/ml and 420 units/ml respectively.

In the present study, the optimal temperature to produce lipase for the selected species was 37 ºC. The results of the current study agreed with a previous study conducted by Hasan *et al.* [22] which showed that the best production of lipase enzyme was at a temperature of 37 ºC. The lipase activity was shown to be most active at pH 7 for the seven bacterial species. The results of the current study were consistent with the results of a previous study conducted by Mazhar *et al.* [19], as the best production of lipase activity for the bacteria *bacillus amyloliquefaciens* was with the value of pH 7 and consistent with the results of [23], as the best production of lipase activity for the bacteria *Bacillus thuringiensis* was also at a pH value of 7.

It was found in the current study that organic nitrogen sources such as peptone and yeast extract significantly affect lipase enzyme production. Peptone was the best source of nitrogen to stimulate the production of lipase enzyme. Inorganic nitrogen sources ($NaNO_3$) had a small or inhibitory effect, and these results are consistent with a previous study performed by Mobarak-Qamsari *et al.* [14], which used olive oil and found that peptone was to be the most suitable substrate for maximum lipase enzyme production by *Pseudomonas aeruginosa.* Four sources of carbon were used in the current study, including glucose as monosaccharide, olive oil, sunflower oil and starch as polysaccharides. The results showed that sunflower oil was the best source of carbon in the production medium for the selected bacterial species, as it significantly increased lipase activity while starch had a small role. The varying effects of different sugars may be due to the nature of their chemical composition and the ease of carbon exploitation by bacteria as it is known that monosaccharides have a simple structure consisting of a single molecule while disaccharides consist of two molecules and disaccharides are less complex than polysaccharides. The study conducted by Al Mohaini *et al.* [24] showed that olive oil used as a carbon source, induced the highest lipase activity (11.0 U/mL) compared to sunflower oil (9.6 U/mL) and cooking oil waste (7.8 U/mL).

The best source of phosphorus in the current study was $NH_4H_2Po_4$ for the production of the lipase enzyme, while KH_2PO_4 was less effective. The difference in the use of phosphorus sources among bacteria is due to the enzymes secreted by bacteria and their ability to use any phosphorus source. This is not consistent with the study conducted by Iboyo *et al.* [25] which showed that KH_2PO_4 is the best source for the production of the lipase enzyme. Different incubation periods were studied, and it was found that an incubation period of 4 days was the best for lipase enzyme production This is consistent with the study conducted by Ikhwani *et al.* [26], which showed that the optimal bacterial production of *Bacillus flexus* InaCC-B486 occurs during an incubation period of 4 days.

CONCLUSION

Seven bacterial isolates exhibited the highest lipase activity among fifty lipolytic strains from Basrah's oil-contaminated sites. Optimization of nutritional (carbon: sunflower oil; nitrogen: peptone; phosphorus: $NH_4H_2PO_4$) and environmental factors (37°C, pH 7, 4-day incubation) significantly enhanced lipase yields by 2.1- to 4.9-fold, with activities reaching 89 U/ml (*Bacillus velezensis* Bac104), 91 U/ml (*Bacillus subtilis* PK9), 86 U/ml (*Bacillus cereus* RB1), 92 U/ml (*Bacillus subtilis* QD517), 88 U/ml (*Bacillus subtilis* SPA N1), 88 U/ml (*Bacillus velezensis* FJ23), and 89 U/ml (*Enterobacter cloacae* YY-2). This research provides a low-cost, scalable framework for lipase production in bioremediation, though future studies must validate industrial applicability through stability assays and statistical robustness testing.

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