

## Research Article

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# Purification and characterization of lipase produced by indigenously isolated bacteria

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### Abstract

Bacteria including *Bacillus* species often produce extracellular enzymes to degrade substances such as proteins, lipids, and starch. Glycerol and long-chain fatty acids are hydrolyzed by lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) to generate esters. Bacterial strains were isolated from rotten fruits and vegetables and cultured at 20 to 50 °C with different types of substrates (Olive oil, Mustard oil and Tributryne). Extracellular enzymes produced from bacteria with different physical parameters and also using different substrates. Only two strains, strain 1 and strain 2 show results when provided substrate Olive oil and Mustard oil at pH 7 and at temperature 30°C but Tributryne show result at pH 9 and at temperature 40°C. Addition of maltose and fructose increased the lipase activity to 28.53U/ mL and 30.17U/mL respectively. The activity enhanced to 50.83U/mL by the addition of yeast extract as nitrogen source. Addition of 60% ammonium sulfate results in maximum precipitation with activity 59.46 U/mL. The results concluded that isolated bacterial strains are good producers of lipase enzyme. The purified enzyme could be a good candidate for various industrial applications.

**Keywords:** Ammonium sulfate precipitation; *Bacillus* species; Lipase activity; Rotten fruits; Tributryne agar Plate

### Introduction

The earlier cultures can be used to check the enzyme-mediated processes. Now a days, around 4000 enzymes were discovered from which 200 are used in various industries. The source of most of the manufacturing enzymes are different bacterial species. Overall, few million dollars trades of enzymes per annum till 1960, but later enormously developed taken

placed in market. For the convenience of better development of biochemistry, many processes like fermentation recovery or producing many number of enzymes at reasonable cost. Similarly, the improvements in methods of using enzymes on request have significantly increasing. Besides, Enzymes can catalyze due to many diverse revolutions, in market enzymes number continues to proliferate. World

enzyme demand is fulfilled by 400 unimportant sellers and 12 main manufacturers. World supply of 60% enzymes manufactured is taken placed by Europe. By hydrolytic action of lipase, free fatty acids are produced enzyme on triacylglycerol. Lipolytic enzymes that carry out oil water interface and are soluble in water [1]. Many type of reactions like Trans esterification; acidolysis, aminolysis, hydrolysis and esterification all are occurred by these enzymes. These enzymes are extensively present in animals, plants and microorganisms. Microorganisms are the most fundamental and commercial source of getting these enzymes lipases. Microbial lipases are the most steady form of lipases according to. Flooded, fine disturbed and situation aerated [2, 3].

Report have also been given that lipases are produced both by synthetic and non-synthetic substrates. In 1997, Benjamin and Panday studied that maximum production of lipase is done by *Candida rugose* due to organisms containing on cultured medium urea (1%), maltose (5%), peptone (3%), and olive oil (10%). Submerged fermentation, with complex culture media that comprising compounds like corn steep liquor, egg yolk, and yeast extract, and soy meal produces fungal lipases [4]. Lipases can be easily produced and secreted by pathogens and highly selective, so it makes best enzymes, their crystal structure is well illustrated [5] the usage of lipases involves generation of cosmetics, production of biopolymers, utilize an herbicide and as a best solvent [6]. The main objective of the current study was the isolation of lipase producing bacterial strains from the rotten fruits. Improving lipase production and its partial purification.

## Materials and methods

### Sample collection

Rotten fruits and vegetables were collected from the markets of Rawalpindi and Islamabad during summer season for separating bacteria producing extracellular enzyme [7]. The samples were properly labeled and preserved.

### Culturing of bacteria

Normal saline solution (0.9% NaCl in distilled water) was used to dissolve the rotten parts of the samples and spread it on a medium comprising 2 % agar, 1 % yeast extract, 1% Peptone and 0.5% sodium chloride (Yeast Extract Peptone (YEP) medium) and then grown at temperature 50-70°C [8].

### Screening of bacteria

When the bacteria cultured, then they undergoes screening on Tributyrin Agar Plates (TBA) comprising 5g beef extract, 3g tributyrin, per liter of peptone, 10 mL and agar-agar, 20 g followed by incubation for 24-48 hrs at 37 °C for screening of lipase producing microorganisms.

### Innoculation of bacteria

For the preparation of inoculums, a loopful of cells from fresh slant was transferred into conical flask containing minimal media without agar (NH<sub>4</sub>Cl 2.0 g, NaCl 5.0 g, Na<sub>2</sub>HPO<sub>4</sub> 6.0 g, KH<sub>2</sub>PO<sub>4</sub> 3.0 g, MgSO<sub>4</sub> 0.1 g in 1L of distilled water) and placed at 37 °C in shaking incubator at 180 rpm for 24 h [9].

### Lipase assay

Lipase assay Lipase activity was assayed quantitatively by using para-nitro phenyl acetate (pNPA) as the substrate. One milliliter of methanol containing 0.2 mg pNPA and sixty milliliter of acetonitrile was mixed with 50 mM phosphate buffer containing 0.087g of K<sub>2</sub>H<sub>2</sub>PO<sub>4</sub> mg and 0.06 mg K<sub>2</sub>H<sub>2</sub>P0<sub>4</sub>. A total volume of 0.9 ml freshly prepared substrate solution in the ratio of 1:4:95 Acetonitrile (1 ml) with PNPA, Ethanol (4 ml) and Phosphate buffer (95 ml) was mixed with 0.3 ml enzyme solution. After 15 min incubation at 60°C in water bath, absorbance at 405 nm was measured against the blank. The amount of enzyme that liberates 1µg pNPA per minute is considered as one unit. Lipase activity was detected by measuring the hydrolysis of p-nitrophenyl esters [10].

### Effect of pH on Lipase activity

To check the effect of pH on lipase, activity was performed at 37 °C at pH ranging from 5 to 9 and using three different substrates.

pH was maintained by using Tris-HCl buffer having pH 7.0 to 9.0, acetate buffer pH 5.0 to 6.0, and phosphate buffer pH 6.0 to 8.0.

#### **Effect of temperature on Lipase activity**

Temperature effect the enzyme by increasing or decreasing its stability and its activity. Adjustment of temperature at its optimum point, results into higher and efficient production of enzymes. Both isolated strains were incubated from 20 °C to 50 °C, to find the effect of temperature on lipase.

#### **Effect of carbon source**

To check and enhance the lipase production, various readily available carbon sources other than media were used. Their impact on lipase activity was check through enzyme assay as described earlier. The carbon sources used were glucose, fructose, lactose, manifold, maltose, sucrose and starch [11].

#### **Effect of nitrogen source**

Various nitrogen sources were also added during the incubation of bacteria with media, to find their effect on bacterial growth and on lipase activity. Peptone, tryptone, beef extract, yeast extract, ammonium sulphate, ammonium chloride and ammonium nitrate were used [12].

#### **Ammonium sulphate purification**

Ammonium sulfate is a salt with high ionic strength, causes the precipitation of proteins at higher concentrations. For the partial purification of lipase, various concentrations of ammonium sulfate were added including 1g, 1.5g, 2g 2.5g 3g and 3.5g in 5 mL of crude extract produced under optimum conditions [13]. These concentrations gave final concentration of ammonium sulfate to 20, 30, 40, 50, 60 and 70% in the crude extract. Each of the test tubes were placed at 4 °C for overnight and next day were centrifuged at 10000 rpm for 10 minutes. Lipase activity assay was performed for each concentration of ammonium sulfate [14]. Both supernatant

and pellete were subjected to lipase assay, after suspended the pellete in phosphate buffer.

### **Results and discussion**

#### **Isolation of bacteria from rotten fruits and vegetables**

High amount of bacteria is present in contaminated sites with organic waste revealed by microbiological analysis of rotten fruits and vegetables. These types of samples were diluted in normal saline solution (0.9% NaCl in distilled water) and then plated in Nutrient agar to check the total viable count.

#### **Screening of Lipolytic bacterial strains**

For the study two potential isolates were selected, forming clear zone. The isolates were further cultured in Tri-butyryn broth at pH (5, 6, 7, 8 & 9) for the production of lipase for two days. It was reported by Pogaku *et al.* [15] that two days for *Staphylococcus* spp is better time for production of extracellular enzymes.

#### **Effect of Temperature on Lipase**

The results obtained during this research shows that lipase produce by isolated bacterial strains have good activity from 30 °C to 40 °C with varying substrate. The maximum activity was obtained at 30 °C with olive oil and Mustard oil as substrates and 40 °C with trybutryne as a substrate. These finding suggests the affinity of lipase vary towards different substrates with varying activities (Fig. 1-3).

*Apergillus oryzae* gives maximum lipase at 40°C using olive oil as a substrate and by using 4Nitropheyl palmitate as substrate its thesis completed and was reported by Toida *et al.* [16]. The maximum enzyme activity was taken by *Penicillium simplicissimum* at 60°C reported by Szejter *et al.* [17]. It was reported in later study by Kim *et al.* [18] that the lipase obtained from hemophilic strain was the majority vigorous at 40°C taken by *Bacillus stearothermophilus*.C10-C6 P-nitro phenyl esters used as substrates.

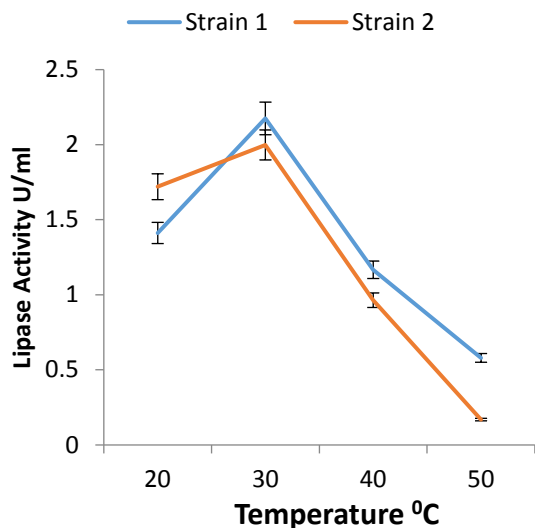


Figure 1. Temperature range of strain 1 and strain 2 with olive oil use as substrate

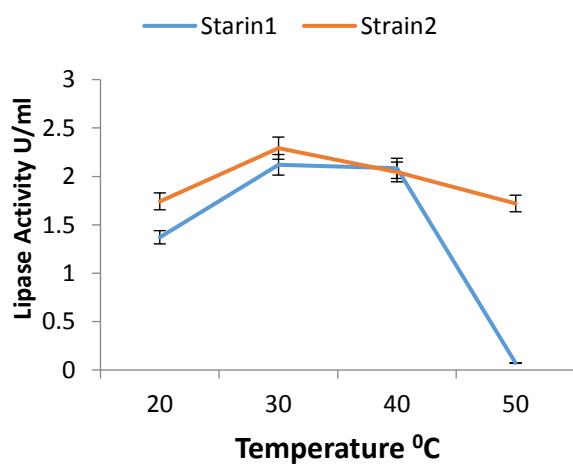


Figure 2. Temperature range of strain 1 and strain 2 with Mustard oil use as substrate

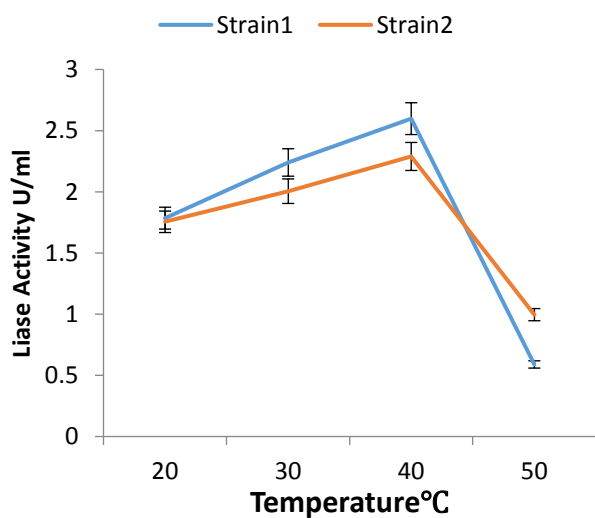


Figure 3. Temperature range of strain 1 and strain 2 with Trybutryne use as substrate

### Effect of pH on lipase

The lipase enzyme from the both strains having very good catalytic activity at neutral pH with different substrates. Lipase from both strain has maximum activity at pH 7 with mustard oil and olive oil as substrates. Only strain 2 has higher activity in alkaline range with trybutryne as a substrate (Fig. 4-6).

Motai *et al.* [19] studied that the *Torulopsis* pH is 6.5 by using different pH buffer at (PNP) ester and temperature is 40°C for optimum enzymes. On the other hand, Sztajer *et al.* [17] studied that *Penicillium Simplicissimum*, best possible pH is 5. Lee *et al.* [20] reported that best possible pH for lipase from 35 K strain is 8.0-9.0. For *Pseudomonas putida*, according to Lee *et al.* [20] study a pH 6.5 P-Nitro phenyl.

### Effect of carbon source

Some carbohydrates were added in the culture media to study their effect on lipase production and activity. Lipase activity increases by maltose and fructose, 28.53U/mL and 30.17U/ml respectively (Fig. 7).

Lipase main source was carbon observed by Lotti *et al.* [21].

### Effect of Nitrogen source

The results shows that yeast extract and peptone are the better nitrogen sources for bacteria. These enhance the division of bacterial cell and cause increases in the production of lipase. There was maximum activity (50.83 IU/mL) by the addition of yeast extract as additional nitrogen source (Fig. 8). Many others chemicals like ammonium chloride and peptone also effected the production of enzyme. On the other hand, lactose from *Cryptococcus* sp increase lipase synthesis reported by Kamini *et al.* [22].

### Partial purification

Ammonium sulfate causes the precipitation of lipase produced by the isolated bacterial strains. Its concentration increases in pellete and in the presence of 60 % ammonium sulfate maximum precipitation was achieved with increased activity (59.46/IU/ml/min) as shown in (Fig. 9).

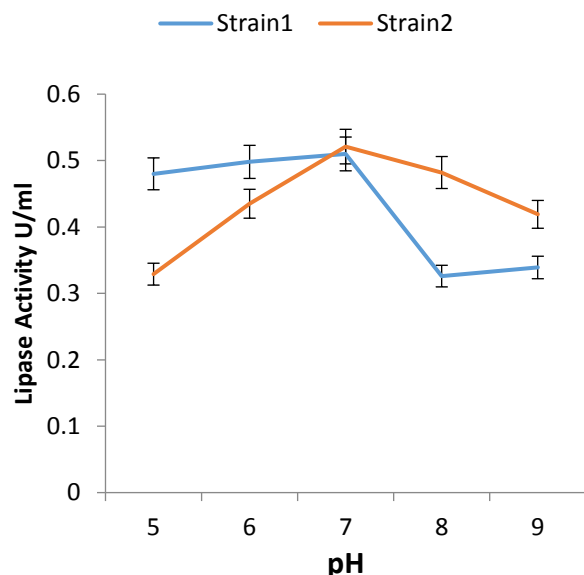


Figure 4. pH range of strain 1 and strain 2 with Olive Oil use as substrate

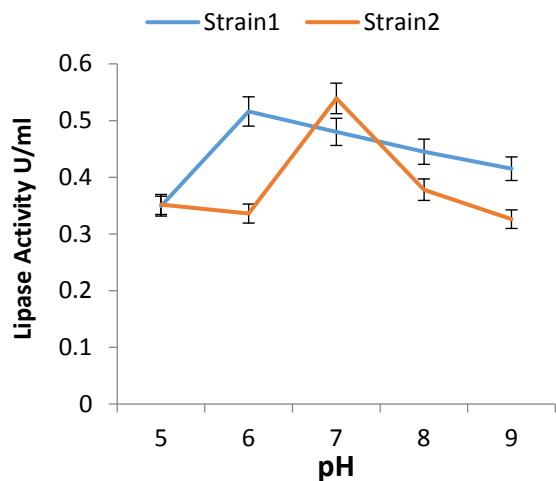


Figure 5. pH range of strain 1 and strain 2 with Mustard oil use as substrate

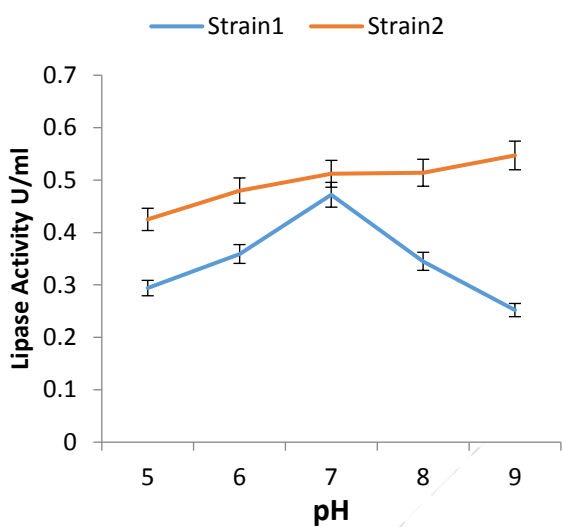


Figure 6. pH range of strain 1 and strain 2 with Trybutryne use as substrate

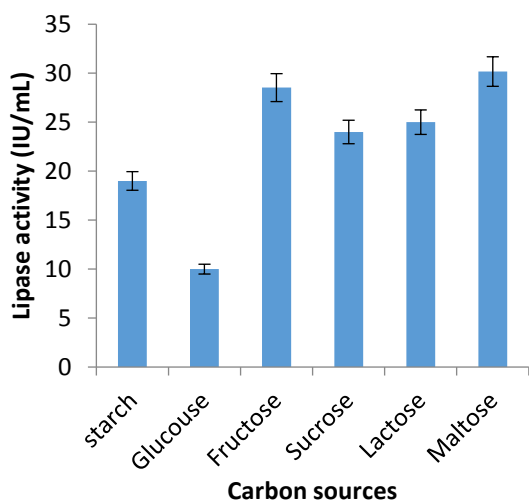
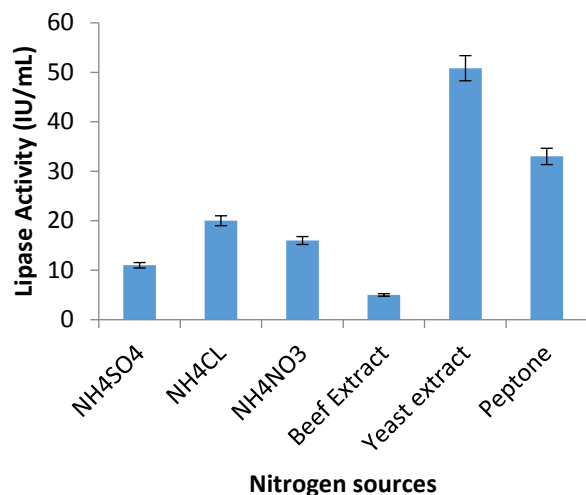
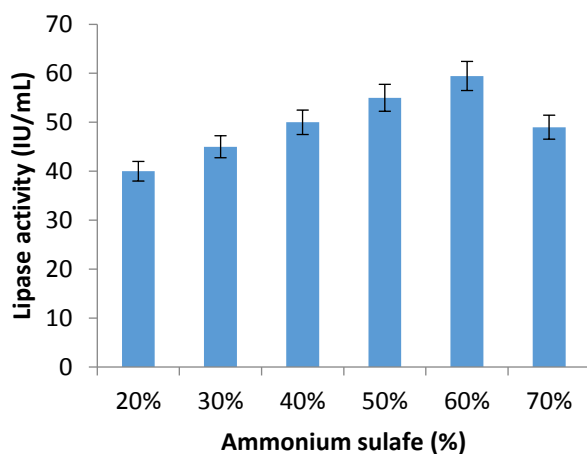


Figure 7. Effect of carbon sources on Enzyme production



**Figure 8. Effect of Nitrogen sources on production of enzymes**



**Figure 9. Purification of bacterial lipase by Ammonium Sulphate (%age)**

### Conclusion

Isolated bacterial strains produce extracellular lipases with good activities. The extracellular lipase shows thermophilic nature with increasing activity during optimizing parameters. Purification procedures enhance the activities indicating the significance of techniques. The bacterial extracellular lipases could be a good candidate for various industrial applications.

### Authors' contributions

Conceived and designed the experiments: MJ Asad & RT Mahmood, Performed the experiments: MA Sultan & S Nazir, Analyzed the data: MJ Asad & M Mahmood, Contributed materials/ analysis/ tools: MB Shah & S Nazir, Wrote the paper: MA Sultan & RT Mahmood.

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