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# Isolation, Characterization and Lipase Production Using Bacterial Isolates from Sewage Sample

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**ABSTRACT**

*Lipases are the hydrolytic enzymes produced by bacterial & fungal species. The present study includes the isolation of effective lipase producer from sewage sample and to standardize various parameters like pH, temperature and incubation period for effective lipase production. Isolated four bacterial species were characterized on the bases of morphological & biochemical characteristics. To check the effectivity, bacterial isolates were exposed to UV light from the distance of 1.5 feet for 30 & 60 seconds which increases the production compared to control set. pH 7.0 was found to be better for lipase production for LP-3 & LP-4 while for LP-1 & LP-2 effective pH was 7.5. 30 °C was effective temperature for LP-1 & LP-2 while 37 °C for LP-3 & LP-4. Incubation period of 48 h was suitable for LP-1, LP-2, LP-3 & 72 h for LP-4.*

**KEYWORDS : Lipase, Ultra violet light, pH, Temperature**

**Introduction:**

A large number of enzymes are being produced and sold for various purposes and the blooming industrial enzyme market is one of the major revenue generators in the life sciences-industry sector. Bacterial lipases may be intracellular, membrane-bound or extracellular. Strains which produce only intracellular lipase can grow only on glycerol and simple lipids but not on long chain triglycerides. Bacterial lipases are secreted in the unfolded state via the Sec-dependent pathway into the periplasmic space where folding takes place with the assistance of chaperone called lipase-specific foldase (Lif). The folded lipases are then transported out into the external medium by a transporter complex (Angkawidjaja and Kanaya, 2006; Buist *et al*., 2006).

Lipases are hydrolases, which act under aqueous conditions on the carboxyl ester bonds present in triacylglycerols to liberate fatty acids and glycerol. The natural substrates of lipases are long-chain triacylglycerols, which have very low solubility in water; and the reaction is catalyzed at the lipid–water interface. Microbial sources are superior to plants and animals for enzyme production and this can be attributed to great variety of catalytic activities available, the possibilities of high yield, ease of genetic manipulation, regular supply due to absence of seasonal fluctuations and rapid growth of micro organisms and inexpensive media. Lipases act on lipids which form aggregates in water and require a water-lipid interface for its catalysis.

In recent times, lipases have emerged as key enzymes in swiftly growing biotechnology, owing to their multifaceted properties, which find usage in a wide array of industrial applications, such as food technology, detergent, chemical industry and biomedical sciences (Jaeger *et al.* **1994, 1999**; Pandey *et al*. **1999).** The catalytic potential of lipases can be further enhanced and made selective by the novel phenomena of molecular imprinting and solvent engineering and by molecular approaches like protein engineering and directed evolution (Reetz and Jaeger **1999**; Jaeger *et al*. **2001**).

**Materials & Method:**

**Sample collection and processing:**

Sewage sample was collected from oil contaminated water source from Surat, with the help of sterile screw cap tube hanging with thread. After collection of sample, it was brought to the laboratory and was allowed to settle down for 30 minutes. After settlement, supernatant was proceeded for enrichment process.

**Screening of microorganism:**

Enriched sample was used for plating to get more lipolytic isolates using TBA agar plates containing (g/L): peptone 5.0, sodium chloride 5.0, Yeast extract 3.0, tributyrate 10 mL and pH was set to 7.5. After incubation period of 48 h, total 12 colonies which showed clear zone were picked and assayed for lipase production. All isolates were preserved on tributyrin agar slant at 4°C for further research. The culture was examined for various morphological and biochemical characteristics.

**Inoculum preparation:**

Inoculum was prepared by inoculating microorganisms in 50 mL of tributyrin broth consisting of (g/L): peptone- 5.0 g, yeast extract- 3.0 g, NaCl- 5.0 g, tributyrin oil- 1 mL having pH 7.5 and flask was incubated at 30 °C, 120 rpm for 48 h.

**Fermentation medium preparation:**

Fermentation medium consists of tributyrin broth supplemented with 10 mL of tributyrin oil and pH was set to 7.5. 95 mL fermentation medium was taken in 250 mL Erlenmeyer flask and were inoculated with 5% inoculum to carry out fermentation. All the flasks were incubated at 30 °C, 120 rpm for 96 h.

**Effect of pH:**

To study the effect of pH on lipase production, various pH ranging from 6.5 to 10.0 were selected. HCl and NaOH were used to set the different pH.

**Effect of Temperature:**

To study the effect of temperature on lipase production, fermentation broths were placed at different temperatures viz. 25 °C, 30 °C, 37 °C and 40 °C.

**Effect of Incubation period:**

To study the effect of incubation period on lipase production, flasks were incubated at 30°C at 120 rpm for 96 h and production of lipase was measured after every 24 h.

**Effect of Ultra Violet:**

To study the effect of UV on lipase production all the isolates were exposed to Ultra Violet light at the distance of approximately 1.5 feet in the laminar air flow having UV lamp of 30 volts for different exposure time.

**Lipase assay:**

- 2 mL of supernatant was taken into 2 different micro centrifuge tubes.
- One tube was kept in water bath at 80°C for 15 to 20 minutes to set blank (for thermal denaturation).
- Other tube was kept at room temperature.
- Spinning was carried out at 4°C for 10 minutes at 5000 rpm.
- Substrate emulsion was prepared by mixing 75 mL of 2% polyvinyl alcohol and 25 mL of tributyrin oil.
- 5 mL substrate emulsion was added to 4 mL of phosphate buffer having pH 7.8.

- Whole system was incubated at 30˚C for 10 minutes.
- For experimental set up, 1 mL enzyme was taken to the flask and incubate at same temperature for 30 minutes at 120 rpm on shaker.
- Reaction was terminated by adding 20 mL of acetone and was titrated against 0.1N NaOH.

**Enzyme activity:** One unit of enzyme activity was defined as the amount of enzyme releasing one µmole of free fatty acid in one minute under standard assay condition.

**Results & Discussion:**

**Isolation and Screening of microorganism:**

When enriched sample was plated on tributyrin agar plate, total 12 isolates which showed clear zone were obtained. Finally four isolates which showed larger zone of hydrolysis were checked for different biochemical tests. LP-1 showed positive tests for indole, methyl red, citrate utilization, nitrate reduction, catalase and ferment various sugars like glucose, maltose, mannitol. LP-2 & LP-3 showed positive tests for nitrate reduction, catalase and ferment glucose as well as mannitol while LP-4 showed positive tests for methyl red, catalase and ferment glucose as well as maltose.

**Effect of pH:**

| pH | LP-1 | LP-2 | LP-3 | LP-4 |
|---|---|---|---|---|
| **6.5** | 3.8 | 3.8 | 3.6 | 3.2 |
| **7.0** | 3.8 | 4.0 | **4.0** | **4.2** |
| **7.5** | **4.2** | **4.4** | 3.9 | 4.0 |
| **8.0** | 3.6 | 4.0 | 3.8 | 4.0 |
| **8.5** | 3.4 | 3.6 | 3.8 | 3.6 |
| **9.0** | 3.4 | 3.2 | 3.6 | 3.6 |
| **9.5** | 3.2 | 3.2 | 3.2 | 3.2 |
| **10.0** | 3.0 | 3.2 | 3.0 | 3.2 |

**Table-1: Effect of pH on Lipase Production**

All the isolates showed the growth between wider pH ranges of 6.5 to 10.0. LP-1 and LP-2 showed highest lipase activity i.e. 4.2 U/mL and 4.4 U/mL at pH 7.5 & LP-3 and LP-4 showed 4.0 U/mL and 4.2 U/mL at pH 7.0 respectively.

**Effect of Temperature:**

| Temperature | Lp-1 | LP-2 | LP-3 | LP-4 |
|---|---|---|---|---|
| **25 °C** | 3.2 | 3.5 | 3.4 | 3.0 |
| **30 °C** | **4.0** | **4.2** | 3.8 | 3.6 |
| **37 °C** | 3.8 | 3.8 | **4.0** | **4.0** |
| **40 °C** | 3.6 | 3.8 | 3.6 | 3.6 |

**Table-2: Effect of Temperature on Lipase Production**

Experiments on effect of temperature indicated that the lipase production was maximum 4.0 U/mL for LP-1 and 4.2 U/mL for LP-2 at the optimum temperature of 30°C while 4.0 U/mL for LP-3 as well as for LP-4 at temperature of 37°C. But at low temperature i.e. 25°C as well as at high temperature above 37°C, the lipase production recorded low.

**Effect of Incubation period:**

| Incubation Time (h) | LP-1 | LP-2 | LP-3 | LP-4 |
|---|---|---|---|---|
| **24** | 2.8 | 3.2 | 2.6 | 2.4 |
| **48** | **4.0** | **4.2** | **4.0** | 3.4 |
| **72** | 3.6 | 3.8 | 3.6 | **4.0** |
| **96** | 3.2 | 3.0 | 3.4 | 3.6 |

**Table-3: Effect of Incubation Period on Lipase Production**

Incubation period plays an important role in lipase production. LP-1, LP-2 and LP-3 showed highest lipase production i.e. 4.0 U/mL, 4.2 U/mL, 4.0 U/mL, respectively during the incubation period of 48 h while LP-4 showed highest lipase production i.e. 4.0 U/mL during the incubation period of 72 h.

**Effect of Ultra Violet light:**

When isolates were exposed to the UV light at the distance of approximately 1.5 feet for 30 and 60 seconds in the laminar air flow, it was observed that lipase activity increases in both the case compared to control set but maximum lipase activity were observed during the exposure of 60 seconds. LP-1, LP-2, LP-3 and LP-4 produced 4.5 U/mL, 4.6 U/mL, 4.3 U/mL and 4.5 U/mL lipases respectively during the exposure period of 60 seconds.

**Conclusion:**

As Lipases have many applications in the field of food industries, pharmaceutical industries, agrochemical industries, in the preparation of cosmetics and personal care products, biofuel production etc. effective producers must be screened and utilized at industrial level for optimum production of lipases. During the isolation, 12 isolates were able to show clear halo zone on tributyrin agar plates but 4 isolates were selected for the further study because of their larger halo zone compared to other isolates. Two isolates were found to be Gram negative and 2 isolates were Gram positive. LP-2 was found to be non motile while all the other three were motile. From the data, it was concluded that lipase production was maximum at pH 7.5, at temperature 30°C, incubation period of 48 h and UV exposure of 60 seconds. Gram positive cocci LP-2 produced maximum lipase compared to other isolates.

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