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**R**ESEARCH **P**APER

# Isolation and optimization of lipase producing bacteria from oil contamination soils

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Lipolytic bacteria were isolated from oil contaminated soil and grown on glycerol tri-butyrate media. The lipase activity is shown by reacting with various chemicals. Lipases are glycerol ester hydrolases that catalyze the hydrolysis of triglycerides to free fatty acids and glycerol. Bacterial lipase producers were isolated from oil spilled soil from vegetable oil processing factories. The effect of incubation time, medium pH, temperature, agitation, inoculums concentration, carbon source and nitrogen source for the lipase production was studied. The lipase production was maximum at pH 7, temperature by the lipase producing bacteria *Staphylococcus*. Increased enzymatic production was obtained when the organisms were cultured in medium supplemented with 1per cent protease peptone by *Staphylococcus*. The results of the present study was to demonstrate that the micro-organism is ideal for extracellular lipase production at industrial level. Different media parameters were optimized for maximal enzyme production. Lipases activity shown on starch containing agar media at pH-7.0 and temperature at $37^0$C for 24-48 h.

Key words : Lipase, Glycerol tributyrate, Extracellular, Oil, Dialysis

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## INTRODUCTION

Lipases (triacylglycerol acylhydrolases EC 3.1.1.3) are a class of hydrolase which catalyze the hydrolysis of triglycerides to glycerol and free fatty acids over an oil–water interface (Benjamin and Pandey, 1996; Chen *et al*., 2001). Microbial lipases have gained special industrial attention due to their stability, selectivity, and broad substrate specificity (Fariha *et al*.,2006 and Hasan *et al*., 2006). Bacterial lipases have been used extensively in food and dairy industry for hydrolysis of milk fat, for instance of biotechnology company has brought recombinant lipases enzyme to market for use in such application ex. bleaching, laundry detergents and even as biocatalysts in alternative energy strategies to convert vegetable oil into fuel (Fujii *et al*., 1986).

Lipases of microbial origin represent the most widely used class of enzymes in biotechnological applications and industrial application of microbial lipases (Seitz,1974; Falch, 1991 and Godfrey and West, 1996). The present review is focused on lipase production discussing the main micro-organisms, substrates and process operations used in this specific field.

## RESEARCH METHODOLOGY

Oil contaminated soil samples were collected from two different places in Allahabad, Dairy technology department, SHIATS ($D_1$), Petrol pump near SHIATS

($D_2$) and self prepared sample of coconut oil contaminated soil in laboratory of Molecular and Cellular Engineering Department ($C_1$).

**Isolation and screening of bacteria from soil :**

Isolation of bacteria from oil contaminated soil was done by serial dilution technique and plated on glycerol tri butyrate base.

**Identification and characterization of microorganisms on the basis of biochemical tests :**

Identification of lipase producing bacterial strains was done on the basis of various biochemical tests (Gram staining, Starch hydrolysis, Catalase test, Indole test, Urease test, Citric test, Methyl red test, Voges-proskauer test, Oxidation and fermentation of glucose [O-F-Test.

**To perform precipitation of submarged enzyme by salt and solvent precipatation method:**

Taken a beaker containing 80ml crude enzyme and surrounded it in a containing ice at 4°C. Added to the ammonium sulphate $(NH_4)_2SO_4$, pinch to pinch added to beaker on magnetic stairer. Overnight stored at 4°C.Transferred the sample solution in ependroff tube. Centrifuged at 10000 rpm for 10 mins at4°C. Discarded supernatant and stored pallet in tris HCL buffer at 4°C. For further used in extraction process by Lowry's method.

**Lowry's method:**

Protein was estimated using Lowry's method.

**Dialysis of lipase enzyme:**

Filled the sample in dialysis bag and tied the both side with thred. shown in Fig. 2. Dipped the bags in beaker which was filled with tris-buffer 100mm, for 1 to 1. 1/2 h stored at 4°C. Changed the buffer and stored at 4°C, over night. Changed the buffer and taken O.D at 660nm.

## RESEARCH FINDINGS AND ANALYSIS

Three different soil sample collected from different area showed high bacterial count. The colony labeled as $D_1$,$D_2$,$C_1$, showed maximum zone of clearance when placed on glycerol trybutyrate agar. Bacterial lipases are

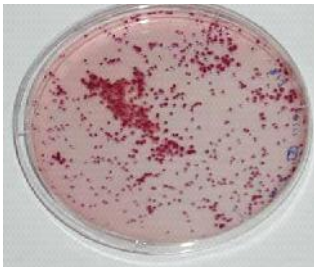

**Fig. 1:** ***Staphylococcus***

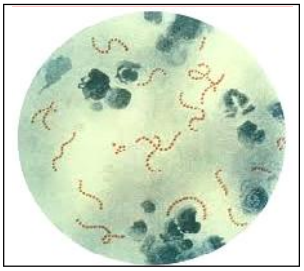

**Fig. 2:** ***Staptococci***

| Table 1: Morphological characterization | | | |
|---|---|---|---|
| Si No. (parameters) | Diary ($D_1$) | Diesal ($D_2$) | Coconut ($C_1$) |
| Microorganism | Staphylococcus | Staphylococci | Staphylococci |
| Gram staning | positive | Positive | Positive |
| Shape | Cocci | Cocci | Cocci |
| Cell arrangement | cluster | Chain form | Chain form |
| Colony texture | mucus | Mucus | Mucus |
| Physical apperence | white | Creamy type | Creamy type |
| Aerobic | positive | Positive | Positive |
| Anaerobic | negative | Negative | Negative |

| Table 2 : Biochemical characterization | | | |
|---|---|---|---|
| Test parameters | Diary | Diesal | Coconut |
| Starch hydrolysis | Negative | Positive | Positive |
| Catlase | Positive | Positive | Positive |
| Indole | Negative | Negative | Negative |
| Urease | Negative | Negative | Negative |
| Citrate | Positive | Positive | Positive |
| Methyl red | Positive | Positive | Positive |

one of the enzyme having huge market demand. The isolated *staphylococcus* spp. isolate have shown in (Fig.1 and Fig.2) the production of extracellular lipases. Optimization studies on media parameters for maximum lipase activity were done on isolated lipolytic bacteria. The isolates shown a broad range of pH and temp. in previous studies but after that we used lowery's method for enzyme extraction and dialysis method for maximum lipase activity.The extracellular lipases can be further purified in less time and used in different industrial applications.

## Morphological characterization :

The isolated strains were screened for extracellular lipase using tributyrin agar media. Two of the isolates $D_1$, $D_2$, $C_1$ produced larger clear zone than the others, indicating higher lipase activity. These two isolates were identified based on morphological (Table 1) and biochemical characterization (Table 2 and 3). Both the bacterial strains were Gram positive and coccoid in shape. In accordance with the Bergey's manual of systematic bacteriology, the isolates were likely to be belonging to genus *Staphylococcus* optimum temperature for the production of lipases, isolated lipolytic bacteria (Qamsari *et al.*, 2011; Sayari *et al.*, 2001 and Sharma *et al.*, 2003). The isolate had shown a broad the temperature, pH and agitation speed. The maximum enzyme production was obtained from $C_1$(Table 4).

Experimental results suggest that various media compositions influenced enzyme (lipase) production by

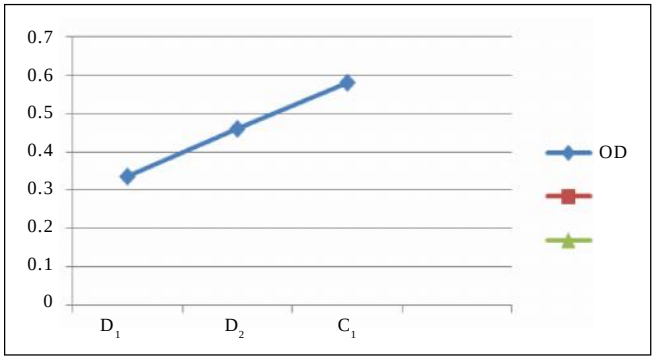


**Fig. 3 : Crude enzyme activity**

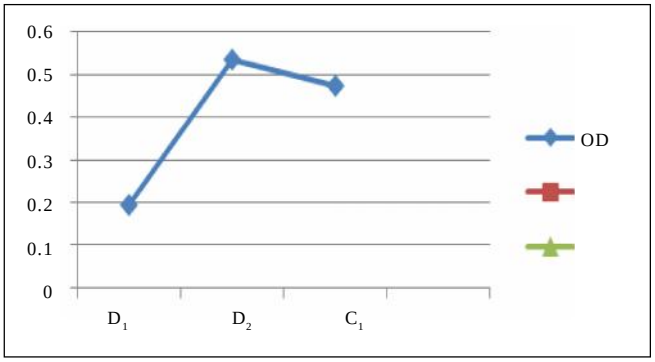


**Fig. 4 : Purified enzyme activity**

**Table 3: O-F test for identification of sugar utilization**

| Si No. | Diary | Diesal | Coconut |
|---|---|---|---|
| Glucose | Positive | positive | Positive |
| Sucrose | Positive | positive | Positive |
| Lactose | Positive | positive | Negative |

**Table 4: Estimation of crude enzyme by Lowry's method**

| Test tube | C.E | D/W | Reagent C | Incubation | Reagent D | Incubation | O.D. (660nm) |
|---|---|---|---|---|---|---|---|
| Blank | ------- | 1ml | 5ml | 10mins | 0.5ml | 30 mins | 0.000 |
| $D_1$ | 0.5ml | 0.5ml | 5ml | At | 0.5ml | In | 0.336 |
| $D_2$ | 0.5ml | 0.5ml | 5ml | room | 0.5ml | Dark. | 0.460 |
| $C_1$ | 0.5ml | 0.5ml | 5ml | Temp. | 0.5ml | - | 0.580 |

**Table 5: Estimation of purified enzyme by Lowry's method**

| Test tube | C.E | D/W | Reagent C | Incubation | Reagent D | Incubation | O.D (660nm) |
|---|---|---|---|---|---|---|---|
| Blank | ------- | 1ml | 5ml | 10mins | 0.5ml | 30 mins | 0.000 |
| $D_1$ | 0.5ml | 0.5ml | 5ml | At | 0.5ml | In | 0.196 |
| $D_2$ | 0.5ml | 0.5ml | 5ml | room | 0.5ml | Dark. | 0.534 |
| $C_1$ | 0.5ml | 0.5ml | 5ml | Temp. | 0.5ml | - | 0.470 |

indigenously isolated bacterial strains $D_1$ and $D_2$,$C_1$ (Table 5). Optimization of growth parameters *viz.*, temperature, pH, agitation (rpm), carbon and nitrogen source etc. had significant effect on lipase activity. However, the present study requires greater research capacities (further purification of the crude enzyme) for comparison with commercial lipase with regard to specific activity. The study also suggests that waste contaminated sites by fat, which are usually comprised of numerous lipid remnants from cooking and non-cooking processes) may serve as excellent breeding grounds for the isolation of lipolytic bacteria of industrial significance (Sharma *et al.,* 1990; Sharma *et al.*, 2001; Sugihara and Tominaga, 1991 and Vohra *et al*., 2002).

The quantitative determination of triacylglycerol is of great importance in clinical diagnosis and in food industry. The lipid sensing device as a biosensor is rather cheaper and less time consuming as compared to the chemical methods for the determination of triacylglycerols. An analytical biosensor was developed for the determination of lipids for the clinical diagnosis lipase biosensor from *Candida rugosa* has been developed as a DNA probe (Linko *et al.*,1998 and Noureddini *et al.*, 2005).

Oil spills in refinery, shore sand and processing factories could be handled by the use of lipases from different origins. It has been also used for the degradation of waste water contaminants such as olive oil from oil mills. Another important application has been reported for the degradation of polyester waste, removal of biofilm deposits from cooling water systems and also to purify the waste gases from factories.

## LITERATURE CITED

**Benjamin, S. and Pandey, A. (1996).** Optimization of liquid media for lipase production by *Candida rugosa*, *Bioresource Technol.,* **55** : 167-170.

**Chen, S. J., Cheng, C. Y. and Chen, T. L. (2001).** Production of an alkaline lipase by Acinetobacter radioresistens, *J. Ferment. & Bioengg.* **86** : 678 -679 .

**Falch**, **E. A.** (**1991).** Industrial enzymes - Developments in production and application, *Biotechnol. Adv.,* **3** : 1200.

**Fariha, Hasan, Shah, Aamer Ali and Abdul, Hameed (2006)**. Industrial applications of microbial lipases. *Enzyme & Microbial. Tech.,* **39**(2): 235-251.

**Fujii, T., Tatar, T. and Minagawa, M.** (**1986).** Studies on applications of lipolytic enzyme in detergency I. Effect of lipase from Candida cylindraceaon removal of olive oil from cotton fabric. *J. American Oil Chem., Soc.*, **63** (6) : 796-799.

**Godfrey T. and West, S. (1996).** Introduction to Industrial Enzymology, In: Industrial Enzymology, T. Godfrey, and S. West (Eds). 2nd Ed., Stockholm Press, NEW YORK, U.S.A.

**Hasan F., Shah A.A. and Hameed, A.** (**2006).** Industrial applications of microbial lipases. *Enzyme & Microbial. Technol.,* **39** : 3678.

**Higak, S. and Morohashi, M. (2003).** Propionibacterium acnes lipase in seborrheic dermatitis and other skin diseases and Unsei-in. *Drugs Under Exp. & Clinic. Res.,* **29 :** 23.

**Illanes, A.** (**1999).** Stability of biocatalysts. *Electro. J. Biotechnol.,* **2** : 786 – 789.

**Jaeger, K.E., Ransac, S. and Dijkstra, B .W.** (**1994).** Bacterial lipases. *FEMS Microbiol. Rev.,* **15** : 567 -570.

**Jensen, R.G. (1983).** Detection and determination of lipase (acylglycerol hydrolase) activity from various sources, *Lipids*, **18** : 45-48.

**Linko, Y.Y., Lamsa, Wu, M., Uosukainen, E., Seppala, J. and Linko P. (1998).** Biodegradable products by lipase biocatalysis. *J. Biotechnol.*, **66** (1) : 41-50.

**Noureddini, H., Gao X. and Philkana, R . (2005).** Immobilized *Pseudomonas cepacialipase* for biodiesel fuel production from soybean oil. *Bioresource Technol.,* **96** : 234.

**Qamsari, E. W., Kermanshahi, R. K. and Nejad, Z. M.** (**2011).** Isolation and identification of a novel, lipase-producing bacterium, *Pseudomonas aeruginosa* KM110, *Iranian J. Microbiol.,* **3** : 342 – 345.

**Sayari, S. AgrebiJaoua, C.Y. and Gargouri, Y. (2001).** Biochemical and molecular characterization of Staphylococcus simulanslipase, *Biochimie*, **83** : 234 - 235.

**Seitz, E.W. (1974).** Industrial application of microbial lipases. *J. American Oil Chem. Soc*., **51** : 456 – 460.

**Sharma, R., Chisti, Y. and Banerjee, U.G. (2003).** Production, purification, characterization, and applications of lipases, *Biotechnol. Adv.,* **19** : 32.

**Sharma, R., Soni, S.K. and Bajpai, P. (1990).** Application of enzymes in the pulp and paper industry. *Biotechnol. Prog.,* **15** : 23 - 25.

**Sharma, R.Y., Christi, Y. and Banerjee, U.C. (2001).** Production, purification, characterization and applications of lipases, *Biotechnol. Adv.,* **19** : 54.

**Sugihara, T. and Tominaga, Y. (1991).** Purification and characterization of a novel thermostable lipase from *Bacillus* sp., *J. Biochem.,* **109** (2) : 211-216.

**Vohra, R.M., Jolly, R.S., Gupta, R.R. and Gupta, J.K.(2002).** Production of extracellular alkaline lipase from a *Bacillus* sp. RSJ1 and its application in ester hydrolysis. *Indian J. Microbiol.,* **42** : 456 - 458.