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Comparative Study for Lipase Production by Using *Pseudomonas Aeruginosa* and *Pseudomonas Fluorescens*

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Abstract

Lipases occur widely in nature, but only microbial lipases are commercially significant. The present work focuses on screening and production of extracellular laccases by *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. The lipase was assayed by tributyrin agar plate method and the activity of the enzyme was further confirmed by titrimetric method. The uses of lipases are enormous and increasing and so there is need to screen and isolate potential species capable of producing large quantities of the enzyme to use for various Industrial applications.

Keywords: Lipase activity, Extracellular lipase, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*.

Introduction

Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are part of the family of hydrolases that acts on carboxylic ester bonds. The physiologic role of lipases is to hydrolyze triglycerides into diglycerides, monoglycerides, fatty acids, and glycerols (Prazeres *et al.*, 2006; Babu and Rao, 2007). Microbial enzymes are often more useful than enzymes derived from plants and animals because of the great variety of catalytic activities available, the high possible yields, ease of genetic manipulation, regular supply due to absence of seasonal fluctuations and rapid growth of microorganisms on inexpensive media and the reaction that are mediated by lipases are reversible (Davranov, 1994). Microbial lipases are more stable than lipases from plants and animals and even the production is more convenient (Wiseman, 1995).

Many microorganisms such as bacteria, yeast and fungi are known to secrete lipases (Sztajer *et al.*, 1998). Lipase producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contamination with oil, etc. The oily environment (oil mill effluent) may provide a good environment for lipase producing microorganisms. Bacterial lipases are mostly extracellular and are greatly influenced by nutritional and physico-chemical factors. Typical substrates are vegetable oil, animal fat, fish oil, olive oil, butter oil (milk fat) and synthetic TAG such as triolein.

The unique characteristics of lipase include substrate specificity, stereospecificity, regiospecificity, and ability to catalyse a heterogeneous reaction at the interface of water soluble and water insoluble systems (Macrae and Hammond, 1985). Microbial lipases have wide application in the processing of food, leather, domestic, industrial wastes, dairy, pharmaceutical, detergent and cosmetic industries (Kathiravan *et al.*, 2012).

Materials and methods

Procurement of Microorganism

The isolates were procured from the Microbial Culture Collection Bank (MCCB), Department of Microbiology and Fermentation Technology, Jacob School of Biotechnology and Bioengineering, SHIATS, Allahabad.

Microorganism	Strain no.
<i>Pseudomonas aeruginosa</i>	0035
<i>Pseudomonas fluorescens</i>	0217

The isolates were routinely sub-cultured on Nutrient agar slants at 15 days of interval maintained at pH 7.4, incubated at 30°C for 24-48 h.

Lipase assay

Tributyryl agar plates were prepared using nutrient agar and Tributyrin (1%). The pH of the medium was

adjusted to 7.3-7.4 using 0.1 N NaOH. The isolates were streaked on the Tributyrin agar plates and incubated at 30°C for 24-48 h. The plates were then examined for the zone of clearance around the colonies.

Production of lipase

For lipase production, 10 ml Nutrient broth was prepared. A loopful culture of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* was inoculated using a sterile loop and incubated at 30°C at 120 rpm. The culture was served as seed culture after 24 h for further inoculation. A 100 ml sterile production media was prepared, sterilized and inoculated with 5% seed culture (v/v) and 1% olive oil was added to it and was incubated at 30°C at 120 rpm for 7 days.

Extraction of crude enzyme

After incubation the cells were harvested by centrifugation at 10,000 rpm at 4°C for 10 min. The supernatant obtained was filtered with whatman No.1 filter paper contained in a funnel. The clear supernatant obtained was used as crude enzyme and was stored in vials for further use.

Lipase Assay by Titrimetric method

The lipase activity was assayed using the method of Yadav *et al.*, (1993). This involves incubating a reaction mixture containing 5.0 ml of olive oil emulsion, 20 ml of 0.1 M phosphate buffer (pH 6.5) and 1.0 ml of the culture filtrate (lipase crude extract) at 40 °C for 30 min with shaking at 130 rpm. After incubation, the reaction mixture was terminated by adding 15.0 ml of acetone-ethanol (1:1) mixture to liberate free fatty acid. The mixture (free fatty acid solution) was then titrated with 0.05 N NaOH in the presence of phenolphthalein indicator. All the tests were conducted in triplicates and mean activities were determined. Blank assays were conducted adding the enzyme just before titration. One unit (u) of lipase activity was defined as the amount of enzyme which produces 1 μ mol of fatty acids per minute.

Hydrophobicity test of culture

25 μ g of congo red (25 μ g/L) was taken in a test tube and 4 ml of phosphate buffer saline was added to it. And then 1 ml of 24 h old culture inoculated in nutrient broth was added and incubated for 15 min. After incubation the solution was centrifuged at 6000 rpm for 10 min and the supernatant was filtered with whatman No. 1 filter paper. The clear supernatant

was obtained and the optical density was measured at 540nm and the blank was set with phosphate buffer.

Result and discussion

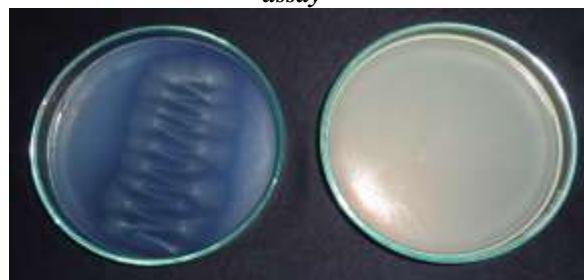
Lipase assay

Pseudomonas aeruginosa and *Pseudomonas fluorescens* were selected to study their lipase enzyme activity. Lipase activity was determined initially by Tributyrin agar method (As shown in Fig:1 and Fig:2) and a quantitative titrimetric assay was done to further estimate the amount of extracellular lipase produced (As shown in Table:1 and Fig:3). Zone of clearance was observed around the colonies of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* indicating the lipase test positive. Ni'matuzah *et al.*, (2012) in his study has screened the *Bacillus subtilis* for biosurfactant activity and production as in present investigation. Lipase enzyme can catalyse lipid hydrolysis reaction on interfacial oil and water.

Fig:1 Screening of *Pseudomonas aeruginosa* for lipase assay



Fig:2 Screening of *Pseudomonas aeruginosa* for lipase assay



Production of lipase and Extraction of crude enzyme

Submerged production of lipase enzyme was conducted in 250 ml capacity Erlenmeyer Flasks in rotary shaker incubator at 120 rpm at 30°C for 7 days. The downstream processing of the lipase enzyme

involved harvesting at crude level. As in present study, similar kind of study was conducted by several workers (Suseela *et al.*, 2014; Brooks and Asamudo, 2011 and Mobarak-Qamsari *et al.*, 2011).

Lipse Assay by Titrimetric method

Pseudomonas aeruginosa showed the lipase activity 3.77 U/ml whereas *Pseudomonas fluorescens* showed the lipase activity 3.49 U/ml by the titration method. Similar kind of study was conducted by Brooks and Asamudo, (2011) in which the enzyme activity was measured by Yadav *et al.*, (1993). Ugochukwu *et al.*, (2008) also determined the lipase activity by the titration method. Kathivaran measured the lipolytic activity of *Pseudomonas aeruginosa* by Titrimetric assay method according to Jagtap *et al.*, (2010). The present data on extracellular lipase producing profile of isolates analyzed could be used for different purposes as the demand of lipase is a great deal in many industries like in synthesis of pharmaceuticals, agrochemicals, house hold detergents, dairy industries, waste water treatment and they are also useful in many organic synthesis (Macrae *et al.*, 1985). Apart from these applications, lipases are also used in bioremediation. As the use of lipases are enormous and increasing there is every need to develop improved screening methods like recombinant DNA methods and much more fungal *spp* need be isolated to solve many environmental problems.

Table:1 Lipase producing activity by titrimetric method

S. No.	Organism	Lipase activity (U/ml)
1.	<i>Pseudomonas aeruginosa</i>	3.77
2.	<i>Pseudomonas fluorescens</i>	3.49



Fig:3 Lipase assay by titrimetric method

Table:2 Hydrophobicity test of culture

Culture	Sample (Congo red)	Phosphate buffer saline	Bacterial suspension inoculated in NB	Incubate for 15 min and centrifuge at 6000 rpm supernatant	O.D (540 nm)
Control	25µg/l	4ml	-		0.75
<i>Pseudomonas aeruginosa</i>	25µg/l	4ml	1ml		0.58
<i>Pseudomonas fluorescens</i>	25µg/l	4ml	1ml		0.56

Conclusion

Since there is growing demand for lipases industrially, rapid and persistent screening for new microorganisms and their lipolytic enzyme activities will open new insights for synthetic processes and provides faster ways to solve environmental problems. The study directly highlights the application of lipases in detergent, food, leather, pharmaceutical and cosmetic industry. Lipases have led considerable interest for present and future application.

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