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# Modification of Process Parameters for Enhanced Lipase Induction from *Bacillus* SR1

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## ABSTRACT

The enzymes catalyze the cleavage of triacylglycerols into fatty acids and glycerols are referred to as lipases (EC 3.1.1.3). Lipases are widely distributed in flora and fauna. Microbial lipases are of great importance than lipases from plants and animals due to their catalytic activity, ease of production and optimization. Lipases have tremendous industrial applications such as in the processing of fats and oils, detergents and degreasing formulations, food processing, the synthesis of fine chemicals, paper manufacture, and production of cosmetics, and pharmaceuticals. Therefore, a potential lipase producing bacterial strain was isolated and identified as gram +ve *Bacillus* SR1. Among different oils tested, olive oil was found to be the favorable substrate for lipase induction. Additionally, lipase induction was observed highest in 24 hours of fermentation at 37ºC and pH 7.5.

Keywords
*Isolation, lipase, Bacillus, fermentation, triacylglycerol.*

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## INTRODUCTION

Triacylglycerol and fatty acids require lipases (EC 3.1.1.3) for their degradation. Lipases have been defined as carboxylesterases that catalyze the hydrolysis, esterification and transesterification of acylglycerides. Lipases are serine hydrolases and hence not required addition of cofactors[1]. The active site consists of three catalytic residues: a nucleophilic residue (serine, cysteine, or aspartate), a catalytic acid residue (aspartate or glutamate), and a histidine residue[2]. Lipases are stereoselective as well as regioselective biocatalysts[3]. Consequently, lipase reactions show more selectivity under mild conditions and become the factor for increasing demands of lipases. Lipases are produced by animals, plants and microorganisms, the majority of lipases used for biotechnological purposes have been isolated from bacteria and fungi[4]. Microbial lipases are more stable and their recovery is comparatively easy[4]. Among microbial lipases, bacterial lipases are of great interests due to easy and inexpensive production[5]. Fermentation is the classical bioprocess used for lipase production by bacteria. Bacterial lipases can be produced by submerged fermentation (SmF) in addition to solid state fermentation (SSF)[6]. Fermentation is used to produce enzymes on industrial scale[7]. Economic fermentation techniques, low energy consumption and greater productivity are the reasons for preference of microbial lipases in industrial sector. Lipases have several industrial applications[7,8]. Bacterial lipases can be used in many processes such as in the processing of fats and oils, detergents and degreasing formulations, food processing, the synthesis of fine chemicals, paper manufacture, and production of cosmetics, and pharmaceuticals[8,9]. Besides this well established role; still the demand for novel lipase with specific properties such as specificity, stability, pH, and temperature is increasing. This has drawn the greater interest in isolation of new and unique lipase producing micro-organisms from diverse habitat and modulation of the process for maximum lipase

yield[10-12]. The present study was therefore aimed to isolate novel lipase producing bacterium, its cultivation using submerged fermentation and optimization of fermentation parameters for maximum lipase yield.

## MATERIALS AND METHODS

### Collection of Sample

For present research, greasy scrapping of kitchen was selected as it is greasy and has a long term oil exposure. Greasy scrapping was collected from a depth of 5-10cm by means of a sterilized spatula and stored in a sterilized vial. Sample was transferred to the laboratory soon after collection and processed immediately[13].

### Isolation of Lipolytic Bacteria

Collected sample was enriched in nutrient broth supplemented with 1% olive oil. For this purpose 1g of sample was suspended in 50 ml of enrichment medium and then agitated at 120 rpm; 37ºC for 30 minutes on a rotary shaker. The sample was then incubated at 37ºC for 48 hours. Consequently, serial dilutions ($10^{-1}$-$10^{-4}$) of the sample were prepared using sterilized 0.9% NaCl. 200µl of each dilution were inoculated on nutrient agar plates by spread plate method and incubated at 37ºC for up to 72 hours. Isolated microbial colonies were picked and examined for lipolytic activity and further subjected to strain identification[14].

### Screening of Bacterial Isolates for Lipolytic Activity and Identification

Screening media used for lipolytic activity consisted of nutrient agar with olive oil (1%) and tween 80 (0.1%). Isolated colonies showed growth on screening medium were selected on the basis of hydrolytic zones were further purified and identified through gram staining[15].

### Storage and Maintenance of Culture

The isolated strain was stored at 4ºC and subcultured after 15 days. The culture was revived on weekly bases in enrichment medium.

### Lipase Production

Extracellular lipase from the isolated strain was harvested through submerged fermentation. The process was carried out in 100ml Erlenmeyer flask. A 10% v/v seed culture was inoculated in medium and incubated at 37ºC for 48 hours. Consequently, the fermented media was centrifuged at 0ºC and 10,000xg to pallet cells and the cell free extract was served as source of crude lipase.

### Lipase Assay

Lipase activity was monitored spectrophotometrically by using para-nitrophenyl palmitate (pNPP) as substrate[16] with slight modifications. The reaction was initiated with 1ml of substrate (40µM) and 0.1 ml of crude enzyme. After 30 minutes incubation NaOH (5%) was added to cease the reaction and absorbance was monitored at 410nm for release of para-nitrophenol. One unit of lipase activity was defined as µmoles of para-nitrophenol released in one minute per assay conditions.

### Optimization of Fermentation Parameters

Lipase production was optimized with variation in one factor at the constant level of other variables. The parameters tested were time course, pH, temperature and different oil substrates.

### Effect of Time on Lipase Production

In order to observe time period for maximum lipase production, isolated strain was allowed to ferment for different time periods (18, 24, 48 and 72 hours)

### Effect of pH on Lipase Induction

Different pH values ranging from 6-8.5 were tested to select the one with higher lipase yield.

### Effect of Temperature on Lipase Production

Temperature effect on lipase production was detected by varying temperature from 30 to 60ºC.

### Evaluation of Different Substrates

Additionally, Lipase Production was also examined with different oil substrates such as castor oil, mustard oil, canola oil, palm oil, almond oil, olive oil.

The selected oil substrate with maximum lipase induction was further tested in presence of tween 80 in order to examine the further enhancement in lipase production.

### Influence of Substrate Concentrations on Lipase Induction

The selected substrate was further tested with different concentrations (1-5%) to optimize the suitable concentration for enhanced lipase yield.

## RESULTS AND DISCUSSION

The colony represent greater zone of hydrolysis was selected for this study. The isolated strain was observed as gram positive, rod shaped and spore former bacterium and identified as *Bacillus* sp. SR1 (Figure **1A** and **B**). The production process of lipase from *Bacillus* sp.

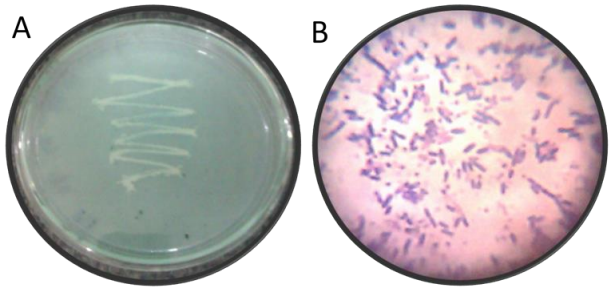


**Figure 1.** Isolation and identification of *Bacillus* SR1. A: Growth of isolated strain on tributyrin agar. B: Gram staining of isolated strain bacillus SR1 showing gram positive rods.

SR1 was further modified with respect to time, pH, temperature and different substrates. Time course study of lipase production revealed that *Bacillus* sp. SR1 produce maximum lipase (32.6 U/mL) in 24 hours of fermentation. Figure **2** describes that after 24 hours of fermentation lipase activity got decreased gradually up to 72 hours of fermentation. *Bacillus* sp.

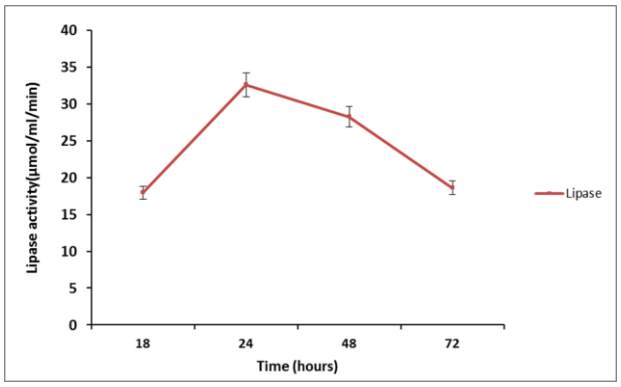


**Figure 2.** Effect of fermentation time on lipase synthesis. Results are expressed as Mean±SD (n=3).

SR1 produce highest lipase yield in its exponential growth phase. According to current study lipase activity decreased subsequently 24 hours which may be due to concomitant production of various proteases after log phase. Similar results were obtained in bacteria isolated from palm oil contaminated waste[17]. The production of proteases after 20 hours of fermentation and release of ammonia from deamination of amino acid caused alkalinisation of the media. Together these two factors are responsible for decreased lipase activity after 24 hours of fermentation and above pH 7.5[18,19].

Influence of various pH values on hyper production of lipase was presented in Figure **3**. It was observed that greater lipase yield was achieved at pH 7.5. This is in accordance with the reported literature[16,17] and reflected the neutral nature of *Bacillus* sp. SR1 lipase.

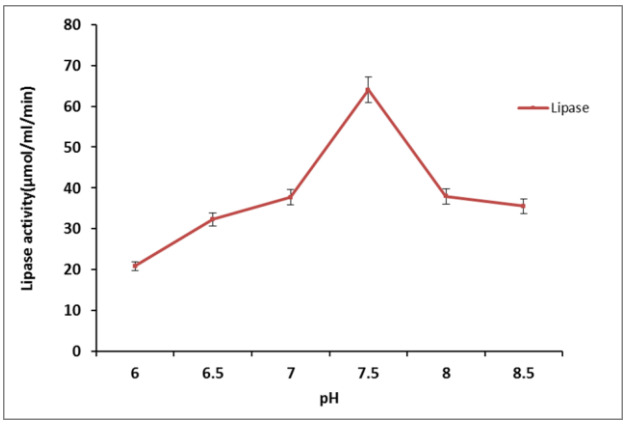


**Figure 3.** Effect of pH on lipase synthesis. Results are expressed as Mean±SD (n=3).

Reported literature showed that usually bacteria preferred pH 7.0 for lipase production[20]. Temperature has a profound effect on a protein and bacterial growth. In present study it was observed that the lipase yield was highest (40.987 U/mL) at 37ºC and gradually decreased after it (Figure **4**) which indicative of enzyme unstability at higher temperature ranges[17,19].

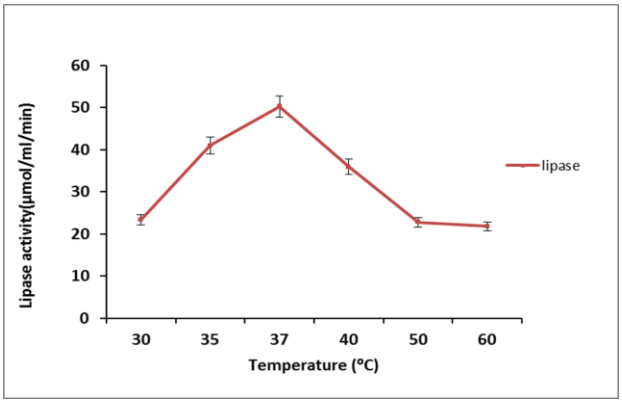


**Figure 4.** Effect of temperature on lipase synthesis. Results are expressed as Mean±SD (n=3).

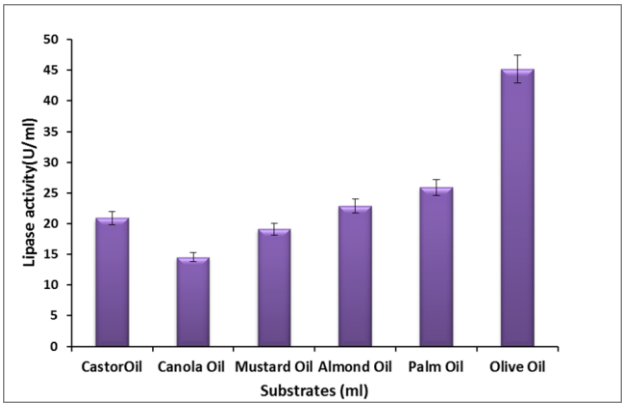


**Figure 5.** Lipase synthesis with different substrates. Results are expressed as Mean±SD (n=3).

*Bacillus* lipase is an inducible enzyme and its induction is directly affected by oil substrates[21]. Therefore, different oil substrates were tested to assess their effect on lipase induction. It was observed that all the tested oils were induced lipase synthesis by *Bacillus* sp. SR1 varyingly (Figure **5**). However, lipase synthesis reaches to its peak i.e. 45.23 U/mL when olive oil was used as substrate. Utilization of olive oil and simultaneous production of lipase indicates the stimulation of lipase operon by olive oil and also the preference of *Bacillus* sp. SR1 for lipase production. However, palm oil, almond oil and castor oil were also found to stimulate lipase production by *Bacillus* sp. SR-1. Lipase induction was also affected by concentration of substrate and for this purpose; different concentrations of olive oil (0.5-5%) were also investigated. Lipase yield was found to be greater i.e. 57.97 U/ml at 1% olive oil concentration and further increase in concentration showed significant decrease in lipase synthesis by *Bacillus* sp. SR1 which may be due to increase in viscosity of the medium which in turn is a cause of low aeration[20].

## CONCLUSION

A potential lipase producing bacterial strain was isolated and identified as *Bacillus* sp. SR1. It produces a neutral enzyme with 57.97 U/ml of lipolytic activity in presence of 1% olive oil. On the basis of results obtained the isolated enzyme seems a good addition in industrially important bacterial lipases. Future research will focus on the further characterization of lipase according to industrial needs.

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