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# PRODUCTION AND OPTIMIZATION OF LIPASE FROM *Bacillus tequilensis* NRRL B-41771

**KALYANA CHAKRAVARTHY BONALA AND LAKSHMI NARASU MANGAMOORI***

Centre for Biotechnology, Institute of Science and Technology, Jawaharlal Nehru Technological University Hyderabad, Hyderabad-500085, AP, India.
*Corresponding Author: Email- mangamoori@gmail.com

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**Abstract-** Extra cellular lipase production by the bacterial strain, *Bacillus tequilensis* was investigated in submerged shake flask fermentation. This work was intended to optimize culture conditions of Lipase production *Bacillus tequilensis with* submerged fermentation using 1% (w/v) carbon source, Nitrogen source and different oils along with physical parameters like pH, temperature, inoculum concentration, agitation speed. The optimum pH and temperature for Lipase production in submerged fermentation were found to be 7.0 and 30°C. Maximum activity was observed with 1% inoculum concentration, at incubation period of 48hrs and agitation speed of 200rpm.
**Key words-** Extra cellular Lipase, *Bacillus tequilensis*

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## Introduction

Enzyme mediated processes have been under usage from ancient times, out of approximately 4000 known enzymes about 200 enzymes are of commercial usage. Majority of these enzymes are microbial in origin, with the advancement of technology, and wide application of these enzymes, many enzymes are brought in to use by various industries [1].

Lipases (E.C.3.1.1.3) are a group of hydrolytic enzymes that catalyze the degradation of triacylglycerols to diacylglycerol, monoacylglycerol, fatty acids and glycerols at the interface between aqueous and the lipid phase [2-3]. They are widely sread in nature, they were isolated from different source such as plants animals and microorganisms, Lipase have immense potential application in various industries like cosmetic, food, detergent, paper and pharmaceutical industries [4-7].Enantiomers of lipase are used for resolution of chiral drugs ,Biofuels, personal products and flavour enhancers [8].

## Material and Methods

**Microorganism-** *Bacillus tequilenses* strain was isolated from oil mill waste. The organism was identified on the basis of morphological and biochemical analysis following Bergey's manual of determination of bacteriology.

**Qualitative assay-** The ability of *Bacillus sp* to produce lipase was tested in tributyrin agar medium containing tributyrin oil 1 % ( w/v). The pH was maintained at 7. Lipolytic activity of *Bacillus* strain was determined by measure in the diameter of hydrolytic zones around each colony [9].

## Lipase assay

The hydrolytic activity was tested by titrimetric method as described in (ACS Specifications, 1993) with slight modifications [10-12]. 20mM Phosphate buffer, Arabic gum (1% w/v) and the substrate (olive oil) 1:1 (v/v) were made up to a total volume of 10 ml. The reaction cocktail was thoroughly mixed and equilibrated at 20°C and 1 mL of crude enzyme was added after being previously incubated at the same temperature. The reaction was left at room temperature for exactly 30 min and stopped by adding 3 mL of 95% ethanol. The released fatty acids from oil substrate during enzymatic hydrolysis were titrated to neutralization with 50 mM NaOH in the presence of thymolphtalein as an indicator. A blank was prepared for each sample, where the enzyme was inactivated by heating at 95 °C for 15 minutes.

One unit of lipase activity was expressed as micro equivalents of fatty acid released from a triglyceride in 30 min at pH 7.0 at 20°C.

**Optimization of Medium Components for Lipase Production**
The optimizations of the following parameters were done for the production medium and they are checked for the lipase activity.

**Incubation Period**
*Bacillus tequilensis* was grown in Tribuytrin broth containing yeast extract, NaCl, peptone and 1% (w/v) olive oil at 36°C in an in orbital shaker at an agitation speed of 200rpm. The culture broth was harvested at 8h intervals by centrifugation at 8,000 g for 20 min at 4°C. Supernatant was collected and used as crude enzyme solution. It was assayed for lipase activity.

**pH**
To check the effect of pH on the production of enzyme, fermentation was carried out at different pH ranging from 3 to 10 with an interval of 1 and the lipase activity was assayed.

**Temperature**
To study the temperature optima of enzyme production, the fermentation was carried out at different temperatures 20°C, 25°C, 30°C, 35°C, and 40°Cwith an interval of 5°C for 12 hrs. The enzyme extracted from these cultures was assayed to check the activity.

**Agitation Speed**
To evaluate the impact of agitation speed on lipase production by *Bacillus tequilensis*, experiments was carried out at different agitation speeds ranging from 120 to 180 rpm at 30°C, pH of 7.0 with 1% (v/v) of inoculum. The cultures were incubated for 48 h and the lipase production was studied.

**Different Carbon Source**
To determine the effect of carbon source on lipase production by *Bacillus tequilensis*, carbon sources mainly carbohydrates were screened for their efficiency to support lipase production like Glucose, sucrose, maltose, lactose, starch were used as the basal carbon source in basal medium and was assayed to check for the lipase activity.

**Different Nitrogen Sources**
Optimization was carried out by using different organic nitrogen as nitrogen sources. Different nitrogen source used were tryptone, Ammonium sulphate, beef extract, yeast extract, Soya bean Meal and various combinations of them as nitrogen sources. and was assayed to check for the lipase activity

**Results and Discussion**
**Qualitative assay**

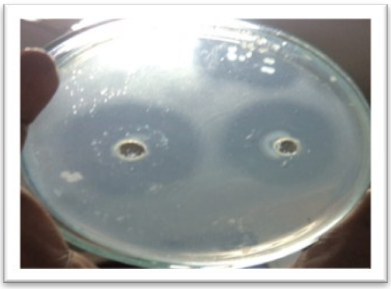

**Fig. 1-** Hydrolytic activity on tributyrin agar plate

**Growth Vs. Enzyme activity**
*Bacillus tequilensis* was cultured in 250 ml Erlenmeyer flasks containing 100 ml of medium at 30°C on in orbital shaker. The growth of the culture and lipase activity was monitored at a regular time interval of 4 hrs.

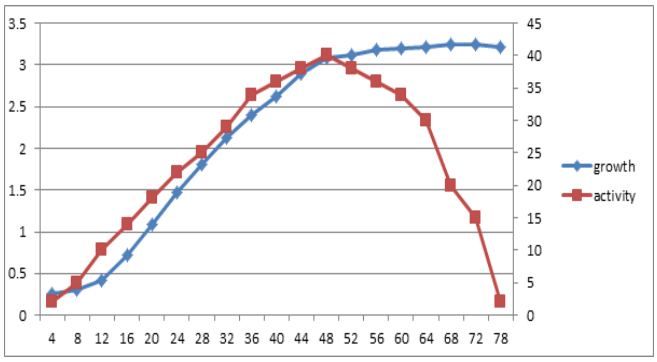


**Fig. 2-** Maximum enzyme activity of 4U/ml was obtained at the end of log phase (48 hrs incubation period). After 48hrs of incubation, the *Bacillus tequilensis* entered the stationary phase and a decrease in enzyme activity was observed

**Effect of different pH on extracellular lipase by solid submerged fermentation using *Bacillus tequilensis***

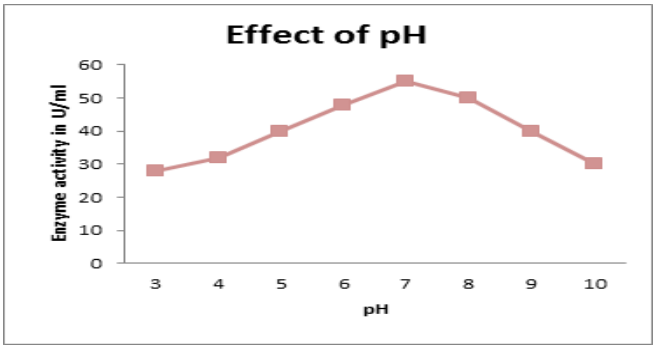


**Fig. 3-** Assay of the Lipase activity at different pH values within the range of 3-10 was done maximum activity was observed at pH 7.0 more than 80% of activity was observed at pH has indicated 7.0 as the optimum pH 6.0 to 8.0

**Effect of different Temperature on extracellular lipase by solid submerged fermentation using *Bacillus tequilensis***

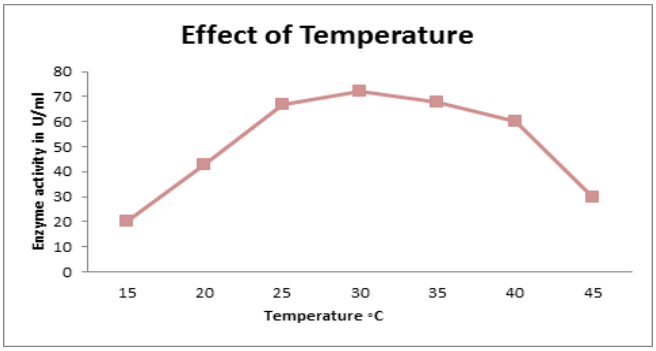


**Fig. 4-** Assay of was Lipase activity at different temperatures within the range of 15-45° C was carried has indicated 30° C as the optimum temperature.

**Effect of different Agitation Speed on extracellular lipase by solid submerged fermentation using *Bacillus tequilensis***

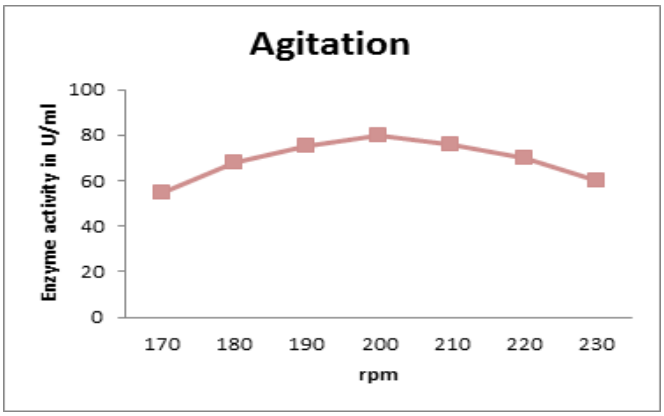


**Fig. 5-** Assay of the Lipase activity at different Agitation Speed within the range of 170-230 has indicated 200 as the optimum Agitation Speed

**Effect of different Carbon Source on extracellular lipase by solid submerged fermentation using *Bacillus tequilensis***

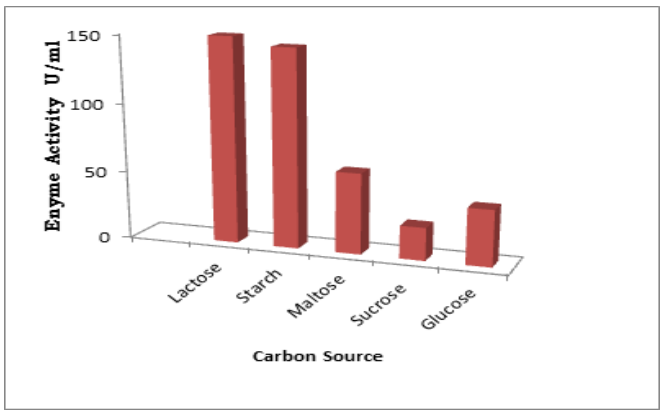


**Fig. 6-** Effect of different Carbon Source on extracellular lipase by solid submerged fermentation using *Bacillus tequilensis,* Lactose is high lipase activity

**Effect of different Nitrogen Source on extracellular lipase by solid submerged fermentation using *Bacillus tequilensis***

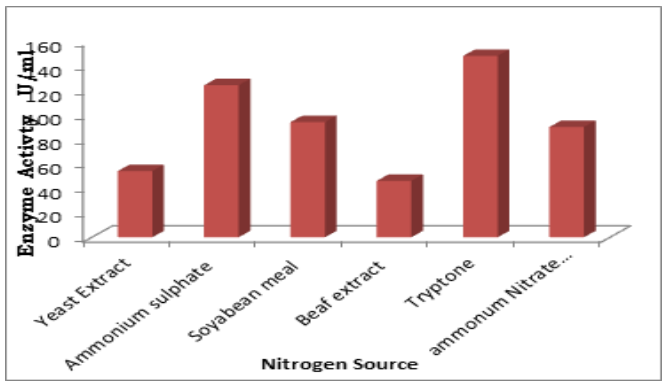


**Fig. 7-** Effect of different Nitrogen Source on extracellular lipase by solid submerged fermentation using *Bacillus tequilensis,* tryptone is high lipase activity

**Effect of different Oil Source on extracellular lipase by solid submerged fermentation using *Bacillus tequilensis***

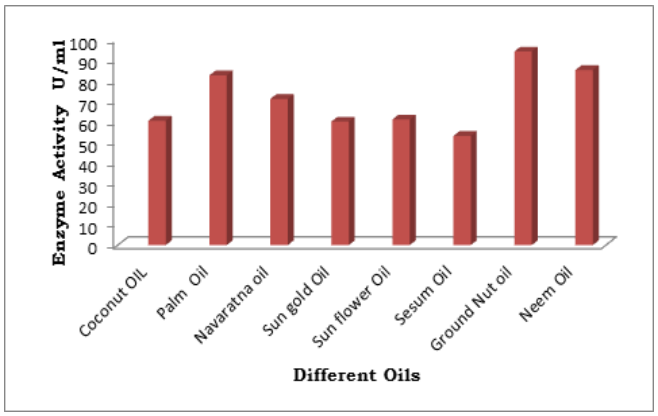


**Fig. 8-** Effect of different Oil Source on extracellular lipase by solid submerged fermentation using *Bacillus tequilensis,* Ground nut oil is high lipase activity

## Conclusion

This strain yielded the maximum enzyme activity and the activity of lipase is 150U/ml under optimized media conditions as 1% of Lactose and ground nut oil as the carbon source and Tryptone as the nitrogen source. The optimal cultivating conditions for better yield of Lipase production are 7.0 initial pH of medium, 30°C incubation temperature, 48 hrs incubation periods of seed, 1% inoculum volume and agitation speed of 200rpm.

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