**International Journal of Pharmaceutical Applications**
ISSN 0976-2639.Online ISSN 2278 – 6023
Vol 3, Issue 2, 2012, pp 352-359
http://www.bipublication.com

# FERMENTATION OF VEGETABLE OILS IN THE LIGHT OF LIPOLYTIC ACTIVITY WITH LIPASE PRODUCING BACTERIAL ISOLATES FROM SOIL SAMPLES

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[Received 05/05/2012, Accepted27/06/12]

## ABSTRACT:

Four bacterial lipase isolates, B1, B3, B4 and G2 from soil samples were used at 30$^{o}$C, 150 rpm, pH 7.0 for 92hrs to ferment the vegetable oils viz. coconut oil, groundnut oil, mustard oil, olive oil, sunflower oil and soybean oil, in the light of lipolytic activity for the degradation of different fatty acid esters of trihydroxy alcohols found in the nature. The respective maximum lipase activity (U/ml) was observed among all the isolates after 24hrs of fermentation. The lipase isolate B4 emerged as the best growth associated lipase producing bacteria by fermenting olive oil as C-source with lipase activity of 38.5 U/ml. Although lipase isolate B3 produced non-growth associated lipase with an activity of 15.5 U/ml by fermenting groundnut oil; the lipase isolate B1, produced growth associated lipase with an activity of 15 U/ml along with an excellent growth of 8.3 g/l. On the lower side, isolate G2 produced the lipase with its best activity as 10.5 U/ml by fermenting coconut oil with minimal growth. Hence the isolates B1, B3 & B4 may further be explored to optimize and improve their lipolytic activity for their possible usage in industry, environmental disasters and the research.

**Keywords:** Fermentation, Vegetable oils, Lipolytic activity, Growth associated lipases, Non-growth associated lipases, lipase isolates

## [I] INTRODUCTION

The soluble lipases (Triacylglycerol lipases, EC 3.1.1.3) with their remarkable hydrolytic ability, at the interfacial area of hydrophilic and hydrophobic solvent emulsions, on triacylglycerol to release free fatty acids and glycerol play a vital role in the recycling of lipids within the biomass of the earth. Considering this special property of lipase as a biocatalyst found in various biological sources with varied catalytic activity, the research has been carried out on its sources to screen & isolate the better lipase

producing sources to produce and commercialize the lipases. These lipases possess vital solicitations in the industries of pharmaceutical, textile, food, detergent, paper, cosmetics, dairy, bakery, leather processing, medicinal, chemical and biofuel production [1-11].

Although it is known that edible fats and oils are predominantly the mixtures of fatty acid esters of the trihydroxy alcohol or glycerol, they are mostly extracted from biological sources such as fruits, nuts, seeds and roots of plants and vegetables [12-13]. Among these lipid molecules, vegetable oils are the widely extracted and exploited lipids for many food preparations, medicines, coatings, paints, cosmetics, pastes, preservatives, fuels and many more applications in domestic, industrial and research domains [14 - 16]. When it comes to the research and development of lipase and its related activities, the vegetable oils such as olive oil, groundnut oil, palm oil, corn oil, sunflower oil, coconut oil, soybean oil, sesame oil and mustard oil have been the first choice as a means of substrate and medium for the growth of microbial organisms and the production of lipase enzyme [17-21]. On the other hand, lipases are found in animals, plants and the microbial bio-systems, but then it was found that microbial (bacterial and fungal) lipases have a broad spectrum of industrial and research applications due to their relatively better adaptability to variable ambient conditions and the affordability [22-26].

Considering the above facts, the present study illustrates the fermentation of vegetable oils with the bacterial lipase producing isolates from soil samples, in the light of lipase production and its activity along with the microbial growth over the period of four days of fermentation process.

## [II] MATERIALS AND METHODS

### 2.1. Isolation of Lipolytic bacteria

Soil samples were collected from the outskirts of Guntur (Andhra Pradesh), Nagpur (Maharashtra) and Udaipur (Rajasthan), India and these soil samples were processed in the Microbiology Laboratory, Department of Biotechnology, Sir Padampat Singhania University, Udaipur. The best lipase producing bacterial isolates from these soil samples were obtained using, serial dilution, pour plate, agar well diffusion and lipase titrimetric methods and named them as lipase producing bacterial isolates, B1, B3, B4 and G2 [27]. These bacterial isolates were subjected to physiological and biochemical tests for their characterization. All the isolates were sub-cultured routinely in the microbiology laboratory and preserved in agar slants at $4^{o}$C in the refrigerator.

### 2.2. Fermentation of vegetable oils with lipase producing bacterial isolates:

The lipase bacterial isolates B1, B3, B4 & G2 of 1ml each were inoculated separately with microbial concentration adjusted to 0.5 McFarland standard and cultured in sterile nutrient broth (HiMedia) added with 1% oil emulsified in the broth as carbon source for inducing lipase activity. Oil content was varied in the broth as coconut oil, groundnut oil, mustard oil, olive oil, sunflower oil and soybean oil for each lipase isolate. Other culture conditions such as temperature ($30^{o}$C) and agitation speed (150 rpm) were maintained as constant optimal physiological conditions [27] whereas incubation period was varied as 24h, 48h, 72h & 96h. Broth was harvested aseptically at an interval of 24hrs from all the samples and obtained the crude enzyme for further processing.

### 2.3 Preparation of crude lipase enzyme and estimation of biomass

Harvested broth samples were centrifuged at 10000g and $4^{o}$C for 10 minutes and the supernatant was collected aseptically as a source of the respective crude enzyme for lipase assay. The excess supernatant was discarded and the respective residual biomass pellets were dried in the oven at $50^{o}$C for 24hrs and weighed the residual dry biomass for all the bacterial lipase isolated at the respective incubation periods.

### 2.4 Lipase assay by titrimetric method

Lipase activity for each sample with the combination of vegetable oil and lipase isolate at every interval was measured by titrimetric method [28] using olive oil as substrate at pH 7.0. The reaction cocktail was prepared by 10% (w/v) olive oil emulsified in the solution of 5% (w/v) gum acacia dissolved in 100mM sodium phosphate buffer, pH 7.0. The crude lipase enzyme (1ml) from each sample was added to the substrate reaction cocktail of 10ml separately, except the blank at room temperature and then incubated at 30°C and 100 rpm for 15 min. in an orbital shaker incubator. The lipase reaction was quenched and fatty acids were extracted from the reaction mixture by adding 1ml of acetone: ethanol solution (1:1) and swirling the contents swiftly. Phenolphthalein indicator of 2-3 drops was added to each reaction mixture and the control. The contents of each reaction mixture were titrated with 0.05M NaOH solution to an end point of pink color at pH 10.0 [26, 29]. Lipase activity was calculated as micro moles of free fatty acids formed from olive oil per ml of crude lipase enzyme [30] given by equation (1).

$$Activity = \frac{(V_S - V_B).N.1000}{S} \quad (1)$$

Where, $V_S$ is the volume of 0.05M NaOH solution consumed by the enzyme-substrate cocktail (ml); $V_B$ is the volume of 0.05M NaOH solution consumed in the titration by the substrate (Control) cocktail (ml); $N$ is the molar strength of the NaOH solution used for titration (0.05) and $S$ is the volume of substrate cocktail solution (10ml).

One unit (U) of lipase enzyme is defined as the amount of enzyme required to liberate 1µmol of fatty acids from triglycerides. Hence the activity of the enzyme is expressed as U/ml of crude enzyme extract.

## [III] RESULTS

### 3.1 Growth and lipase profiles

The biomass growth profiles of bacterial lipase isolates at 30°C over a period of 96hrs on the vegetable oils viz. coconut oil, groundnut oil, mustard oil, olive oil, sunflower oil and soybean oil were depicted in Figures 1-4; whereas the corresponding lipase activity profiles for each isolate were depicted in Figures 5-8.

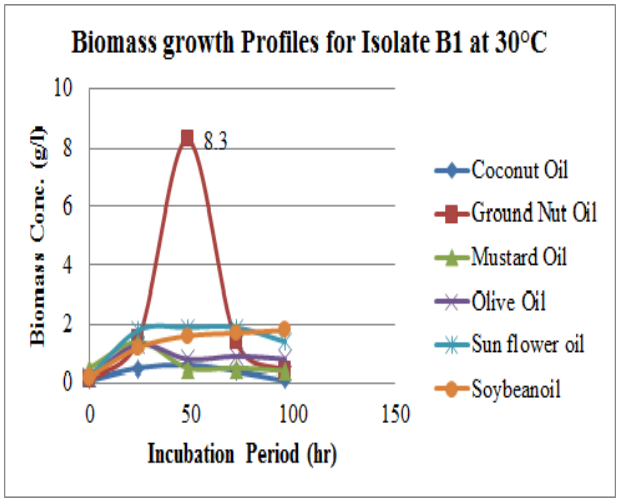


**Fig: 1**. Biomass growth profiles of *isolate B1* from the vegetable oil fermentations

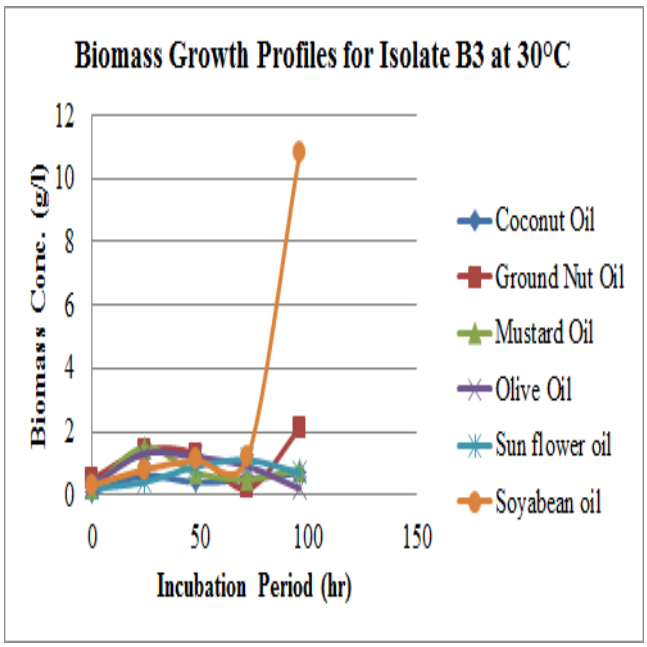


**Fig: 2.** Biomass growth profiles of *isolate B3* from the vegetable oil fermentations

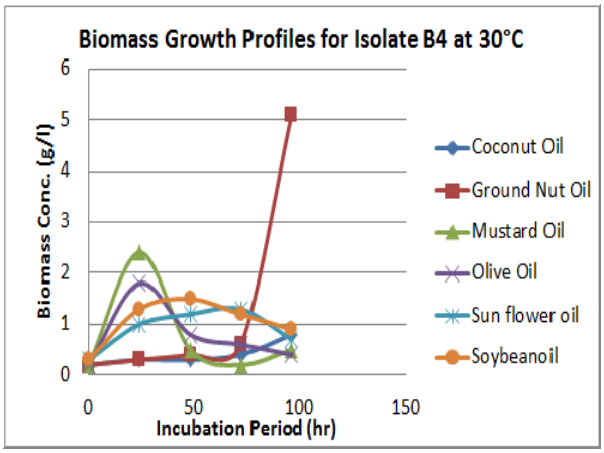


**Fig: 3**. Biomass growth profiles of *isolate B4* from the vegetable oil fermentations

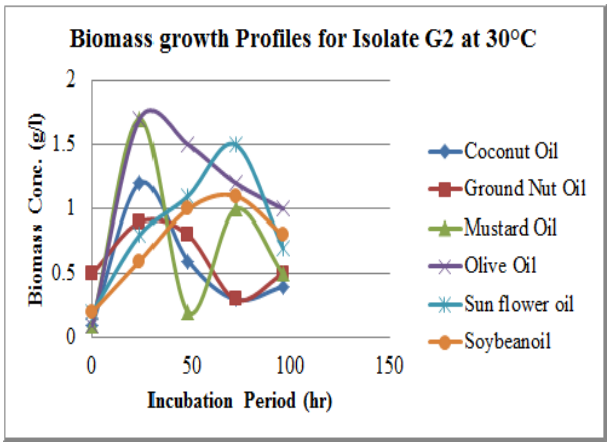


**Fig: 4.** Biomass growth profiles of *isolate G2* from the vegetable oil fermentations

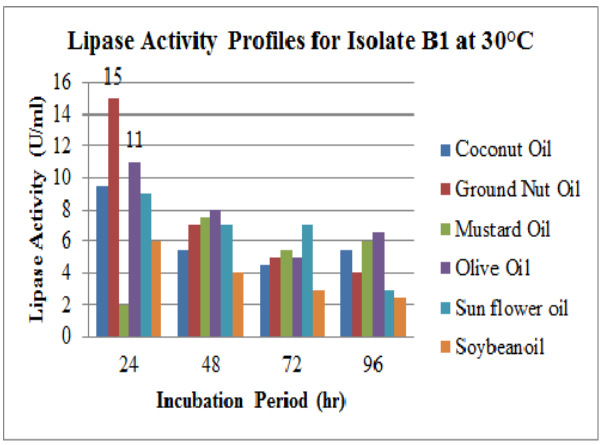


**Fig: 5**. Lipase activity profiles from the fermentation of vegetable oils by *isolate B1*

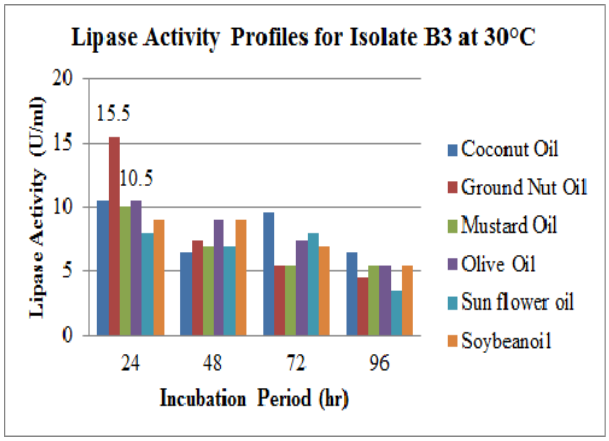


**Fig: 6.** Lipase activity profiles from the fermentation of vegetable oils by *isolate B3*

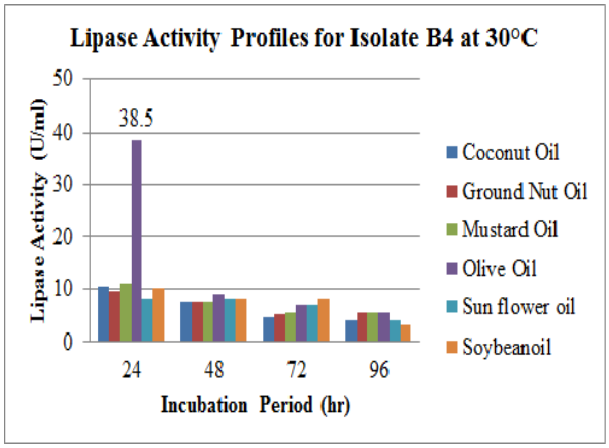


**Fig: 7.** Lipase activity profiles from the fermentation of vegetable oils by *isolate B4*

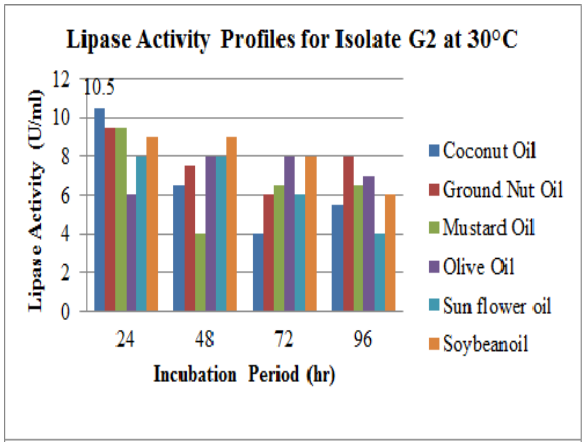


**Fig: 8.** Lipase activity profiles from the fermentation of vegetable oils by *isolate G2*

## [IV] DISCUSSION

Analysis of Figures 5 & 1 indicates the capability of lipase isolate B1 to produce the maximum lipase activity, 15U/ml, using ground nut oil as a C-source for its growth and metabolic activities, by 24hrs of fermentation; whereas the maximum growth (8.3 g/l) of isolate B1 was observed by 48hrs. The surge of exponential growth here could have been the result of lipolytic activity of lipase, break down of ground nut oil, leading to the creation of smaller fatty acids that could be assimilated easily by the lipase isolate B1, after 24hrs of incubation. In the later periods of fermentation with ground nut oil the lipase activity diminished gradually, leading to the gradual reduction in the growth of isolate B1 and vice versa. Further, the second best fermented vegetable oil with the lipase activity of 11U/ml by 24hrs of fermentation period was olive oil; but for the later period, the growth was almost static around 0.8g/l with decreased lipase activity. All other vegetable oils recorded moderate to lower lipase activity (6.5 – 2.5 U/ml) and oscillating growth in the range of 0.5 – 2.5 g/l all through the fermentation period.

Fermentation of ground nut oil with lipase isolate B3 (Figures 6 & 2) had produced the lipase of 15.5 U/ml by 24hrs and in the later periods, diminishing lipase activity was observed and all this while the growth of lipase isolate B3 was trailing around 1.5g/l, which may be due to the adoption of isolate B3 to assimilate or feed on non-fatty nutrient sources; however the higher lipase activity by 24hrs of fermentation may be to eliminate the obstructing oily layer between the aqueous nutrients and the cell boundary. Hence steady growth was observed for more than 48hrs and then after 76hrs, the isolate B3 started to grow on the fatty acids released till then. Similar to isolate B1, the second best vegetable oil for the fermentation of isolate B3, was olive oil, with lipase activity of 10.5U/ml after 24hrs of incubation, and then in the later periods, the activity was gradually decreasing, but there was a moderate growth of 1g/l all through the fermentation period till 72hrs and then declined, implying that the isolate B3 was feeding on the both fatty and non-fatty nutrients simultaneously. Another peculiar observation was made from the fermentation of soybean oil recording an above average lipase activity of 8.0U/ml throughout the incubation period and an accelerated growth profile after 76hrs of incubation period, which could be due to the primary growth on non-fatty nutrients till 76hrs and then the surge of secondary growth was observed, due to the metabolic shift in feeding on to fatty acid nutrients liberated till then. However all other vegetable oils were fermented steadily with an average lipase activity of 6U/ml throughout the fermentation period with an average growth of 1g/l.

The highest lipase activity, 38.5U/ml for the fermentation of olive oil by isolate B4, by 24hrs of incubation period was recorded from Figure-3, whereas by the same duration, higher growth of 1.8g/l was observed from Figure-7. In the later period of fermentation, the lipase activity declined below 10U/ml for olive oil and the biomass growth also declined to an average of 0.5g/l. These observations indicate the ability of isolate B4 to feed simultaneously on both fatty acids and non-fatty nutrients. Unlike the previous isolates, the isolate B4 had the second best choice for fermentation as mustard oil with recorded lipase activity above 10U/ml by 24hrs of fermentation and the highest growth was observed to be 2.4g/l; subsequently the growth as well as lipase activity declined, which also refers to the remarkable ability of isolate B4 to assimilate both fatty acids and non-fatty nutrients simultaneously. Another interesting observation was made from Figures 3&7 with the fermentation of ground nut oil by isolate B4, where the growth was slow & steady till 48hrs and then the growth accelerated; however lipase activity was 9.5U/ml by 24hrs of incubation and then steadily declined. This Phenomenon could

be due to the mixed pool of fatty acids and non-fatty nutrients till 76hrs, where the metabolic pathways might have been oscillating to equilibrium in the isolate B4 and then the growth has taken a leap to reach the accelerated growth. The other two vegetable oils sunflower oil & soybean oil recorded a moderate and stable growth throughout the fermentation period with moderately declining lipase activity. On the other side the coconut oil recorded lipase activity of 10.5U/ml by 24hrs of fermentation and then the lipase activity declined constantly with slower growth rate below 1g/l, which indicates unfavorable growth conditions with lower lipase activity of isolate B4.

From Figures 8 & 4, the respective maximum lipase activity of 10.5U/ml for the fermentation of coconut oil with the maximum growth of G2, 1.2 g/l by 24hrs of incubation was observed; but later the lipase activity declined along with the declined growth of biomass. Similar to isolate B4, isolate G2 has the second best fermentable nutrient as mustard oil with lipase activity of 9.5U/ml and the higher growth of 1.7g/l by 24hrs of fermentation and then the lipase activity as well as growth were oscillating at reduced values, which can be inferred to the switching of metabolic activities to assimilate fatty and non-fatty nutrients in an alternate way and it also suggests lipase as a growth associated secondary metabolite for isolate G2. Another inference can also be made from figures 8 & 4 that the isolate G2 has the higher growth of 1.8g/l by 24hrs incubation but the lipase activity was lowest 6U/ml and then the growth declined whereas the lipase activity increased to 8U/ml; this indicates the preferential growth of G2 on non-fatty nutrients. Similar results were also observed for the fermentation of ground nut oil with isolate G2. In case of sunflower oil and soybean oil, the lipase activity was steady for 48hrs and then it was declining whereas the growth was increasing steadily for 72hrs and then declined; which indicate the steady feeding of isolate G2 on both fatty and non-fatty nutrients up to 72hrs and then as the nutrients exhausted, the growth also started to decline. All the bacterial isolates were found to be gram positive rod shaped bacteria, and they are more likely to be of the genus, *Bacillus*.

## [V] CONCLUSION

Lipase isolate B4 emerged as the best isolate to ferment olive oil with highest lipase activity of 38.5U/ml by 24hrs of fermentation and the lipase was produced as growth associated product. Although isolate B3 produced the lipase activity of 15.5U/ml, as the second best lipase activity in the study, the isolate B1 may be the best isolate with the fermentation on ground nut oil producing lipase activity of 15U/ml along with an excellent growth of 8.3g/l by 24hrs of fermentation. Isolate B1 produced lipase as growth associated product whereas isolate B3 produced lipase as non-growth associated product. So these three isolates B1, B3 & B4 with higher lipase activity may further be explored to optimize and enhance their lipolytic activity as growth associated and non-growth associated products by using various optimization techniques & methods for their possible usage in industry, environmental disasters and the research.

## ACKNOWLEDGEMENT

The authors are grateful to Prof. P. C Deka, the Vice Chancellor, Sir Padampat Singhania University, Udaipur, Rajasthan, India, for his help, encouragement and insights at various stages of the research work. Dr. Archana Gajbhiye, Head, Department of Biotechnology, School of Engineering, Sir Padampat Singhania University, Udaipur, Rajasthan, India, is also gratefully acknowledged for the help and suggestions at various stages of the research work carried out.

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