**International Journal of Science and Research (IJSR)**
**ISSN: 2319-7064**
**Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296**

# Characterization and Cultural Optimization of Lipase Producing Bacteria Isolated from Oil Contaminated Soil

**Kyaw Swa Lynn[1], Tin Tin Hla[2]**

[1]Mandalay Technological University, Department of Biotechnology, Mandalay Region, Republic of the Union of Myanmar

[2] Mandalay Technological University, Department of Biotechnology, Mandalay Region, Republic of the Union of Myanmar

**Abstract:** ***The present study involves isolation and characterization of lipase producing bacteria from oil-contaminated soils and comparative studies of their lipase activities by two quantitative methods: titrimetric and spectrophotometric assay methods. Totally, 17 bacterial lipolytic strains were screened on tributyrin agar medium. Five isolates which possessed higher lipase activity were further confirmed by Rhodamine B-olive agar for true lipase activity. After quantitative studies in fatty acid formations, the isolate 8a was selected as the best lipase producer strain. Morphological, biochemical characteristics and 16s rRNA sequence analysis had been carried out. Potent isolate was identified as Bacillus subtilis. Growth conditions of Bacillus subtilis for maximum lipase productivity were optimized. The optimum temperature and pH for lipase productivity were 38˚C and pH 6 at 5 days incubation time. The optimum condition for enzyme productivity was obtained when 2% olive oil substrate was used, and olive oil was found as the best substrate for lipase synthesis.***

**Keywords:** lipase producing bacteria, Rhodamine B-olive oil agar, tributyrin agar, characterization, sequence analysis, optimization

## 1. Introduction

A large part of the earth's biomass was composed of lipids and they are very important things in our daily life [1]. Lipolytic enzymes, grouped into three main categories, (esterases, phospholipases and lipases) catalyze the hydrolysis of triacylglycerols into diacyglycerols, monoacylglycerols, free fatty acids (FFAs) and glycerol by acting at oil water interface and they also breakdown lipids and make them mobile within the cells of individual organisms [2], [3]. Lipases, which can both hydrolyze lipids or synthesize esters, are the most important class of hydrolytic enzymes [4] and its function is presented as the following equation;

Triglycerides ↔glyceride + fatty acids [5]

Although lipases are widely occurred in nature from animals, plants and microbes, microbial lipases have received special attention because of their substrate specificity, selectivity, stability and a variety of enzymatic properties, such as wide pH, wide range of temperature [6]. Lipase-producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, oilseeds, decaying food, hot springs, coal tips, and compost heaps [7], [8]. Lipase has a wide range of applications in various industries because of their specific properties. They have many biotechnological applications such as, food technology, fats and oils industries, detergents and degreasing formulations, the synthesis of fine chemicals and pharmaceuticals, paper manufacture, production of cosmetics, biosurfactants and biomedical science [9]-[11]. Bacterial enzymes have higher activities and neutral or alkaline pH optima over fungal enzymes [12]. Bacterial lipases are mostly secreted out in the culture medium as extracellular enzymes and they are affected by nutritional and physico-chemical factors; such as temperature, pH, nitrogen and carbon sources, presence of lipids, inorganic salts, stirring conditions, dissolved oxygen concentration [13].

Several methods have been developed to screen and measure for lipase activity and production. Most of qualitative methods are agar plate assay methods. The most useful and reliable method for lipase activity is Tributyrin Agar (TBA) plate assay method by using tributyrin as a substrate [14], [15].

Several colorimetric methods were used for detecting lipase activity. Rhodamine B plate assay is used to detect true lipase and the formation of orange fluorescent halos around the colonies visible under UV radiation confirms lipase production [16]. The most widely used quantitative methods for determining lipase activity are titrimetric method [17] using olive oil as a substrate and colorimetric copper soap method spectrophotometrically [18].

Currently, a large number of bacterial lipase has been found from various sources, but industries need more of noble lipase with greater specificity, activity and different characteristics from different sources. The present study was designed to isolate and characterize lipase producing bacteria from oil contaminated soil and choose the potent strain with high lipolytic activity by qualitative and quantitative methods, optimize its cultural conditions for lipase productivity.

## 2. Materials and methods

### 2.1 Collection of samples

Oil spilled soil samples were collected from oil mills located


Licensed Under Creative Commons Attribution CC BY

at Nwar Htoe Gyi Township and Maharaungmyay Township, Mandalay Region, Myanmar. These mills were operated and produced plant oils commercially. The contaminated soil samples were collected using aseptic technique in sterile plastic bags for the isolation of lipase producing bacteria under laboratory conditions.

### 2.2 Isolation and screening of lipolytic bacteria

To begin with, lipolytic bacteria from collected soil samples were firstly screened by using Tributyrin Agar (TBA) plate assay. After 48 hr incubation at 37˚C on TBA plates, colonies showing clear zones formation were restreaked as lipolytic strains and five isolates with greater clearance zones were further examined by Rhodamine B- olive oil agar (ROA) plate method according to C. H. Collins, and his colleagues. After 24 -48 hr incubation period, the lipase producing activities were identified by formation of orange fluorescent halos around the colonies visible upon UV irradiation at 350 nm these five strains were used for further studies.

### 2.3 Characterization of selected lipolytic bacteria

The selected five strains were characterized based on microscopic morphology, gram staining, and some biochemical tests according to Bergey's manual of determinative bacteriology [19].

### 2.4 Production media and crude enzyme preparation

Firstly, inoculums of screened bacteria were prepared in the culture media broth composed of (0.5% olive oil emulsified with 0.2% tween 80, 0.3% yeast extract, 0.2% NaCl, 0.04% $MgSO_4$, 0.07% $MgCl_2$, 0.05% $CaCl_2$, 0.03% $KH_2PO_4$, 0.03% $K_2HPO_4$, 0.05% $(NH_4)_2SO_4$) in 50 mL conical flask and incubated in water -bath shaker for 2 days at 37˚C and 150 rpm agitation. Then, the enzyme production media was prepared in the above media except with 1% olive oil + 0.3% tween 80 and 0.1% yeast extract according to L. Dong-Woo and his partners [20]. Triplicate flasks were inoculated for each isolates with 1% v/v of inoculum to a 100 mL of medium of 500 mL flasks. The inoculated shake flasks were subjected in a water-bath shaker at 37˚C, 150 rpm agitation for 6 days. Samples were withdrawn at every 24 hr intervals and collected samples were centrifuged at 9000 rpm for 15 min. The supernatant were filtered by filtration and used as crude enzyme to determine lipase assay.

### 2.5 Lipolytic enzyme assays

In order to select a best lipase producer strain, five bacterial isolates were investigated for the production of lipase by determining free fatty acids (FFAs) formation released from enzyme-substrate reaction during enzymatic hydrolysis of olive oil substrate. In this study, two kinds of assay methods were used to estimate FFAs formation; (i) titrimetric method, and (ii) colorimetric copper soap method using UV spectrophotometer.

#### 2.5.1 Titrimetric method for lipase productivity

The lipase activity of crude extracellular lipase enzyme for various incubation times of five isolates was assayed by titrimetric estimation of FFAs liberated during hydrolysis of olive oil emulsion. The reaction mixture was prepared with some modifications of the reference [21]: in brief, 1 mL of crude enzyme solution was mixed with 3 mL of olive oil emulsion (1% w/v gum arabic), 1 mL of 0.1 M tris-buffer (pH 7.2), 2 mL of deionized water and 0.02 mL of 20 mM CaCl2 solution. It was subjected into water bath-shaker at 37˚C, with 150 rpm agitation for 30 min. The reaction was stopped by placing it at 4˚C and added 4 mL of ethanol into it. Then, the free fatty acids liberated during the reaction was titrated against 0.05 N NaOH using phenolphthalein as indicator. The control containing the reaction mixture and ethanol without the addition of enzyme was titrated against 0.05 N NaOH similarly. One unit of lipase enzyme was defined as the amount of enzyme that released 1 μmole of fatty acid per ml of enzyme solution under assay conditions, using the following equation:

$$\text{Amount of enzyme} = \frac{\Delta \text{ Vol. of NaOH} \times \text{Molarity of NaOH} \times 1000 \times 2 \times \text{DF}}{1 \text{ mL of enzyme used}}$$

ΔVol. of NaOH = volume of NaOH for sample – volume of NaOH for blank,
1000 = conversion factor for miliequivalent to microequivalent,
2 = time conversion factor from 30 minutes to 1 hour (unit definition),
DF = dilution factor

#### 2.5.2 Spectrophotometric method for lipase productivity

Liberated free fatty acids (FFAs) were determined spectrophotometrically by using colorimetric copper soap method. The reaction mixture was prepared as described by Kwon and Rhee with slight modifications. The reaction was initialized by addition of 1 mL of crude enzyme into 2.5 mL olive oil (emulsified with 1% w/v gum arabic), 300 μL of 1 M tris –buffer pH 7 and 0.02 mL of 20 mM $CaCl_2$ solution. The mixture was incubated in water -bath shaker at 50˚C, 150 rpm agitation rate for 30 min. The reaction was terminated by adding 1 mL of 6 N HCl and followed by addition of 5 mL of benzene and the mixture was vortexed for one minute. The upper layer containing FFAs was separated into which 1 mL of cupric acetate/pyridine reagent was added. The two phases thus formed were vortexted for one minute. The reagent was prepared by adjusting the solution of 5% (w/v) copper (II) acetate to pH 6.1 with pyridine. Free fatty acids dissolved in benzene layer were determined by measuring the absorbance of benzene solution of green colour at λ max = 715 nm. To determine lipase activity, standard curve of oleic acids was drawn with samples containing different concentrations of oleic acid (20, 40,-, 140 in μmoles). The samples were prepared in 5 mL of benzene and 1 mL of cupric acetate/pyridine reagent (CAPR) solution and the two phases formed were mixed by vortexing one minute. Then, the standard curve of oleic acid and absorbency was plotted by measuring the absorbance of benzene layer at λ max = 715 nm against the control which contains no free fatty acids.


Licensed Under Creative Commons Attribution CC BY

### 2.6 Identification and sequence analysis of lipase producing bacteria

After investigation on lipase production of five isolates (2, 5,8a, A, and B) by two quantitative assay methods, isolate 8a was selected as the best lipase producer strain. To identify the isolate 8a, isolation of genomic DNA from the bacteria was done by the DNA extraction method [22]. Then, genomic DNA was amplified with a set of universal bacterial primers, 27F (5' AGAGTTTGATCMTGGCTCAG 3') as forward primer and 1492R (5' TACGGYTACCTTGTTACGACTT 3') as reverse primer to get 16s rRNA gene of strain 8a. Amplification of purified DNA was done in a thermocycler: 35 cycles of 95ºC for 15 seconds, 56ºC for 15 seconds and 72ºC for 30 sec [23]. After that, PCR products were visualized by agarose gel electrophoresis and DNA sequencing was performed in a highly automated gene sequencer. These sequences were compared to other sequences in GenBank database (BLASTN) to identify the bacterial classe and species.

### 2.7 Study on the growth pattern of the selected bacteria

The growth pattern of the potent strain was determined by incubating it on nutrient broth medium and collecting samples at every four hours for the first 16 hours and, at every six hours for the rest till to 62 hours. The optical density (OD) of collected samples were determined for the bacterial growth by using UV-VIS spectrophotometer at 600 nm and the growth curve was drawn against the absorbance of OD values over incubation period.

### 2.8 Optimization of cultural conditions for lipase productivity

Some parameters of lipase production media were investigated for lipase activity by varying one at a time method to optimize culture conditions. They were pH, temperature, lipid substrates and substrate concentrations. The optimum incubation time was observed at day 5 incubation time in previous experiments. The lipase production was measured spectrophotometrically using colorimetric copper soap method.

#### 2.8.1 Effect of pH and temperature

Lipase production of *bacillus* sp. was investigated on production media with different pH (5- 9) and three different wide range of temperature {30, 40, 50 (°C)} in a water bath shaker at 150 rpm agitation, 1% (w/v) olive oil concentration. Optimal lipase productivity was occurred at 40˚C and then optimum temperature was studied again with a short range at around 40˚C (38˚C, 41˚C, 44˚C).

#### 2.8.2 Effect of substrate concentration

To observe which substrate was the best for lipase production, different substrates (olive oil, groundnut oil, sesame oil, mustard oil and sunflower oil) with 1% (w/v) were used and studied for lipase productivity while other parameters were remaining keep the same.

The effect of various concentration of olive oil (0.5% to 2.5% w/v) on lipase production was investigated to get optimum substrate concentration at temperature 37˚C, 150 rpm agitation, and pH 7 for 5 days.

## 3. Results and discussions

### 3.1 Isolation and Screening of Lipolytic Bacteria

The lipase producing microbes were isolated from two different soil samples by plating method on TBA media containing 1% tributyrin (Figure. 1(a)). The hydrolysis of tributyrin caused clear zone around the colonies. The bacterial colonies showed clear zones were screened for lipolytic activity. Totally, 17 isolates were collected from the soil samples. Among them, five isolates (2, 5, 8a, A and B) showed high lipolytic activity. Further studies were performed on these five isolates. The production of lipase enzyme was confirmed with 0.1% Rhodamine B -olive oil agar (ROA) (Figure. 1(b)). In this experiment, orange colour did not appear on agar plate after incubation time at room temperature, it was observed after storing the agar plates overnight at 4°C refrigerator. Orange fluorescent colour was found when the plates were determined under UV light. ROA plate assay was used to confirm true lipase activity because lipolysis can be made by other enzymes like esterase. Among 5 isolates, the color of orange fluorescent formation of strain A and B were more significant than other three strains.

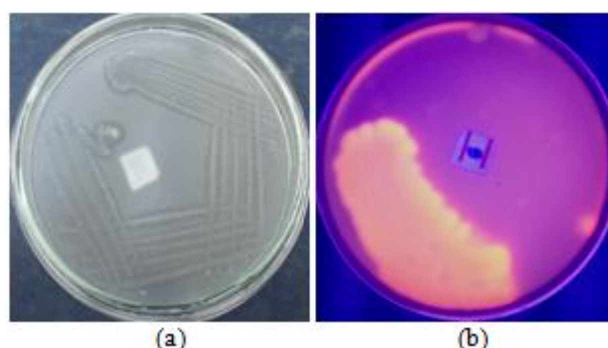


**Figure 1**: Screening of lipolytic activity of bacterial isolates on (a) Tributyrin agar medium (b) Rhodamine B agar medium under UV light

### 3.2 Characterization of selected lipolytic bacteria

After screening, four isolated strains were characterized as gram positive and one was gram negative. All of five strains were rod shaped. Some biochemical tests of the selected bacteria were also shown in (Table 1).

**Table 1:** Some biochemical properties of lipase producing bacteria

| *Strains No.* | *2* | *5* | *8a* | *A* | *B* |
|---|---|---|---|---|---|
| Gram' stain | - | + | + | + | + |
| Catalase | + | + | + | + | + |
| Indole | - | - | - | - | - |
| Methyl red | - | + | - | - | - |
| Voges-Proskauer | + | - | + | + | + |
| Citrate | + | + | + | + | + |
| strach | - | + | + | + | + |
| Casein | + | + | + | + | + |
| gelatin | - | - | - | - | - |


Licensed Under Creative Commons Attribution CC BY

### 3.3 Assays for lipolytic activity

To choose a best lipase producer strain out of five strains, comparative studies of lipase production on these isolates were done by measuring fatty acids formations released from enzyme-substrate reactions. FFAs formation was determined by two quantitative assay methods; titrimetric method and UV spectrophotometric method.

#### 3.3.1 Lipase producing activity and free fatty formation by titrimetric method

Figure 2 shows the lipase enzyme activity of five isolates over a period of 6 days incubation time using titrimetric enzyme assay. In this figure, strains B gave the best activity than other strains at day 5 although it slightly dropped at day 4 incubation time, whereas strain 2 showed the lowest activity. According to the line graph, strain B, A and 8a revealed a better pattern and higher activity than the rest two strains. FFAs formation of strain A increased till to day 6. So, from this experiment, isolates A, B and 8a were picked up to choose the best strain by comparing their lipase activity in spectrophotometric assay method.

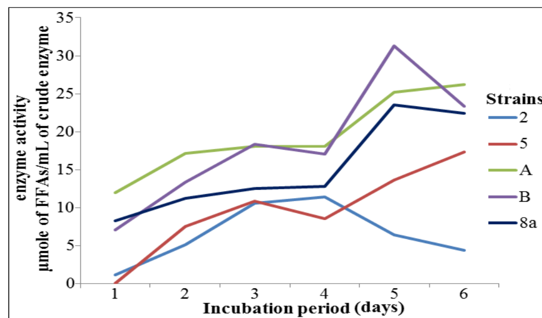


**Figure 2:** Lipase enzyme activity (µmoles of FFAs/mL of crude enzyme) of five isolates over six days incubation period by titrimetric assay

#### 3.3.2 Lipase producing activity and free fatty formation by spectrophotometric method

**(a) Oleic acid standard curve**

Figure 3 shows the oleic acid standard curve drawn to determine the amount of FFAs released by enzyme –substrate reaction as a reference. In spectroscopic assay, the reaction mixture and procedure were performed by Kwon and Rhee method. But, in this study, benzene was used instead of isooctane to cover the cost. So filtration step was needed because cupric acetate does not dissolve completely in benzene.

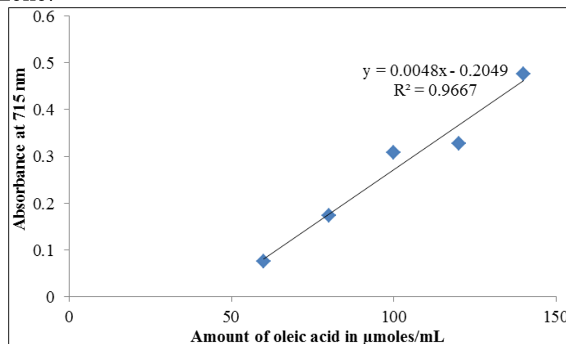


**Figure 3:** standard calibration curve of oleic acid with benzene, CAPR at 715 nm

**(b) Enzyme Assay**

Lipase activity was determined spectrophotometrically by using colorimetric copper soap method. Three strains (A, B, and 8a) were incubated in production media for 8 days. The absorbent values of the benzene layer were calculated using the equation, y = 0.0048x-2049, which was resulted from oleic acid standard curve shown in figure 3. Lipase activities of the three strains (A, B, and 8a) for 8 days incubation period were shown in figure 4. According to the results, lipase activities of three strains were not different significantly from each other. Estimate amounts of enzyme activity were 36 µmole/mL for isolate A on day 7, 36.2 µmole/mL for isolate 8a on day 5, and 32.73 µmole/mL for isolate B on day 5 respectively. But strain 8a was showed highest activity and most reliable results in the pattern of FFAs formations over incubation time. So, isolate 8a was chosen as the best lipase producer strain for further studies.

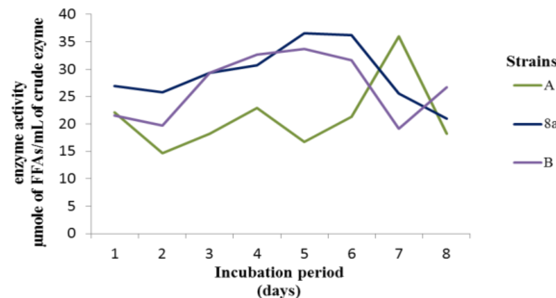


**Figure 4:** lipase activity (unit/ mL) of three isolates (A, 8a, and B) at different time intervals (day 1-8) by spectrophotometric method

### 3.4 Identification of potent strain 8a

16S rDNA sequence of the potent strain was amplified by 27F and 1492 primers, the amplified products were shown in Figure 5. They were sequenced and compared to the Genbank databases with the help of BLASTN program. The potent strain, 8a, had 99% identity to *Bacillus subtilis* (Table 2). So, according to the data observed on biochemical tests, molecular and morphological identification, the potent strain, 8a, was identified as *Bacillus subtilis.*

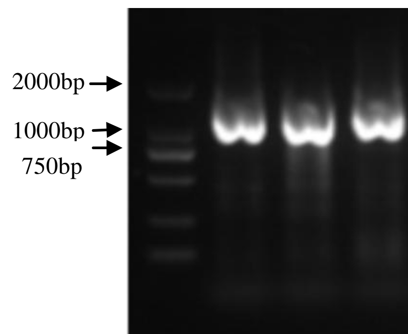


**Figure 5:** PCR results: MarkerDL2000、8a、2、5

**Table 2:** Sequencing result

| *strain's name* | *Primer (27-1492bp)* | *Sequence accession description* | *similarity* |
|---|---|---|---|
| 8a | 27F 1492R | *Bacillus subtilis GenBank: FJ435215.1* | 99% |


Licensed Under Creative Commons Attribution CC BY

### 3.5 Growth condition of *Bacillus subtilis* on nutrient agar

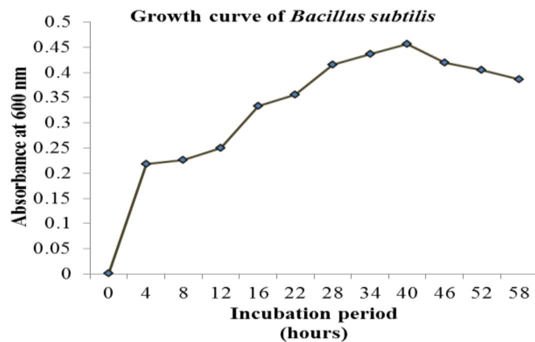


**Figure 6:** Growth pattern of *Bacillus subtilis* on nutrient agar by measuring OD values at 600nm over incubation period

Figure 6 shows the growth condition of *Bacillus subtilis* for 58 hr incubation period on nutrient media. Maximum growth of culture was detected at 40 hr. it indicated that the growth of *Bacillus subtilis* reached maximum within 48 hr in lipid free media, but in growing on lipid media, lipase synthesis was at optimum at day 5. So, it can be interpretable that the growth may be slow and/or exponential phase may be too long in olive oil containing media.

### 3.6 Optimization of cultural conditions for lipase productivity

Lipase activity was assayed by spectrophotometric method. Optimum incubation time was observed at day 5 incubation time according to the results of two quantitative experiments.

#### 3.6.1 Effect of pH on lipase productivity in terms of FFAs formation

The optimum pH for the activity of the enzyme is 6 and it is active over a broad range of pH 5–9 (figure 7). As described in the graph, it suggested that the organism more preferred in acidic pH conditions.

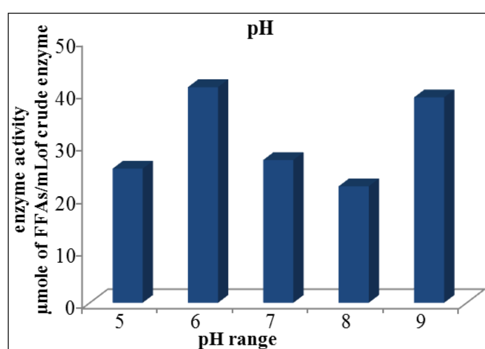


**Figure 7:** Effect of pH on lipase productivity using 1% olive oil as substrate

#### 3.6.2 Effect of temperature on lipase productivity in terms of FFAs formation

Optimum temperature for lipase activity of *Bacillus subtilis* was investigated by incubating it at three different temperatures (30˚C, 40˚C, and 50˚C). It observed that the optimum temperature for enzyme synthesis was at around 40 ˚C as described in figure 8. But temperature range was wide, so determination of optimum temperature was repeated again with a narrow range at around 40˚C. It was evident that stimulation of lipase synthesis was at temperature between 34˚C and 45˚C and the optimum temperature for lipase production was at 38˚C (Figure 9).

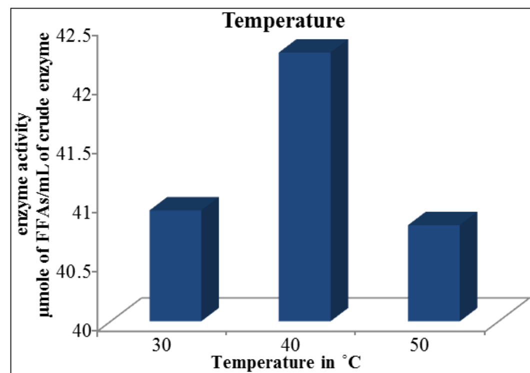


**Figure 8:** Effect of temperature on lipase productivity using 1% olive oil as substrate

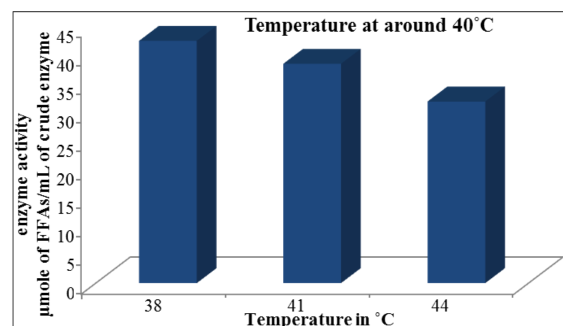


**Figure 9:** Effect of temperature on lipase productivity using 1% olive oil as substrate

#### 3.6.3 Effect of substrate concentration on lipase productivity in terms of FFAs formation

Various edible oils were used to determine the best carbon source for the bacterial lipase production. Figure 10 reveals lipase activity of bacteria by the effect of various substrates. It suggested that olive oil was the best substrate for bacteria to produce lipase enzyme. It can also be seen that sesame oil was the second favorite oil, nearly the same as olive oil and it was surprising because the soil source for *Bacillus subtilis* was collected near from sesame producing oil mill.

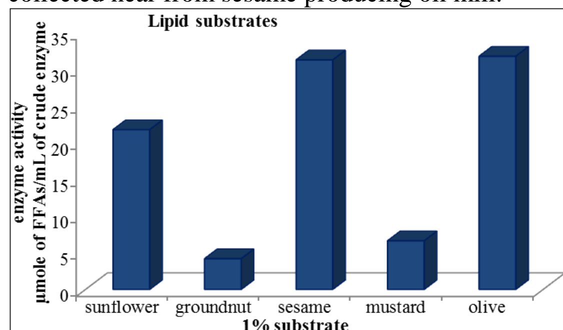


**Figure 10:** Effect of different lipid substrates (1%) on lipase productivity

The optimum substrate concentration for lipase activity was studied by incubating of bacteria at various concentrations of olive oil substrate (0.5%-2.5%). It was observed that lipase activity was at maximum level when 2% olive oil was used (Figure 11).


Licensed Under Creative Commons Attribution CC BY

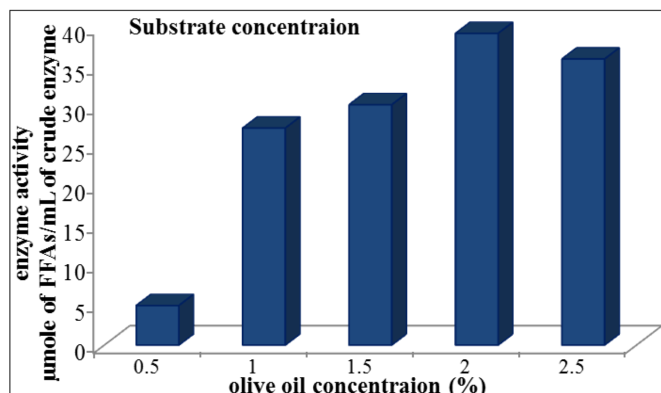


**Figure 11:** Effect of substrate concentration on lipase productivity using different concentrations of olive oil

Enzyme activity depends on assay conditions such as (CAPR) ratios, buffer type, emulsion system, and many other factors. It can also fluctuate along with time due to culture conditions such as bacterial population, metabolites produced by microbes, and substrate depletion. In this study, optimization was done only on cultural conditions. Growth profile of bacteria in lipase production media and enzyme assay conditions were needed to be studied to determine more the effect of parameters on lipase activity.

## 4. Conclusions

All the experiments in this paper were performed to compare lipase producing activities of the selected strains and to choose the best strain for further studies. Both qualitative and quantitative methods were used to examine lipase activity. In this study, 17 bacterial strains were screened by TBA assay method and ROA assay method for lipase producing activity. Five bacterial strains were potent strains. According to FFAs formations, isolate A, 8a and B were more enzyme producer strains, which were determined by titrimetric method. Among them, isolate 8a was selected as the best lipase producer strain by spectrophotometric method. The isolate 8a was identified as *Bacillus subtilis* and the optimum cultural conditions for enzyme production were 5 days incubation period, pH 6, temperature 38˚ and 2% olive oil was the best substrate and concentration. It was proved that the potent strain, 8a, (*Bacillus subtilis*) was good enough as a lipase producer strain to use in industrial processes.

## 5. Acknowledgements

The author would like to express his thanks to Dr. Sint Soe, Rector, Mandalay Technological University, for his kindness, help, permission and suggestion for completion of this paper. The author would like to express his gratitude to his supervisor, Dr. Myo Myint, Professor and Head, Department of Biotechnology and his co-supervisor, Dr. Tin Tin Hla, Professor, Department of Biotechnology at Mandalay Technological University. The author also appreciates and thanks to corresponding people who helped directly and indirectly for this research.

## References

[1] A.B. Salleh, R .Musani, and Razak, "Extra and Intracellular Lipase from Themophlic Rhizopus oryzae and Factors Affecting their Production," Can. J. Microbiol., 39: 978-981, 1993.

[2] D. Gilham, and R. Lehner, Techniques to Measure Lipase and Esterase Activity *in vitro*, Methods 36: 139-147, 2005.

[3] J. L. Arpigny, and K.E. Jaeger, "Bacterial Lipolytic Enzymes: Classification and Properties," Biochem. J., 343: 177-183, 1999.

[4] R. Sharma, S. K. Soni, R. M. Vohra, L. K. Gupta, and J.K. Gupta, "Purification and Characterization of a Thermostable Alkaline Lipase from a New Thermophilic Bacillus sp.," RSJ-1, Process Biochem., 37:1075–1084, 2002.

[5] Allan Svendsen. "Lipase Protein Engineering," Biochimica et Biophysica Acta 1543.223-238, 2000.

[6] H. Treichel, D.D. Oliveira, M.A. Mazutti, M. D. Luccio, and J.V. Oliveira, "A Review on Microbial Lipase Production," Food Bioprocess Technol., 3: 182-196, 2010.

[7] R. K. Saxena, W. S. Davidson, A. Davidson, and B. Giri, "Purification and Characterization of an Alkaline Thermostable Lipase from *Aspergillus carneus*. Process Biochem., 39:239–247, 2003.

[8] Y. Wang, K. C. Srivastava, G. J. Shen, and H. Y. Wang, "Thermostable Alkaline Lipase from a Newly Isolated Thermophilic *Bacillus* Strain, A30-1 (ATCC 53841)," J Ferment Bioeng, 79:433–438, 1995.

[9] R. J. Kazlauskas, and U.T. Bornscheuer, "Biotranformations with Lipases," in Pihler G, Stadler A, Kelly PJW, Rehm HJ (eds) Biotechnology. Wiley-VCH, New York, pp 137–192, 1998.

[10] T. Chaiyaso, A. H-Kittikun, and W. Zimmermann, "Biocatalytic Acylation of Carbohydrates with Fatty Acids from Palm Fatty Acid Distillates," J Ind Microbiol Biotechnol., 33:338–342, 2006.

[11] M. A. Robles, P. A. G. Moreno, L.E. Cerdán, and E. M. Molina, "Bio Catalysis: Towards Ever Greener Biodiesel Production," J. Adv. Biotechnol. 27(a), 398-408, 2009.

[12] S. J. Choi, J. M. Hwang, and S. I. Kim, "A Colorimetric Microplate Assay Method for High Throughput Analysis of Lipase Activity," Journal of Biochemistry and Molecular Biology, Vol. 36, No. 4, pp. 417-420, July 2003.

[13] F. Rosenau, and K. E. Jaeger, "Bacterial Lipases from *Pseudomonas*: Regulation of Gene Expression and Mechanisms of Secretio," Biochimie; 82:1023-1032, 2000.

[14] C. H. Collins, P. M. Lyne, and J. M. Grange, Microbiological Methods. 7th ed., Butterworth-Heinemann, UK, 1995.

[15] M.P. Prasad, and K. Manjunath, "Comparative Study on Biodegradation of Lipid Rich Waste Water using Lipase Producing Bacterial Species," Indian J Biotechnol., Vol. 10 .Issue 1, pp. 237-245, 2011.

[16] G. Kouker, and K. E. Jaeger, "Specific and Sensitive Plate Assay for Bacterial Lipases," Applied and


Licensed Under Creative Commons Attribution CC BY

Environmental Microbiology, Vol. 53(1), pp. 211-213, Jan 1987.

[17] F. Beisson, A. Tiss, Rivi-ere and R. Verger, "Methods for Lipase Detection and Assay: A Critical Review," Eur. European Journal of lipid science and technology, Vol. 102, No.2, pp 115-133, 2000.

[18] D. Y. Kwon, and J. S. Rhee, "A Simple and Rapid Colorimetric Method for Determination of Free Fatty Acids for Lipase Assay," Department of Biological Science and Engineering, Korea Advanced Institute of Science and Technology, Seoul, Korea, 1986.

[19] J. G. Holt, N.R. Krieg, P.H.A. Sneathm, J.T. Staley, and S.T. Williams, Bergey's Manual of Determinative Bacteriology, (9th Edn.), Baltimore. MD: Williams and Williams. 49-59, 1994.

[20] L. Dong-Woo, K. You-Seok, K. Ki-Jun, K., Hak-Jong, C. Byung-Chan, K. Doo-Sik, M. T. Suhartono, and, P. Yu-Ryang, "Isolation and Characterization of A Thermophilic Lipase from Bacillus Thermoleovorans ID-1,' FEMS Microbiology Letters, 179. pp 393-400, Aug 1999.

[21] Lipase Olive Oil- Sigma-Aldrich. [online]. Available: http://www.sigmaaldrich.com.› Sigma.

[22] J. Hosek, P. Svastova, M. Moravkova, I. Pavlik, and M. Bartos, "Methods of Mycobacterial DNA Isolation from Different Biological Material: A Review," Veterinarni Medicina. 51. 180–192, 2006.

[23] Pissamai Powedchagun, Hideyuki Suzuki, and Sirirat Rengpipat, "Characterization of A Probiotic *Bacillus* S11 Bacterium of Black Tiger Shrimp *Penaeus monodon*," J. Sci. Technol., 33(1),1-8, 2011.

## Author Profile

**Mr. Kyaw Swa Lynn** is a PhD student at the department of Biotechnology, Mandalay Technological University. He was born in Taung Dwin Gyi Township, Magway Region, Republic of the Union of Myanmar. His date of birth is June 2, 1983 and he is now 35 years old. He received the Degree of Bachelor of Science (Biotechnology) and Master Degree (Industrial Biotechnology) from Yangon Technological University, Myanmar in 2004 and 2006. Currently, He is doing his PhD research at the department of Biotechnology, Mandalay Technological University.


Licensed Under Creative Commons Attribution CC BY