

## IDENTIFICATION AND OPTIMIZATION OF LIPASE PRODUCING BACTERIA FROM PALM OIL CONTAMINATED WASTE

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### ABSTRACT

Bacteria isolated from semi-solid waste, SS2B1 exhibited a greater zone of clearance, 9mm with higher lipase activity. SS2B1 isolate demonstrated a Gram-positive and rod shape arrangement under microscope observation belong to *Bacillus* sp based on biochemical characteristics. The effect of carbon source, nitrogen source, medium pH and temperature for the lipase production was studied. The lipase production was maximum (0.1228  $\mu\text{g/ml/min}$ ) at pH7, temperature 37<sup>0</sup>C by the lipase producing bacteria SS2B1, *Bacillus* sp. Increased enzymatic production was obtained when the organisms were cultured in medium supplemented with 1% tryptone and palm oil as substrate with 53.58% optimization process. The results of the present study demonstrate that the *Bacillus* sp. is ideal for extracellular lipase production at industrial level such as detergent, leather and fine chemical industries.

**Keywords:** bacteria, lipase; optimization; palm oil contaminated oil; screening; production.

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### 1. INTRODUCTION

Lipases (EC 3.1.1.3) are ester hydrolases which catalyze the hydrolysis of triacylglycerol to liberate fatty acids and glycerols [1] Lipases are ubiquitous enzymes



and currently attracting an enormous attention widely used enzyme in biotechnological applications such as food, detergent, cosmetic, organic synthesis and pharmaceutical industries [2]. Lipase-producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, oilseeds and decaying food, compost heaps, coal tips and hot springs [3]. Lipase are produced by many microorganisms include bacteria, fungi, yeasts and actinomyces.

Microbial lipases have gained special industrial attention due to their selectivity, great variety of catalytic activities, easy to manipulate genetically and capable of rapid growth on inexpensive media [4]. Microbial enzymes are also more stable, convenient and safer than animal and plant enzymes [5]. Generally, bacterial lipases are glycoproteins but some extracellular bacterial lipases are lipoproteins [6]. Previous study had reported the isolation of extracellular lipase from various type of bacterial species, including *Bacillus sp.* and *Pseudomonas sp.* [7-8]. Among them, *Pseudomonas sp.* is gained particular attention due to the first studied and used in biotechnological production but also because of their involvement in bacterial pathogenesis [9].

A simple and reliable method for detecting lipase activity in microorganisms has been described such as the uses of surfactant Tween 80 in a solid medium to identify a lipolytic activity. The formation of opaque zones around the colonies is an indication of lipase production by the organisms. The production of extracellular lipases from bacteria is greatly influenced by medium composition especially carbon and nitrogen sources besides physicochemical factors such as temperature, PH and dissolved oxygen [5]. Considering the importance of lipase enzyme, lipase producing bacteria have been identified and optimized in the present study from palm oil contaminated soil samples.

## **2. METHODOLOGY**

### **2.1. Collection of Soil Sample**

Soil samples were obtained from the contaminated soil from a palm industry, Koh Foh Sime Darby Plantation in Bahau, Negeri Sembilan. Three types of samples were collected; solid waste (processed in steam and turibines), semi solid waste (pool) and liquid (treated anaerobic pool for daily consumption).

## 2.2. Isolation of Lipase Producers

A serial dilution was carried out to the soil samples and were plated on Nutrient Agar (NA) and incubated at 37°C for 24, 48 and 72 hours. They were aseptically subculturing and subjected to phenotypic characterization based on morphological, biochemical and physiological characters according to Bergeys Manual of Systematic Bacteriology.

## 2.3. Screening for Lipase Activity by Tributyrin Clearing Zone (TCZ)

Lipolytic activity was screened on predominant bacteria isolated in the nutrient agar plate. Tween 80 was used a substrate and lipolysis is observed directly by changes in the appearance of the substrate, which are emulsified mechanically in various growth media and poured into a petri dish. Lipase production is indicated by the formation of clear halos around the colonies grown on tween 80 containing agar plates.

## 2.4. Lipase Assay

The lipase activity in the supernatant was determined by the titrimetric method. The composition of production medium used in this study was: (% w/v) peptone 0.2;  $\text{NH}_4\text{H}_2\text{PO}_4$  0.1; NaCl 0.25;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.04;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  0.04; olive oil 2.0 (v/v); pH 7.0; 1-2 drops Tween 80 as emulsifier. Fatty acids were extracted by adding acetone: ethanol reagent (1:1). Amount of fatty acids liberated were estimated by using universal indicator with 0.05M NaOH. One unit of lipase activity is defined as the amount of enzyme that liberated 1 $\mu\text{mol}$  FFA in 1min at 37°C. The bacterial isolate that produced maximum lipase was selected for further work.

## 2.5. Optimization of Fermentation Conditions

### 2.5.1. Effect of Carbon Source

Effect of carbon source on the lipase production was analyzed by replacing the olive oil with different carbon sources palm oil, ghee oil, coconut oil, groundnut oil, sunflower oil and mustard oil at a concentration of (1% w/v) were added into the production medium in 500 ml Erlenmeyer flasks containing 100 ml of liquid medium on a rotary shaker (150 rpm) and incubated at 36°C for 24 hours and the enzyme was assayed.

### 2.5.2. Effect of Nitrogen Sources

Effect of nitrogen sources on the lipase production was studied by replacing the nitrogen source with yeast extract,  $\text{NaNO}_3$ , tryptone and peptone at a final concentration of 1% (w/v) were added to the medium and incubated at 36°C for 24 hours in a rotary shaker (150 rpm).

### 2.5.3. Effect of the Medium pH and Incubation Temperature

The effect of pH and temperature of the fermentation medium for lipase production was performed by varying pH of the medium from 1, 4, 7, 10 and 13 whereas the other parameters were unaltered. For selection of optimum temperature for the production of lipases, the temperatures varying from 4°C, 25°C, 37°C, 48°C and 60°C were selected by keeping the remaining parameters same.

### 2.5.4. Data Analysis

Data were analyzed by computed all the calculation obtained from triplicate titration. Graph was plotted based on the result obtained.

## 3. RESULTS AND DISCUSSION

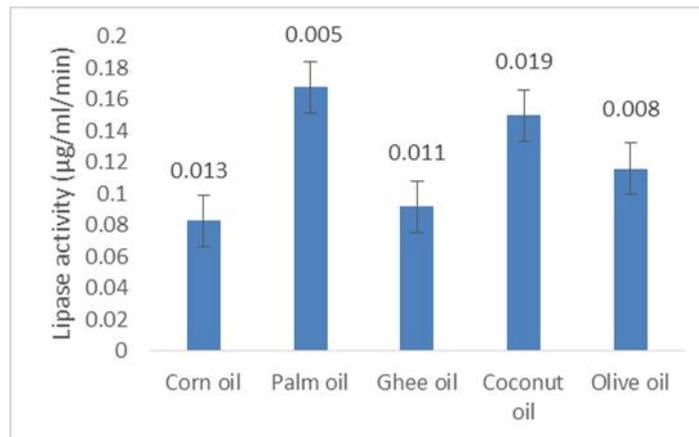
Industrial demands for a new source of lipase with different enzymatic characteristics that could create novel applications, stimulate the isolation and selection of new strains of lipolytic microorganisms. In the present study, we aimed to isolate, screen and identified lipase-producing bacteria from three types of palm oil contaminated wastes. Palm oil contaminated wastes had been selected due to the potential of their environmental conditions that rich of lipid which suitable habitat for lipid degrading bacteria.

A total of seven bacterial isolates were obtained from different palm oil contaminated wastes. Among them, semi-solid sample demonstrated the highest bacterial isolated with 37.5% frequency isolation. Based on the morphological and biochemical characteristic, all bacterial isolates demonstrated Gram positive characteristic when observed under microscopic analysis. Among them, five bacterial isolates showed a rod shape while one isolate demonstrated a cocci shape in bunches. *Bacillus* sp. was the most identified bacteria isolated from the three type of palm oil contaminated waste. *Staphylococcus* sp. is the only bacteria identified in semi solid waste. Present study showed a similar finding with the study made by [10-12], which found *Staphylococcus* and *Bacillus* in majority of bacterial isolate in oil contaminated soil. Lipolytic activity was further screened and characterized by the formation of opaque zones around the colonies as an indication of lipase production by the organisms. Screening of lipase producers on agar plates frequently done by using tributyrin as a substrate [13]. However, other version of this methods have been reported by [14] using Tween 80. SS2B1

isolated was demonstrated the highest diameter of halozone with 9mm compared to others isolates. This bacterial isolate was further used to determine the optimization of lipase activity.

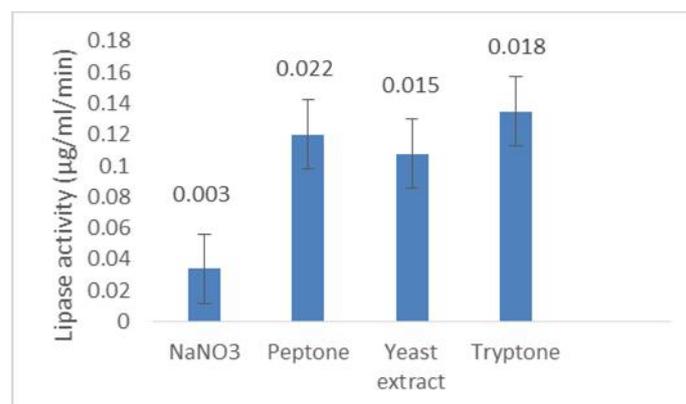
**Table 1.** Morphological identification and biochemical reaction of isolate from three palm oil contaminated sample

Sample	Solid		Semi-Solid			Liquid	
	S1B1	S2B2	SS2B1	SS2B2	SS1B3	L1B1	L2B2
Cultural characteristic	White, irregular colonies, raised, dry	Dull, irregular shape, wavy margin, rough texture	Golden yellow, circular, large, opaque, convex smooth and shiny.	Whitish, irregular shape, dry texture and wavy margin	White, large shape, umbonate, dry texture	Dull, large shape, undulate margin, dry texture	White, wavy margin, rough texture, irregular shape
Gram reaction	+	+	+	+	+	+	+
<b>Biochemical Characteristics</b>							
Indole (I)	-	-	-	-	-	-	-
Methyl red (M)	-	-	+	-	-	-	-
Voges Proskauer (VP)	+	+	+	+	+	+	+
Citrate (C)	+	+	-	+	+	+	+
Halo zone	3mm	2mm	4mm	9mm	4mm	3mm	4mm
Expected bacteria	<i>Bacillus sp.</i>	<i>Bacillus sp.</i>	<i>Staphylococcus sp.</i>	<i>Bacillus sp.</i>	<i>Bacillus sp.</i>	<i>Bacillus sp.</i>	<i>Bacillus sp.</i>



**Fig.1.** Effect of different carbon sources on lipase activity

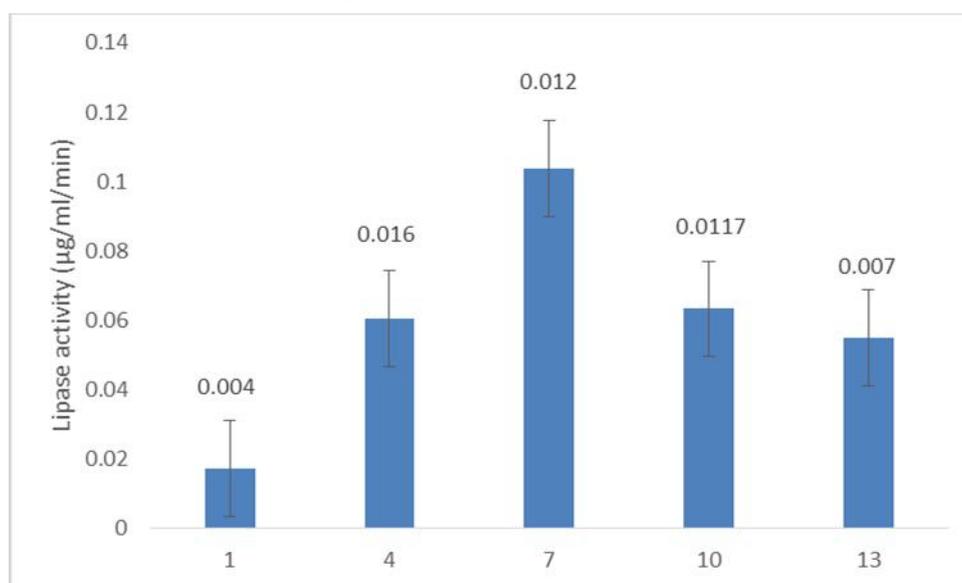
Both amount and type of carbon sources can influence the yield of lipase production. Various types of carbon sources including corn, starch, glucose and molasses are commonly used as growth substrate to produce enzymes by fermentation [15]. However, carbon catabolite regulation (CCR) in microbial system will catabolize the best carbon sources (the one which most rapidly supplies carbon and energy for growth [16]. In this study, isolated *Bacillus* sp has maximum lipase yield in palm oil ( $0.168\mu\text{g/ml/min}$ ) among various carbon sources. Palm oil contains an equal amount of saturated and unsaturated fats with mixture of triglycerides, monoglycerides, diglycerides and free fatty acids. In [17] reported that majority of fatty acids found in palm oil (myristic, palmitic, stearic, oleic and linoleic acid) were the mostly favarouble for lipase hydrolysis. Result was aligning with [18] who found palm oil is the best carbon sources for isolated *Staphylococcus* sp.



**Fig.2.** Effect of nitrogen sources on lipase activity

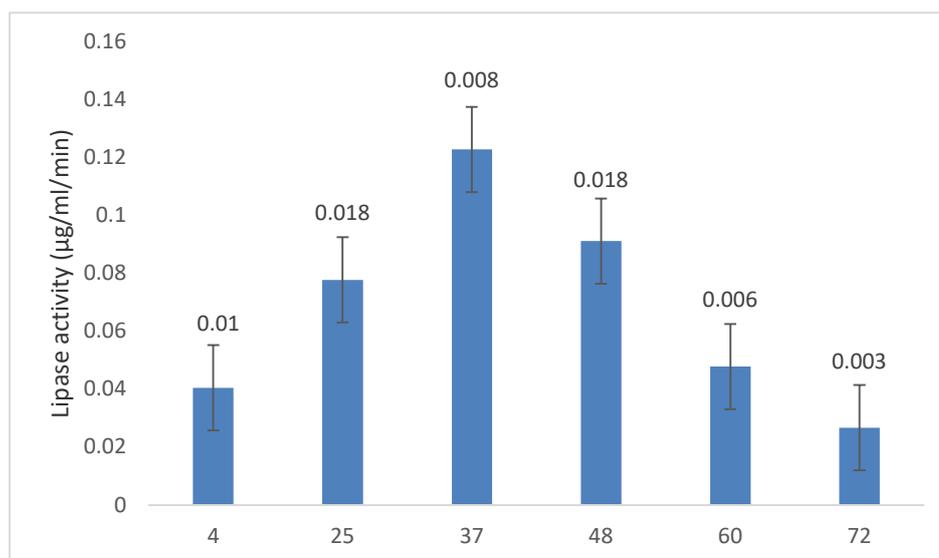
Organic nitrogen source is well known to affect enzyme synthesis since it can supply amino acids and many cell growth factors which needed for cell metabolism and protein synthesis

[19]. Bacterial strains were grown in growth medium with various organic nitrogen sources in order to determine the effect on lipase production. Highest levels of lipase activity and bacterial growth were detected ( $0.135\mu\text{g/ml/min}$ ) when growth medium supplemented with tryptone. A similar result reported that tryptone was the best nitrogen source for lipase production by *Burkholderia* sp. [20]. However, a study by [21] on enzyme activities by *Bacillus* sp. demonstrated the lipase activity were highest produced when only there is a combination of yeast extract and tryptone as nitrogen sources.



**Fig.3.** Effect of various pH values in lipase activity

Optimum activity of enzymes is pH dependent and varies from one enzyme to another. The pH of the environment influences the growth of organisms to a greater extent. The effect of pH is caused by protonation or deprotonation of one of the participant molecules in one or more of the reaction steps [22]. The results on the effect of medium pH on the tested organism indicated that the lipase production were maximum ( $0.012\mu\text{g/ml/min}$ ) at pH 7 and found to be less at pH 1, 4, 10 and 13 respectively. In [23-24] revealed a similar result which demonstrated a highest lipase activity at pH 7 by using *Bacillus* and *Pseudomonas* sp. Another finding also recorded lipase activity was maximum in *Pseudomonas aerogionosa* and *Bacillus licheniformis* between pH 7 to 10 [25-26].



**Fig.4.** Effect of various temperature values in lipase activity

Temperature is a critical parameter that has to be controlled, it varies from organism to organism and it influences secretion of extra cellular enzymes. Effect of temperature on lipase activity was evaluated by assaying lipase activity at different temperature levels (4-72°C). The lipase activity of was high 0.1228µg/ml/min at 37°C (Fig. 4) from SS2B1 when grown at the medium temperature of at the optimum pH of 7.0, but it has been found less at incubation temperature 4°C, 60°C and 72°C. Results align with [27] whom reported that *Pseudomonas aeruginosa* showed enhanced lipase activity at incubation temperature 35°C to 40°C. In contrast with the study done by [28] shown that bacterial lipase used in his study favor slightly higher temperature and recorded highest enzyme activity at 50°C. Study showed that bacteria SS2B1 can tolerate a wide range of temperature and still survive and produce lipase activity.

#### 4. CONCLUSION

Present study showed one lipase producing bacteria isolated from palm oil contaminated wastes. Isolate SS2B1 identified as *Bacillus* sp. demonstrated the maximum activity of lipase and the optimum activity were produced in the mixture of minimal media supplied with palm oil and trytone at 37°C and pH of 7.

## 5. ACKNOWLEDGEMENTS

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