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To cite this article: W Astuti *et al* 2019 *J. Phys.: Conf. Ser.* **1277** 012019

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# Characterization of lipase bacteria from water in Mahakam river port Samarinda

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**Abstract.** Lipase is a hydrolase enzyme that is widely used in industry. The waters in Mahakam river port become a conducive place for the growth of bacteria that are able to hydrolyze lipids, so that bacteria are thought to be able to produce lipases. This research was conducted to screen bacteria that produced extracellular lipase and characterize the lipase. Screening of lipase-producing bacteria has been done with solid media containing Rodhamin B and the activity of lipase was conducted by titrimetricly. The characterization of isolated bacteria indicated that this bacteria belongs to Gram positive cocci shaped bacteria. Lipase-producing bacteria was successfully selected from the water taken in Mahakam river port Samarinda. The lipase was produced for 36 h. The optimum working conditions of lipase at 40°C and substrate concentration of 6%. Specific activity of bacterial lipase in Mahakam river water at Samarinda Port is 0.7212 U / mg.

## 1. Introduction

Various efforts have been explored to isolate lipase because this enzyme is extensively served in wide variety of biotechnological processes of fields such as detergent, pharmaceutical, oleo-chemical, food, textile, perfume, cosmetic industries and industrial wastes management [1]. Furthermore, the increased demand for industrial lipases from microbial especially, has caused to the identification of novel enzymes from new sources with unique properties.

Specifically, lipase (triacylglycerol hydrolases E.C 3.1.1.3) is an enzyme that hydrolyzes triacylglycerol into fatty acids glycerol and fatty acids that commonly proceed with high chemo-, regio- and/or enantioselectivity at the interface between the nonpolar substrate and water [2]. These reactions are useful to substitute the conventional and complex chemical techniques. In industry, lipase-catalyzed product shows efficiency improvement and energy saving compared to the conventional high-temperature, high-pressure-steam splitting methods. Thus, new class of lipases from great available sources have been most important stage in a lipases isolation to improve and increase the efficiency of the industry process without increasing the cost [3].

Generally, lipases are isolated from extracellular spaces of plants, animals and various microorganisms. However, lipases from microorganism have been obtained high demand than other lipase types (plants and animals) because chemical catalytic activities, less energy consumption [3]. Moreover, lipase from microorganisms especially bacteria are easy to manipulate genetically, inexpensive in treatment cost because of simplicity and ease of mass production, activity in extreme



conditions (high/low temperature and pH), no requirement for cofactors, and environmental friendly. Lipase-producing bacteria have been attained in diversified habitats such as hot spring water, household wastes, soil contaminated with oil, etc [4]. Therefore, it is still high demand to investigate new habitat for potential lipases.

Here we introduce the objective of the present investigated is to identification and isolation of new strain of microorganisms located in Mahakam river port Samarinda. Also, Lipase from this strain was prior characterized. This study can identify new lipase with temperature and pH stability and good specificity.

## 2. Materials and methods

### 2.1. Collection of sample and bacterial isolation

Water samples were collected from 3 sampling points of Mahakam river port, Samarinda. Water samples then homogenized to get represent sample the water body.

Microorganisms isolation from water samples 1 mL of samples were homogenized in 5 mL of LB (1% tryptone, 1% NaCl and 0.5% yeast extract) sterile and incubated on a shaker incubator at 37°C for 20 h. The culture diluted ten-fold serial dilution in aquabidest sterile. Each dilution tube inoculated to sterile nutrient agar plate was and incubated at 37°C for 24 h to obtain isolated colonies. The single colony was selected and sub-cultured for screening lipase producing bacteria [5].

### 2.2. Screening of bacteria producing extracellular lipase

Screening of lipase-producing bacteria were detected on Rhodamine B (2%)-olive oil (10%)-Nutrient agar (2.5%) medium. Then each colony of bacteria was inoculated into screening medium and incubated at 37°C for 48 h. The plate was observed to UV light (350 nm). Bacteria colony that extracellular lipase producing was present orange fluorescence.

### 2.3. Lipase production

The selected colony bacteria was produced the extracellular lipase in Luria Bertani medium (1% tryptone, 0.5% yeast extract and 1% NaCl). The bacteria were inoculated at 37°C, on a rotary shaker at 200 rpm. The samples were taken after every 12 h until 96 h. Cell density was measured by taking the OD at 600 nm. The culture cell were centrifuged at 6,000 rpm for 15 min, and the supernatant was extracellular lipase.

### 2.4. Lipase activity assay

Activity of lipase was evaluated titrimetrically employing olive oil hydrolysis [6]. Substrate for lipase activity assay containing 5 mL phosphate buffer 0.2 M pH 7, 0.05 g gum arabic, and 0.2 mL olive oil. The emulsion of substrate were added 1 mL lipase. The enzyme - substrate was incubated at 37°C for 1h. The reaction stoped with 15 mL ethanol-acetone (1 : 1). The fatty acids producing identified by titration with 0.1 M NaOH. One unit of lipase activity is defined as 1 micromol free fatty acid produced by 1 mL enzyme per minute.

### 2.5. Protein determination

Protein concentration was determined by the method of Bradford [7] employing albumin as a standard.

### 2.6. Effect of temperature

The lipase activity was performed in varied temperature from 30-60°C in increments of 5°C.

### 2.7. Effect of substrate concentration

Lipase was assayed in pH 7 and optimum temperatur (40°C) with different concentrations (0.5-3.0 % v/v) of olive oil.

### 3. Results and discussion

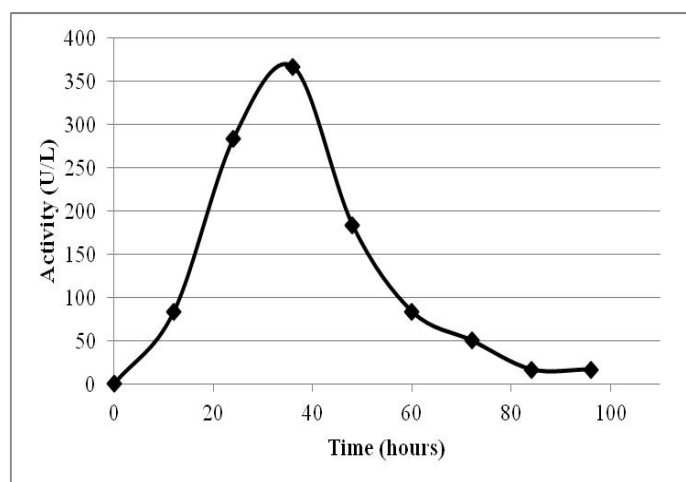
#### 3.1. Screening bacteria that produced lipase

Bacteria that produced lipase were isolated from water of Mahakam river port in Samarinda, East Kalimantan Indonesia. Screening of bacteria that produced lipase using Rhodamine-B agar plates containing olive oil. The colonies that produced lipase were recognized by formation orange fluorescence color around the colonies when exposed to Ultraviolet light at 350 nm after 24 h incubation. From the total of 23 single colonies bacteria, there were 4 colonies that producing lipase. One of the 4 colonies that producing lipase was selected to produced and characterized the lipase. The isolate was identified as Gram positive cocci shaped bacteria.

#### 3.2. Growth and lipase production

The lipase were produced in Luria Bertani (LB) medium including 2% olive oil. The selected bacteria were cultivated in shaking flask at 37°C, 200 rpm for 96 h and the lipase activity was observed at various incubation time (0–96 h with 12 h intervals). Olive oil in this medium were substrate to inducing lipase and another carbon source.

The lipase activity was gradually increased on 0–36 h (figure 1) and decreased significantly after 48 h. The optimum time for producing lipase was 36 h yielding an activity of 366.66 U/L. This could be due to consumption of carbon source available in the media which results in large biomass production. In short supply of the carbon source forces the bacteria to employ olive oil as an alternative carbon source for the production and maintenance of biomass which resulting more lipase production [5].

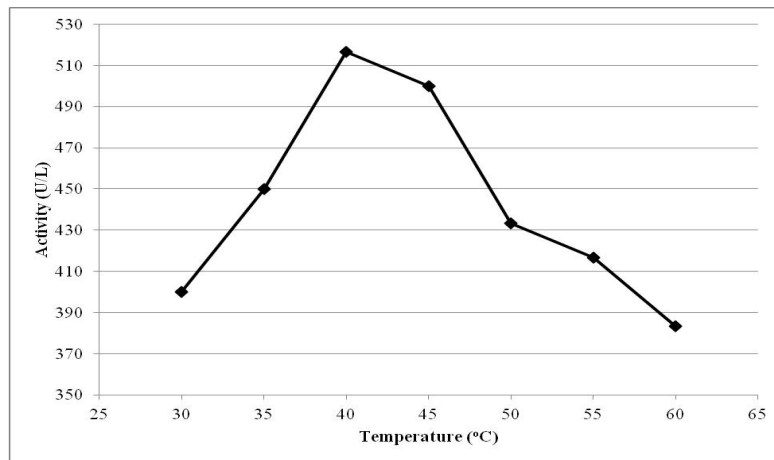


**Figure 1.** Time of lipase producing.

#### 3.3. Effect of temperature on lipase activity

The temperature effect on crude lipase activity is shown on figure 2. Crude lipase of bacteria from water Mahakam river displayed maximal activity at 40°C is 516.67 U/mL. At temperatures in the range of 30–45°C the enzyme showed significant increase activity and at higher than optimum temperatures the activity was drastically decrease.

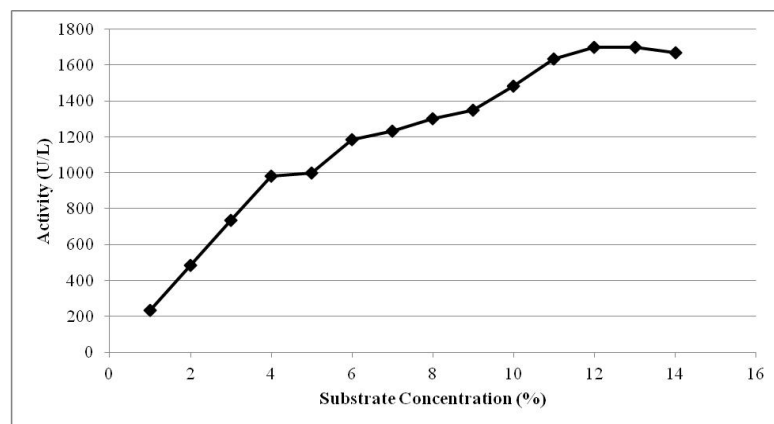
The properties of high enzyme activity at low temperatures is a advantage for energy saving in various industries that using lipase. The lipase, especially implemented at low temperatures, have great potential in industries, for example cheese and dairy production, transesterification of triglycerides and conventional process of biodiesel production is performed at 50–60°C [8].



**Figure 2.** Effect temperature on lipase activity.

The growth temperature and environment of the each microorganism related to optimum temperature for lipase activity. For example, lipase from *Rhizomucor endophyticus* most active at 40°C [3], lipase of *Bacillus subtilis* from oil contaminant wastewater the optimum temperature was 50°C [9], crude lipase from *Rhizopus arrhizus* maximal catalytic activity temperature of 35°C [8]. The enzyme retained its full activity at 35 to 50°C.

### 3.4 Effect of different concentration of substrate on lipase activity



**Figure 3.** Effect of different concentration of substrate on lipase activity.

Measurement lipase activity were analyzed by different olive oil concentrations. Increasing concentration of olive oil from 1 to 12% showed an increase in activity lipase. At 12% olive oil, activity lipase was maximum activity (figure 3). It could be due to a certain ability of the organism to use it as a carbon source and high concentration of such substrate could become toxic for the organism. Specific activity of bacterial lipase in Mahakam river water at Samarinda Port is 0.7212 U / mg

## 4. Conclusion

Lipase from bacterial isolated in the Mahakam River Water in Samarinda Port worked optimally at 40°C and optimum substrate concentration of 6% (v/v). The specific activity of Lipase is 0.7212 U/mg.

## Acknowledgment

Authors would like to thank Rita Hairani and Zihnil Adha Islamy Mazrad for their assistance in proofreading.

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