Lipase Derived from Bacteria Isolated from The Seawater of Muara Badak, East Kalimantan

Winni Astuti*, Safridah Hanum Nasution, Djihan Ryn Pratiwi

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Mulawarman Jln. Barong Tongkok No. 4 Kampus Gunung Kelua Samarinda, East Kalimantan

***Corresponding Author**

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ABSTRACT

Halophilic bacteria are extremophilic microorganisms that can optimally grow in environments with high salt concentrations. Sea water is one of the habitats of halobacteria. Isolation, characterization, and determination of the activity of crude extracts of lipase under various working conditions, the effect of metal ions, and variation of NaCl concentrations were performed. Lipase activity was determined using titrimetry. Four bacterial isolates were obtained from seawater Muara Badak, East Kalimantan, which showed fluorescent orange around the colony under UV light, with the highest activity in isolate 5 at 2.86 U/mL. The optimum working conditions of the crude extract of lipase were pH 6, temperature of 50°C, and substrate concentration of 2%.

Keywords: Lipase, Seawater bacteria, Optimum pH, Optimum temperature, Muara Badak East Kalimantan

INTRODUCTION

Enzymes play an important role in various aspects of life, such as in the food, medical, and chemical industries (Mandik, 2024). Enzymes for industrial purposes can be isolated from various microorganisms. Microorganisms are the most potential source of enzymes, compared to plants and animals. The use of microorganisms is advantageous because they grow rapidly, can grow on inexpensive substrates, and their yield can be easily increased by regulating growth conditions and through genetic engineering (Sholeha, R., & Agustini, R., 2021). In addition, enzymes with high stability under specific conditions are required. Such enzymes can be obtained from bacteria that live in extreme environments, such as halophilic bacteria.

Lipase is a crucial enzyme in biotechnology, with numerous industrial applications in the food, dairy, detergent, and pharmaceutical industries. Several genera of lipase-producing bacteria include Bacillus, Pseudomonas, Burkholderia, and Serratia. Lipase is typically produced in media containing lipids, such as oils. The lipase enzyme produced by bacteria is generally extracellular (Pramiadi 2014).

This study aimed to characterize lipase by testing its activity under various working conditions. Preliminary studies have shown that seawater from Muara Badak contains lipase-producing bacteria, as indicated by the presence of orange fluorescence around the colonies under UV light and their ability to break down oil into fatty acids and glycerol. Therefore, further research is needed on lipase-producing bacteria from Muara Badak seawater to determine the lipase caracterization . This study also aimed to determine the factors that influence enzyme performance, namely substrate addition, the effect of pH, temperature, and substrate concentration.

METHODOLOGY

Sampling Technique

Samples of seawater from Muara Badak were collected at three points. At each point, 500 mL of water was collected and placed into bottles, which were then stored in a cool box filled with ice for further analysis (Rahayu, 2014).

Preparation of Bacterial Isolation Media

The medium used for selection was Nutrient Agar (NA) with the following composition: peptone 5 g/L, beef extract 3 g/L, NaCl 8 g/L, and agar 12 g/L. The NA medium was supplemented with 2.5% olive oil dissolved in 100 mL of seawater. Next, the medium was sterilized for approximately 30 min at 121°C using an autoclave. After sterilization, 1 mL of sterile Rhodamine B solution was added until the medium turned pink. The NA medium was then poured into sterile Petri dishes and allowed to solidify.

Selection of Lipase-Producing Bacterial Isolates

Bacterial isolates were diluted up to 1000 times. Then, 40 µL of the diluted isolates were spread on the surface of solid NA media using the spread plate method and incubated for 24 h at 37°C. Single colony isolates grown on the media surface were then re-cultured using the streak plate method on solid NA medium and incubated for 24 h at 37°C. Single-colony isolates showing lipase activity were prepared for quantitative testing using titrimetry. Qualitatively, lipase activity was indicated by fluorescence around the colonies when exposed to UV light.

Lipase Production from Bacterial Isolates

Lipase production was carried out by inoculating 10 µL of the selected bacterial isolates into 10 mL of liquid LB medium and shaking at 37°C for 72 h on a water shaker. The culture was then centrifuged at 12,000 rpm and 4°C for 30 min to separate the supernatant and pellet. The resulting supernatant, which served as a crude enzyme extract, was quantitatively tested for lipase activity.

Quantitative Measurement of Lipase Activity

Lipase activity was measured by titrimetry. Olive oil (0.05 mL) and gum arabic (0.05 g) were added to 4 mL of phosphate buffer at pH 7.5. Next, 1 mL of the enzyme solution was added to the substrate mixture and homogenized. The mixture was then incubated for 30 min at room temperature. After incubation, 10 mL of an acetone:ethanol solution (1:1) was added to the sample. The solution was then titrated with 0.02 M NaOH using one drop of phenolphthalein indicator until the color turned pink and remained stable. The titrated enzyme volume was recorded. Titration was performed in triplicate. For the control, the crude enzyme extract was replaced with distilled water. 3.4.7 Determination of Optimum Conditions

Optimum pH

The optimum pH was determined by measuring the lipase activity at various pH levels: 4, 5, 6, 7, 8, and 9. Olive oil (0.05 mL) and gum arabic (0.05 g) were added to 4 mL of buffer at each pH variation. Subsequently, 1 mL of the enzyme solution was added to the substrate mixture. The mixture was then incubated for 30 min at room temperature. Next, 10 mL of an acetone:ethanol solution (1:1) was added to each sample. The solution was titrated with 0.02 M NaOH using one drop of phenolphthalein indicator until the color turned pink and remained stable. The volume of enzyme titration was recorded.

Optimum Temperature

The optimum temperature for lipase activity was determined by measuring its activity at various incubation temperatures: 30, 40, 50, 60, and 70°C. Then, 0.05 mL of olive oil and 0.05 g of gum arabic were added to 4 mL of buffer at the optimum pH. Then, 1 mL of enzyme solution was added to the substrate mixture. The mixture was incubated for 30 min at each temperature. Subsequently, 10 mL of an acetone:ethanol solution (1:1) was added to the mixture, which was titrated with 0.02 M NaOH using one drop of phenolphthalein indicator until the color turned pink and remained stable. The enzyme titration volume was recorded.

Optimum Substrate Concentration

The optimum substrate concentration for lipase was determined by measuring its activity at various substrate concentrations under optimal pH and temperature conditions. Gum arabic (0.05 g) and olive oil at varying

concentrations (1, 1.5, 2, 2.5, and 3% v/v) were added to 4 mL of buffer at optimum pH. Then, 1 mL of enzyme solution was added to the substrate mixture. The mixture was incubated for 30 min at the optimum temperature. Subsequently, 10 mL of an acetone:ethanol solution (1:1) was added. The solution was then titrated with 0.02 M NaOH using 1 drop of phenolphthalein indicator until the color turned pink and remained stable. The volume of enzyme titration was then recorded.

Effect of NaCl on Lipase Activity

The effect of NaCl on lipase activity was determined by adding 120 µL of NaCl at various concentrations of 2, 4, 6, 8, 10, 14, and 18% (b/v) to the enzyme and incubating for 1 h at room temperature. Gum arabic (0.05 g) and olive oil at the optimum concentration were added to 3880 mL of buffer at the optimum pH. Next, 1 mL of the enzyme solution that had been incubated with NaCl was added to the substrate mixture. The mixture was then incubated for 30 minutes at the optimum temperature. After incubation, 10 mL of an acetone:ethanol solution (1:1) was added. The solution was titrated with 0.02 M NaOH using 1 drop of phenolphthalein indicator until the color turned pink and remained stable. The enzyme titration volume was then recorded.

Data Analysis

Lipase activity was determined by titrimetry using olive oil as the substrate. One unit of lipase activity was equivalent to 1 µmol of free fatty acid produced from substrate hydrolysis catalyzed by the crude enzyme extract per minute. The equations are as follows:

$$\text{Lipase Activity (U/mL)} = \frac{(V_a - V_b) \times N\ NaOH \times 1000}{VE \times t}$$

Where: Va = Titrated sample volume (mL) Vb = Titrated blank volume (mL) VE = Enzyme volume (mL) t = incubation time (min) (Nopiani et al., 2016).

RESULT AND DISCUSSION

Selection of Lipase-Producing Bacterial Isolates

Lipase-producing bacterial isolates were selected qualitatively. Six bacterial colonies were obtained through the isolation process. These isolates were regrown on NA media containing Rhodamine B. From the selection results, four bacterial colonies showed lipase activity, as indicated by orange fluorescence around the colonies. The selection results are presented in Figure 1.

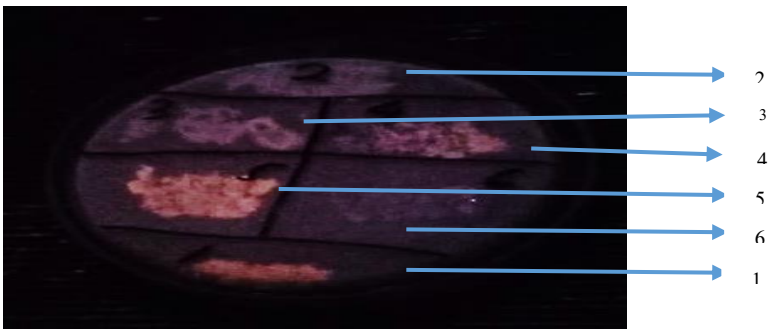

Figure 1 Qualitative test results of lipase activity from bacterial isolates under UV light.

Based on Figure 1. isolates 1, 3, 4, and 5 displayed lipase activity, as indicated by the orange fluorescence around the bacterial colonies; however, in isolates 2 and 4, the fluorescence intensity was low. No fluorescence was observed around the colonies of isolates 2 and 6. This indicated that isolates 1, 3, 4, and 5 were positive for lipase activity. According to Telussa (2013), the orange fluorescence is caused by a reaction between Rhodamine B and fatty acids, which are the products of olive oil hydrolysis by the lipase produced by the bacteria. These fatty acids form a complex with Rhodamine B, which is orange in color and fluoresces under UV light. The enzymes produced by these bacteria are extracellular.

The lipase activities of colonies 1 and 5 were quantitatively tested using the titrimetry method. The crude lipase extract from bacterial colony 1 showed an enzyme activity of 2.8 U/mL, whereas the crude lipase extract from colony 5 showed an enzyme activity of 2.86 U/mL. These results indicated that the lipase from colony 5 had the highest activity; thus, colony 5 was chosen for further lipase production and testing.

Optimum Acidity Level (pH)

The optimum pH was determined to understand the effect of pH on the enzyme, substrate, and enzyme-substrate complex. The optimum acidity level (pH) of the crude lipase extract was determined by testing lipase activity at various pH levels (4, 5, 6, 7, 8, and 9) using titrimetry. The data on the effect of pH variation on lipase activity are presented in Figure 1.

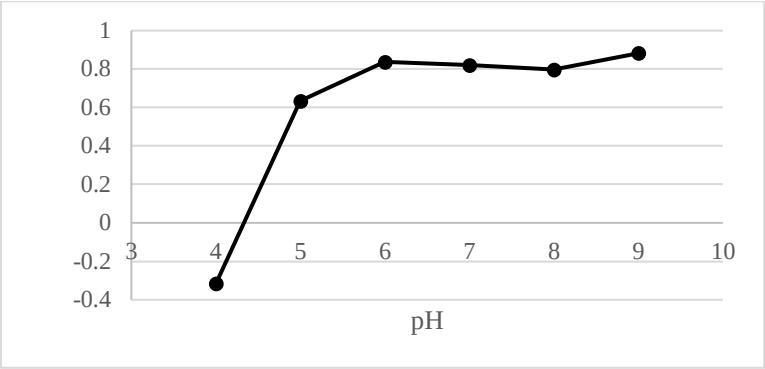

Figure 2. pH Effect on Lipase Activity

Enzymes require a specific pH to carry out their activities. Changes in environmental pH can affect the active site of the enzyme in the formation of the enzyme-substrate complex, thereby reducing enzyme activity. Each enzyme has a different optimum pH (Irawati, 2016). Based on the data above, lipase exhibited optimum activity at pH 6. At pH 4–6, lipase activity increased significantly. After surpassing the optimum pH, the activity declined as the pH increased. Enzymes are proteins that are composed of various amino acids. Enzyme activity is pH-regulated. When the pH decreases or increases, the properties of the amino acid side chains change, resulting in an overall change in the structural shape of the enzyme. Habibie (2014) explained that at optimum pH, the active site structure of the enzyme matches the substrate, allowing the formation of a proper enzyme-substrate complex and producing the maximum amount of product. Conversely, at suboptimal pH, the enzyme undergoes structural changes, resulting in loss of activity.

Optimum Temperature

This study was conducted to examine the effects of temperature on enzymes, substrates, or both. Enzymes can perform their activities within a certain temperature range. The optimum temperature is the most suitable temperature for an enzyme. Both decreases and increases in temperature can reduce enzyme activity. The optimum temperature of the crude lipase extract was determined by testing its activity at various incubation temperatures: 30, 40, 50, 60 °C, and 70°C. The data showing the effects of temperature variation on lipase activity are presented in Figure 3.

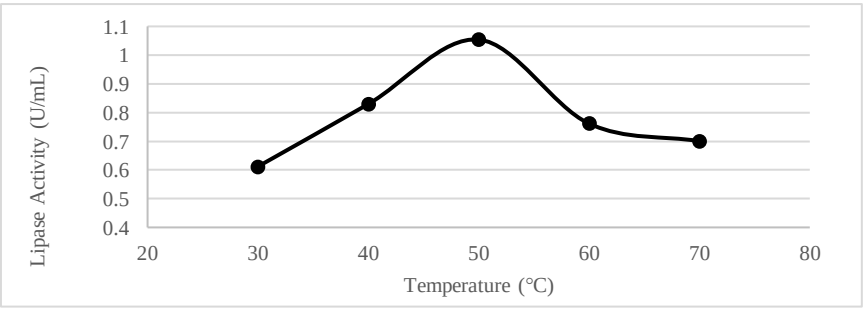

Figure 3. Effect of Temperature on Lipase Activity.

Based on the results of this study, the optimum temperature of the crude lipase extract was 50°C, with lipase activity of 1.054 U/mL. At 30°C, lipase activity was low; as the temperature increased, lipase activity also increased until the optimum temperature was reached. Further increasing the temperature to 70°C caused lipase activity to decline again. Zusfahair (2009) noted that, in general, lipase-producing bacteria have an optimum temperature in the range of 30–60°C. All enzymes function within different temperature ranges depending on the organism. In most cases, each increase of 10°C will double enzyme activity until optimum conditions are reached (Purwanti, 2015). In this study, the enzyme activity increased as the temperature was raised until it reached the optimum point. This is because higher temperatures increase the kinetic energy. Increased kinetic energy in the enzyme and substrate raises the activation energy available for the enzyme to perform the reaction (Purwanti, 2015). Increasing the environmental temperature beyond the optimum, that is, at 60–70°C, disrupts hydrogen bonds, ionic bonds, or hydrophobic interactions, altering the secondary, tertiary, or quaternary structure of the enzyme, and thus the folding of the enzyme.

Optimum Substrate Concentration

The optimum substrate concentration for the crude lipase extract was determined to understand the effect of substrate concentration on lipase activity. Lipase activity was assessed at various substrate concentrations. The substrate used was olive oil, with concentration variations of 1.0; 1.5; 2.0; 2.5; and 3.0%. The data showing the effect of substrate concentration variation on lipase activity are presented in Figure 4.

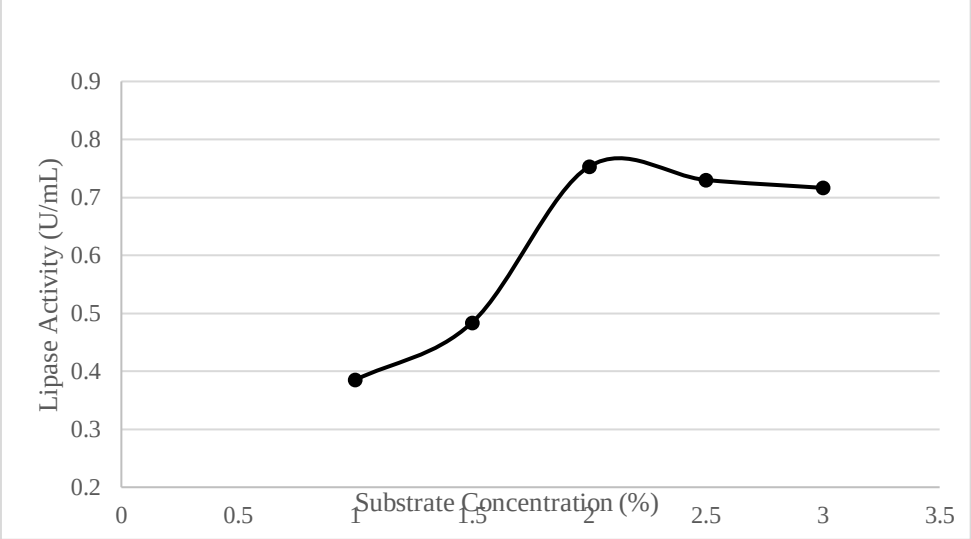

Figure 4. Effect of Substrate Concentration on Lipase Activity

Based on the data obtained, the optimum substrate concentration for crude lipase extract is at a substrate concentration of 2% with an activity of 0.753 U/mL. Lipase activity was low at 1% concentration. This is because fewer enzyme active sites interact with the substrate at substrate concentrations lower than the optimum. However, as the substrate concentration increased to 2%, the enzyme activity increased significantly. Increased substrate concentration results in more substrate interacting with the enzyme's active sites, leading to greater formation of the enzyme-substrate complex and, thus, increased enzyme activity (Irawati, 2016). At 2.5–3% concentration, the enzyme activity tended to plateau and did not show significant changes. When the substrate concentration is increased, the reaction rate increases until it reaches a certain value (Vmax). If other conditions remain constant, above a certain threshold, further increases in the substrate concentration do not significantly change the reaction rate. This is because the active sites of the enzyme molecules are already saturated by the substrate (Saryono, 2011).

CONCLUSIONS

This study showed that bacteria have been obtained from the seawater of Muara Badak, East Kalimantan, which are capable of producing extracellular lipase. The optimum working conditions for the crude lipase were pH 6, temperature of 50°C, and substrate concentration of 2%.

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