PRODUCTION OF LIPASE ENZYME BY MARINE ACTINOBACTERIA WITH VARIOUS PH AND TEMPERATURE

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Abstract: The demand for enzymes as biocatalysts in industry is very high. Research and development of different types of enzymes from different sources has started. One very important enzyme to study is the enzyme lipase. Lipase enzymes are enzymes of the hydrolase class that catalyze the hydrolysis of triglycerides to glycerol and free fatty acids. Lipases are found in a variety of sources including animals, plants, and microorganisms. Marine microorganisms, including marine actinobacteria, cannot be separated from this enzyme source's research and development process. The purpose of this study was to obtain a test protocol and optimal pH and temperature conditions for the hydrolysis reaction by lipase enzymes from marine actinobacteria. Optimal pH and temperature conditions for hydrolysis reactions by lipase enzymes from marine actinobacteria using spectrophotometry at different pH values and different temperatures of 3, 4, 5, 6, 7, 8, 9, and 10. Temperatures of 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C and 90 °C are measured at a wavelength of 405 nm. The results showed that the activity of the lipase enzyme at pH 9 with Tris-HCl buffer was the optimum pH, and the temperature of 70°C was the optimum temperature for the lipase hydrolysis reaction of marine actinobacteria.
Keywords: *Actinobacteria*; Marine; Hydrolysis; Lipase; pH; Temperature.

Abstrak: Kebutuhan enzim sebagai biokatalisator dalam bidang industri sangat tinggi. Berbagai macam enzim dari beragam sumber sudah mulai diteliti dan dikembangkan. Salah satu enzim yang sangat penting untuk diteliti adalah enzim lipase. Enzim lipase merupakan enzim golongan hidrolase yang mengkatalis proses hidrolisis trigliserida menjadi gliserol dan asam lemak bebas. Lipase dapat ditemukan dalam berbagai sumber seperti pada hewan, tumbuhan, dan mikroorganisme. Mikroorganisme laut tidak terlepas dari proses penelitian dan pengembangan sumber enzim ini termasuk Actinobacteria laut. Tujuan penelitian ini adalah untuk memperoleh protokol uji dan kondisi pH dan temperatur optimum reaksi hidrolisis oleh enzim lipase dari Actinobacteria laut. Kondisi pH dan temperatur optimum reaksi hidrolisis oleh enzim lipase dari Actinobacteria laut dilakukan secara kuantitatif dengan metode spektrofotometri pada variasi pH 3, 4, 5, 6, 7, 8, 9, dan 10 dan variasi suhu 30°C, 40°C, 50°C, 60°C, 70°C, 80°C, dan 90°C diukur pada Panjang gelombang 405 nm. Hasil menunjukkan bahwa aktivitas enzim lipase pada pH 9 menggunakan buffer Tris HCl merupakan pH optimum dan temperatur 70°C merupakan temperature optimum reaksi hidrolisis enzim lipase dari

Actinobacteria laut.
Kata kunci: *Actinobacteria*; Laut, Hidrolisis; Lipase; pH; Temperatur.

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Introduction

Actinobacteria are Gram-positive bacteria that have a filamentous shape similar to fungi (Muazi et al., 2023). Actinobacteria (order *Actinomycetales*) have been recognized as an important source of natural bioactive compounds. In fact, This order produced around 45% of metabolites, 34% produced by *Streptomyces*, and 11% by other genera (Sharma et al., 2014; Selim et al., 2021). Actinobacteria are commonly found in soils (Sapkota et al., 2020), and sediments (Rozirwan et al., 2021). Apart from that, Actinobacteria can also be found in marine areas. Subramani & Sipkema (2019) report that since 2007-2017, there have been 177 new species of Actinobacteria obtained from marine environments. Marine habitats include coastal, deep-sea sediment, seawater, and mangrove forests (Ngamcharungchit et al., 2023). Marine actinobacteria can also be obtained from marine organisms such as sea grass, sponges, fishes, mollusks, and corals (Sarkar & Suthindhiran, 2022). Various conditions found in marine areas such as differences in pH, tidal gradients, and high salinity make them produce bioactive products that may be unusual in their adaptation. Actinobacteria also produce enzymes that have high economic and biological value for industrial applications. The high G+C content in Actinobacteria DNA (Sheshadri et al., 2022) as well as the adaptation effect made to their extreme habitat, allows them to produce enzymes that are also stable and active in these conditions.

Enzymes are complex polyproteins of approximately 1000-2000000 grams/mole in size and can catalyze specific biological reactions by lowering their activation energy (Mukhtar et al., 2017). Enzymes can speed up the chemical reaction to obtain the final valuable products (Fasim et al., 2021). One of the enzymes produced is lipase. Lipase is a serine hydrolase enzyme that breaks down triglycerides into glycerol and fatty acids (Murtius et al., 2022). Lipase itself belongs to the class of hydrolases (Chandra et al., 2020). As the name suggests, lipases hydrolyze lipids in the presence of water to form fatty acids and glycerol (Lim et al., 2022). Several studies have succeeded in finding marine Actinobacteria that produce lipase enzymes. Vertygo et al. (2021) and Agustriana et al. (2023) stated that the six isolates of marine Actinobacteria they obtained produced lipase enzymes activity. Lipase activity is affected by many factors especially pH and temperature (Amaturrahim et al., 2020). Therefore, determining lipase activity at each specific temperature and pH range is necessary to determine optimal lipase activity. Previous studies revealed the optimization of pH and temperature on the

activity of the lipase enzyme produced by marine Actinobacteria. Priya et al. (2012) stated that two strains among 30 Actinobacteria screened from sediments of Tiruchendhur coastal areas of Tamil Nadu showed higher lipase activity at pH 10.0 and 50°C. However, the optimization of pH and temperature on the lipase enzyme produced by other marine Actinobacteria is less reported.

Methods

Materials

The materials used in this research were buffer solutions (Merck), Gum arabic powder (Liberty Scientific), p-nitrophenyl acetate (Merck), Tris-HCl (Fisher Scientific), sodium acetate (Merck), Glycine NaOH (Merck), p-nitrophenyl acetate (Himedia), isopropanol (Merck), aluminum foil (Klin Pak), microfuce tube (Ependorf), tube (Falcon), ethanol (Merck), and Triton X-100 (Merck).

Equipment

The equipment used in this research were **an** autoclave (Hirayama HVE-50) spectrophotometer (Thermo Helios Omega UV-VIS), weighing beaker (Pyrex), centrifuge (Blow N Glow Scientifick), vortex (Thermolyne 37600 Mixer), and waterbath (Memmert WPE45).

Procedures

Tool Sterilization

Sterilization of the tools was performed by autoclaving using an autoclave at a temperature of 121°C for 15 minutes. Tools used during the testing period are provided clean and dry. Tools are wrapped in opaque paper and clear plastic and secured with tape prior to autoclaving. This is intended to avoid friction and high pressure during the sterilization process and minimize the risk of contamination prior to tool use. Additionally, these tools can be used and placed in an autoclave. The correct temperature and time are set and the autoclave can be opened after sterilization is complete and the temperature has cooled. Sterilization of tools is important. This is because microbiological testing must be performed aseptically to avoid contamination of tools, media, particulates, and air.

Buffer Preparation

Buffers were prepared by diluting the buffers with varying pH from a 1 M solution concentration to 50 mM. 57.15 mg of 0.1% gum arabic powder was placed in a weighing beaker, 57.15 µl of 0.1% Triton X-100 solution and 57.15 ml of buffer solution were added and mixed until uniform.

Substrate Preparation

36.23 mg of *p*-nitrophenyl acetate (pNPA) substrate was prepared from the refrigerator, placed in a weighing beaker, dissolved by adding 20 mL of isopropanol, mixed until homogeneous, placed in a flask, and covered with aluminum foil.

Enzyme Preparation

The crude enzyme was prepared in a volume of 75 µl, diluted using 675 ml of reaction buffer, and stored at 20° C in clear microfuge tubes.

Sample Incubation

Prepare a test tube, add 250 µL duplo substrate and 750 µL duplo reaction buffer, vortex, and incubate at 60 °C using a waterbath for 3 minutes, then add 250 µL enzyme and incubate again. After 5 minutes, ethanol was added to 1 ml to stop the kinetics and the samples were vortexed before transfer to the falcon. Samples loaded onto the falcon are centrifuged at 20°C and 12.000 rpm for 5 minutes. The supernatant was then collected and placed in a dark microfuge tube for observation. The effect of pH on lipase production was inspected by assaying the enzyme activity using various buffers including sodium acetate (pH 6.0), Tris-HCl (pH 8.0), Tris-HCl (pH 9.0), and glycine-NaOH buffer (pH 8.0). Meanwhile, the effect of temperature on lipase production was inspected by incubated samples at various temperatures (30°C, 40°C, 50°C, 60°C,70°C, and 80°C) for 24 hours and the activity of lipase produced was evaluated.

Calculation and Interpretation of Results

Absorbance values of samples and controls were observed and calculated using a spectrophotometer at a wavelength of 405 nm. Duplicate absorbance values were recorded for later processing in an Excel spreadsheet.

Result and Discussion

Optimization of pH for Lipase Enzyme Activity

Enzymes can catalyze reactions under optimal conditions if they are in the optimal environment. Enzymes are unique because they have different properties for optimal pH conditions. PH treatment affected the activity of the lipase enzymes shown in Table 1.

Table 1. Effect of pH Treatment on Lipase Enzymatic Activity

Buffer	Lipase Enzyme Activity (U/ml)	Standard Deviation
Sodium Acetate pH 6	27.388	3.38
Tris HCl pH 8	0.288	2.16
Tris HCl pH 9	92.589	9.06
Glycine NaOH pH 8	48.307	4.03

Table 1 shows that pH treatment affected the activity of the lipase enzyme. The highest lipase activity was obtained from Tris-HCl buffer pH 9 treatment which is 92.589 U/ml, and the lowest lipase enzymatic activity was obtained from Tris-HCl buffer pH 8 treatment which is 0.288 U/ml. This is in line with Kumar (2020) who found that the lipase enzyme was observed to be active in the pH ranging from 7 to 9.

A decrease in pH to acidic conditions causes a decrease in activity, and an increase in pH to base can damage the enzyme structure. If the pH is too low, H^+ ions bind to $-NH_3^+$ in the amino acid structure of proteins to form $-NH_4$. The bonding process breaks bonds between nitrogen atoms and other hydrogen atoms, denaturing the enzyme. The high pH condition causes the -OH ions to bond to the hydrogen atoms of the COO enzymatic groups to form HO. This breaks the bond between the hydrogen atom and nitrogen or oxygen, thereby damaging the structure of the enzyme (Lehninger, 1995).

Optimization of Temperature for Lipase Enzyme Activity

Optimal conditions for enzymes depend on the temperature changes that occur. It is affected by changes in ambient temperature. Temperature treatment affected the activity of the lipase enzymes shown in Table 2.

Table 2. Effect of Temperature Treatment on Lipase Enzymatic Activity

Temperature (°C)	Lipase Enzyme Activity (U/ml)	Standard Deviation
30	8.195	1.73
40	13.874	5.39
50	18.115	4.74
60	68.794	2.66
70	72.533	8.12

Table 2 shows that temperature treatment affects the activity of the lipase enzyme. The average value of lipase enzyme activity in the table shows that the highest lipase enzyme activity was obtained at a 70°C temperature treatment of 72.533 U/ml and the lowest activity was obtained at 30°C temperature treatment of 8.195 U/ml.

Optimum conditions for enzymes depend on the temperature changes that occur. Raising the temperature to the optimum limit speeds up the reaction and slows it down as the enzyme denatures. Temperatures that are too low can adversely affect enzymatic activity (Lehninger, 1995). The lipase enzyme is a protein that denatures (damages) secondary proteins. The secondary structure consists of hydrogen bonds formed by the polar ends of protein chains. Damage to secondary structure can lead to changes in protein structure and impair the protein's function as a catalyst (Hutasoit, 2017).

Conclusion

Based on the findings, it could be concluded that pH greatly influences lipase enzyme activity with the optimum pH obtained being pH 9 using Tris HCl buffer, which is 92.589 U/ml, and temperature also affecting lipase enzyme activity where the optimum temperature obtained is 70°C, which is 72.533 U/ml.

Conflict of Interest

We have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Role of the author:

Author 1 (Muhammad Rizqi Aulia): Conceptualization, methodology, writing, review, and editing.

Author 2 (Eva Agustriana): Data curation, checking, and evaluating.

Author 3/corresponding author (Lenni Fitri): validation, evaluating, review, editing, and submitting journal.

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