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Optimization of Lipase Production from Local Bacteria Isolate with Palm Oil Inducer

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Abstract

A lipase is a group of hydrolase enzymes with multi-functional abilities such as catalyzing hydrolysis, alcoholysis, esterification, interesterification, and synthesis of organic compounds. The purpose of this study was to obtain the optimum conditions for lipase production and to study the effect of adding a palm oil inducer on the production of lipase from local bacterial isolates. The method used in this study included the rejuvenation of local bacterial isolate cultures, optimization of production, which included pH, palm oil inducer, and incubation time. The lipase activity test was carried out by using the acid-base titration method with the phenolphthalein indicator. The results showed that the optimal conditions for lipase production occurred at pH 5 with an activity value of 7.5 U/mL and an incubation time of 18 hours with an activity value of 13.83 U/mL. The addition of a 10% (v/v) palm oil inducer increased the value of lipase activity to 8.75 U/mL.

1. Introduction

Lipase is a group of hydrolase enzymes with the IUPAC name triacylglycerol ester hydrolases (E.C.3.1.1.3), which function to hydrolyze triglycerides to become fatty acids and glycerol. Other reactions that lipase can catalyze are alcoholysis, esterification, interesterification, and synthesis in organic reactions [1]. Lipase also plays a role in separating fatty acids and dissolving the oil in industrial equipment to mix with water [2]. Due to the extensive function of lipase catalysis, lipase is widely marketed and used in various fields of industry and biotechnology [3, 4].

Lipase can be isolated and purified from fungi, yeast, bacteria as well as plants and animals. However, lipase derived from microbes is most widely used in the industrial world because it is more economical, stable, easy to cultivate, and generally resistant to heat, although the bacteria that produce it are not resistant to heat [4]. Lipase produced by microbes can come from different environments, including industrial waste disposal environments, factories processing vegetable oil, oil-contaminated soil, oil derived from seeds, food spoilage processes, coal, and compost piles [5].

Initial studies conducted on eight isolates, obtained from the mesophilic phase of the domestic waste composting process with a composting temperature of 37°C, found that there were isolates with lipase activity, which was $LKMA_3$ isolates. However, this isolate still had a relatively low lipase activity of 0.1666 U/mL [6]. To obtain lipase with relatively better activity, optimum growth media is needed to develop lipase-producing microbes. The influencing factors include the media's nitrogen content and the physicochemical factors such as pH, temperature, and incubation time [2]. Lipase production will also increase if a suitable inducer is present in the medium. An inducer is a substance added to the bacterial growth medium, which aims to stimulate bacteria's growth in producing lipase. Without an inducer, lipase is still produced but in small amounts [5]. Therefore, to get a relatively better value of enzyme activity, it is necessary to optimize the conditions, including pH, inducer addition, and incubation time.

In this study, the optimization of lipase production from local bacterial isolates - $LKMA_3$, was obtained from the mesophilic phase of the composting process of domestic waste. The optimization phases carried out include pH optimization, incubation time, and the addition of palm oil inducers. The measurement of lipase activity resulting from each optimization stage was determined by the titration method [7].

2. Methodology

This research is an experimental study conducted to obtain the optimum conditions for lipase production and study the effect of adding palm oil inducers in lipase production from local bacterial isolates.

2.1. Equipment and Materials

The tools used in this study were: glassware, autoclave (Centro Clave model), Bunsen burner, Laminar Air Flow/LAF (CURMA model 9005-FL), STUART SSL2 water bath shaker incubator, HAAKE water bath incubator, magnetic stirrer, refrigerator, oven, Eppendorff micropipette, incubator shaker, centrifuge, pH meter, burette, Statif, Genesys spectrophotometer.

The materials used in this study were local isolate lipolytic bacteria from the domestic waste composting process with the $LKMA_3$ code, nutrient agar (NA) and Nutrient Broth (NB) for bacterial growth media, distilled water, olive oil (Herborist brand), Tween 80, phenolphthalein indicator, palm oil as the inducer, NaOH, 70% alcohol, phosphate buffer, Na_2CO_3, $CuSO_4$, acetone, and ethanol.

2.2. pH optimization

The pH optimization was carried out by growing $LKMA_3$ isolates on NB media using 0.1 M phosphate buffer with a pH range of 4-6. Isolates were grown for 24 hours in an incubator shaker at a speed of 150 rpm at 37°C, then centrifuged. The supernatant obtained was determined by its lipase activity by the titration method [4].

2.3. Optimization of additional inducers

The addition of an inducer was carried out at optimum pH conditions. Each bacterial growth medium was added with palm oil inducer with a concentration of 6%, 8%, 10%, 12%, 14%, 16%, 18%, 20% (v/v). LKMA3 isolates were grown in NB media that had been given an inducer and incubated in the same way as in Section 2.2.

2.4. Optimization of incubation time

$LKMA_3$ isolate bacteria were grown on NB media with optimum pH and inducer concentration. Optimization of growth time was carried out for 48 hours in an incubator shaker at 37°C at 150 rpm. Each 6-hour interval sampling was carried out to determine the OD (Optical Density) value at a wavelength of 600 nm. Lipase activity testing was carried out by the titration method [4].

2.5. Lipase activity test

The lipase activity test was carried out using the acid-base titration method by mixing 2 mL of olive oil, 1 mL of 0.1M phosphate buffer, and 1 mL of an enzyme, then incubated at 37°C for 30 minutes. The enzyme-substrate was inactivated with 1 mL of a mixture of acetone: ethanol (1: 1), then two drops of 1% phenolphthalein indicator were added, then titrated with 0.1 M NaOH. Titration was stopped when the solution changes color to pink [6]. Activity measurements were performed in duplicate for each sample. Determination of the blank was carried out with the same solution composition. After the enzyme was added, 1 mL of acetone: ethanol mixture (1: 1) was immediately added to inactivate the enzyme and then incubate at 37°C for 30 minutes. The same procedure performed titration as for sample analysis [4].

3. Results and Discussion

The local isolate $LKMA_3$ bacteria were isolated from the mesophilic phase in the composting process of domestic waste at 37°C [6]. The results of the morphological test showed that the isolates obtained were gram-negative, bacilli-shaped, and motile. Preliminary test results on the crude extract of the enzyme product before optimization showed a relatively low lipase activity of 0.166 U/mL. One way to increase the number of lipase products produced by bacteria is to add an inducer. The study results reported that the addition of palm cooking oil as an inducer was proven to increase the production of lipase from *Aspergillus niger* [7].

In this study, the optimization process of lipase production was carried out through variations in pH, incubation time, and the addition of an inducer to the $LKMA_3$ bacterial growth medium. The pH variation is carried out in the pH range 4-6. Determination of the optimum pH for optimization of lipase production is presented in Figure 1.

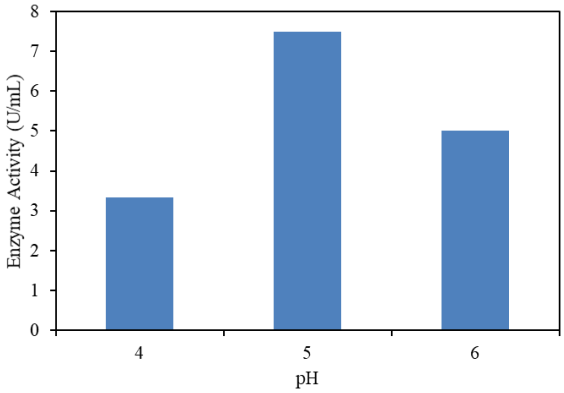

Figure 1. Effect of pH on lipase activity of a local bacterial isolate - $LKMA_3$

Figure 1 shows that the optimum pH of $LKMA_3$ isolate bacteria originating from the mesophilic composting phase in producing lipase is at pH 5 with a lipase activity value of 7.50 U/mL. Meanwhile, at pH 4 and 6, the lipase activity values were 3.33 U/mL and 5.00 U/mL. An increase in bacterial lipase activity originating

from the mesophilic environment of oil-contaminated industrial areas has also been reported. There was an increase in lipase activity from 7.5 U/mL to 8 U/mL at pH 6 after optimization [8].

In the optimization stage of lipase production, palm oil inducer was also added with a concentration range of 6%, 8%, 10%, 12%, 14%, 16%, 18%, and 20% (v/v). Each inducer was added to the production medium at pH 5 and incubated for 24 hours at 37°C at 150 rpm. The lipase activity results by the acid-base titration method at various inducer concentrations are presented in Figure 2.

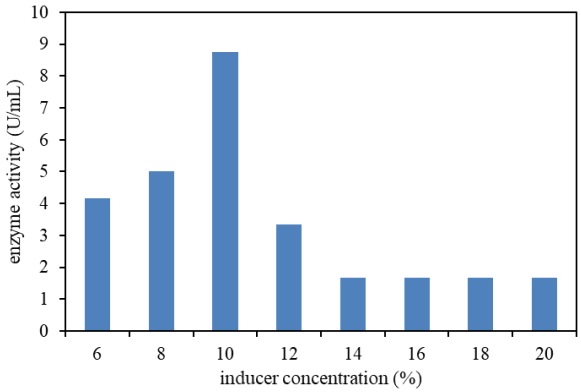

Figure 2. Effect of palm oil inducer concentration on lipase activity of local bacterial isolates - $LKMA_3$

Figure 2 shows the effect of palm oil inducer on lipase production. The palm oil inducer concentration of 10% gave the highest lipase activity of 8.75 U/mL. This lipase activity value showed an increase compared to before adding the inducer (lipase activity at pH 5 = 7.50 U/mL). Research on palm oil used as an inducer was also reported to increase the lipase activity of Aspergillus niger by 1.5 U/mL at an inducer concentration of 3% (m/v) pH 7, and a temperature of 30°C [7].

The incubation time's optimization phase aims to obtain the optimum time required by the local isolate bacteria $LKMA_3$ to produce the maximum amount of lipase. In this study, the incubation time range started from the 0th hour to the 48th hour. During the incubation time, every 6 hours, optical density (OD) measurements were carried out at a wavelength of 600 nm, and determination of lipase activity by the acid-base titration method.

Optical density is one way to determine the number of bacterial cells by using the spectrophotometric method by measuring the OD value. The OD value is a measure of turbidity, which indicates the number/density of bacterial cells in the growth medium. Measurement of OD values at certain times during the incubation period aims to determine the profile of bacterial growth [9]. The growth profile of local bacterial isolates-$LKMA_3$ in the conditions without and with the addition of palm oil inducer during the growth period is presented in Figure 3.

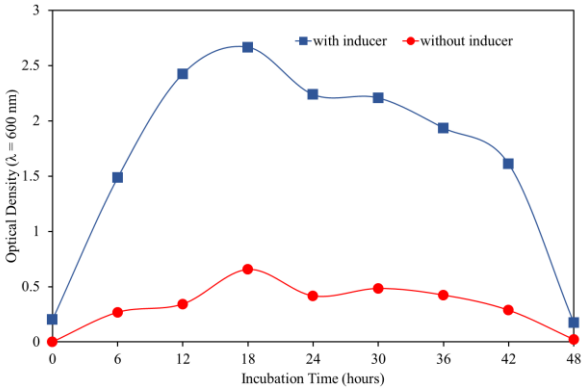

Figure 3. Effect of growth time of local bacterial isolates on conditions with and without the addition of 10% palm oil inducer

Figure 3 shows that the addition of a 10% palm oil inducer significantly increased local isolate bacteria $LKMA_3$ (24.67%). The optimum condition occurs at the 18th hour (OD value = 2.6550), which is an exponential phase of bacterial growth of $LKMA_3$ isolates with the addition of an inducer. The bacterial growth of $LKMA_3$ isolates without palm oil inducers experienced slower growth, as indicated by the lower OD value of the exponential phase (OD = 0.6549). In the exponential phase, bacterial growth is very fast, so that the production of enzymes, including lipase, is very much produced by bacteria [6]. Enzymes function in metabolic processes that support cell growth and have been produced from the beginning of bacterial growth, which increases in the exponential phase and then decreases according to the cell growth phase [7].

The optimum incubation time is determined based on the bacteria's time to produce the lipase with the highest activity. Determination of the incubation time was carried out in a time range of 0 to 48 hours, pH 5, and the addition of a 10% (v/v) palm oil inducer. The effect of incubation time on optimization of lipase production is presented in Figure 4.

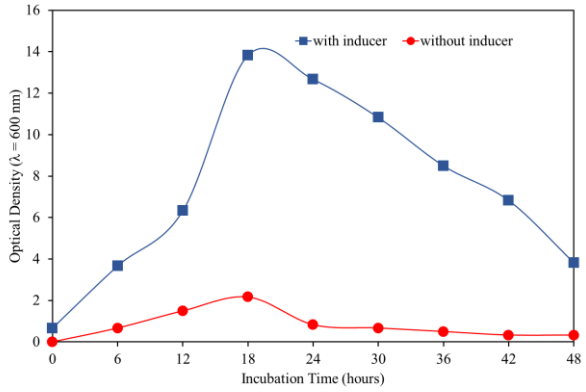

Figure 4. Effect of optimum incubation time on lipase activity at the production optimization stage with and without 10% (v/v) palm oil inducer

Figure 4 shows that the optimum incubation time for bacteria to produce lipase was obtained at the 18th hour. The production of lipase by local isolate bacteria $LKMA_3$ at optimum conditions at 18 hours with 10% palm oil inducer was much more significant than without

inducer. At 18 hours, the lipase activity with an inducer's addition was 13.83 U/mL, an increase of about six times greater than that without an inducer, which was 2.17 U/mL.

This study indicates that the local isolate $LKMA_3$ bacteria can use palm oil as an inducer in increasing lipase production. In general, compounds that can be used as inducers contain long-chain fatty acids such as oleic, palmitic, stearate [10]. Palm oil contains high levels of oleic and palmitic acids to be used as an inducer to produce lipase. The addition of palm oil inducers can increase the growth and number of cells which causes the production of lipase to increase.

4. Conclusion

Based on this study's results, it can be concluded that the local isolate bacteria $LKMA_3$ can produce lipase optimally at pH 5, an inducer concentration of 10%, and an incubation time of 18 hours. Local isolate bacteria can also use palm oil as an inducer to produce lipase with an optimum concentration of 10%.

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References

[1] Malihe Masomian, Raja Noor Zaliha Raja Abd Rahman, Abu Bakar Salleh, Mahiran Basri, A new thermostable and organic solvent-tolerant lipase from Aneurinibacillus thermoaerophilus strain HZ, *Process Biochemistry*, 48, 1, (2013), 169-175 https://doi.org/10.1016/j.procbio.2012.11.002

[2] Novriyanti Hutasoit, Putu Timur Ina, I Dewa Gede Mayun Permana, Optimasi pH dan Suhu pada Aktivitas Enzim Lipase dari Biji Kakao (*Theobroma Cacao* L.) Berkapang, *Jurnal Ilmu dan Teknologi Pangan*, 5, 2, (2017), 95-102

[3] Abhijit Ray, Application of lipase in industry, *Asian Journal of Pharmacy and Technology*, 2, 2, (2012), 33-37

[4] Haniya Mazhar, Naaz Abbas, Sakhawat Ali, Amir Sohail, Zahid Hussain, Syed Shahid Ali, Optimized production of lipase from Bacillus subtilis PCSIRNL-39, *African Journal of Biotechnology*, 16, 19, (2017), 1106-1115 https://doi.org/10.5897/AJB2017.15924

[5] Dian Pratiwi, Firman Sebayang, It Jamilah, Produksi dan Karakterisasi Enzim Lipase dari *Pseudomonas aeruginosa* dengan Menggunakan Induser Minyak Jagung Serta Kofaktor Na^+ dan Co^{2+}, *Jurnal Saintia Kimia*, 1, 2, (2013), 1-5

[6] Leony Fransiska, Isolasi dan Identifikasi Bakteri Lipolitik pada Proses Pengomposan Limbah Domestik, *undergraduate thesis*, Department of Chemistry, University of Lampung, Bandang Lampung, 2019

[7] Sri Wahyu Murni, Siti Diyar Kholisoh, DL Tanti, EM Petrissia, Production, Characterisation, and Isolation of Lipase from Aspergillus niger by using Palm Oil as Inducer, *Eksergi*, 12, 1, (2015), 1-4 https://doi.org/10.31315/e.v12i1.1034

[8] A Jaiswal, M Preet, B Tripti, Production and optimization of lipase enzyme from mesophiles and thermophiles, *Journal of Microbial and Biochemical Technology*, 9, 3, (2017), 126-131 http://dx.doi.org/10.4172/1948-5948.1000355

[9] Neviaty P. Zamani, Moh. Muhaemin, Penggunaan Spektrofotometer Sebagai Pendeteksi Kepadatan Sel Mikroalga Laut, *Maspari Journal*, 8, 1, (2016), 39-48

[10] R. Gupta, N. Gupta, P. Rathi, Bacterial lipases: an overview of production, purification and biochemical properties, *Applied microbiology and biotechnology*, 64, 6, (2004), 763-781 https://doi.org/10.1007/s00253-004-1568-8