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Original Article

OPTIMIZATION OF *LACTOBACILLUS PLANTARUM* ACTIVITIES IN THE BIOSINTHESIS OF LIPASE ENZYMES

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ABSTRACT

Objective: Lipase was protein compounds that can be used for many human activities. Its main function was to degrade fat including 'wrapping' cholesterol which make easily flowed in the blood. The presence lipase was important because can help the digestive healthy. These enzyme can catalyze a variety of reactions including hydrolysis, alcoholysis, esterification and aminolysis. Lipase was utilized in various sectors, such as fat, oil, milk and pharmaceutical industries. This enzyme biosynthesis can be carried out by *Pseudomonas aeruginosa, Lactobacillus plantarum* and *Aspergillus niger*.

Methods: The process through fermentation techniques in lipid containing substrates under optimal conditions required by microorganisms. The fermentation products produced were tested for the presence of lipase enzymes qualitatively and quantitatively. The biosynthesis process can be influence by changes in pH, temperature and the presence of glucose. This study aimed to determine the ability of *L. plantarum* to produce lipases with vegetables oil substrates. The research used *L. plantarum* carried out at 37 °C for 24-48 h and pH 6-8 in the vegetable oil substrates.

Results: The fermentation products showed hydrolysis reaction to the test media containing oil lipid with lipase levels of 2.708-3.3000 U/ml

Conclusion: The results showed that *Lactobacillus plantarum* can synthesize the lipase enzyme in palm oil and corn oil as substrates.

Keywords: *Lactobacillus plantarum*, Biosynthesis, Lipase, Palm oil, Corn oil

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INTRODUCTION

Lactobacillus plantarum is a broad member of the genus Lactobacillus. It is one of the most studied species that is used in the food industry as a probiotic microorganism and microbial starter. *Lactobacillus plantarum* has been identified from a variety of traditional foods and is characterized by systemic and molecular taxonomic purposes and produces enzymes such as α-amylase, esterase, and lipase [1]. Lipase is an enzyme whose use is very broad and is found in animals, plants, fungi and bacteria. Lipases catalyze the hydrolysis of long-chain acyl glycerol at the aqueous oil interface [2].

Lipase has substrate specificity and variety different enzymatic properties, like broad source, short cycle, wide pH range, and a wide temperature range. Microbes that can produce lipases have important role than animals and plants. Lipase used in enzymatic theoretical research and practical applications for example in the process hydrolysis and esterification. Lipases from microorganisms for industrial are more attractive because lipases can be produced in large quantities and can be used as a catalyst and easily manipulated genetically. Lipases produced by microbes are heat-stable and play a role in the process of spoilage of dairy products. Factors to be considered when using microbes to produce lipases are microbial growth, the conditions of lipase production in the corresponding, test the lipase activity of the enzymes produced. Microbial growth conditions that affect lipase synthesis and production include carbon sources. Carbon sources can come from olive oil or other vegetable oils [2]. Vegetable oil has been used as an inducer for lipase production by adding vegetable oil to the media. Vegetable oils are used, such as peanut oil, sunflower oil, olive oil, palm oil and coconut oil [3]. Bacteria, yeast, and mould are known to secrete lipases to break down lipids. *C. rugosa* produces lipases that function to reduce serum cholesterol levels. *S. marcescens* has been used for the synthesis of lovastatin, a drug in which lipases are widely used for asymmetric hydrolysis of 3-phenylglycidic acid esters that are very important in the synthesis of diltiazem hydrochloride [4, 5].

MATERIALS AND METHODS

Materials

Lactobacillus plantarum from Microbiology and Bioprocess Technology Laboratory, Institut Teknologi Bandung, Nutrient Agar (Merck), Nutrient Broth (Merck), Aquadest sterile, 95% alcohol (Smart Lab), methyl red (Merck), palm oil (Tropical®), corn oil (KCO®) tween 80 (Merck), NaOH (Merck), phenolphtalein indicator (Merck), phosphate buffer pH 7 (Merck), acetone (JT baker), ethanol (Smart Lab).

Methods

Preparation of starter

The bacterial culture of *Lactobacillus plantarum* made turbidity of the suspension which was arranged into a population of±1 million colonies/ml in medium nutrient agar. Then made a series that is equivalent to the turbidity of the suspension, the absorbance value of 0.8 to 1.2. Subsequently incubated at 37 °C for 24-48 h. This suspension is made as a fermentation starter [6].

Making a bacterial growth curve

Nutrient Broth medium was put into the 500 ml Erlenmeyer as much as 225 ml, adding a suspension of bacteria that had been made as much as 25 ml (10% v/v). Then incubated in a shaker incubator with a speed of 150 rpm for 48 h at a temperature of 35-37◦C. Sampling was carried out every 1 hour for 48 h, then absorption measurements were carried out using UV-Vis spectrophotometry at a wavelength of 660 nm to see the turbidity level [6].

Production of bacterial metabolites

Lactobacillus plantarum bacterial suspension was inoculated as much as 25 ml (10% v/v) into a 500 ml Erlenmeyer glass containing 225 ml of Nutrient Broth medium with palm oil substrate (with 1%, 3% and 5% substrate concentration variation)). Then incubated at 37 °C using a shaker incubator with a speed of 150 rpm for 48 h. Sampling is done at a certain time, namely at the optimum time of secondary metabolite production based on the growth curve (until the end of the stationary phase). The fermentation results are centrifuged at 4000 rpm for 30 min to obtain a supernatant, a metabolite of the bacterium *Lactobacillus plantarum* [7].

Qualitative test of lipase enzyme activity

Identification was carried out by inoculating bacterial metabolite fractions into 20 ml Nutrient Agar media containing 10% (2 ml w/v)

sterile palm oil and corn oil and adding 0.1% (0.2 ml) Rhodamine B. Then, it was incubated at 37°C for 24 h. Lipolytic activity is indicated by orange fluorescence in Petri dishes when seen in 365 nm UV light [8, 9].

Semiquantitative test of microbiological methods

Lipolytic tests were carried out using 20 ml Nutrient Agar media containing 2.5% Tween-80 (0.5 ml w/v) and 5% palm oil (1 ml w/v) and 0.01% methyl red added to medium. The medium that has been dense made 3 µm holes using perforators. The metabolite fraction was inoculated 10 µl in the perforated media, then incubated for 24 h at 37 °C. The lipolytic activity of *Lactobacillus plantarum* grown on specific media is indicated by the appearance of a yellowish-white area that is considered a clear zone that appears around the formed colonies. The clear zones that emerge and bacterial colonies that grow are measured in diameter and calculated semiquantitatively to obtain the lipolytic index [7, 8].

$$\text{Lipolytic index} = \frac{\text{DZ} - \text{DK}}{\text{DK}}$$

DK: Diameter of microorganism colony

DZ: Diameter of clear zone

Quantitative test of chemical methods

A total of 1 ml fraction plus 1 ml of sterile palm oil and 2 ml phosphate buffer pH seven were incubated at 37 °C for 30 min. After incubation, 5 ml of acetone-ethanol (1: 1) solution was added, and two drops of phenolphthalein (PP) indicator was added and then titrated with 0.05 N sodium hydroxide (NaOH) until it began to turn pale pink [10–12].

The activities of lipolytic be calculated as follow.

$$\text{Activity of lipase (U/ml)} = \frac{(\text{A} - \text{B}) \times \text{N NaOH} \times 1000}{\text{VE} \times \text{t (incubation)}}$$

A= Volume (ml) NaOH for sampel

B= Volume (ml) NaOH for blanko

1000= Conversion from mmoles to micromoles

t= incubation time

VE = Volume supernatant volume.

RESULTS AND DISCUSSION

The growth and metabolite production of bioactive compounds selected for qualitative, semi-quantitative, and quantitative analysis in the lipase enzyme activity test was at 9th, 25th, 30th hours, the log and stationary phases, based on the results of the growth curve. The growth curve is significant in determining the characteristics of the bacterium *Lactobacillus plantarum* because it describes the bacterial population within a certain period time and knows its activity in the metabolic process.

The growth curve of the *Lactobacillus plantarum* was carried out using the turbidimetry measurement. The growth of *Lactobacillus plantarum* was characterized by an increase in the medium's density value (turbidity). The value of the density of the medium was measured at a wavelength of 660 nm using spectrophotometer. Growth curves are used to determine the optimal time of *Lactobacillus plantarum* in producing metabolite compounds [13].

The results of the growth curve (fig. 1) the phases that occur in the growth of the *Lactobacillus plantarum* bacteria in the Nutrient Broth medium include the adaptation phase (lag phase) occurring in the 0 to 2 h. The density in 0 to 2 h has not increased significantly. The population of *Lactobacillus plantarum* in this adaptation phase has not experienced significant growth because it has just adapted to its new medium or environment. In this phase of adaptation, *Lactobacillus plantarum* increase in cell size, but the number is very little population growth occurs even though cell metabolism continues. A significant increase in population characterizes the logarithmic phase (exponential phase). The number of cells increases after the 2nd hour to the 26th hour, during which phase the *Lactobacillus plantarum* bacterium grows and divides at maximum speed. New cells were formed at a constant rate, and mass increases exponentially [14].

The next phase was the stationary phase, which was marked by the growth of *Lactobacillus plantarum* bacteria stops, and there is a balance between the numbers of cells that divide with the number of cells that die. The phase starts at the 27thhour with a graph that is horizontal [15].

The next phase is the death phase but is not seen in the growth curve because the growth curve of *Lactobacillus plantarum* for 48 h consists of three phases, namely the lag phase, the log phase, and the stationary phase. These three phases correspond to growth curves with the growth curve of bacteria in general. The death phase is when the population of the bacterium *Lactobacillus plantarum* begins to die because the energy reserves in the cell are depleted the nutrients in the medium are exhausted [14].

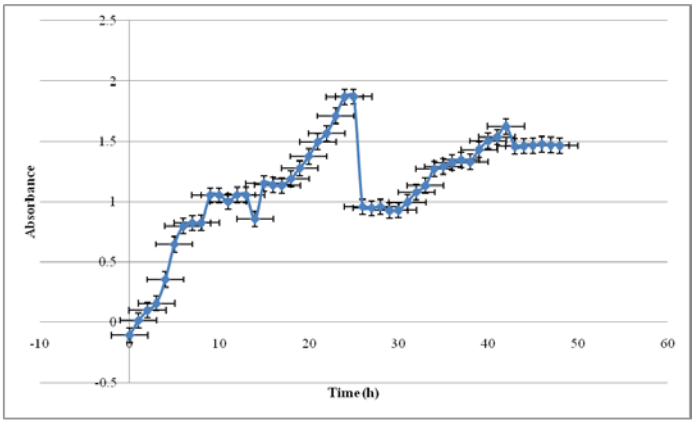

Fig. 1: Growth curve of *Lactobacillus plantarum* with exponential phase: 2-26 h; stationary phase: 27-48 h

Palm oil and corn oil as substrates added to optimize lipase production in the fermentation process. The concentration of the substrate is varied. The substrates variations were made to determine the effect of the substrate concentration on *Lactobacillus plantarum* of producing lipase to the maximum. There are three variations in the concentration of palm oil and corn oil used, namely 1%, 3%, and 5% of the solution's total volume.

Fermentation substrate concentration greatly affects the bioactive metabolites produced. Palm oil is a substrate in producing lipase enzymes because it contains triglycerides. Triglycerides are one of the crucial components that play a role in optimizing the bioactive metabolites produced. Triglycerides are inducers for bioactive metabolites that can increase the production of bioactive metabolites. High substrate concentration will increase the enzyme reaction so that the lipase enzyme obtained is more

optimum compared to low substrate concentration [16]. Corn oil has the same fat content as olive oil and palm oil, although the amount is different [17]. Palm oil and olive have used to induce maximum lipase production by *Bacillus aryabhattai* SE3-PB [18]. Corn oil has been used as a substrate to produce lipases by *Bacillus cereus* HSS [19]. The presence of lipase is indicated by an orange glow when viewed under UV light at a wavelength of 365 nm (table 1).

Table 1: Data of qualitative lipase enzyme testing results

Substrate concentration	Qualitative test results		
	9th hours	25th hours	30th hours
1 %	(+) orange fluorescence	(+) orange fluorescence	(+) orange fluorescence
3 %	(+) orange fluorescence	(+)orange fluorescence	(+)orange fluorescence
5 %	(+) orange fluorescence	(+) orange fluorescence	(+) orange fluorescence

The lipolytic index (fig. 2) obtained the highest ratio of palm oil and corn oil with a concentration of 5% at the fermentation time for 25 h. The higher the substrate concentration added, the greater the lipolytic index, meaning that the lipase formed to break down more fat. The fat contained in the media degrades into simpler molecules to form a clear zone. The wide diameter of the yellow area can indicate the amount of the lipase enzyme produced.

Metabolites that have large lipolytic index values are assumed to have high lipolytic activity. Lipases will hydrolyze media containing lipids to fatty acids and glycerol. The fatty acids that formed then react with rhodamine B to form complex bonds. This form complex bond happens because there is a reaction between uranyl ions from fatty acids with cationic on rhodamine B. The complex bonds that cause the appearance of orange glow when viewed under UV light [20].

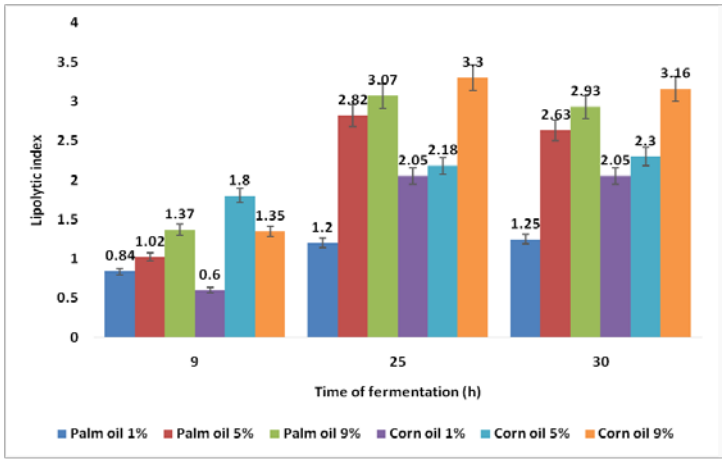

Fig. 2: Lipolytic index

Test the lipase activity quantitatively (fig. 3), based on the titration method with NaOH, palm oil and corn oil as a substrate and phenolphthalein as an indicator. The lipase enzyme activity was indicated by changes in the solution from colourless to pink when titrated with NaOH due to changes in pH. The pink colour appears when NaOH can no longer bind to fatty acids, thereby giving the alkaline nature of the solution and giving rise to a pink colour as an indicator of the pH change of the solution [21, 22]. The NaOH volume needed during titration is calculated to determine the amount of lipase activity. The metabolite of *Lactobacillus plantarum* showed the highest lipase activity after fermentation for 25 h, which contained a 5% palm oil concentration of 3.112 U/ml and 5% corn oil concentration is equal to 3.9 U/ml. The higher the volume of NaOH during titration, the higher the fatty acids produced from the lipid hydrolysis process [11]. Previous studies using *Acinetobacter baylyi* selected for maximum lipase production obtained after 12 h of culture with three evaluated substrates: 0.358 U/ml. min^{-1} in olive oil, 0.352 U/ml. min^{-1} in grapeseed oil, and 0.348 U/ml. min^{-1} with canola oil [23].

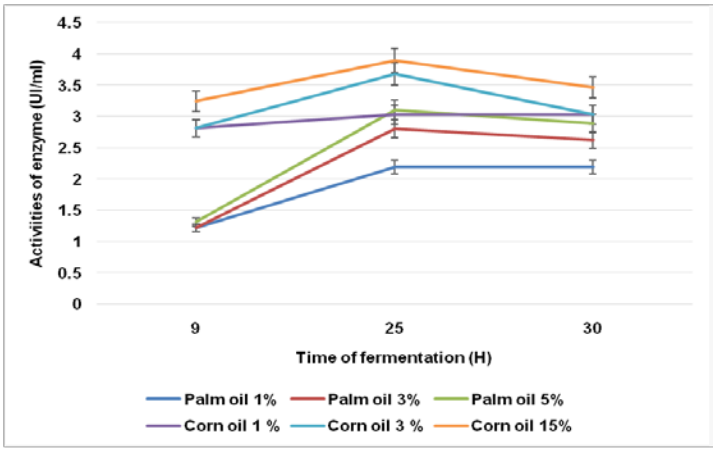

Fig. 3: Activities of lipase (UI/ml)

CONCLUSION

The results showed that *Lactobacillus plantarum* can synthesize the lipase enzyme in palm oil and corn oil as substrates.

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AUTHORS CONTRIBUTIONS

All authors contributed equally.

CONFLICT OF INTERESTS

Declared none

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