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Screening Lipolytic from Soil Bacterial Contaminated Oil

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Abstract. The research aims to study the lipolytic activity of bacteria isolated from soil contaminated with oil. Screening of lipolytic bacteria was performed in Rhodamin-B agar plate containing olive oil. The lipolytic activity was determined by spectrofotometry method toward p-nitrofenil palmitat as a substrate. The result this research showed that there are 15 of 150 isolates bacteria from soil contaminated with oil was confirmed by observing the zone of hydrolysis formed around the growth of colony on Rhodamin B agar plate containing olive oil. One of the lipolytic bacteria identified as higher activity was produced lipase with activity of 7,41 U/mL

Keywords : *Enzime activity, Lipolytic, Olive oil, Rhodamin-B*

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Introduction

Lipases occur widely in nature, but microbial lipases are commercially significant because of low production cost. Most of the industrial microbial lipase is derived from fungi and bacteria. The majority of enzymes used in food industry are for food processing, mainly for modification and breakdown biomaterials. Lipase (triasilglycerol acylhydrolase, E.C, 3.1.1.3) is an enzyme can hydrolyze long chains of triglycerides [1]. Lipase is a water-soluble enzyme and naturally lipase catalyze the hydrolysis of ester bonds in water insoluble lipid substrate and long-chain acylglycerol synthesis, diacylglycerol, monoacylglycerol, and glycerol [2]. Hydrolase enzyme lipase can be used potentialy produced fatty acid which precursors in the chemical industry [3]. Other applications of lipase enzymes include being used in fat and oil processing, food processing, chemical synthesis, pharmaceuticals, paper synthesis, cosmetic production and also in the biodiesel industry [4]. POME waste soil is the land around the disposal of pressing the palm kernel. POME waste is currently used only as a substitution for fertilizer (land application), ground moisture guards, as well as retaining palm oil erosion. The soil surrounding POME's waste disposal is thought to have an opportunity as a source of lipolytic bacteria because it has unique environmental conditions where the soil still contains a lot of palm oil residue, this is in a way lipase works that can hydrolyze the triglyceride chain. The soil that is around the waste is presumably still contains many palm oil that can as a source of carbon for still growing.

Experimental

Sampling Site and Collection

Soil samples from Palm Oil Mill Effluent were taken from palm oil processing factory by PT. Agro Bukit Central Kalimantan which is located on Sudirman road Km 106 Sampit, Central Kalimantan (2 ° 33'55.3 "S, 112 ° 46'03.5" E) in January 2016. The soil is taken from the bottom of the waste treatment as deep as 3-5 cm from ground.

Isolation of bacteria from oil-contaminated soil samples with olive oil enriched Luria Bertani media

The liquid media used had the following compositions of 0.2 grams of triptone, 0.2 grams of NaCl, and 0.1 grams of yeast extract, and 20 L of cooking oil (50 L/mL) dissolved in 20 mL of distilled water. The liquid medium is then poured into the Erlenmeyer, preferably filling 1/5 of the Erlenmeyer.

Liquid media that is ready to be added 5 grams of soil samples contaminated with POME. The liquid medium was then homogenized by shaking at a speed of 1200 rpm, for 24 hours and at a temperature of 37^{o}C.

Isolation of Bacteria from Oil Contaminated Soil Samples with Solid Luria Bertani Media

The media used is Luria Bertani (LB) media. 20 mL solid media, weighing 0.4 grams of bacto agar, 0.2 grams of triptone, 0.2 grams of NaCl, and 0.1 grams of yeast extract, dissolved in 20 mL of distilled water, sterilized by autoclaving at 121ºC for 15 minutes. The warm sterile media was then poured into a petri dish.

Solid media that is ready to be added 1000 ul of enriched soil sample. The solid media was then incubated for 24 hours at 37^{o}C.

Rejuvenation of Bacterial Isolates Growing on Solid Luria Bertani Media

The media used is Luria Bertani (LB) media. 20 mL solid media, weighing 0.4 grams of bacto agar, 0.2 grams of triptone, 0.2 grams of NaCl, and 0.1 grams of yeast extract, dissolved in 20 mL of distilled water, sterilized by autoclaving at 121ºC for 15 minutes.

Cultures of isolates from solid LB media were previously taken using a sterile toothpick. Then a sterile toothpick containing this culture was applied to the prepared new solid LB medium. Solid LB media containing these cultures were stored at low temperatures.

Lipolytic Bacteria Screening Using Rhodamine-B . Test Media

The test medium (Rhodamine B plate agar) consisted of LB agar medium containing cooking oil, Rhodamine B, and tween 20. The method of preparation is to divide the LB agar medium into culture bottles, each as much as 20 mL and add 600 L (3 %) cooking oil, 40 l Rhodamine B, 0.1% H2O,

and 50 L tween 20. The media was then sterilized using an autoclave at 121 °C, 1 atm, for 15 minutes. After cooling down, media poured into petridish.

To test for the presence of lipolytic bacteria, a sterile toothpick of bacteria that had been isolated was taken and then grown by spotting on Rhodamine-B agar media and incubated for 24-48 hours at 37 °C. Lipolytic activity is characterized by the presence of a halo zone around the colony. The lipolytic index was measured by the comparison of the halo zone and the colony diameter.

Lipolytic Activity

Lipolytic activity was determined by a spectrophotometric method toward p-nitrophenylpalmitate as a substrate [5]. The reaction mixture consisted of 0.1 mL enzyme extract, 0.8 mL of 0.05 M Tris buffer (pH 8) and 0.1 mL of 0.01M of p-NPP (disolved in isopropanol). The reaction mixture was incubated at 37°C for 10 min. the reaction was added with 0.25mL of 0.1M mixture of cold acetone-ethanol (1:1) Na2CO3 to stop the reaction. The reaction mixture was centrifuged at 11,000 g for 15 min and the absorbance was measured by spectrophotometer UV-Vis at 410 nm. The enzyme activity expressed in units of units/ mL (U/ mL) One unit of lipase activity was defined as the amount of enzyme which liberated 1 µmol of p-nitrophenol per minute.

Results and Discussion

The results of the isolation of POME soil samples grown on solid media LB (Luria Bertani) obtained as many as 150 colonies that could grow on that medium. These colonies were obtained from solid LB media which was enriched with liquid isolates on liquid LB media with olive oil added as an inducer using the spread plate method (Figure 1). After the colonies grew, the next step was the selection of a single colony to be transferred to a new solid LB medium using a sterile toothpick (Figure 2).

The results of the measurement of the diameter of the hydrolysis zone of bacterial isolates are presented in Table 1 and Figure 3.

The clear zone was measured based on the lipolytic hydrolysis index which is the ratio between the diameter of the clear zone and the diameter of the colony. Where the clear zone produced is directly proportional to the activity produced by the isolate [6]. Table 1 shows the lipolytic index produced by bacterial isolates with a clear zone range of 0.32 to 1.42 cm.

Lipases produced by bacteria hydrolyze olive oil in the medium. Free fatty acids released are detected on the agar plate which forms a complex with rhodamine B [7,8,9]. The orange glow around lipolytic bacterial colonies is due to the formation of dimer complexes between rhodamine B and fatty acids and monoglycerides or diglycerides [10]. In addition to producing a luminescent zone

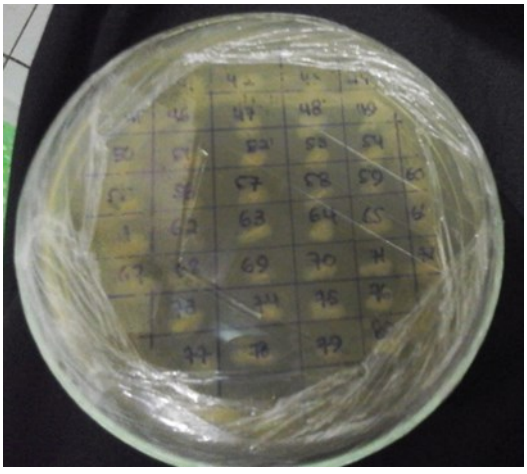

Figure 1. Single colonies that have been transferred to solid Luria Bertani media

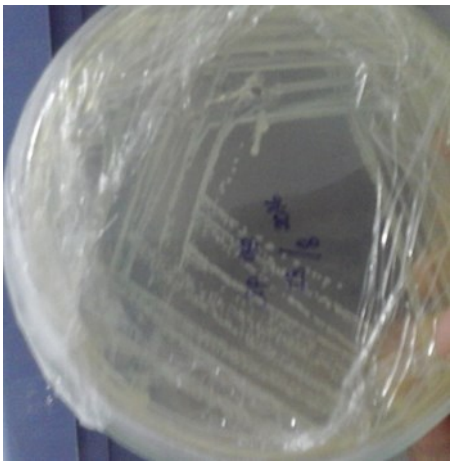

Figure 2. Bacterial isolates that have been scratched onto solid Luria Bertani media

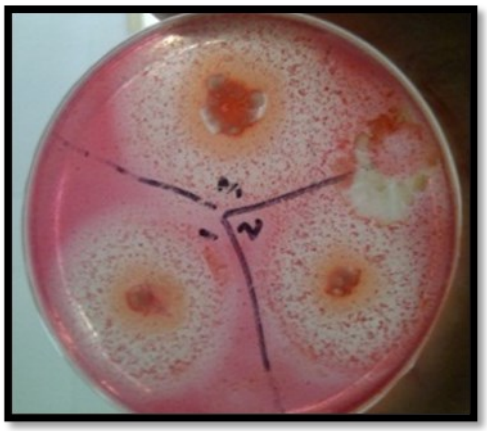

Figure 3. Clear zone around bacterial isolate colonies on Rhodamine B agar media added with 1%

when detected under UV light, lipolytic bacteria can also produce a clear zone. The clear zone formed showed more olive oil from the medium hydrolyzed by the lipase enzyme from 15 bacterial isolates and the hydrolysis product was utilized by bacteria as a carbon source in their growth and development. This is reinforced by the statement of Zusfahair that the bacterial colonies selected for lipase production were the colonies with the largest clear zone which showed more triglycerides than the medium hydrolyzed by the lipase enzyme [11]. This is because the clear zone produced is directly proportional to the activity produced by isolated bacterial isolates [6].

In Table 1 it is known that the hydrolysis index is between 0.57 to 2.55. In addition to the visual appearance, the lipolytic activity of a bacterial isolate can be measured based on the resulting lipolytic hydrolysis index. The hydrolysis index is the ratio between the diameter of the clear zone and the diameter of the colony. Colonies producing lipase enzymes whose lipolytic index was measured were expressed as the ratio between the diameter of the clear zone and the diameter of the colony [12].

Conclusion

The results showed that there were 15 isolates out of 150 isolates that had a hydrolysis zone around the colony. The enzyme activity expressed in units of units/ mL (U/ mL) One unit of lipase activity was defined as the amount of enzyme which liberated 1 µmol of p-nitrophenol per minute. One of the lipolytic bacteria that has the highest activity is 7.41 U/ml.

Table 1. Data on the measurement of the diameter of the hydrolysis zone and the diameter of the lipolytic bacteria colony from POME soil samples

Isolate	Diameter of clear zone (cm)	Diameter colony (cm)	Hydrolisis Index
L8	0,32	0,20	0,60
L11	0,52	0,30	0,73
L12	0,45	0,25	0,80
L14	0,61	0,37	0,65
L15	0,52	0,22	1,36
L21	0,37	0,20	0,85
L31	0,53	0,31	0,71
L34	0,44	0,28	0,57
L49	0,58	0,20	1,90
L69	1,42	0,40	2,55
L87	0,54	0,30	0,80
L92	0,47	0,30	0,57
L114	0,45	0,27	0,67
L115	0,43	0,22	0,95
L127	0,35	0,19	0,84

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