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Screening of Lipolytic Bacteria from Cemetery Soil

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Abstract

Background: The public cemetery is an area for the burial of corpses so that active decomposition of bodies occurs. One of the decomposition bacteria that might be found is lipolytic bacteria because the human body comprises 21,33-32,51 % lipids. Explorations of lipolytic bacteria from cemeteries in Indonesia have not been found, so this research needs to be done. This non-experimental study aimed to select and identify lipolytic bacterial isolates from the Pracimaloyo cemetery, Surakarta. **Method:** A total of 36 bacterial isolates were selected for their lipolytic activity using tributyrin media. The lipolytic index determines lipolytic activity. Identification of lipolytic bacteria based on colonial morphology and Gram staining. **Result:** The study showed that 22.2% (8 isolates) showed a lipolytic positive, with the largest lipolytic index (LI) value of 2.5 (isolate P36). Bacterial colonies are circular-shaped, have entire edges, flat elevation, and are yellow or white. Gram staining results showed that the isolated is a group of Gram-negative bacteria in the form of coccus. **Conclusion:** The conclusion of this study suggests that bacterial isolates from Pracimaloyo cemetery that have potential lipolytic activity, which is likely to be from the genera Pseudomonas and Klebsiella, were obtained.

Keywords: Cemetery; clear zone; lipolytic bacteria; screening; Tributyrin agar

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Introduction

The Public Cemetery (TPU) or Public Cemetery is a land area provided for burying bodies without distinction of religion and class, managed by the Regional Government (Government, 2013). The depth of burial of bodies varies from country to country. In Indonesia, the burial depth is around 1.5 m (Kaysi, 2018); in Nigeria, 0.79-1.32 m (Turajo et al., 2019); in Italy, 1-2 m (Yousef et al., 2022); in South Africa 1.8 m (Dippenaar, 2014), and in Egypt 0.95-3 m (Evans et al., 2015).

The process of decomposing a corpse begins when a person is declared dead. After death, microorganisms in the body will attack the body's tissues, and the decomposition process begins (Upayogi, 2019). Composing a corpse consists of three phases: autolysis, decay, and bone decomposition (Siregar et al., 2022). Fat is the second largest component in the human body after water. The composition of the human body consists of 55.13-67.85% water, 12.51-23.6% fat, and 14.4-18.62% protein (Forbes et al., 1953). In the corpse decomposition process, the microorganisms that are thought to play a role in decomposing fat are mold or lipolytic bacteria (Suyanto et al., 2015).

Lipolytic bacteria are microorganisms that produce lipase enzymes that can degrade fat into fatty acids and glycerol (Ethica, 2018; Widiastuti et al., 2019). Lipase enzymes have received much attention due to their ability to participate widely in hydrolysis, inter-esterification, alcoholysis, acidolysis, esterification, and aminolysis reactions. Apart from that, lipase enzymes also have a role in several industrial applications, namely milk processing, detergents, medical and pharmaceutical industries, fats and oleo-chemicals, food, cosmetics, and perfumes (Lanka & Latha, 2015).

Lipase can be isolated and purified from mold, yeast, bacteria, plants, and animals;

however, economically valuable and more stable lipase comes from bacteria (Bestari & Suharjono, 2015). Research has been conducted on the isolation of lipolytic bacteria, including from mangrove soil samples (Remijawa et al., 2020), palm oil wastewater (Chairunnisa et al., 2020), polluted river water (Dahliaty et al., 2012), and cashew nut shell (Syah, 2022). Research from oil waste has found three lipolytic bacteria, namely Bacillus lichen forms, Bacillus coagulan, and *Pseudomonas diminuta* (Kawuri & Darmayasa, 2022), from the final processing site (TPA) obtained five lipolytic bacteria in the form of Gram-negative streptococcus, diplococcus, coccobacillus, and two of them. Coccus (Tsani, 2021) and Nitrococcus mobilis have a lipolytic index value 0.98 (Rizky et al., 2017).

Research on the isolation of lipolytic bacteria in Indonesia has been carried out with various kinds of samples as previously described, but from the results of searching published articles, samples originating from TPU have not been found, except for research Putra et al., (2023) on rhizosphere bacterial populations of Cambodian plants. at Pracimaloyo TPU, Surakarta. One of the largest TPUs managed by the Surakarta City Government is Pracimaloyo TPU, located on Jalan Slamet Riyadi Number 361 Makamhaji, Kartasura, with an area of 14.5 ha divided into 19 blocks (Zainuddin et al., 2022). Therefore, this research was carried out to select and identify bacterial isolates from TPU Pracimaloyo capable of degrading lipids.

Method

This research was conducted at the Biology Laboratory of Muhammadiyah University, Surakarta, from February to April 2023.

Bacterial Rejuvenation

The TPU Pracimaloyo bacterial isolate was rejuvenated by inoculating it onto a new slanted agar medium with a loop needle and then incubating it for 24 hours before being used for testing. Bacterial rejuvenation aims to obtain active bacterial isolates to optimize bacterial growth.

Selective media creation

The selective media used in this research refers to Oktavia and Wibowo (2017) to detect lipolytic microorganisms, namely tributyrin agar, with modifications (Suyanto et al., 2015). The method for making a selective medium with a volume of 1 L is as follows: weigh 5 g of peptone, 3 g of yeast extract, 10 g of tributyrin, and 20 g of bacteriological agar, then put it in an Erlenmeyer flask containing 1 L of distilled water and homogenize using a magnetic stirrer hotplate. The prepared media was then sterilized using an autoclave for 15 minutes at 121^{o}C and poured into a petri dish.

Screening of lipolytic bacteria and calculation of lipolytic index

Each bacterial isolate aged 24 hours was taken using a loop needle, inoculated onto tributyrin selective media, then incubated at 37^{o}C for seven days. Observations were made every day for seven days to see the formation of a clear zone around the colony. Lipolytic activity is characterized by a clear zone on the tributyrin medium (Dinçer & Kıvanç, 2018). The lipolytic index can be calculated using the following formula (Rizky et al., 2017):

$$\text{Lipolytic Index} = \frac{\text{Diameter of Clear Zone (cm)}}{\text{Diameter of Bacterial Colony (cm)}}$$

Identify bacteria

Identification of bacteria is carried out by observing colony morphology, which includes shape, margins, color, and elevation, as well as Gram staining. Data on bacterial grouping and cell shape were obtained from Gram staining results. The Gram staining procedure follows Cappuccino & Sherman (2014): 1) clean the glass object with 70% alcohol, then drip on sterile distilled water to dilute the bacterial cells and spread them evenly to form a thin layer. Next, fix it by passing it over the fire several times; 2) drip crystal violet dye onto the

preparation, leave for 1 minute, then rinse with running water; 3) drip the preparation with iodine solution and leave for 1 minute; 4) wash the preparation with 95% alcohol solution for 30 seconds; 5) finally, add safranin dye to the preparation for 1 minute, wash with running water and dry. The indicator that a bacterium is said to be Gram-positive is the result of purple-colored cells, while Gram-negative bacteria are marked with red cells. In general, the shape of bacteria is divided into three types, namely round (coccus), rod (bacillus), and spiral (spirilium) (Jamil et al., 2022).

Results

The ability of lipolytic bacteria to hydrolyze fat is determined by calculating the lipolytic index (IL), the results of which can be seen in Table 1.

Table 1. Screening results for the lipolytic ability of burial soil bacterial isolates

Isolate Code	Lipolytic Index (IL) Day to-					
	2	3	4	5	6	7
P8	1,67	1,38	1,24	1,21	1,37	1,38
P31	1,50	1,56	1,64	1.67	1,75	1,92
P36	1,67	2,00	1,88	2,38	2,50	2,44
P14	-	1,24	1,48	1,61	1,68	1,71
P17	-	1,18	1,27	1,36	1,55	1,46
P18	-	1,22	1,44	1,40	1,60	1,82
P29	-	-	1,14	1,25	1,50	1,44
P32	-	-	1,40	1,33	1,38	1,43

Lipolytic-positive bacterial isolates produce clear zones around the colonies, as shown in Figure 1.

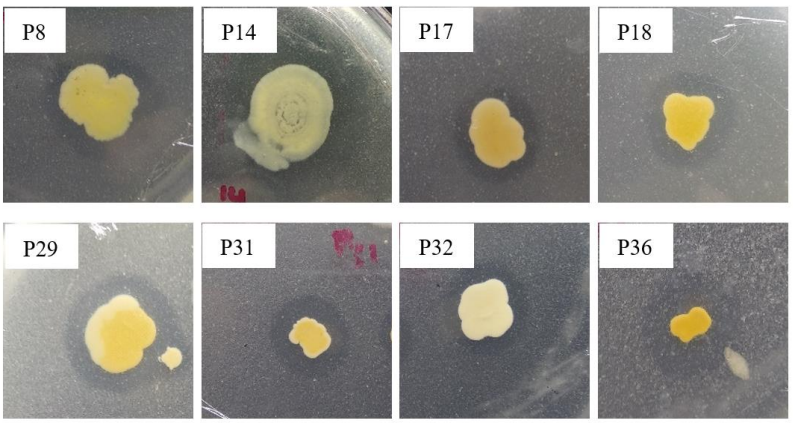

Figure 1. Screening results for the lipolytic ability of burial soil bacterial isolates

Lipolytic-positive bacterial isolates were observed by observing the morphology of bacterial colonies, which was then carried out by Gram staining, and the results can be seen in Table 2.

Table 2. Observation of bacterial colony morphology and Gram staining

Isolate Code	Colony Morphology				Gram Stain	
	Form	Edge	Elevation	Color	Cell Shape	Grams
P8	*Circular*	*Entire*	*Flat*	Yellowish	*Coccus*	Negative
P14	*Irregular*	*Entire*	*Flat*	Yellowish	*Coccus*	Negative
P17	*Circular*	*Entire*	*Flat*	Yellow	*Coccus*	Negative
P18	*Circular*	*Entire*	*Flat*	Yellow	*Coccus*	Negative
P29	*Circular*	*Entire*	*Flat*	Yellowish	*Coccus*	Negative
P31	*Circular*	*Entire*	*Flat*	White	*Coccus*	Negative
P32	*Circular*	*Entire*	*Flat*	White	*Coccus*	Negative
P36	*Circular*	*Entire*	*Flat*	White	*Coccus*	Negative

Lipolytic activity was observed for one week to determine its growth, as shown in Figure 2.

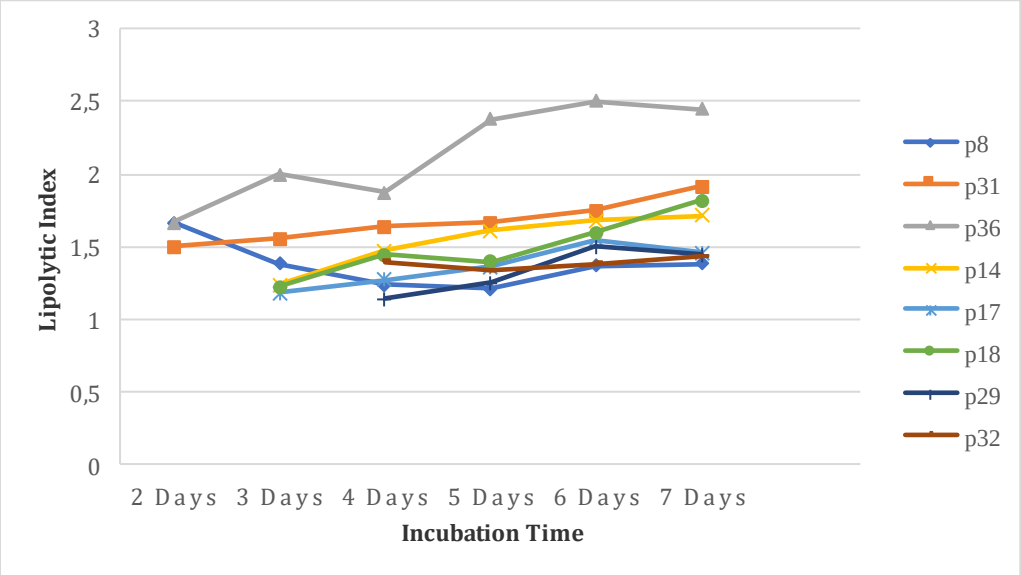

Figure 2. Lipase enzyme activity as measured by the lipolytic index based on incubation time

Discussion

Based on research that has been carried out from 36 collections of TPU Pracimaloyo bacterial isolates, approximately 22.2% (8 isolates) were lipolytic. Table 1 shows that isolate P36 has the highest lipolytic index, namely 2.50, while isolate P32 has the lowest lipolytic index, 1.43. This shows that isolate P36 can degrade fat to a greater extent than other isolates. The lipolytic index value obtained from burial ground is relatively large compared to mangrove soil (1.05) (Remijawa et al., 2020), palm oil liquid waste (0.48) (Khairani & Manalu, 2023), landfill soil (1, 51) (Tsani, 2021), and isolates of Azotobacter spp. (Firdausi and Zulaika, 2015). The larger the clear zone area that is formed, the greater the ability of the bacteria to produce lipase enzymes to degrade lipids.

Tributyrin is one of the most superficial lipids in natural animal and plant fats and oils. Tributyrin agar media is a substrate for detecting lipase production and carbon sources (Suyanto et al., 2015). To support bacterial metabolism, a nitrogen source is needed in the form of peptone (Nasikhin & Shovitri, 2013) and yeast extract (Mazhar et al., 2018), which is then added to the media. Tributyrin is insoluble in water and forms a cloudy culture medium (Yousef et al., 2022). The bacterial lipase enzyme will hydrolyze tributyrin, turning it into water-soluble butyric acid. This hydrolysis will make the media transparent. Therefore, lipolytic bacteria can produce lipase enzymes and hydrolyze tributyrin, thus forming a clear zone around the bacterial colony (Figure 1).

Each bacterial isolate tested showed lipolytic activity at different times. Only three bacterial isolates were lipolytic positive during the two-day incubation, namely P8, P31, and P36. After three days of incubation, there were additional lipolytic-positive bacteria, namely P14, P17, and P18, and on the 4th day, there were additional lipolytic-positive bacteria, namely P29 and P32 (Table 1, Figure 1). This can happen because the ability of each bacterial isolate to produce the lipase enzyme is greatly influenced by the type of microbe and the incubation time related to the microbial growth phase. This is in line with research Sumarlin et al., (2013) that shows that differences in lipase activity are influenced by several factors, namely the presence of substrate and type of microbe. The different types of microbes in each sample will influence the different levels of lipase production and activity.

Lipolytic activity in producing lipase enzymes follows bacterial growth. For example, isolate P36 showed optimal lipolytic activity on day 6, then decreased on day 7 (Figure 2). This can occur due to a reduction in the ability of bacteria to produce the lipase enzyme, thereby causing a reduction in hydrolyzed tributyrin. It can also be caused by the nitrogen

nutritional needs of the bacteria being met or the bacteria starting to lyse. According to Asnawi et al. (2014), the decrease in enzyme activity is also due to the reduced amount of substrate broken down or the enzyme product that is formed, which can inhibit the formation of the substrate enzyme complex.

Table 2. shows that the eight positive isolates for lipolytic had the following morphological characteristics: circular or irregular colony shape, entire edges, flat elevations, and various colors, namely white or yellowish. The Gram staining showed that all isolates positive for lipolytic belonged to the Gram-negative group with coccus cell form. Bacteria with isolate codes P31, P32, and P36 are thought to belong to the genus Klebsiella, while bacteria with codes P8, P14, P17, P18, and P29 are thought to belong to the genus Pseudomonas. Other research has isolated and identified Klebsiella and Pseudomonas bacteria found in liquid petroleum waste (Sayuti & Suratni, 2015) and liquid palm oil waste (Khairani & Manalu, 2023), it is known that Klebsiella and Pseudomonas are a group of lipolytic bacteria that can hydrolyze lipids.

Conclusions

TPU Pracimaloyo obtained bacterial isolates that have potential lipolytic activity in degrading fats, which are thought to belong to the genus Pseudomonas and Klebsiella. The highest lipolytic activity was found in isolate P36, while the lowest was in isolate P32.

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Declaration statement

The authors reported no potential conflict of interest.

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