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SCREENING ACTIVITY OF LIPOLYTIC BACTERIA: BIOFOULING COMPLEX ON SURAMADU CONCRETE BRIDGE SUBSTRATE

Abdus Salam Junaedi*, M. Atho'illah, Friska Rachmawati Tasya, Akhmad Farid
Program Studi S1 Manajemen Sumberdaya Perairan, Jurusan Kelautan dan Perikanan, Fakultas Pertanian, Universitas Trunojoyo Madura
Jl. Raya Telang, Perumahan Telang Indah, Telang, Kecamatan Kamal, Kabupaten Bangkalan, Jawa Timur 69162
Email*:* abdus.salamj@trunojoyo.ac.id

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ABSTRACT

Biofouling is an aquatic biota that lives by attaching to a harmful substrate. Bacteria are one of the factors in the formation of biofouling communities on the substrate. Lipolytic bacteria are microorganisms capable of degrading lipid (fat) compounds. The purpose of this study aimed to determine the characteristics of lipolytic bacteria that were successfully isolated from a sample of the Suramadu concrete bridge substrate (Surabaya-Madura waters). There are 3 sampling stations doing 3 repetitions at each station. The isolation method used was a pour plate using selective Stone Mineral Salt Solution Extract Yeast Agar (SMSSEYA) + 2% bacto agar media . The results showed that the characterization of bacterial colonies in Surabaya-Madura waters obtained 5 types of bacterial colonies namely BL1, BL2, BL3, BL4, and BL5. The lipolytic indexes of bacteria BL2, BL3, and BL4 were respectively 0.51; 1.31; 0.78 mm and types of bacteria BL1 and BL5 with a value of 0 mm each. Bacteria types BL2, BL3, and BL4 are lipolytic bacterial isolates because they are capable of producing extracellular lipase enzymes characterized by the formation of clear zone areas surrounding the bacterial colonies.

Keywords: Suramadu Bridge; Biofouling ; Lipolytic Bacteria; Lipolytic Index

INTRODUCTION

The construction of the Suramadu Bridge which directly connects Madura Island with Surabaya in East Java Province has had positive and negative impacts. Susanto (2011) states that one of the positive impacts of the construction of the Suramadu Bridge is faster access to Madura Island. At the same time the negative impact is reducing public interest in using sea transportation. In addition, based on an ecological review, the bridge's construction impacts the number and diversity of biota forming the biofouling complex (Sonjaya, 2016).

Attached biota or also known as biofouling is the accumulated growth of marine organisms that live and attach to substrates that are submerged by seawater (Syahputra &Almuqaramah, 2019). Biofouling is an aquatic biota that lives by attaching to a substrate that is submerged by water either temporarily or permanently. The existence of Biofouling has a detrimental impact. Sonjaya (2016) reported that the attachment of Biofouling caused damage to the construction of concrete bridge piers and became brittle and corrosion. The condition of the foot of the concrete bridge will be easily brittle due to the attachment of Biofouling due to the impact of the acidic environment around the foot of the bridge which comes from the metabolic process of Biofouling biota. Besides that, attachment to the ship's hull can also lead to wasteful use of fuel .

Embedding of Biofouling can occur in various submerged infrastructures such as bridge piles, harbor piles and other coastal structures. Biofouling can grow and develop quickly on man-made construction substrates that are submerged in seawater, one of which is bridges (Syahputra and Almuqaramah, 2019). Sonjaya (2016) explained that the presence of biofouling on concrete piles or bridge legs around seawater can cause damage to the bridge structure due to corrosion caused by biofouling .

Based on its size, biofouling is divided into microfouling and macrofouling (Syahputra & Almuqaramah, 2019). Mujiyanto & Satria (2011) explained that the process of biofouling can go through several processes, initially forming a biochemical layer on a clean surface. This event was then followed by the attachment of microfouling (group of bacteria and diatoms) and macrofouling in the last stage (group of macroalgae and invertebrates). Thaeraniza *et al.* (2020) explained that microfouling such as groups of bacteria is the initial stage in the biofouling formation because bacteria can form biofilms. Alimah & Ariyanto (2011) explained that microfouling (bacteria) forms biofilms on the surface of the substrate, the greater the number of bacterial colonies, the more extracellular polymeric substances (EPS) will be produced.

The biofilm matrix functions as a protector for bacteria from various environmental influences and is composed of extracellular polymer substances (EPS). EPS production is controlled by gene expression that plays a role in maintaining cohesion between bacterial cells and determining the structure of the biofilm. The biofilm matrix itself consists of three main components with varying proportions, namely extracellular polysaccharides, extracellular nucleic acids, and proteins, all of which have a high air content (Yadav *et al*., 2020).

Microfouling and macrofouling can occur due to physical and chemical factors. The condition of the waters and the nutrients around the waters of the substrate at the foot of the Suramadu concrete bridge make the conditions of the

attached biota diverse. Waste originating from households (domestic waste) also contributes to pollution, including is oil pollution. Ziah & Farid (2020) report that the Kamal area contributes approximately 4.2 tonnes of domestic waste per week originating from the area and also carried by ocean currents. Oil and grease pollution in waters can originate from community domestic waste. In addition, the port in the Kamal area, which is west of the Suramadu concrete bridge, also contributes to water pollution caused by oil spills as ship fuel (Sari *et al* ., 2021). Water conditions like this can allow lipolytic bacteria to grow well, because lipolytic bacteria need a substrate that contains oil and fat to grow correctly (Nurdini 2010).

Lipolytic bacteria are microbes that contain lipase enzymes and are able to degrade oil and grease pollution in the environment. Oil and grease pollution can come from domestic waste and ship fuel oil spills (Chairunnisa *et al.,* 2019). Nurhasanah & Dian (2008) explained that the content of the lipase enzyme present in lipolytic bacteria can assist in the rapid degradation of organic compounds such as oil by breaking the ester bonds of triacylglycerols into fatty acids and the glycerol compounds will dissolve in water. Devi & Jha (2020) reported that *Bacillus licheniformis* isolated from oil refinery waste has lipolytic and proteolytic abilities that have the potential to biotechnologically decompose complex compounds such as fats and proteins. Isolated *Bacillus licheniformis* showed high lipolytic and proteolytic activities under aerobic conditions at a temperature of 40ºC and pH 8 with the best carbon source for lipase production being vegetable oil, while for the best protease production being peptone as a nitrogen source.

Chairunnisa *et al.* (2019) reported that lipolytic bacteria will produce lipase enzymes which will catalyze the hydrolysis of fats into fatty acids and glycerol compounds. Lipolytic bacteria have the ability to degrade lipid compounds up to 25% (Januar *et al.*, 2013). The bacterial strain *Bacillus cereus* 103PB has the ability to degrade pollution originating from the palm oil industry by producing extracellular lipase enzymes that can reduce oil and grease levels originating from industrial waste (Bala *et al.*, 2014).

While macrobiofouling is often more visible and immediately problematic, addressing microfouling and lipolytic bacterial activity provides long-term, cost-effective, and environmentally friendly solutions to biofouling across various industries. Their study enables early intervention, advanced biotechnological applications, and sustainable antifouling innovations. Romeu & Mergullhao (2023) concluded that various strategies have been developed to address the problem of biofouling in the marine environment, including the use of nanotechnology, biomimetic models, and the integration of natural compounds, peptides, bacteriophages, or specific enzymes on surfaces. This approach emphasizes the importance of controlling biofouling-forming microorganisms in preventing more detrimental macro biofouling.

Romeu & Mergullhao (2023) concluded that various strategies have been developed to address the problem of biofouling in the marine environment, including the use of nanotechnology, biomimetic models, and the integration of natural compounds, peptides, bacteriophages, or specific enzymes on surfaces. This approach emphasizes the importance of controlling biofouling-forming microorganisms in preventing more detrimental macrobiofouling. Gizer *et al*. (2023) provide a more in-depth review of the exploration of physical methods in biofouling control, such as surface modification to reduce microbial adhesion. Emphasis is placed on the importance of preventing the formation of microbial biofilms as a first step in avoiding more severe biofouling activity.

Based on the above problems, the research entitled screening and testing the activity of the lipase enzyme lipolytic bacteria forming a biofouling complex on the Suramadu concrete bridge substrate (Surabaya-Madura waters) necessary. Judging from the several studies that have been carried out, they are still focused on the amount and diversity of macro Biofouling attached to the Suramadu concrete bridge substrate. This study aims to determine the results of screening and identification of lipolytic bacteria which are indicated as initial attachment organisms at the foot of the Suramadu concrete bridge (Surabaya-Madura waters).

RESEARCH METHODS

Materials and Tools

The sample used is a sample of macrofouling *substrate scraping* attached to the concrete pillars of the Suramadu Bridge (Surabaya-Madura side). Station 1 is the location around the first pillar, station 2 is the location around the fifth pillar, station 3 is the location around the tenth pillar of the Suramadu Bridge from the Surabaya-Madura side. The distance between the pillars is about 3 meters.

Sampling was carried out at three stations (Surabaya-Madura side) with three sampling points, namely one, two, and three. The materials used in this study included Nutrient Agar (NA) media, methylated spirits, 70% alcohol, distilled water, and 0.90% NaCl solution, selective media for lipolytic bacteria: olive oil 2%, *yeast extract* 0.01%, 0.5 g $CaCO_3$, 0.25 g NH_4NO_3, 0.1 g Na_2HPO_4, 0.05 g KH_2PO_4, 0.05 g $MgSO_4.7H_2O$, 0.02 g $MnCl_2.7H_2O$, rhodamine B 0.01% (dissolved in 200 ml distilled water).

The tools used on study this is micropipette 1000 μL, autoclave, balance analytics, hot plate, oven, Erlenmeyer, volumetric pipette, petri dish, beaker glass, tube reaction, bunsen flame, shelf tube reaction, needle ose, aluminum foil, masking tape, cotton, paper brown, tissue, magnetic stirrer, blue and yellow tip, spatula, porcelain cup, dropper, vortex, disk, tongs, and calipers.

Time and Place of Research

This research activity was carried out in September-November 2021. Sampling was carried out on the Suramadu concrete bridge on the Madura side and Surabaya side (Figure 1.), especially on the substrate samples attached around the diameter of the concrete bridge piers using a 30 x 30 cm squared transect. Observation of research variables was carried out at UPT Basic Chemistry Laboratory, University of Trunojoyo Madura as isolation and identification as well as a place for screening lipolytic bacteria. Study conducted a number of activities as activity isolation, characterization, and screening bacteria. Activity isolation bacteria carried out in the Laboratory Biotechnology Sea, activities characterization

carried out in the Laboratory Resource Waters, and activity screening bacteria conducted on Laboratory Basic.

Bacterial Isolation

Sampling of the barnacle substrate originating from the Suramadu concrete bridge piles was carried out by scraping the substrate using a spatula. Samples originating from three sampling points at one station are then mixed together to form a composite sampleSubstrate samples were taken as much as 10 g and then diluted into 90 mL of 0.9% NaCl solution. Dilution was carried out by taking 1 mL of the main sample solution and then diluting it into 9 mL of 0.9% NaCl solution with a dilution series of 10^{-1}. Dilution was carried out gradually until a 10^{-4} dilution was obtained to reduce the concentration of bacteria to facilitate isolation (Yunita *et al*., 2015). Bacterial isolation was carried out using the pour plate method. The pour plate technique was carried out by taking 1 mL of sample at dilutions of 10^{-2}, 10^{-3}, and 10^{-4}, then inoculated into a petri dish. The next step was to pour 10 mL of NA media into a petri dish and homogenize it by slowly moving the petri dish to form a figure of eight and repeating it 6 times. This aims to ensure that the samples in the petri dish can be mixed evenly and can be grown by single bacterial colonies. The petri dish was then wrapped using plastic wrap and incubated at room temperature for 1 x 24 hours.

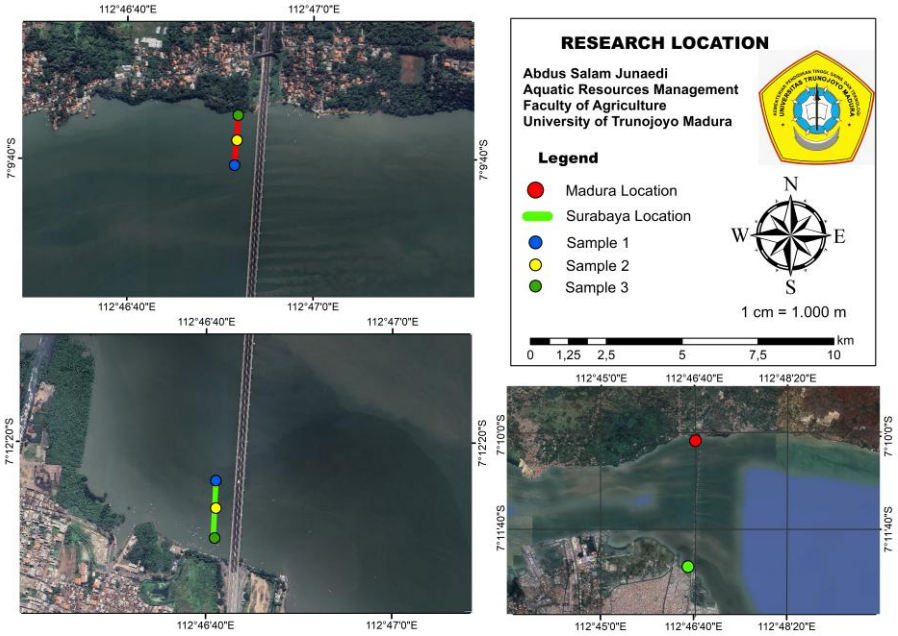

Figure 1. Map of Sampling Locations

Morphological Characterization

The morphological characteristics of bacterial colonies observed as studied by Junaedi *et al*. (2019) such as color, shape, edges, elevation, surface, size, and diameter of bacterial colonies growing on the media based on Bergey's Manual of Determinative Bacteriology, ninth edition (Holt *et al*., 2000).

Lipolytical Activity Screening

Screening lipolytic bacteria that have ability dissolution ingredients organic from lipid groups (lipolytic) are performed with use method point (spot), ie take 1 ose isolate pure bacteria in a manner aseptic then inoculate it to in selective media for lipolytic bacteria: olive oil 2%, *yeast extract* 0.01%, 0.5 g $CaCO_3$, 0.25 g NH_4NO_3, 0.1 g Na_2HPO_4, 0.05 g KH_2PO_4, 0.05 g $MgSO_4.7H_2O$, 0.02 g $MnCl_2.7H_2O$, rhodamine B 0.01% (dissolved in 200 ml distilled water), then sample incubated at 37ºC for 5 x 24 hours (Ghasemi, 2011).

This research was conducted by finding the value of the lipolytic index (IL) of each sample of lipolytic bacteria that is able to grow on selective media with the result that the clear zone is formed and will be calculated by the formula. Nurdin *et al.* (2015) explained that to determine the lipolytic index (IL) can be calculated using the following formula:

$$IL = \frac{\text{Clear Zone Area} - \text{Colony Area}}{\text{Colony Area}} \quad \text{..... (1)}$$

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RESULT AND DISCUSSION

Morphological Characterization

The results of the morphological characterization of bacteria from samples that were able to be isolated showed different results and characters. Parameters for observing the morphological characterization of bacteria used include color, shape, edges, and elevation, and size colony growing bacteria on isolates. This is in accordance with the observations made by Diarti *et al.* (2017), who used morphological observations to characterize bacteria based on color, shape, surface, elevation, consistency, and bacterial edges.

The morphological characterization of the types of bacterial colonies that were successfully isolated from the Suramadu concrete bridge substrate (Surabaya-Madura waters) showed different results. In Surabaya waters, the number of morphological characterization of 3 different types of bacterial colonies was obtained. Madura waters obtained the number of morphological characterization of the types of bacterial colonies for 5 different types of bacterial colonies. The results obtained indicated that the types of bacterial colonies found in Surabaya waters also contained the same types of bacterial colonies in Madura waters. Morphological characterization of the types of bacterial colonies was carried out based on several observations including shape, edges, color, and elevation (Yolantika *et al.* 2015).

The names of the types of lipolytic bacterial colonies (BL) sequentially, namely the types of bacterial colonies 1, 2, 3, 4, and 5 are BL1, BL2, BL3, BL4, and BL5. The results of the morphological characterization of the types of bacterial colonies can be seen in Table 1 and Table 2. Surabaya waters as a result of the morphological characterization of the types of bacterial colonies obtained were 3 types of bacterial colonies. Determination of the morphological characterization of the type of bacterial colony is carried out by several observation factors including color, shape, edge, elevation, surface, size, and diameter. The shape obtained in the BL1 bacterial colony type is white, round shape, irregularly rounded edges, *convex elevation* , smooth surface, small size, and 4 mm in diameter. The shape obtained in the BL2 bacterial colony type was fluorescent white, spherical in shape, irregularly rounded edges, flat elevation, smooth surface, medium size, and 7 mm in diameter. While the shape obtained in the BL3 bacterial colony type is white, irregular shape, curved edges, flat elevation, smooth surface, large size, and 18 mm in diameter. The results of the morphological characterization of the type of bacterial colonies that were successfully isolated from the Suramadu concrete bridge substrate (Surabaya waters) can be seen in Table 1 .

The results of the morphological characterization of the types of bacterial colonies in Madura waters. obtained were 5 types of bacterial colonies. The shape obtained in the BL1 bacterial colony type was white, round shape, irregularly rounded edges, convex elevation, smooth surface, medium size, and 5 mm in diameter. The shape obtained in the BL2 bacterial colony type was fluorescent white, spherical in shape, irregularly rounded edges, flat elevation, smooth surface, small size, and 4 mm in diameter. The shape obtained in the BL3 bacterial colony type was white, irregular in shape, grooved edges, flat elevation, smooth surface, large size, and 14 mm in diameter. The shape obtained in the BL4 bacterial colony type was transparent white, irregular shape, irregular edges, flat elevation, smooth surface, large size, and 15.7 mm in diameter. The shape obtained in the BL5 bacterial colony type was white, irregular shape, irregular edges, convex elevation , smooth surface, large size, and 20 mm in diameter.

This is in accordance with the results of Sabdaningsih *et al*. (2013) showed that the morphological observations of bacterial colonies obtained irregular round shapes, the edges of the bacterial colonies were different (serrated, flat, and wavy), all bacterial isolates had a flat elevation, the colony colors were different (white, cream, and orange). Another study was also conducted by Holderman *et al.* (2017) who showed that the results of observing the morphology of bacterial colonies obtained round shapes, the edges of the bacterial colonies varied (regularly rounded and jagged), the colors of the bacterial colonies varied (white, yellow, and red), and the elevation of the bacterial colon grew on the surface. Characterization of the types of bacterial colonies needs to be observed with the aim of facilitating the identification of bacteria, because the nature of bacterial colonies can determine the type of bacteria. The results of the morphological characterization of the type of bacterial colonies that were successfully isolated from the Suramadu concrete bridge substrate (Madura waters) can be seen in Table 2.

Table 1. Characterization of The Types of Bacteria that Were Successfully Isolated from The Suramadu Concrete Bridge Substrate (Surabaya Waters).

Macroscopic characteristics	Types of bacterial colonies on the Suramadu concrete bridge substrate sample (Surabaya waters)		
	BL1	BL2	BL3
Color	White	Fluorescent white	White
Shape	Round	Round	Irregular
Edge	Regular round	Regular round	Notched
Elevation	*Convex*	Flat	Flat
Surface	Fine	Fine	Fine
Size	Small	Currently	Big
Diameter	4 mm	7 mm	18 mm

Note: BL = lyoplitic bacteria; 1-3: lipolytic bacteria type code

Table 2. Characterize The Types of Bacteria That Have Been Isolated from the Suramadu Concrete Bridge Substrate (Madura Waters).

Macroscopic characteristics	Types of bacterial colonies on the Suramadu concrete bridge substrate sample (Madura waters)				
	BL1	BL2	BL3	BL4	BL5
Color	White	Fluorescent white	White	Transparent white	White
Shape	Round	Round	Irregular	Irregular	Irregular
Edge	Regular round	Regular round	Notched	Irregular	Irregular
Elevation	*Convex*	Flat	Flat	Flat	*Convex*
Surface	Fine	Fine	Fine	Fine	Fine
Size	Currently	Small	Big	Big	Big
Diameter	5 mm	4 mm	14 mm	15.7 mm	20 mm

Note: BL = lyoplitic bacteria; 1-5: lipolytic bacteria type code

The variation of microbial communities found in the waters of Surabaya and Madura, based on the observed morphological characteristics, can be influenced by several environmental factors, such as salinity (Mufida, 2019), water temperature (Rahmawati & Arisandi, 2020), and the acidity or pH level of the water (Wahyuni, 2017). According to Mufida (2019), the difference in salt content between the two waters has an impact on the abundance and types of bacteria that grow, which ultimately forms differences in the microbial communities found in each location. Furthermore, research conducted by Rahmawati & Aprisandi (2020) revealed that variations in water temperature affect bacterial metabolism and activity. The difference in temperature in the two waters can have an impact on the structure of the bacterial community, where the sea surface temperature that changes spatially and temporally is influenced by various complex factors, such as the intensity of sunlight and the dynamics of mixing water masses due to currents. In addition, Wahyuni (2017) also added that the acidity or alkalinity of water plays a role in determining the survival of various types of bacteria. Therefore, the difference in pH between Surabaya and Madura waters can result in variations in the bacterial communities that develop in each region.

The differences in bacterial communities in the waters of Surabaya and Madura can be influenced by the varying levels of pollution in each region. Water pollution from domestic waste, industry, and various other activities plays a role in determining the composition and number of bacteria that develop in the aquatic ecosystem. Badriani (2016) reported that Surabaya waters tend to receive a higher pollution load than Madura waters. This is because Surabaya is a city with intensive domestic waste sources that pollute the waters of Surabaya and is a metropolitan city that is densely populated and filled with various industrial activities. Domestic waste contributes greatly to pollution in Surabaya waters. However, an interesting fact found in the Surabaya waters in this study is that the variation of bacterial types found was not as abundant as that found in the Madura waters. Al Fatah (2018) reported that Madura waters, although less industrialized, still face the challenge of pollution due to local activities such as ship cutting and rapid socio-economic development, which contributes to heavy metal pollution such as cadmium (Cd), copper (Cu), and zinc (Zn). Lailiyah *et al*. (2022) added that heavy metal pollution can affect the structure of bacterial communities, by encouraging the dominance of species that have tolerance or bioremediation capabilities to these metals. Therefore, it can be concluded that increasing levels of heavy metals in Madura waters have the potential to cause changes in the composition of bacterial communities, with an increase in the proportion of bacteria that are able to survive or metabolize these heavy metals.

Lipolitical Activity Screening

The results showed different results from a total of 5 types of bacterial colonies that were able to grow on selective media totaling 5 types of bacterial colonies. Meanwhile, there were only 3 types of bacteria that were able to grow in clear zones and there were 2 types of bacterial colonies that were unable to grow in clear zones on selective lipolytic media. Measurement of the lipolytic index was carried out by means of two repetitions (duplo) by naming lipolytic bacteria (BL) and duplo lipolytic bacteria (BLD). The lipolytic index calculation value can be seen in Table 3.

Based on Figure 2, it can be seen that the value of the calculation of the lipolytic index for each type of bacterial colony is different. All types of bacteria that have been isolated from the Suramadu concrete bridge substrate (Surabaya-Madura waters) can grow on selective media, but not all types of bacteria are able to produce clear zones on selective media. There are 3 types of bacteria that can produce clear zones, namely BL2, BL3, and BL4 bacteria. Types of bacteria that cannot produce clear zones on selective media are BL1 and BL5 bacteria.

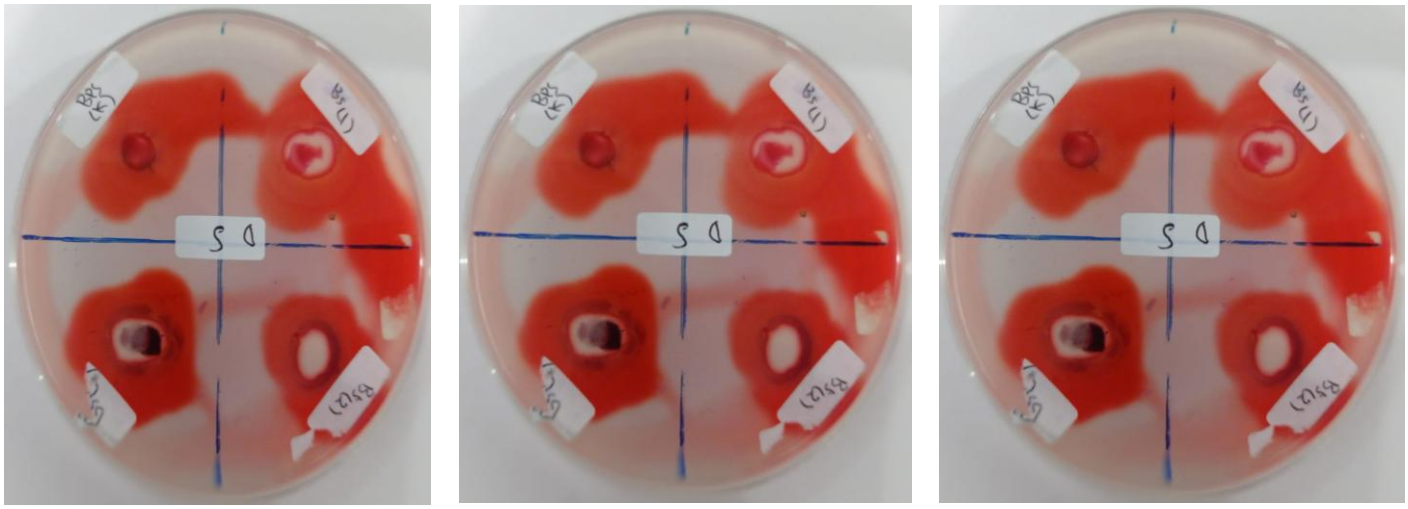

Figure 2. Results of The Characterization of The Type of Bacterial Colonies Isolated from The Suramadu Concrete Bridge Substrate (Surabaya-Madura Waters)

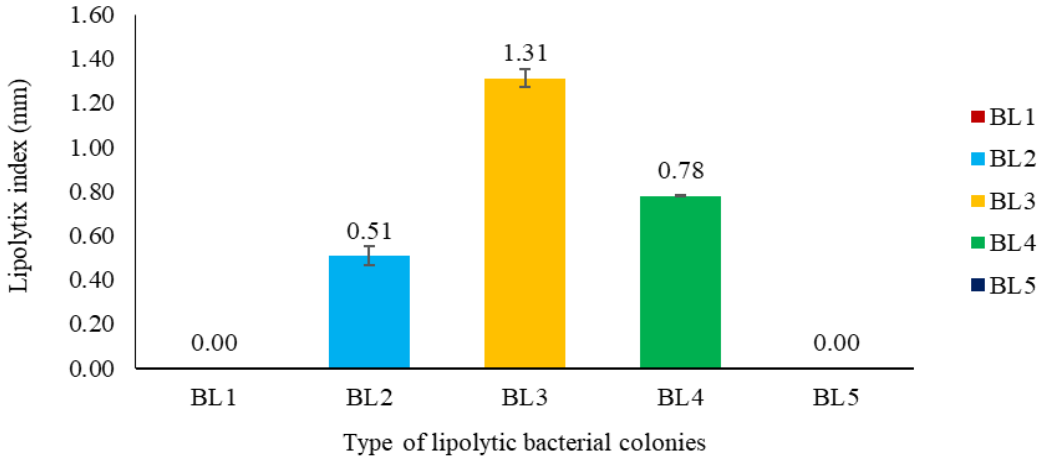

Figure 3. The Results of The Calculation of The Lipolytic Index

Table 3. Measurement of The Lipolytic Index of Bacterial Colonies on The Suramadu Concrete Bridge Substrate (Surabaya-Madura Waters).

No	Sample	Test	Colony diameter(mm)	Clear zone diameter (mm)
1	BL1	1	9.00	0.00
		2	10.98	0.00
		3	12.32	0.00
		Control	12.18	13.32
2	BL1 D	1	12.98	0.00
		2	11.50	0.00
		3	12.00	0.00
		Control	17.18	18.72
3	BL2	1	6.98	11.28
		2	7.00	0.00
		3	7.32	0.00
		Control	13.00	16.00
4	BL2 D	1	7.32	0.00
		2	8.08	0.00
		3	7.00	10.00
		Control	14.00	16.18
5	BL3	1	8.00	11.00
		2	6.98	8.72
		3	8.18	10.00
		Control	13.00	14.18
6	BL3 D	1	9.08	12.50
		2	7.72	10.98
		3	8.32	10.18
		Control	14.72	15.00
7	BL4	1	7.08	0.00
		2	7.00	9.00
		3	10.50	10.98
		Control	8.32	10.28
8	BL4 D	1	9.00	10.00
		2	6.72	8.32
		3	10.98	0.00
		Control	10.00	12.98
9	BL5	1	18.00	0.00
		2	14.00	0.00
		3	13.72	0.00
		Control	14.18	16.32
10	BL5 D	1	13.00	0.00
		2	11.00	0.00
		3	12.98	0.00
		Control	12.00	14.98

Calculation of the dissolving activity of organic matter (fatty compounds) on selective media showed different results. The results of the screening calculation for the types of bacteria BL2, BL3, and BL4 were respectively 0.51; 1.31; 0.78 mm. The highest calculation results were obtained for the type of BL3 bacteria of 1.31 mm. The lowest calculated values were obtained for the types of bacteria BL1 and BL5 with a value of 0 mm each. The types of bacteria BL2, BL3, and BL4 are types of lipolytic bacterial isolates because they are able to produce extracellular lipase enzymes which are characterized by the formation of clear zone areas surrounding the bacterial colonies.

Dewi (2023) emphasized that lipolytic bacteria use fat or oil as the main specific carbon source. If the growth medium does not provide a suitable carbon source, such as olive oil or other lipid substrates, then bacterial growth can be inhibited. It is possible that lipolytic bacteria types 1 and 5 (BL1) and (BL5) are still unable to utilize the olive oil provided in the growth medium as a carbon source properly. Hutagalung (2009) reported that bacteria that can live in palm oil industry liquid waste are bacteria that are tolerant to oil and bacteria that utilize carbon sources from the oil. This is emphasized by Bala *et al*. (2014) who stated that the ability of microorganisms to degrade depends on their ability to adapt to the environment.

Simamora & Sukmawati (2020) explained that lipolytic bacterial isolates that have the best ability to produce extracellular lipase enzymes are lipolytic bacterial isolates that are able to grow well in isolation media containing lipids. Research conducted by Ervina *et al.* (2020) with a sample of *Bacillus* bacteria with code T2, the results of the highest lipolytic index were 6.01. Another study was also conducted by Oktavia & Wibowo (2016) who obtained the result that the highest lipolytic index value was obtained for a type of bacterial isolate with an SKn code of 2.6 mm using a sample in the form of surimi processing wastewater. The lowest lipolytic index value was found in bacterial isolates with RGB, RGBt, RGKnt, and RGBP codes with each value of 1.1 mm, the sample used was crab meat canning wastewater. The parameter that can be used to determine the type of lipolytic bacteria is the formation of clear zones on the tributyrin agar media which can be observed directly with the naked eye.

The results of this study (BL3) compared to the IL values from the two previous studies did show a lower lipolytic index value. Therefore, the area of the clear zone produced in this study (BL3) was not as wide as the area of the clear zone produced in the two previous studies. This may be influenced by the differences in the types of lipolytic bacteria used. In addition, it is also possible that there are differences in the ability to express lipase activity derived from the coding genes owned by the lipolytic bacteria. Sumarlin *et al*. (2013) explained that differences in lipase activity produced by microbes can be influenced by several factors, one of which is different microbial strains that can affect differences in production levels and various lipase activities. However, the findings of this study provide an alternative type of lipolytic bacteria originating from the local area around Suramadu (Suramadu-Madura).

Lipolytic bacteria type 3 (BL3) which showed the best lipolytic activity in this study can then be retested for its ability in the food industry (such as fermented milk products) as reported by Cano *et al*. (2019) that several strains of lactic acid bacteria isolated from several dairy products showed significant enzymatic activity so that they could increase functionality in food applications. In addition, the application of lipolytic bacteria type 3 (BL3) can also be tested for its application in the manufacturing industry (detergent products that can remove oil stains) as reported by Kang *et al*. (2024) that pure lipase enzyme derived from *Lacticaseibacillus rhamnosus* IDCC 3201 has the potential to be used as a detergent in industrial applications because it can maintain more than 80% of its lipolytic activity and stains from the tested cotton were successfully removed with simulated ventilation settings.

The next application that can be applied to lipolytic bacteria type 3 (BL3) is in the pharmaceutical industry (antibiotic production) as reported by Es *et al*. (2007) related to lipase enzyme extracts derived from *Lactobacillus delbruckii* subsp *lactis* bacteria isolated from freshwater fish processing waste showed antagonistic properties against several pathogens including *Listeria monocytogenes*, *Bacillus cereus*, *Yersinia enterocolytica*, and *Escherichia coli*. The oil waste processing industry has also been of interest in recent years. Lipolytic bacteria type 3 (BL3) are also expected to be able to decompose oil waste components in the waste processing activity process. Lee *et al*. (2015) stated that bacterial species isolated from oil spill areas in conventional night markets contain lipase enzymes that can provide industrial-based applications and solve environmental problems polluted by oil waste. Biodiesel production from lipolytic bacterial activity has also been interesting to study in recent years. The application of lipolytic bacteria type 3 (BL3) found in this study is also expected to be applied in biodiesel production activities. Chow *et al*. (2021) reported that of the 114 lipase enzymes tested, 22 of them were able to produce methyl oleate from triolein in the presence of methanol, indicating the potential of bacterial lipase enzymes in producing biodiesel.

CONCLUSION

Bacterial characterization In Surabaya waters, the types of bacterial colonies BL1, BL 2, and BL3 were obtained. Meanwhile, in Madura waters, the types of bacterial colonies BL1, BL2, BL3, BL4, and BL5 were obtained. Characterization observations include white color, round shape, edges (regular and notched), elevation (flat and *covex*), smooth surface, size (small, medium, and large) and different diameters . Calculation of the lipolytic index of BL2, BL3, and BL4 bacteria respectively was 0.51; 1.31; 0.78mm. The highest calculation was obtained for the type of BL3 bacteria of 1.31 mm. While the lowest calculated values were obtained for the types of bacteria BL1 and BL5 with a value of 0 mm each. The types of bacteria BL2, BL3, and BL4 are types of lipolytic bacterial isolates because they are able to produce extracellular lipase enzymes which are characterized by the formation of clear zone areas surrounding the bacterial colonies.

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