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Lipase activity and identification of lipolytic bacteria from leather industrial liquid waste in Magetan Small Industrial Area, Indonesia

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Abstract. *Mehola JA, Setyaningsih R, Susilowati A. 2024. Lipase activity and identification of lipolytic bacteria from leather industrial liquid waste in Magetan Small Industrial Area, Indonesia. Asian J Trop Biotechnol 21: 61-67.* Lipolytic bacteria can produce the lipase enzyme, potentially a lipid biodegradation agent. The bacteria can be found in places that contain lots of fat, one of which is the leather industry wastewater. This research aimed to obtain lipolytic bacteria that have the potential as biodegradation agents and observe the lipase enzyme activity from small industrial lather wastewater in Magetan, Indonesia. This research was conducted in several steps, including isolation, screening, lipase enzyme activity assay, and identification of lipolytic bacteria. Isolation of lipolytic bacteria using minimal media enriched with olive oil. The screening of lipolytic bacteria was carried out using Rhodamine B agar, a method that has proven effective in such studies. The enzyme activity was determined using a spectrophotometric method, with p-nitrophenyl palmitate (p-NPP) as the substrate at $\lambda = 410$ nm. The lipolytic bacteria that exhibited high enzyme activity in each treatment were identified based on the 16S rRNA gene encoding sequence using the Basic Local Alignment Search Tool (BLAST) on the NCBI website https://www.ncbi.nlm.nih.gov/. The research showed significant findings; four isolates of lipolytic bacteria were discovered, each with unique characteristics. The highest lipase activity was observed in the form of crude enzyme. Further precipitation with ammonium sulfate revealed that isolate Q exhibited the highest enzyme activity among the other isolates in the 30-45% fraction. The 16S rRNA gene sequence analysis indicated that isolate Q shared a 100% similarity with *Stenotrophomonas maltophilia*.

Keywords: Identification, leather industrial wastewater, lipase, lipid biodegradation, lipolytic bacteria

INTRODUCTION

Magetan District, East Java, Indonesia is famous for its leather craft products. The leather tanning industry is in the Magetan Small Industrial Area (SIA). At Technical Implementation Unit SIA Magetan, there are 35 tannery industries, all of which depend on the Same Wastewater Treatment Plant (WWTP). The leather industry uses large amounts of chemicals and water. The leather industry's operational process produces liquid, solid, and gas wastes; the three wastes, liquid waste, are the most generated. The tanning industry grew rapidly; however, the Magetan leather industry activities' negative impact is the Gandong River's environmental pollution (Perdana 2018). The leather tanning industry can potentially cause physical and chemical environmental changes. Environmental pollution from this type of waste can be identified quickly from changes in odor, color, clarity, and taste. This pollution ultimately threatens the living things and the environment around the industrial area (Azwar 1996). The remaining fat will cover the water's surface and prevent light from entering the water, inhibiting biological processes. One effort that can be made to overcome waste problems is with environmentally sound wastewater treatment technology through a biodegradation process by bacteria.

Furthermore, to obtain lipid biodegrading bacteria naturally, this can be done by isolating them from the waste (Fidiastuti and Suarsini 2017). Bacteria that can degrade oil and fat can produce lipase enzymes. Based on its physiological function, lipase plays an essential role in the hydrolysis of fats and oils into fatty acids and glycerol, which are needed in metabolic processes (Gupta et al. 2011). Lipase (Triacylglycerol acylhydrolase EC 3.1.1.3) is a hydrolase class enzyme that catalyzes the hydrolysis reaction of triglycerides into glycerol and free fatty acids on the surface of water and oil. Bacteria-producing extracellular lipase is very important for commercial purposes because mass production is relatively easy (Treichel et al. 2010). Lipolytic bacteria that produce lipase enzymes are necessary to degrade fats in wastewater. Lipolytic bacteria can eliminate the pre-treatment sewage process or at least reduce the burden on the pre-treatment process in the waste processing process (Oktavia and Wibowo 2016). Wardani et al. (2015) studied the isolation of the diversity of indigenous bacteria in Malang's leather tanning industry's wastewater treatment plant. The indigenous bacteria found in the WWTP were proven to utilize pollutants in the waste. Because they can use pollutants, these bacteria have the potential to act as biodegradation agents. However, the identification of lipolytic bacteria and lipase enzyme activity of lipolytic bacteria from leather industry waste has not yet been carried out. Several types of lipolytic bacteria include *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Chromobacterium*, and *Pseudomonas* (Gupta et al. 2004).

The promising potential of the lipolytic bacteria identified in this research is a milestone for leather industry waste management, which is necessary in the metabolic process. These bacteria, capable of thriving in waste conditions, can be introduced to the activated sludge tank at WWTP Magetan SIA. Their production of lipase enzymes can significantly enhance the waste processing process, particularly in reducing lipid levels in leather industry waste. The significance of this research lies in its potential to isolate and identify lipolytic bacteria from Magetan SIA liquid waste. By the 16S rRNA coding gene sequence, we can determine the specific species of lipolytic bacteria and test the activity of the lipase enzyme that it produces. These findings were innovations in the leather industry's waste management, offering a sustainable solution to environmental challenges.

MATERIALS AND METHODS

Material

The bacteria source was meticulously selected from the liquid waste of the WWTP Magetan SIA. The carefully prepared media used in this research consisted of three types: Nutrient Agar, minimal media with olive oil, and Rhodamine B agar. Morphological observations, conducted with utmost precision, used materials such as 0.85% NaCl, crystal violet, iodine, acetone alcohol, safranin, distilled water, and immersion oil.

Media for lipase production consisting of 0.5% (w/v) peptone, 0.3% (w/v) yeast extract, 0.625% (v/v) NaCl, 0.01% (v/v) $MgSO_4•7H_2O$, 0.01% (v/v) $CaCl_2•2H_2O$ and 1% (v/v) olive oil. Materials for enzyme activity testing are ammonium sulfate ($(NH_4)2SO_4$), 4-Nitrophenol, distilled water, p-nitrophenyl palmitate (p-NPP), isopropanol, Gum Arabic, Triton X-100, and 50 mM Tris-HCl buffer pH 8.0. The molecular identification stage of lipolytic bacteria uses the FavorPrepTM Tissue Genomic DNA Extraction Mini Kit, DNA marker, loading dye, TAE buffer (Tris base, Acetic acid, and EDTA), DNA polymerase, agarose, PCR mix, forward primer 63f (5'-CAG GCC TAA CAC ATG CAA GTC-3'), reverse primer 1387r (5'-GGG CGG WGT GTA CAA GGC-3'), GelRed, and nuclease-free water.

Instruments used

Waste collection: Measurement cups, Duran bottles, pH meters, and thermometers are the tools used for sampling liquid waste from the leather industry.

Isolation and screening of lipolytic bacteria: The tools used at this stage, including the versatile Biology Safety Cabinet (BSC), Petri dishes, test tubes, beakers, Erlenmeyer, measuring cups, Duran bottles, spatulas, stirring rods, Drigalsky rods, inoculation needles, vortexes, analytical balances, Bunsen burners, UV lamp, micro-pipette (P2, P20, P200, P1000), and incubator, are designed to maximize efficiency and accuracy in our procedures.

Lipase enzyme activity test: The tools used include an incubator shaker, photometer, spectrophotometer, centrifuge, Eppendorf tube, analytical balance, cuvette, Erlenmeyer, ice pack, magnetic stirrer, and freezer.

Identification of lipolytic bacteria: This stage uses a stereo microscope, binocular microscope, glass slide, cover glass, spin column, micropipette, PCR tube, gel electrophoresis, gel documentation, incubator, microcentrifuge, thermal cycler, chiller, and freezer.

Procedures

Sample preparation

Waste is collected using the grab sampling method, and one bag of waste is collected directly from the homogenization tank at a time. Temperature and pH parameters were measured.

Isolation and screening of lipolytic bacteria

A total of 1 mL of waste sample was dissolved in 9 mL of saline solution (0.85% NaCl), then homogenized until mixed, and a 10-1 dilution was obtained. Dilution was carried out in a 10-5 dilution series. Microorganisms were grown (plated) on nutrient agar media using the spread plate method by adding 0.1 mL of microorganism source to each Petri dish and then leveling using a Drigalsky. Isolates were incubated for 24-48 hours at 37°C (Susanty et al. 2013). Each colony that grows and has a different morphology is then streaked back onto a minimal agar medium enriched with olive oil using the streak plate method. Isolates were incubated for 48 hours at 37°C. According to Kumar et al. (2012), screening was done by taking one isolate and then streaking it on Rhodamine B agar media enriched with olive oil. The bacterial isolate was then incubated at 37°C for 48 hours. Positive results can be seen by the hydrolysis of olive oil into fatty acids, which then interact with Rhodamine B so that pink or orange colonies appear, which fluoresce when illuminated using a UV lamp with a wavelength of 365 nm.

Morphological observations of lipolytic bacteria

Pure colonies grown on Nutrient Agar media were observed using a stereo microscope. Macroscopic morphological observations are carried out by looking at bacterial colonies' shape, edges, elevation, and color at appropriate magnification. The gram staining method was used for these observations. The preparations were observed from the smallest to the largest magnification under a microscope. Gram-positive bacteria will be blue or purple, and negative bacteria will be red (Yulvizar 2013).

Lipase enzyme activity test

According to Saadatullah et al. (2018), there are several stages in measuring lipase enzyme activity, namely:

Creating of lipolytic bacterial growth curves: One mL of bacterial culture in an enzyme production medium was put into the cuvet. The absorbance value was measured using a spectrophotometer with a wavelength of 600 nm. Absorbance was measured every three hours during 48 hours of observation to obtain a growth curve for lipolytic bacteria (Oktavia and Wibowo 2016).

Lipase enzyme production: The media composition for lipase production is 0.5% peptone, 0.3% yeast extract, 0.625% NaCl, 0.01% $MgSO_4•7H_2O$, 0.01% $CaCl_2$, $2H_2O$, and 1% olive oil at pH 7. A total of 5 mL Overnight culture

in NB media was added to 50 mL of lipase production media and incubated in an incubator shaker at a speed of 120 rpm and a temperature of 37°C. After reaching the optimal incubation time, the culture was centrifuged at 4,500 rpm for 20 minutes at 4°C, and the supernatant was used as a source of extracellular lipase enzyme (crude enzyme).

Precipitation of lipase enzyme with ammonium sulfate: Duong-Ly and Gabelli (2014) stated that the lipase enzyme was precipitated using the enzyme precipitation method by adding ammonium sulfate $(NH_4)2SO_4$. The crude extract of the lipase enzyme was precipitated in stages using ammonium sulfate with precipitation fractions of 0-30%, 30%-45%, and 45%-60%. For 0-30% precipitation, 2.46 g of ammonium sulfate was added to 15 mL of crude lipase extract to obtain supernatant I and pellet I. For 30-45% precipitation, 1.118 g of ammonium sulfate was added to supernatant I to obtain supernatant II and pellet. II. Then, for 45-60% precipitation, 1.125 g of ammonium sulfate was added to supernatant II to obtain supernatant III and pellet III. Ammonium sulfate was added slowly and then homogenized using a magnetic stirrer. The mixture was left overnight in a 4°C freezer. The protein precipitate was separated by centrifugation at 4,500 rpm at a temperature of 4°C for 20 minutes. Each pellet in each fraction was resuspended in 1 mL 50 mM Tris HCl buffer pH 8 and used for enzyme activity assays. Lipase activity was measured using p-nitrophenyl palmitate (p-NPP) as a substrate. Substrate preparation using solution A (30 mg p-NPP dissolved in 10 mL isopropanol) was added to 90 mL solution B (0.1-gram Gum Arabic and 0.4 mL Triton X-100 dissolved in 50 mM Tris-HCl buffer pH 8.0), all ingredients were stirred until wholly dissolved with a final volume of 100 mL (Ertuğrul et al. 2007). Enzyme activity measurements were performed by mixing 1.8 mL of substrate solution and 0.2 mL of crude enzyme or pellet resulting from precipitation and incubating at 30°C for 15 minutes. Absorbance was measured using a UV-Vis spectrophotometer with a wavelength of λ = 410 nm.

Identification of lipolytic bacteria

Lipolytic bacteria with the most stable lipase enzyme activity will be identified molecularly. Lipolytic bacterial genomic DNA extracted from the screening process was extracted using the FavorPrepTM Tissue Genomic DNA Extraction Mini Kit. Amplification of the 16S rRNA gene encoding lipolytic bacteria using primers, namely 63 forward primers (63f: 5'CAGGCCTAACA CATGCAAGTC-3') and 1387 reverse primer (1387r: 5'-GGGCGGAWGTGTACAAGGC-3') (Marchesi et al. 1998). The PCR method was carried out by mixing 1 µL 63 forward primer with a concentration of 10 pmol, 1 µL 1387 reverse primer with a concentration of 10 pmol, 25 µL MyTaqTM HS Red Mix 2x, and 2 µL DNA template, and 21 µL ddH2O. Pre-denaturation was carried out at 95^0C for 1 minute. One PCR cycle of 35 cycles consists of denaturation at 95°C for 15 seconds, annealing at 55°C for 15 seconds, elongation at 72°C for 15 seconds, and finalizing at 72°C for 7 minutes. Next, PCR was stopped, and the PCR amplification product was stored at 4°C. PCR amplification products were observed using gel electrophoresis. Sequencing of PCR amplification results of the 16S rRNA gene encoding lipolytic bacteria was carried out at 1st Base Apical Scientific Malaysia. The 16S rRNA sequence obtained was analyzed using the Nucleotide BLAST device on the NCBI website (www.blast.ncbi.nlm.nih.gov/blast.cgi) (Waturangi et al. 2008).

Data analysis

The bacterial isolates and the lipase enzyme activity data for each bacterium are explained descriptively. The 16S rRNA sequences obtained were analyzed using the BLAST Nucleotide tool on the NCBI website. The higher the identity percentage, the higher the similarity of the sequence being searched for with the reference sequence in Gene Bank (Hall 2001). The criteria for species identification are a minimum of 99% sequence similarity, and the ideal percentage is ≥99.5% (Janda and Abbot 2007).

RESULTS AND DISCUSSION

Waste characteristics

The liquid waste taken is from the homogenization tank at the Wastewater Treatment Plant Magetan SIA. Waste has a pH of 9 with a waste temperature of ± 30°C. The liquid waste looks cloudy brown; there is a layer of fat on the surface of the wastewater; there is still hair, skin, and animal flesh remaining, and it also observed fat foam clumping in several corners of the homogenization tank (Figure 1). The remaining fat in the waste will appear on the water's surface. This layer of fat can prevent sunlight from entering the water and the environment becomes aerobic and inhibits water biological processes.

Lipolytic bacteria isolate

The process of isolating the lipolytic bacteria was conducted with scientific rigor. In the initial stage, 44 bacterial isolates were obtained from the 10^{-5} serial dilution and then inoculated onto minimal agar media. This meticulous process yielded 22 bacterial isolates that demonstrated growth. Each isolate was carefully labeled, resulting in the isolates' identification A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, and V. Olive oil was selected as the substrate for the minimal medium due to its easy availability and digestibility by bacteria (Susilowati et al. 2018).

Four positive lipolytic bacterial isolates were isolate D, isolate H, isolate M, and isolate Q. Positive lipolytic activity was characterized by the appearance of isolates that glowed brightly and were orange or pink when exposed to UV light with a wavelength of 350 nm (Figure 2). Rhodamine B Agar was chosen because it is a sensitive fluorescent indicator for free fatty acids. Olive oil was used as a lipase substrate, and rhodamine B indicated lipase activity. The lipase enzyme is different from other hydrolytic enzymes because it can work on interfacial substrates. Lipase works actively on substrates that cannot be dissolved in water, such as triglycerides, composed of long-chain fatty acids. The interaction of rhodamine B with fatty acids released during triglyceride hydrolysis causes fluorescence around the colony under ultraviolet radiation (Rahayu 2018).

Table 1. Macroscopic and microscopic morphological characteristics of lipolytic bacterial isolates

Isolate name	Colony morphology				Cell morphology	
	Shape	Color	Elevation	Edges	Grams	Cell shape
D	Circular	Purple	Umbonate	Scalloped	+	Bacillus
H	Irregular	White	Flat	Undulate	+	Coccus
M	Circular	Purple	Raised	Entire	+	Coccus
Q	Punctiform	Cream	Convex	Entire	-	Bacillus

Figure 1. Wastewater treatment plant in a small industrial area in Magetan, East Java, Indonesia. A. Homogenization/equalization tank; B. Homogenization tank wastewater

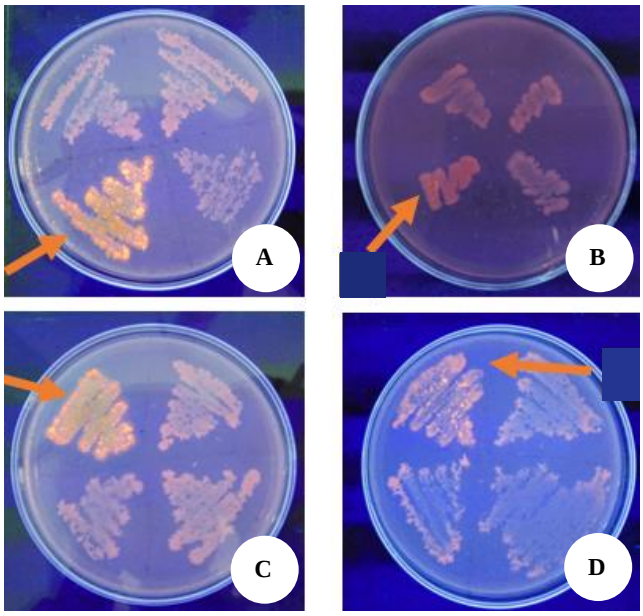

Figure 2. Lipolytic bacterial colonies on Rhodamine B Agar Media under a 350 nm UV lamp exposure. A. Isolate D; B. Isolate H; C. Isolate M; D. Isolate Q

According to Kasipah et al. (2013), the hydrolysis reaction of olive oil by lipase produces free fatty acids. The complex bond between rhodamine and free fatty acids creates a pink glow when irradiated under UV light. According to Sugiharni (2010), one of the reasons why non-lipolytic isolates can grow on rhodamine B agar media is the possibility that these bacteria produce esterase. An orange glow will not form when esterase is detected in the rhodamine B medium.

The morphological characteristics of the four bacterial isolates with lipolytic activity were observed for colony and cell morphology. Colony characteristics include shape, color, elevation, and edges; the cell characters include gram characteristics and shape. The macroscopic form of each isolate was very diverse (Table 1).

Observing the morphological characteristics of bacterial colonies makes identifying the type of bacteria easier. However, further tests, namely molecular identification, need to be carried out to obtain accurate identification.

Sigmoid growth curve of lipolytic bacteria

Our research has led us to optimize the bacterial growth time, allowing us to determine the precise time for lipase enzyme production. The data obtained from this optimization process has revealed that each bacteria has a unique optimal growth time, as demonstrated in the lipolytic bacterial growth curve. The peak of bacterial growth, or the exponential phase, has been identified as an excellent time to produce enzymes due to bacteria's rapid binary division phase, leading to an abundance of bacterial cells. The turbidity level in the enzyme production medium increases with the incubation period. The turbidity of the medium during the growth of lipolytic bacteria indicates cell density during development, which increases the OD absorbance value (Mira et al. 2022). Bacteria can grow until the nutrients available in the medium have been used up.

The sigmoid growth curve (Figure 3) depicts the increase in lipolytic bacterial cells starting from the

adaptation phase (lag) and rapid growth in the logarithmic (exponential), stationary, and cell death phases.

The time required to divide from one cell into two perfect cells is called generation time, and the ability of each bacterium to reproduce varies from one bacteria to another. Isolate Q was the isolate that reached the fastest exponential peak at 12 hours. Even though they are under the same treatment conditions, differences in generation time occur due to genetic factors in each bacterium. Genetics is an intrinsic factor in a microorganism that is not changed (Sariadji et al. 2015). Lipolytic activity is part of the primary bacterial metabolite activity following the microbial growth phase. It increases optimally at the end of the log (exponential) phase or the beginning of the stationary phase, then decreases with microbial activity and decreasing substrate nutrients (Bestari and Suharjono 2015).

Lipase enzyme activity

Lipase enzyme activity was measured when each isolate peaked its exponential phase grown in enzyme production media. Each isolate has four variations of lipase enzyme treatment: crude enzyme, 0-30%, 30-45%, and 45-60% precipitation with ammonium sulfate (Su'i and Suprihana 2013). Lipase activity was measured using a UV-VIS spectrophotometer λ 410 nm and p-nitrophenyl palmitate (p-NPP) substrates. Lipase enzyme activity can be observed in Figure 4; the highest enzyme activity was produced from crude enzyme lipase treatment, with the highest activity from isolate H, namely 705.3 Units/mL. Meanwhile, from the enzyme precipitation, isolate D had the highest enzyme activity of 167.7 units/mL in the 45-60% ammonium sulfate precipitation. The enzyme activity of isolate H when precipitating 45-60% ammonium sulfate had the highest value, 224.5 Units/mL. Isolate M had the highest enzyme activity value at 30-45% precipitation of 344.8 Units/mL. Meanwhile, isolate Q had the highest enzyme activity value of 384.7 units/mL when 30-45% ammonium sulfate was deposited. Apart from that, the enzyme precipitation from isolate Q always had the highest value in each precipitation compared to other isolates. In this study, precipitation with ammonium sulfate did not seem to increase the concentration and activity of the lipase enzyme compared to the crude extract.

Moreover, adding ammonium sulfate salt, called the salting-out process, causes hydrophobic interactions between proteins and reduces protein solubility so that proteins precipitate, including enzymes (Duong-Ly and Gabelli 2014). According to Lehninger (2004), salting-out is an effort to produce enzymes with better specific activity than crude extract and high enzyme yields. The crude enzyme has higher activity than the precipitated lipase enzyme, and the lipase activity after precipitation decreased drastically compared to the lipase activity of the crude extract. This shows that most of the enzymes are necessary to be appropriately deposited. During precipitation, the ammonium sulfate temperature plays a significant role in enzyme stability. The time the enzyme is stored due to multilevel precipitation with ammonium sulfate also affects the enzyme activity value. The study revealed that the enzyme activity resulting from multilevel precipitation with ammonium sulfate decreased because the enzyme was stored in the freezer before its activity was measured (Figure 4). Enzyme activity will decrease with the length of storage time (Ardian et al. 2014). Each protein will precipitate at a different salt concentration. Su'i and Suprihana (2013) states hydrophobic proteins will precipitate at low salt concentrations. Meanwhile, hydrophilic proteins require a high salt concentration to precipitate them. The higher activity of the crude enzyme compared to the precipitated lipase enzyme can be attributed to several factors: Protein Conformation and Activity: The crude enzyme preparation typically contains the enzyme in its native, active conformation. During precipitation, the enzyme may undergo conformational changes or partial denaturation, leading to activity loss. Presence of Cofactors and Stabilizers: Crude enzyme extracts often contain various cofactors, stabilizers, and other proteins that help maintain enzyme stability and activity. These may be lost or removed during precipitation, resulting in lower enzyme activity. Enzyme Inhibitors: The precipitation process might introduce conditions (such as changes in pH, ionic strength, or the presence of precipitating agents) that can inhibit the enzyme's activity. In contrast, the crude extract might maintain a more favorable environment for the enzyme. Enzyme Concentration and Aggregation: Precipitation can lead to enzyme aggregation, reducing the availability of active sites for substrate binding. In the crude extract, enzymes are more likely to be in a dispersed, active form. Loss of Enzyme During Precipitation: Some enzyme activity may be lost during precipitation due to incomplete recovery or losses during washing and handling steps.

Furthermore, the ammonium sulfate salt ion will affect protein solubility. At low concentrations, salt ions surround protein molecules and prevent protein molecules from joining together. Meanwhile, at high salt concentrations, the electrical charge around the protein increases, attracting water molecules from the protein colloid and causing hydrophobic interactions between proteins, which is called salting out (Scopes 1994). The enzymes from salting out in each fraction are free from non-protein contaminants but still contain non-enzyme proteins (Putri et al. 2013).

Lipolytic bacterial species based on molecular identification

This study identified one isolate of lipolytic bacteria with a unique characteristic of stable lipase enzyme activity. This characteristic, whether in the form of a crude enzyme extract or after going through the sedimentation stage, was most pronounced in isolate Q. This discovery opens up exciting possibilities for further research with significant potential for industrial application improvements. The total enzyme activity value from the sedimentation of isolate Q consistently surpassed other isolates at each deposition level, suggesting its suitability for various industrial processes. The electrophorogram of the PCR product, which provides further evidence of these findings, can be seen in Figure 5.

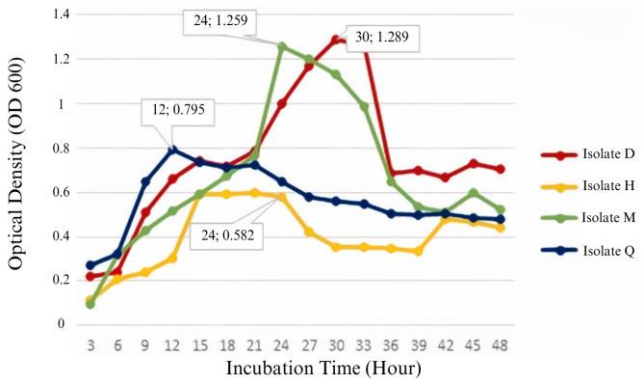

Figure 3. Sigmoid growth curve of lipolytic bacteria in lipase enzyme production media

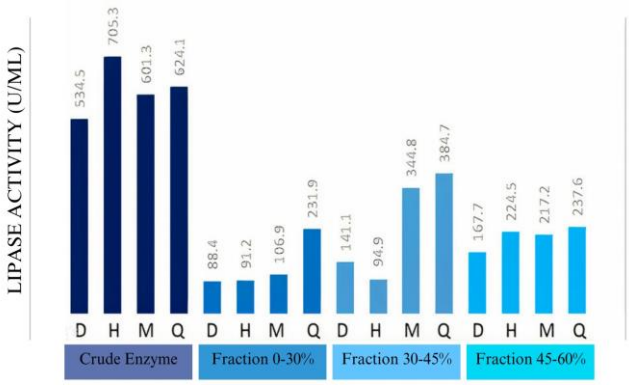

Figure 4. Histogram of lipase enzyme activity from each isolate D, H, M, Q (crude enzyme, ammonium sulfate fraction 0-30%, ammonium sulfate fraction 30-45%, and ammonium sulfate fraction 45-60%)

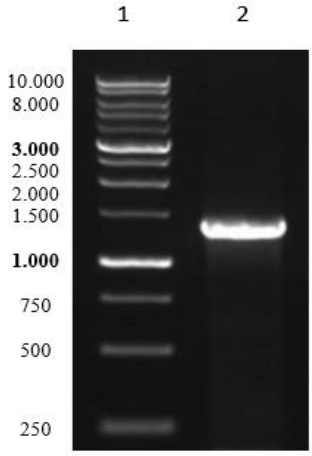

Figure 5. Electrophoregram of PCR products of lipolytic bacteria. Note: 1. 1kb ladder marker, 2. PCR product of isolate Q's 16S rRNA gene

The electrophoregram shows a DNA band measuring approximately 1300 bp. The actual base sequence was 765 nucleotide bases because the sequence was obtained from partial (single-pass) sequencing. These sequences were analyzed using the BLASTN program on the NCBI website and then matched with the Gene Bank database to get the names of the species with the closest relationship and a description of the similarity percentage. Based on the results of BLASTN analysis, isolate Q has a max score of 1411, a total score of 1411, a query cover of 100%, an E value of 0.0, and a percent identity or similarity percentage of 100% with *Stenotrophomonas maltophilia*. A 16S rRNA sequence similarity ≤99% and ≥97% allowed the strain and species identification at the genus level (Drancourt et al. 2000). Isolate Q is Gram-negative and has a bacilli cell shape, creamy white, and small round colonies. This is aligned with the literature stating that *S. maltophilia* is an aerobic Gram-negative bacterium. These bacteria are found widely in aqueous environments, in soil, and on plants and have been used in biotechnological applications (Berg et al. 1999).

Based on Thomas et al. (2014), lipase activity was detected in *S. maltophilia* growth media. The extracellular lipase produced by *S. maltophilia* helps it develop in an environment with limited carbohydrates, making lipids the only carbon source. Research by Larik et al. (2018) also stated that *S. maltophilia* is a lipolytic bacterium that can be used in biotechnology. However, our research went further by isolating and characterizing lipolytic bacteria from a specific environment, making a unique contribution that Stenotrophomonas has many important environmental roles. These species in the Stenotrophomonas genus have an important ecological role as natural elements in biocontrol and bioremediation agent cycles. The *S. maltophilia* bacteria that secrete solvent were organic-resistant lipases isolated from oil-contaminated samples (Li et al. 2013).

Lipase produced by *S. maltophilia* showed stability in environments containing high hydrophobic solvents and pure hydrophilic solvents for seven days. Lipase activity is high at low temperatures and in the alkaline pH range; this characterizes lipase from *S. maltophilia* for industrial applications involving organic solvents such as organic synthesis (Li et al. 2013). Several researchers studied enzyme activity in processing domestic waste using an activated sludge system. Lipase and protease enzyme extraction in activated sludge evaluate the activity of the lipase enzyme in terms of its degradation of organic compounds in wastewater (Kasipah et al. 2013). Based on the references above, lipase from *S. Maltophilia* isolates obtained from this research is expected to be helpful as a waste biodegradation agent. These practical implications of our research significantly contribute to environmental science and biotechnology.

In conclusion, a total of 4 isolates of lipolytic bacteria were successfully isolated and selected from the homogenization tank waste from WWTP SIA Magetan and were proven to have lipase enzyme activity, namely isolate D, isolate H, isolate M, and isolate Q. The highest lipase enzyme activity was produced in the form of crude enzyme, and from the multilevel enzyme precipitation showed that isolate Q had the most stable and highest enzyme activity among the other isolates; the enzyme

activity of isolate Q was optimal at 30-45% ammonium sulfate precipitation. The 16S rRNA gene sequence from isolate Q has a similarity percentage of 100% with *S. maltophilia*.

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