Jurnal Pembelajaran Dan Biologi Nukleus p-ISSN: 2442-9481
Vol 9 (3): 496-505, November 2023 e-ISSN: 2685-7332

Screening for Lipolytic Bacteria from Bonoloyo Cemetery, Surakarta

Heni Sulistyo Rini, Erma Musbita Tyastuti, Yasir Sidiq, Triastuti Rahayu(*)
Biology Education Department, Faculty of Teacher Training and Education, Universitas Muhammadiyah Surakarta. Jl. A. Yani Tromol Pos I, Pabelan, Kartasura, Surakarta 57162, Jawa Tengah, Indonesia

***Corresponding author**: tr124@ums.ac.id

Submitted September 01st 2023 and Accepted October 19th 2023

Abstract

A cemetery is a burial place managed by the government. Inside the TPU, the body is decomposed by microorganisms, one of which is lipolytic bacteria, because the human body is composed of about 12.5–13.60% lipids. Research on lipolytic bacteria from TPU in Indonesia has not been found, even though it has the potential to obtain lipolytic bodies. Therefore, this research was conducted to select lipolytic bacteria from TPU and provide simple assistance. A total of 45 bacterial isolates from TPU Bonoloyo Surakarta were selected for their lipolytic activity using tributyrin agar media. Lipolytic activity was determined using the lipolytic index (LI). Bacterial isolates with lipolytic potential were identified based on colony morphology and Gram staining. The results showed that 30 isolates (67%) showed lipolytic activity, with the highest LI value of 5.43 (BLB 9) after 2x24 hours of incubation. Bacterial isolates that have lipolytic activity are white colonies with circular shapes. The results of Gram staining showed that the bacterial isolates belonged to a group of Gram-negative bacteria in the form of cocci. The conclusion put forward is that public cemeteries (TPU) have the potential to store isolates of lipolytic bacteria dominated by Gram-positive bacteria by as much as 57%

Keywords: *Bonoloyo, Cemetery, Lipolytic Bacteria, Screening, Tributirin*

https://doi.org/10.36987/jpbn.v9i3.4944

INTRODUCTION

A public cemetery (TPU) is an area provided for the burial of corpses. TPU is managed by the government, which is regulated in Government Regulation Number 9 of 1987 concerning Provision and Use of Land for the Purposes of Cemeteries and Minister of Home Affairs Decree Number 26 of 1989 concerning Guidelines for Implementing Government Regulation Number 9 of 1987. Bonoloyo TPU is one of the largest TPUs in Surakarta, with up to 15.6 hectares and 23 blocks of burial areas (Amalia, 2011). This TPU is classified into old and new burial blocks with uneven ground contours.

Corpses that have been buried will undergo decomposition by soil microorganisms, including fungi and bacteria. Corpse decomposition can be physically observed through 5 phases, including the fresh phase, bloat, active decay, advanced decay, and skeletonization (Shreshta R and Kanchan T, 2020). When humans are declared dead, the process of decomposition begun to occur, which begins with autolysis. Autolysis is the first stage of the human decay process, which involves the breakdown of tissue, beginning 24 to 72 hours after death and continuing until the laying of the corpse. Active decay occurs when the corpse's body undergoes rapid decomposition due to insect activity. A decrease in insect activity characterizes advanced decay because the flesh has undergone liquefaction and leaves skin and hair to dry out, as well as bones that are still in the skeletonization phase (Singh et al. 2018). As a result of decomposition, corpse material will move to the surrounding soil when insects and microbes dominate the next decomposition process (Brilliana Putra et al. 2020). The main constituent components of the human body are fat 12.51-23.60%, protein 14.4-18.62%, and water 55.13-67.85% (Dent et al. 2004; FORBES et al. 1953) so that the bodies of decomposers involved are dominated by fat decomposers (lipolytic) and protein decomposers (proteolytic). Therefore, in the cemetery, it is likely that potential lipolytic bacteria can be found.

Lipolytic bacteria are bacteria that play a role in the decomposition of organic remains in the soil, such as dead plants and dead animals that contain fat. In this process, lipolytic bacteria break down fats into fatty acids and glycerol, which can be used as a source of nutrition by other microorganisms and plants (Melati 2020). Many bacteria that are aerobic and proteolytic are also lipolytic. Several lipolytic bacteria were identified from the genera *Pseudomonas, Alcaligenesis, Serratia, Micrococcus*, and *Dyella lipolyca* sp. Nov. (Fardiaz 1992; Tang et al. 2017).

Lipid breakdown by lipolytic bacteria because these bacteria produce lipase enzymes (Ramnath et al. 2017). Lipolytic activity can be tested using the selective medium of tributyrin as a lipid substrate to be decomposed. The strength of lipolytic activity is determined based on the proportion of bacterial colonies with formed clear zones. The larger the clear area formed, the greater the ability of bacteria to produce extracellular lipase enzymes to degrade lipids (Chairunnisa et al. 2019). Therefore, tributyrin agar is a selective and differential medium that can be used to isolate and identify lipolytic bacteria (Carrazco-Palafox et al. 2018). Lipase is an enzyme that has an important role in the industrial field because it can catalyze hydrolysis and synthesis reactions. Lipase can catalyze various reactions, so it is very useful for codification of flavors in the food industry, pharmaceutical products, digestion of oils and fats in food, leather processing, textile production, cosmetic production, paper processing, and synthetic chemical applications, e.g., biopolymer and biodiesel production (Houde et al. 2004; Szymczak et al. 2021).

In Indonesia, isolation and screening of lipolytic bacteria have been widely carried out. Some of them are research on lipolytic bacteria from palm oil liquid waste (Chairunnisa et al. 2019) (Khairani and Manalu 2023), SBE (Spent Bleaching Earth) waste (Elyza et al. 2016), surimi and crab liquid waste (Oktavia and Wibowo 2017), Siak River water (Dahliaty et al. 2012), and landfill (Tsani 2021). From these studies, isolates of lipolytic bacteria were identified as *genera Bacillus, Pseudomonas, and Klebsiella*

(Chairunnisa et al. 2019; Khairani and Manalu, 2023), *Citrobacter, Enterobacter, and Acinetobacter* (Elyza et al. 2016), and *Streptococcus* (Tsani 2021).

Exploration of lipolytic bacteria sourced from burial grounds in Indonesia has not been found, even though burial grounds hold the potential for lipolytic bodies. Bacterial exploration from Indonesian burial grounds that have been carried out are proteolytic (Saputri et al., 2023) and cellulolytic bacteria (Syarifah et al., 2023), respectively with a proteolytic index of 2.08 and a cellulolytic index in the high and moderate categories of 70%. Therefore, this study needs to be done to select lipolytic bacteria from TPU and carry out simple identification. The study results are expected to yield potential lipolytic bacterial isolates that can be developed for various industrial fields.

METHOD

This research conducted in April-May 2023 at the Biology Laboratory of the Faculty of Teacher Training and Education, Universitas Muhammadiyah Surakarta.

Sample

The research samples were in the form of soil bacteria isolates isolated from TPU Bonoloyo of Surakarta totaling 45 isolates. The tools used are *Laminar Air Flow (LAF), petridish (Iwaki),* test tube *(Pyrex),* incubator *(Memmert IN55*), digital scale (Durascale DAB-E223), oven (Maspion), *hot plate magnetic stirrer (Ciramec +),* erlenmeyer *(Pyrex), autoclave (GEA LS-35LJ),* microscope *(Olympus CX21),* refrigerator *(Sharp),* micropipette *(Socorex)*, glass objects, matches, bunsen, stationery, and documentation tools.

The materials used in this study were isolates of soil bacteria originating from the Bonoloyo Public Cemetery Place (TPU), tributyrin agar lipolytic selective media composed of peptone *(Oxoid),* yeast extract *(Oxoid),* tributyrin, bacteriological agar *(Himedia),* aquadest, alcohol, Nutrient Agar *(Merck)* media, Gram dyes (violet crystals, iodine, alcohol, safranin).

The isolation

Rejuvenation of TPU Bonoloyo bacterial isolate is carried out by inoculating into media to make a new tilt using an ose needle, then incubated for 24 hours before being used for lipolytic tests. The purpose of bacterial rejuvenation is to obtain active bacterial isolates so that bacterial growth can be optimized.

Selective Medium Manufacturing and Lipolytic Activity Test

Bacterial selection begins with the manufacture of selective medium tributyrin agar (Suyanto et al, 2015). The lipolytic selective media component with a volume of 1 L consists of yeast extract 3 g, peptone 5 g, bacteriological agar 20 g, and tributyrin 10 g dissolved in 1 L aquades (Ramnath et al. 2017). The medium was sterilized by autoclave at 121°C for 15 minutes (Oktavia and Wibowo 2017).

Lipolytic bacterial selection is carried out by inoculating bacterial isolates to be tested by toting on the surface of tributyrin agar medium (Ramnath et al. 2017). After that, the petridish is incubated for 24 hours and observed bacterial growth and clear zones formed. Bacterial colonies that grow on selective media and have a clear zone around the

colony are lipid degrading colonies. Colony and clear zone mortality were measured after 24 hours to obtain lipolytic index (LI) values (Rosdi et al. 2022), using the formula

$$\text{Lipolytic Indeks} = \frac{clear\ zone\ diameter}{colony\ diameter}$$

The higher the index value produced, the higher the lipolytic activity.

Characteristics of bacterial morphology

Characterization is done by observing the morphology of bacteria macroscopically (colony shape, colony edge, colony surface, and colony color) and microscopic (gram staining and shape). Gram staining is done by making a bacterial smear preparation in the glass of the object followed by staining. Staining begins with fixation on the flame 3 times and cooling. The preparation is dripped with a 0.5% violet crystal solution until it covers, let stand for 30 seconds, rinse with excess dye water. Add lugol solution for 30 seconds, rinse with water. Remove the color by adding 96% alcohol for 10-20 seconds, then rinse with water. Drip the preparation with a 0.25 % safranin solution for 30 seconds, rinse with water and let it dry. After drying, drip 1 drop of immersion oil. Observe under the microscope. If the observation results are colored purple, it means grampositive bacteria, it is possible to reduce the presence of gram-negative bacteria are colored red (Ramnath et al. 2017).

RESULTS AND DISCUSSION

Testing of lipolytic activity

Lipolytic bacteria contain lipase enzymes to break down fats into water-soluble lentic acid and glycerol (Chairunnisa et al. 2019). Lipolytic bacteria screening research that have been isolated from TPU Bonoloyo, Banjarsari District, Surakarta, show lipolytic activity with diverse lipolytic indices **(Table 1).**

From 45 isolates selected, 30 isolates (67%) showed different lipolytic activities with LI. LI values 1-2 are indicated by isolate BLB1, BLB2, BLB4, BLB7, BLB11, BLB12, BLB14, BLB16, BLB18, BLB21, BLB22, BLB27, BLB29, BLB30, BLB31, BLB32, BLB33, BLB37, BLB38, BLB40, BLB41, BLB42, BLB44, BLB45, and LI values >2 are indicated by isolate BLB10, BLB25, BLB26, BLB28, BLB43 with the largest LI value of 5.43 produced by isolate BLB9 **(Table 1).**

(Figure 1A). The lipolytic ability shown by bacterial isolates from Bonoloyo Cemetery is greater than isolates screened from landfill soils with LI 1.51 (Tsani 2021), from crab canning wastewater and surimi treatment wastewater with LI 2.6 (Oktavia and Wibowo 2017), from Sungai Siak water with LI 0.17 (Dahliaty et al. 2012), and from palm oil liquid waste with an LI value 0.48 (Khairani and Manalu 2023). This suggests that lipolytic bacteria from TPU soil can break down fats into fatty acids and glycerol more strongly.

p-ISSN: 2442-9481
e-ISSN: 2685-7332

Table 1. Calculation of Lipolytic Index (LI) Activity

Isolate Code	Diameter Colony (cm)	Clear Zone (cm)	Bacterial Index
BLB1	0.3	0.5	1.67
BLB2	1.15	1.45	1.26
BLB4	0.9	1.4	1.56
BLB7	0.75	1	1.33
BLB9	0.35	1.9	5.43
BLB10	0.7	1.8	2.57
BLB11	0.5	0.75	1.50
BLB12	1.3	1.5	1.15
BLB14	0.6	1.5	2.50
BLB16	1	1.5	1.50
BLB18	0.5	1	2.00
BLB21	0.95	1.2	1.26
BLB22	1.4	1.65	1.18
BLB25	0.55	1.5	2.73
BLB26	0.55	1.6	2.91
BLB27	0.6	1.45	2.42
BLB28	0.5	1.5	3.00
BLB29	0.9	1.35	1.50
BLB30	0.6	1	1.67
BLB31	0.5	0.75	1.50
BLB32	0.65	0.95	1.46
BLB33	0.5	1.15	2.30
BLB37	0.9	1.55	1.72
BLB38	0.6	0.8	1.33
BLB40	0.8	1.7	2.13
BLB41	0.95	1.35	1.42
BLB42	0.55	0.95	1.73
BLB43	0.6	1.6	2.67
BLB44	0.4	0.8	2.00
BLB45	0.25	0.55	2.20

The TPU area is a place where the decomposition of bodies containing fat or lipids occurs, so bacteria must be found lipolytic. Of the 45 bacterial isolates from TPU Bonoloyo tested, 67% of the isolates had lipolytic activity. This proves a strong correlation between the availability of substrates and decomposing bodies, in this case, lipolytic bacteria. This data is in line with research (Chairunnisa et al. 2019), which uses lipolytic bodies to overcome pollution due to oil waste. These lipolytic bacteria can also be utilized for bioremediation agents and in the production of lipase enzymes for industrial applications (Leoni F. 2019);(Student et al. 2021).

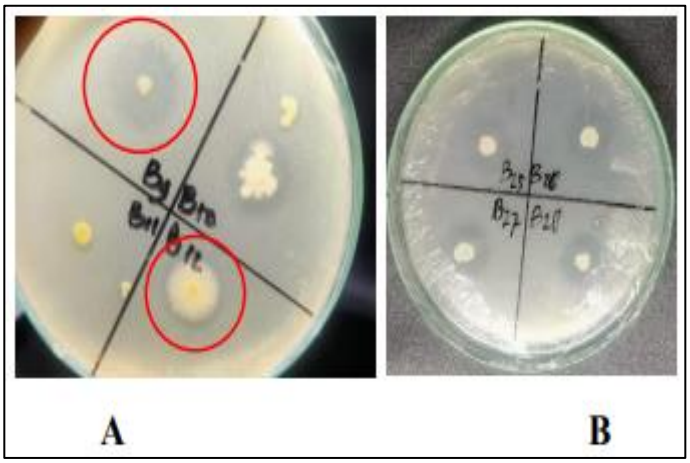

Figure 1. A: the difference in the diameter of the bacterial colony and the clear zone, the largest BLB9=LI and the smallest BLB12=LI; B: lipolytic bacteria screening results

The lipolytic potential is indicated by the formation of a clear zone around the bacterial colony **(Figure 1).** This clear zone is formed due to the degradation of the substrate's degradation in cellulose by the lipase enzyme. In this study, tributyrin is a cellulose substrate that will be broken down by lipase enzymes produced by bacteria (Carrazco-Palafox et al. 2018); (Ramnath et al. 2017), so that bacterial colonies surrounded by clear zones show lipolytic ability (Oktavia and Wibowo 2017). In this study, the prayer that had the largest LI was BLB 9 of 5.43 **(Tabel 1, Gambar 1A).** The isolates form a large clear zone, although the colony is smaller than the other isolates **(Figure 1).** This is in line with research (Chairunnisa et al. 2019), namely the larger the clear zone formed, the bacteria higher the bacteria's potential for degrading lipids.

BLB 9 isolate need to be tested for other lipolytic activity because LI is only measured after two days of incubation. Many factors, such as pH, substrate, incubation time, and incubation conditions, will influence enzyme activity. However, the influence of the substrate on lipolytic enzyme activity can vary depending on the type of bacteria and other conditions (Leoni F. 2019). Bacterial growth patterns also influence enzyme activity. Bacterial isolates show lipase activity by following bacterial growth patterns. Lipase in each isolate is thought to be part of the activity of the primary metabolites of the bacterial isolate. Primary metabolite activity goes hand in hand with the microbial growth phase, increases at the end of the logarithmic phase or the beginning of the stationary phase, and can decrease as microbial activity decreases, and nutrients as substrates decrease (Jeager 2015). Some studies also show that lipolytic activity is best formed at pH 8 (Oktavia & Wibowo 2017).

Macroscopic and microscopical characterization of potential lipolytic bacteria isolates

Bacterial characterization is observed through macroscopic (colony shape, colony edge, colony surface, and colony color) and microscopic (Gram staining) characters. From 45 bacterial isolates, 30 isolates of lipolytic potential bacteria were obtained from TPU Bonoloyo. The characteristics of lipolytic bacteria can be seen in **Table 2**.

Table 2. Characteristics of bacterial colonies and isolate cells that have lipolytic activity

Isolate Code	Colony Form	Edge of the colony	Colony surface	Colony color	Cell Shape	Gram
BLB1	Circular	Entire	Convex	Cream	Coccus	-
BLB2	Circular	Entire	Convex	Cream	Coccus	-
BLB4	Circular	Undulate	Convex	Cream	BacillI	-
BLB7	Circular	Undulate	Convex	White	Coccus	-
BLB9	Circular	Entire	Convex	White	Coccus	-
BLB10	Circular	Undulate	Convex	White	Coccus	+
BLB11	Circular	Entire	Convex	Yellow	Coccus	+
BLB12	Circular	Undulate	Convex	Yellowish white	Coccus	-
BLB14	Circular	Entire	Convex	Yellow	Coccus	+
BLB16	Circular	Undulate	Convex	White	Coccus	+
BLB18	Circular	Entire	Convex	White	Coccus	+
BLB21	Circular	Undulate	Convex	Yellow	Coccus	-
BLB22	Circular	Entire	Convex	White	Bacilli	+
BLB25	Circular	Undulate	Convex	White	Coccus	-
BLB26	Circular	Curled	Convex	White	Coccus	-
BLB27	Circular	Curled	Convex	White	Coccus	+
BLB28	Circular	Curled	Convex	White	Coccus	-
BLB29	Irregular	Undulate	Convex	White	Coccus	+
BLB30	Circular	Entire	Convex	White	Coccus	+
BLB31	Circular	Undulate	Convex	Yellow	Coccus	+
BLB32	Circular	Entire	Convex	Yellow	Coccus	-
BLB33	Circular	Curled	Convex	White	Coccus	-
BLB37	Irregular	Curled	Convex	White	Coccus	+
BLB38	Irregular	Curled	Convex	White	Coccus	+
BLB40	Circular	Entire	Convex	White	Coccus	+
BLB41	Irregular	Undulate	Convex	White	Coccus	-
BLB42	Circular	Entire	Convex	Yellow	Coccus	+
BLB43	Irregular	Undulate	Convex	White	Coccus	+
BLB44	Circular	Entire	Convex	White	Coccus	+
BLB45	Irregular	Curled	Convex	White	Coccus	+

Table 2. shows that the results obtained from macroscopic characterization show bacterial colonies are dominated by circular and irregular shapes with different colony edges, flat and convex shapes dominate colony surface, and white-yellow is the dominant color. The gram staining results obtained 57% gram-positive bacteria and 43% gram-negative bacteria, while the cell form is coccus. Gram staining aims to distinguish between gram-positive bacteria and gramnegative bacteria and the shape of bacterial cells. It is said that gram-positive bacteria are formed when purple forms in bacterial cells, and gram-negative bacteria are said to be gram-negative with the formation of red in bacterial cells (Rahmawati et al. 2021). The results of other studies also showed that lipolytic bacteria isolated also had almost the same colony morphological characteristics, namely circular

and irregular colony shapes, entire and undule margins, and a white to yellowish color (Khairani and Manalu 2023).

CONCLUSION

This study shows that TPU Bonoloyo has the potential to store potential lipolytic bacterial isolates, with the highest lipolytic index (LI) of 5.43 (isolate BLB 9). BLB9 isolates have a colony shape with an entire edge and are gram negative in the form of coccus. Lipolytic bacterial colonies were observed as circular and iregular, while the gram grouping was gram-positive and gram negative bacteria by as much as 57% and 43%, respectively. Based on the results obtained, further research should be done on the type of bacteria so that the bacteria can be exploited based on their function.

REFERENCES

Amalia, G. (2011). *Ketersediaan Lahan Tempat Pemakaman Umum (TPU) di Kota Surakarta*. 8.

Brilliana Putra, A., Fakhrurizal Amin, I., Dini Fitriyasa, R., & Safitry, O. (2020). Memperkirakan saat Kematian (Postmortem Interval) Menggunakan Temuan Mikrobiom pada Setiap Tahap-Tahap Penguraian (Decomposition): An Evidence-Based Case Report. *Journal of Indonesian Forensic and Legal Medicine*, *2*(1), 111–122.

Carrazco-Palafox, J., Rivera-Chavira, B. E., Ramírez-Baca, N., Manzanares-Papayanopoulos, L. I., & Nevárez-Moorillón, G. V. (2018). Improved method for qualitative screening of lipolytic bacterial strains. *MethodsX*, *5*, 68–74. https://doi.org/10.1016/j.mex.2018.01.004

Chairunnisa, C., Riyanto, R., & Karim, A. (2019). Isolasi dan Uji Bakteri Lipolitik dalam Mendegradasi Minyak Pada Limbah Cair Kelapa Sawit di Kebun Marihat, Pematang Siantar. *Jurnal Ilmiah Biologi UMA (JIBIOMA)*, *1*(2), 44–52. https://doi.org/10.31289/jibioma.v1i2.155

Dahliaty, A., Susanti, R., & Haryani, Y. (2012). Skrining Bakteri Lipolitik dari Sungai Siak di Daerah Pelita Pantai Kota Pekanbaru. *J. Ind. Che. Acta*, *3*(1), 1–4.

Dent, B. B., Forbes, S. L., & Stuart, B. H. (2004). Review of human decomposition processes in soil. *Environmental Geology*, *45*(4), 576–585. https://doi.org/10.1007/s00254-003-0913-z

Elyza, F., Gofar, N., & Munawar, M. (2016). Identifikasi Dan Uji Potensi Bakteri Lipolitik Dari Limbah Sbe (Spent Bleaching Earth) Sebagai Agen Bioremediasi. *Jurnal Ilmu Lingkungan*, *13*(1), 12. https://doi.org/10.14710/jil.13.1.12-18

Fardiaz, S. (1992). *Mikrobiologi Pangan*. Gramedia Pustaka Utama.

Forbes, R. M., Cooper, A. R., & Mitchell, H. H. (1953). The composition of the adult human body as determined by chemical analysis. *The Journal of Biological Chemistry*, *203*(1), 359–366. https://doi.org/10.1016/s0021-9258(19)52646-1

Houde, A., Kademi, A., & Leblanc, D. (2004). Lipases and their industrial applications: An overview. *Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology*, *118*(1–3), 155–170. https://doi.org/10.1385/ABAB:118:1-3:155

Jeager, B. (2015). Uji Kualitatif dan Kuantitatif Isolat Bakteri Lipolitik dari Limbah Cair Pabrik Pengolahan Ikan Kecamatan Muncar, Banyuwangi. *Jurnal Biotropika*, *3*(3), 2–6.

Khairani, & Manalu, K. (2023). Isolasi Dan Identifikasi Bakteri Lipolitik Dari Limbah Cair Kelapa Sawit (*Elaeis quineensis* Jacq.). *BIOEDUSAINS: Jurnal Pendidikan Biologi Dan Sains*, *6*(1), 1–13.

Leoni F. (2019). Isolasi dan Identifikasi Bakteri Lipolitik Pada Proses Pengomposan Limbah Domestik. *F. Leoni*, *53*(9), 1689–1699.

Melati, I. (2020). *Pusat Penelitian Limnologi LIPI*. *Rahayu 2005*, 272–286.

Oktavia, D. A., & Wibowo, S. (2017). Penapisan dan Identifikasi Bakteri Lipolitik yang Diisolasi dari Air Limbah Pengolahan Surimi dan Pengalengan Rajungan. *Jurnal Pascapanen Dan Bioteknologi Kelautan Dan Perikanan*, *11*(2), 147. https://doi.org/10.15578/jpbkp.v11i2.262

Rahmawati, L. R., Adlina, S., & Yuliana, A. (2021). Isolasi dan Identifikasi Bakteri Penghasil Protease Ekstraseluler dari Limbah Cair Tahu Putih. *Jurnal Kesehatan Bakti Tunas Husada: Jurnal Ilmu Keperawatan, Analis Kesehatan Dan Farmasi*, *21*(2), 187–193.

Ramnath, L., Sithole, B., & Govinden, R. (2017). Identification of lipolytic enzymes isolated from bacteria indigenous to Eucalyptus wood species for application in the pulping industry. *Biotechnology Reports*, *15*(July), 114–124. https://doi.org/10.1016/j.btre.2017.07.004

Rosdi, A., Dahalan, F. A., Zhan, L. Z., Babakhani, P., Shams, S., Engineering, C., & Area, P. (2022). *Collection of samples*. *2*, 1–5.

Saputri, R. D., Tyastuti, E. M., Sidiq, Y. & Rahayu, T. (2023). Public Cemetery's Potency as The Source of Proteolytic Bacteria. Quagga: Jurnal Pendidikan dan Biologi, 15(2), 201-206. doi: 10.25134/quagga.v15i2.44Shreshta R, Kanchan T, K. K. (2020). Methods of estimation of time since death. In *StatPearls Publishing*. StatPearls Publishing.

Singh B, Minick KJ, Strickland MS, et al. (2018). Temporal and Spatial Impact of Human Cadaver Decomposition on Soil Bacterial and Arthropod Community Structure and Function. *Front Microbiol*, *8*(1), 2616.

Student, M. T., Kumar, R. R., Omments, R. E. C., Prajapati, A., Blockchain, T.-A., Ml, A. I., Randive, P. S. N., Chaudhari, S., Barde, S., Devices, E., Mittal, S., Schmidt, M. W. M., Id, S. N. A., PREISER, W. F. E., OSTROFF, E., Choudhary, R., Bit-cell, M., In, S. S., Fullfillment, P., … Fellowship, W. (2021). *Frontiers in Neuroscience*, *14*(1), 1–13.

Suyanto, E., Soetarto, E. S., & Cahyanto, M. N. (2015). Produk Lipase Kapang Lipolitik

pada Limbah Ampas Kelapa. Bioeksperimen: Jurnal Penelitian Biologi, 1(1), 12–17. https://doi.org/10.23917/bioeksperimen.v1i1.311

Syarifah, S.N., Rahayu, T., Tyastuti, E.M., & Sidiq, Y. 2023. Screening andIdentification of CellulolyticBacteria from PublicCemetery Soil.BIOEDUSCIENCE, 7(2), 192-199.

Szymczak, T., Cybulska, J., Podleśny, M., & Frąc, M. (2021). Various perspectives on microbial lipase production using agri-food waste and renewable products. *Agriculture (Switzerland)*, *11*(6). https://doi.org/10.3390/agriculture11060540

Tang, L., Chen, M. H., Nie, X. C., Ma, M. R., & Qiu, L. H. (2017). Dyella lipolytica sp. nov., a lipolytic bacterium isolated from lower subtropical forest soil. *International Journal of Systematic and Evolutionary Microbiology*, *67*(5), 1235–1240. https://doi.org/10.1099/ijsem.0.002020

Tsani, Fauqi. Falakhus. (2021). Isolation and Characteristic of Lipolytic Bacteria from the Soil of the Final Processing Site (TPA) Talangagung Kepanjen, Malang. Thesys. Maulana Malik Ibrahim Islamic University of Malang.

How To Cite This Article, with *APA style* :

Rini, H.S., Tyastuti, E.M., Siddiq, Y., Rahayu, T. (2023). Screening for Lipolytic Bacteria from Bonoloyo Cemetery Surakarta. *Jurnal Pembelajaran dan Biologi Nukleus*, 9(3), 496-505. https://doi.org/10.36987/jpbn.v9i3.4944