Characteristic Isolation of Bacteria in Lake Water on Campus-A of UIN Sulthan Thaha Saifuddin Jambi

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Submitted January 13 Th 2025 and Accepted May 27 Th 2025

Abstract

Background: *Water bodies are highly susceptible to contamination from various sources, including domestic, agricultural, and industrial waste. These contaminants often introduce pathogenic microorganisms such as Escherichia coli and Salmonella sp., which pose significant risks to human and animal health. Despite this, bacteria also play a vital role in supporting aquatic ecosystems by facilitating the decomposition of organic matter.* **Methodology:** *This study was conducted with the objective of isolating and characterizing bacterial species present in the waters of Campus A Lake. Sampling was carried out by collecting water from several points within the lake. Bacterial isolation was performed using the serial dilution technique, followed by inoculation on Nutrient Agar (NA) media and incubation at 37 °C for 24 hours. The characterization phase included morphological observation of colonies, Gram staining, and biochemical tests such as oxidase reactions.* **Findings:** *The study successfully isolated three distinct bacterial colonies. One isolate exhibited a rod-shaped (bacillus) morphology, while the remaining two showed a spherical (coccus) form. All isolates tested Gram-negative, evidenced by the pink coloration after staining. Importantly, most of the isolates demonstrated an ability to break down organic material, indicating their usefulness in environmental cleanup. Additionally, the introduction of Eco Enzyme into lake water positively influenced the microbial ecosystem by promoting the growth of beneficial decomposers like Bacillus sp. and Lactobacillus sp., while simultaneously suppressing pathogenic bacteria.* **Contribution:** *These findings provide valuable insight for future water quality management and the development of effective, eco-friendly bioremediation strategies.*

Keywords: *Bacterial; Gram; Isolation; Lake; Microbial*

https://doi.org/10.36987/jpbn.v11i2.6846

INTRODUCTION

A study conducted by the water isolation group at the State Islamic University of Sulthan Thaha Saifuddin Jambi identified the presence of *Escherichia coli* in lake water that was treated with *Eco Enzyme* every month. The test results using *NA* culture media showed that all the samples analyzed contained *E. coli*, indicating that pathogenic bacteria remained present despite the regular addition of *Eco Enzyme*.

One of the surface water sources that is vulnerable to pollution due to changes in land use, climate fluctuations, and changes in hydrological patterns is lakes (Randle & Barnes, 2018). Its vulnerability is not only influenced by the level of pollution, but also by the resilience of the lake ecosystem to these pollution factors (Bi & Zhang, 2018). This problem is further complicated when the catchment area experiences increased nutrient levels, which can accelerate algae growth, reduce oxygen levels, and damage habitats (Jeppesen, 2015). These activities can trigger *eutrophication* due to excess nitrogen and phosphorus, which causes algal blooms and the death of fish and other aquatic organisms (He et al., 2019; Lin et al., 2020). This condition will be worse if there is heavy metal content in the water, which can cause *bioaccumulation* in *phytoplankton* (Zheng et al., 2022).

The quality of lake water is influenced by the quality of the flow originating from its catchment area, related to human activities. This can pose a risk of spreading various diseases, one of which is diarrhea caused by *Escherichia coli* (*E. coli*) bacteria and *total coliform* in polluted lakes. *E. coli* bacteria in water usually come from pollution or contamination of human and animal waste. Meanwhile, *total coliform* is a group of bacteria that can be found in the environment, such as in soil and water affected by surface flow and human and animal waste disposal. When it rains, water carries waste from the waste, which then seeps into the soil or flows into water sources (Sumampouw, 2017).

Microscopic organisms that are generally single-celled and do not have a cell nucleus membrane are *bacteria*. These organisms usually have cell walls, but do not contain chlorophyll. Despite their small size, *bacteria* have a very important role in everyday life. Some types of *bacteria* are known to be beneficial, such as those used in the food industry. However, there are also harmful *bacteria*, such as those that can cause food spoilage and even cause infections and diseases in humans (Febriza et al., 2024).

Bacteria play a very important role in the lake water ecosystem, both in *biogeochemical* processes and in the decomposition of organic matter. As *decomposers*, *bacteria* break down dead organic matter into compounds that can be used by other organisms such as *phytoplankton* and aquatic plants (Wu et al., 2020). In addition, *bacteria* are also involved in important nutrient cycles, such as *nitrogen fixation* and *denitrification*, which help regulate nitrogen and phosphorus levels in lake waters, thereby supporting ecosystem balance and preventing *eutrophication* (Zhang et al., 2021). Some *bacteria* also play a role in *sulfate reduction* and more complex organic matter processing (Ma et al., 2022). However, *pathogenic bacteria* in lakes can reduce water quality and risk causing organic matter decay and human disease (Zhao et al., 2020). Therefore, although *bacteria* have a very important role, their presence needs to be balanced so as not to damage the quality of the aquatic ecosystem. Hence, identifying *bacteria*, or monitoring water quality becomes essential to maintain the ecological balance and ensure the sustainability of aquatic environments.

METHOD

Isolation of bacteria from aquatic environments such as lakes is an essential initial step in various microbiological studies. Bacteria that inhabit lakes play a significant ecological role and can be utilized in various fields such as *biotechnology* and *environmental monitoring* (Tortora et al., 2020). In this study, bacterial isolation was conducted by collecting water samples and incubating them at 37 °C for 24 hours. Colonies that grew on the culture media were observed based on their morphology, including shape, color, diameter, and consistency (Madigan et al., 2018).

Samples were taken from Campus A Lake to obtain a more representative picture of the microorganisms present. The collected water was then serially diluted in 0.85 % *NaCl* solution to a final dilution of 10^{-8}, and homogenized using a *vortex mixer*. This dilution series was intended to reduce microbial density and facilitate isolation of single colonies (Anderson & Smith, 2019). From each dilution, 0.1 mL was pipetted onto *Nutrient Agar (NA)* plates and spread with a sterile, cooled spreader. Plates were incubated at 37 °C for 24 hours. Growth was observed only at the 10^{-8} dilution, yielding colonies on four *Petri dishes*. After incubation, macroscopic colony characteristics were recorded, including colony shape (*circular* or *irregular*), colony color (*yellow* or *white*), colony margin (*entire* or *lobate*), and colony elevation (*flat*). These observations provide preliminary clues to bacterial genus or species (Cappuccino & Welsh, 2019; Madigan et al., 2018).

The *Gram staining* was performed to differentiate bacteria by cell-wall structure. Colonies were picked with a sterile loop, smeared onto a glass slide, air-dried, and heat-fixed. *Crystal violet* was applied for one minute, rinsed, then 1% *Lugol's iodine* added and rinsed again. *Decolorization* was performed with alcohol or an alcohol-acetone mixture. Finally, *safranin* was applied as a *counterstain*. Slides were examined under *oil-immersion objective*. *Gram-positive* cells retained the purple *crystal violet* stain, whereas *Gram-negative* cells appeared pink (Sumarmono & Wijayanto, 2019; Martinez et al., 2018).

Sample or Participant

This study used water from *Campus Lake* A as a sample. The location of the lake is at the State Islamic *University of Sulthan Thaha Saifuddin Jambi*. Samples were taken from three different points to reflect the variation in *microbial ecosystem* conditions in the lake. The sampling was carried out in the morning to minimize the influence of fluctuating temperatures. Each point produced 100 mL of sample, which was then stored in a sterile *bottle* to ensure no *contamination* during transportation to the *laboratory*.

Instrument

The tools and materials used in this study consisted of various equipment that supports the process of bacterial isolation and *identification*. Water sampling was carried out using *sterile sample bottles*, equipped with *markers* for identification and *thermometers* to measure the temperature of the water during sampling. *Nutrient Agar (NA)* was used as a medium for bacterial growth, while microbiological equipment such as *sterile ose*, *glass objects*, *binocular light microscope*, and *bunsen* were used for macroscopic and microscopic observation of bacteria. *Gram staining* was carried out with reagents such as *crystal violet*, *iodine solution*, *alcohol*, and *safranin* to determine the type of bacterial cell wall. In addition, other tools such as *micropipettes*, *vortexes*, and *spreaders* were used for *homogenization*, *sample dilution*, and *inoculation* into *culture media*.

Data collection

In this study, data were collected through water sampling from *Lake of Campus* A. *Samples* were taken from three identical points, taking into account depth variations to reflect the diverse *microbial* conditions in various layers of the lake. The selection of sampling points was carried out to obtain a representative picture of the distribution of *microorganisms* in the lake. After sampling, water samples were homogenized with 0.85 % *NaCl* solution to ensure even distribution of *microorganisms*. Observations of bacteria were carried out after the samples were isolated and incubated with *Nutrient Agar (NA)* media at 37 °C for 24 hours, to support the growth of bacterial colonies. The data collected included *macroscopic* characteristics, such as shape, color, edge, and elevation of colonies observed visually, as well as *microscopic* data including cell shape, *Gram* properties, and colony color analyzed under a *microscope*. The purpose of this data collection is to understand the diversity of *microbes* in the lake and the interactions of *microorganisms* in the *aquatic ecosystem.*

Procedure

This procedure is carried out by performing *serial dilution* using 0.85 % physiological *NaCl* solution until reaching a dilution level of 10^{-8}, to reduce the density of *microorganisms* and facilitate the *isolation* of single colonies on the *media*. Each dilution is *inoculated* into *Nutrient Agar (NA)* media using the *spread plate method*, then *incubated* for 24 hours at 37 °C. This procedure is carried out to obtain isolated *bacterial colonies* and to characterize their *macroscopic* and *microscopic* characteristics, which are the basis for further *identification* and *classification*. The staining results show that *Gram-positive* bacteria are purple, while *Gram-negative* bacteria are pink (Sumarmono & Wijayanto, 2019; Martinez et al., 2018).

Data analysis

Data analysis was carried out in two stages, namely *descriptive analysis* and *identification* and classification of microorganisms. First, data from *macroscopic* and *microscopic* observations were analyzed descriptively to describe the main characteristics of *bacteria*, such as colony morphology, cell shape, and *Gram* properties. Furthermore, data from *Gram* staining and morphological observations were used in the identification process. Classification of microorganisms was carried out based on *cell* shape (e.g., *coccus or bacillus*), and *Gram* properties (Madigan et al., 2018).

RESULT AND DISCUSSION

The results of the study on the *Isolation* of *Bacteria* in lake water on *Campus A*. The bacteria obtained were *Gram-negative bacteria*. Based on their *morphological* form, *bacteria* are divided into 3, namely round (*coccus),* long (*bacillus*), *spiral* . While from the results of the research conducted, the form of *bacteria* obtained was only a long form (*bacillus)* . And the results of the bacteria obtained were in the *dilution* stages 10^{-8} that grew only four *petri dishes*.

Table 1. Characterization Results of Bacterial Isolates in Campus-A Lake Water

Observation	Key characters	Isolate KD.1	Isolate KD.2	Isolate KD.3
Macroscopic	Colony form	*Circular*	*Irregular*	*Circular*
	Colony edge	*Entire*	*Lobate*	*Entire*
	Colony color	Yellow	Yellow	White
	Colony elevation	*Flat*	*Flat*	*Flat*
Microscopic	Cell shape	*Bacil*	*Coccus*	*Coccus*
	Properties of grams	-	-	-
	Colony color	Red	Red	Red

Information :
Irregular (irregular shape)
Circular (round)
Flat (flat surface with medium)
Entire (flat edge)
- : negative result
Lobate (notched edge)
Bacill (stem)
Cocus (round)

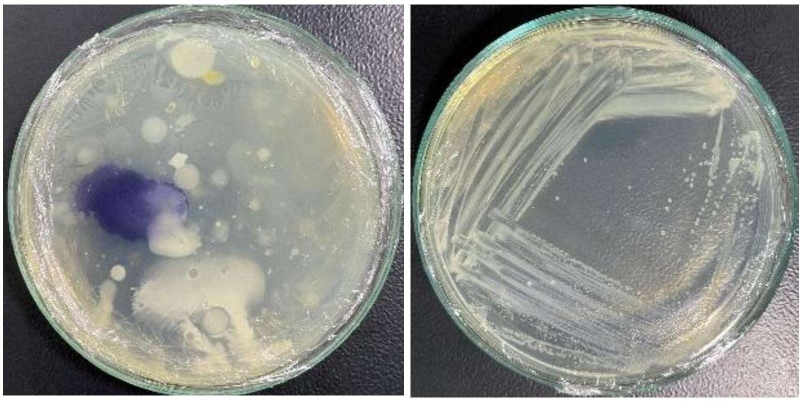

Figure 1. Macroscopic Observation of Bacterial Isolates from Campus-A Lake Water

Based on table 1, the *colony shape*, *colony edge*, *colony color*, and elevation (*colony* surface) have different results. This can be seen in figure 1 above based on *macroscopic* morphological observations. *Colony* morphological observations in the three isolates (KD.1, KD.2, KD.3) have similar *colony* shapes, namely *circular* (round) with Isolate KD.2 in the form of *Irregular* (irregular). The edges of the *colonies* of Isolates KD.1 and KD.3 have similar *Entire* (flat) *colony* edges, while Isolate KD.2 has *Lobate edges* (curved edges). The color of the *colonies* of Isolates KD.1 and KD.2 is yellow while Isolate Kd 3 is white. The elevation (colony surface) of Isolates KD.1, KD.2, KD.3 has a *flat* surface (flat surface with the medium).

Based on Table 1, the results of *microscopic* observations show differences in cell shape, *Gram* properties, and color of bacterial colonies. This is also seen in Figure 2, where observations of isolates KD.1, KD.2, and KD.3 show similarities in *Gram* properties, namely negative. The cell shape of isolates KD.2 and KD.3 is *coccus* (round), while isolate KD.1 has a *bacillus* shape (rod). The colony color of the three isolates is similar, namely red.

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

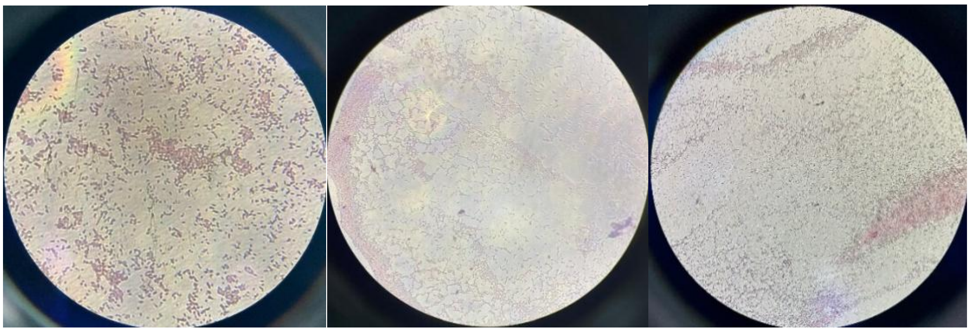

Figure 2.A Figure 2.B Figure 2.C

Figure 2. Figure 2.A shows isolate KD 1, which has a rod-shaped (*bacillus*) cell shape with red-colored colonies, while Figures 2.B and 2.C show isolates KD 2 and KD 3, each having a coccus cell shape and red-colored colonies. *Microscopic* observation of bacterial isolates from Campus A Lake water was carried out using 40x and 100x objective lenses with a 10x ocular lens, resulting in total magnifications of 400x and 1000x, respectively. Figure 2.A was observed using a 100x objective lens with a 10x ocular lens (1000x), while Figures 2.B and 2.C were observed using a 40x objective lens with a 10x ocular lens (400x).

Gram staining technique is used as a method to analyze bacterial characteristics, such as *Gram* properties, cell shape, and cell arrangement patterns (Ely et al., 2017). The results of morphological analysis showed that isolates KD.1, KD.2, and KD.3 have the characteristics of *Gram-negative* bacteria with heterogeneous cell morphology, including *coccus* and *bacillus* forms, and varied cellular arrangement patterns. *Gram-positive* properties occur because the isolate can retain *crystal violet* dye (*Gram A*), so that the bacterial cell wall does not absorb *safranin* dye (*Gram D*). Conversely, *Gram-negative* properties occur because the isolate is unable to retain *crystal violet* dye (*Gram A*), so that its cell wall can absorb *safranin* dye (*Gram D*) (Ely et al., 2017; Laila et al., 2019; Widyaningsih, 2016).

The characteristics of *Gram-negative* bacteria show that they have a complex cell wall with a *lipopolysaccharide (LPS)* layer that provides protection against extreme conditions and some types of antibiotics. According to Ely et al., (2017), this layer makes *Gram-negative* bacteria tend to be more resistant to antimicrobial compounds. The *Gram* staining method provides benefits for identifying the nature of bacterial cell walls, which affect their sensitivity to antibiotics. Martinez et al., (2018) stated that this technique is often used in environmental microbiology research to study water quality.

Water samples from the lake which were given additional *Eco Enzyme* show significant changes in community *microbiota* in aquatic ecosystems. *Eco Enzyme*, produced from the fermentation of organic materials such as fruits, vegetables, and sugar, contains various types of bacteria, enzymes, and bioactive compounds that can affect the microbial community in lake water (Bunyamin, 2020). *Eco Enzyme* contains decomposing bacteria that help accelerate the decomposition process of organic matter in lake water. Bacteria such as *Lactobacillus*, *Bacillus*, and other decomposing bacteria play an important role in decomposing organic matter in lakes. This process can reduce the organic load that damages water quality and increase dissolved oxygen levels (Liu et al., 2018).

According to Pan (2020), the addition of *Eco Enzyme* can affect the composition of the bacterial community in the lake. Several studies have shown that *Eco Enzyme* can increase the number of probiotic bacteria that are beneficial to aquatic ecosystems, while reducing pathogenic bacteria that can harm aquatic organisms. Bacteria such as *Pseudomonas*, *Bacillus*, and *Escherichia* can thrive in the presence of *Eco Enzyme*, because they can utilize organic matter that is decomposed by the enzymes in *Eco Enzyme*.

Eco Enzyme contains various types of enzymes produced by microorganisms in the fermentation process. Enzymes such as *protease*, *lipase*, and *amylase* support bacteria in decomposing organic matter in lake water (Le et al., 2021). These enzymes also affect the metabolism of local bacteria, encouraging more efficient bacterial growth in the decomposition process. *Eco Enzyme* can act as a natural control agent for pathogens in lake water. Some bacteria in *Eco Enzyme*, such as *Lactobacillus* and *Bacillus*, have antimicrobial properties that can inhibit the growth of pathogenic bacteria such as *Escherichia coli* and *Vibrio*, which are often found in polluted waters (Bunyamin et al., 2020).

Thus, *Eco Enzyme* can help stabilize the microbial ecosystem in lakes and prevent ecological disturbances. *Eco Enzyme* can improve the balance of bacteria in lake water by strengthening the activity of beneficial decomposing bacteria, reducing the number of pathogenic bacteria, and improving water quality and the health of aquatic ecosystems.

Bacteria play an important role as decomposers in lake ecosystems, which are responsible for decomposing dead organic matter, such as leaves, dead animals, and other organic waste into simple compounds. These compounds can then be utilized by other organisms such as *phytoplankton* and aquatic plants as a source of nutrients. Research by Wu et al., (2020) shows that bacteria play a key role in the carbon and nitrogen cycles, which are two important biogeochemical cycles for ecosystem balance. In the context of polluted lakes, decomposer bacteria can also help reduce the accumulation of organic matter that can trigger *eutrophication*.

Bacteria that can break down complex organic matter have great potential for use in *bioremediation*, an environmentally friendly water pollution treatment technique. Ma et al., (2022) explained that natural bacteria from the local environment can help break down difficult-to-decompose organic compounds, such as pesticides and hydrocarbons, without the need for chemicals. Bacteria-based *bioremediation* technology includes methods such as *bioaugmentation*, which is the addition of certain bacteria to speed up the decomposition process. He et al., (2019) stated that bacterial *biofilters* are increasingly popular in wastewater treatment.

This study provides important insights for lake management as an essential resource, especially to meet the needs of clean water for humans, the environment, and various economic activities. This study can be a basis for designing sustainable management strategies to overcome water pollution caused by domestic waste, industry, and agricultural activities. In addition, these findings support mitigation measures for the impact of climate change, which worsens water conditions by increasing temperatures, accelerating *eutrophication*, and changing rainfall patterns. The use of natural bacteria and *eco-enzymes* offers an environmentally friendly and more cost-effective solution compared to chemical methods. By involving local communities in its implementation, this strategy not only helps preserve aquatic ecosystems but also encourages public awareness of the importance of protecting the environment. This step can create a positive long-term impact on environmental sustainability and community welfare, as expressed by Bi et al., (2018). The results of this study are an important basis for formulating integrated environmental policies based on scientific evidence to face various global challenges.

CONCLUSION

The results of this study show that three bacterial isolates were successfully obtained from the water of Campus A Lake. The *Gram* test showed a red color in the bacteria, confirming that they are *gram-negative*. The first isolate has a rod-shaped cell (*bacillus*), while the other two isolates are round (*coccus*). All three isolates showed *gram-negative* properties, as indicated by the red color in the *Gram* staining results. Most of the identified bacteria exhibited the ability to decompose organic matter, indicating their potential for *bioremediation* applications. The addition of *Eco Enzyme* to lake water had a positive impact on the microbial ecosystem, promoting the growth of decomposing bacteria such as *Bacillus* and *Lactobacillus*, while reducing the number of pathogenic bacteria. This study provides a deeper understanding of the presence and characteristics of bacteria in Campus A Lake, which can serve as a basis for water quality management and the development of *bioremediation* technology to reduce pollution.

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

Anggriani, L., Manurung, A. Q., Marsa, A.D., Ritonga, N. H., Alinza, C., Ningsih, N. G. A., & Saputra, M. A. J. (2025). Characteristic Isolation of Bacteria in Lake Water on Campus-A of UIN Sulthan Thaha Saifuddin Jambi. *Jurnal Pembelajaran dan Biologi Nukleus*, *11*(2), 546-551. https://doi.org/10.36987/jpbn.v11i2.6846

Conflict of interest : The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions : All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was submited by [**Latusi Anggriani**]. All authors contributed on previous version and revisions process of the manuscript. All authors read and approved the final manuscript.