JOURNAL OF ARTHA BIOLOGICAL ENGINEERING

Journal Homepage : https://jos.joabe.id

Study of the Characteristics of Bacteria Isolated from the Pelus River, Banyumas Regency

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Article Information

Article history :
Received August 08, 2023
Accepted October 11, 2023
Available online November 12, 2023

Keywords :
Enzyme activity, bacterial communities, microbial ecology,

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Abstract

Community activities around the Pelus River cause pollution which affects the bacterial community found in the river. The aim of this research was to determine the characteristics of bacteria isolated from the Pelus river environment in Banyumas Regency. The method used in this research is an exploratory method where bacterial sampling is carried out at 3 different stations. The samples taken were then isolated, observed colony morphology, as well as biochemical and physiological characterization of the bacteria. The research results obtained 10 different isolates. A total of 10 isolates were obtained based on morphological differences. The characteristics of most of the Pelus River bacteria show variations ranging from enzymatic to environmental tolerant which describes the waters of the Pelus River. Bacteria isolated from the water have characteristics that identify them as bacteria capable of degrading compounds.

DOI : https://doi.org/10.62521/6wsyrt43

Introduction

The Pelus River is a river that stretches from the upstream in the Baturaden area to the downstream in the Sokaraja area, Banyumas Regency (Nugrayani et al., 2023). Pelus River originates at the foot of Mount Slamet, which gives it a natural quality. However, the middle and downstream parts of the Pelus River, residential areas and community activities around the river influence the condition of the aquatic environment due to the disposal of waste from community activities (Lukmanulhakim et al., 2023). Several community activities in the Pelus River environment, such as agriculture, fishing and household waste, result in pollution that can affect microorganisms, especially bacterial communities (Yogafanny, 2015).

Bacteria are microorganisms that have a very important role in an ecosystem (Meng et al., 2022). One of the roles of bacteria is to degrade complex organic compounds into simpler ones so that they do not become toxic in the environment (Ayilara & Babalola, 2023). The bacterial mechanism for degrading organic compounds in the environment uses metabolic products in the form of excreted degradation enzymes such as protease, amylase, cellulase and lipase (Indrayani et al., 2022). This ability can be the interaction of bacteria with other organisms which influence each other in an ecosystem, so it is necessary to study the characteristics of bacteria in an environment.

Bacterial characterization is the basic technique used to view bacterial characters. One method that can be used is characteristics based on extracellular enzyme activity and biochemistry (Jackson et al., 2013; Quintin et al., 2021). Several conditions in the aquatic environment can affect the presence and characteristics of bacteria. Some bacteria will perform roles such as degrading aquatic organic compounds. However, under certain conditions it can be pathogenic for other organisms (Cabral, 2010; Joutey et al., 2013). This research aims to determine the characteristics of bacteria isolated from the Pelus river environment in Banyumas Regency.

Materials and methods

Research Materials

The tools used in this research include autoclaves, pipettes, Erlemeyers, Bunsen burners, Petridish, digital scales with an accuracy of 0.0001 grams, measuring cups, measuring needles, pastel pellets, incubators, test tubes, vortexes, water baths, microwaves, sterile glass bottles, thermometer, pH meter, and dropper pipette. Materials used in research on digestive organs include NA (Nutrient Agar), NB (Nutrient Broth) Sterile NaCl 0.9%, sterile skimmed milk powder, tapioca, iodine solution, distilled water, gum arabic, $CaCl_2$, olive oil and congo red 0.1%, TSIA Media, MRVP, SIMA, H_2O_2, Gram Reagent.

Research Methods

Results were analyzed through a descriptive approach using an exploratory methodology. Observations include bacterial inoculation, colony morphology, biochemical and physiological tests.

Bacterial Isolation

Water samples were taken from three stations in the Pelus River area, namely Pabuaran, Karangwangkal and Arcawinangun villages. Then, the water samples obtained were homogenized in one container to describe the bacterial population in the Pelus River. Samples were diluted to a dilution of 10^{-1}-10^{-5}. Each dilution sample was cultured on NA growth media (nutrient agar) using the pour plate method and incubated at 28˚C for 48 hours. Then, the bacteria that grow were isolated based on different colony morphology to obtain a pure culture.

Biochemical and physiological characterization of bacteria

Characterization of bacteria was conducted based on biochemistry and physiology. Biochemical characters include motility, Gram stain, catalase, oxidase, oxidation-fermentation, lactose, glucose, sucrose, proteolytic, amylolytic, lipolytic and cellulolytic. Physiological characteristics include pH (4,7,9), temperature (4℃,30℃,45℃) and osmotic on salt (0.8%, 3%, 5%).

Data Analysis

Data analysis was conducted from morphological and biochemical data to determine the characteristics of bacteria. Aquatic environments were described and tested using physiological data.

Results and Discussion

Descripsion Of Research Location

The location chosen as the sampling location is an area consisting of residential areas because this area is a campus area. The waste from residential areas enters directly into river bodies, causing changes in bacterial communities. Additionally, other activities such as agriculture and fishing also affect the river environment by discharging residual waste or fertilizer.

Isolation and colony morphology

According to the results of bacterial isolation from the Pelus River area cultured on growth media, 10 isolates were found with different colony morphologies. Colony morphology includes shape, edges, elevation, colour and size. Each isolate that shows morphological differences is used as

Table 1. Morphological characteristics of bacterial colonies

Isolate code	Colony morphology				
	Shape	Elevation	Edge	Colour	Size
SP01	Circular	Convex	Entire	Deep yellow	Medium
SP02	Circular	Convex	Entire	Deep yellow	Big
SP03	Irregular	Convex	Lobate	Cloudy white	Big
SP04	Circular	Convex	Entire	Yellow	Medium
SP05	Irregular	Flat	Lobate	Deep yellow	Medium
SP06	Irregular	Flat	Lobate	Yellow	Big
SP07	Circular	Convex	Entire	Transparent	Small
SP08	Circular	Convex	Entire	Yellow	Small
SP09	Circular	Flat	Lobate	Yellow	Medium
SP10	Circular	Convex	Entire	Shiny yellow	Big

a basis for distinguishing bacterial species (Sousa et al., 2013). The morphology of bacterial colonies isolated from the Pelus River is presented in Table 1.

Observations of colony morphology showed that most bacterial isolates had a circular shape, but some had irregular shapes. Convex and flat elevations, entire and lobate edges. Colony color is transparent, white, shiny yellow to dark yellow. Colony sizes vary from small, medium to large. The difference between each isolate is the initial standard in the bacterial identification process. According to (Anbari *et al.*, 2022), the morphological characteristics of different colonies can be used as a starting point for bacterial determination.

Biochemical characteristics of bacteria

A total of 10 isolates were isolated based on colony morphology and then biochemical testing was carried out to determine the biochemical reaction of bacteria to a compound.

The motility tests of isolates SP07, SP09, and SP10 showed a negative result, indicating that these bacteria were not motile. The movement of motile bacteria is characterized by the presence of a means of movement for the bacteria in the form of flagella or gliding motility, while non-motile bacteria do not have flagella or gliding motility. Thus, isolates SP07, SP09, and SP10 do not have movement tools such as flagella or gliding motility. The findings of Damayanti et al. (2018) confirm that the RS1b bacterial isolate showed a negative motility test, which indicates the bacteria are not motile, as they do not spread and accumulate in the middle of the cells, but

Table 2. Bacterial biochemical test

Isolate code	Motility	Gram stain	Oxidase	Catalase	Source of sugar			O/F	Cell shape
					G	S	L		
SP01	+	-	+	+	+	-	-	Fermentative	Coccus
SP02	+	+	+	+	-	+	+	Oxidative	Coccus
SP03	+	+	-	-	-	-	-	Fermentative	Coccus
SP04	+	+	+	-	+	-	+	Oxidative	Coccus
SP05	+	+	+	+	+	-	-	Oxidative	Bacil
SP06	+	+	+	+	-	+	+	Oxidative	Coccus
SP07	-	+	-	+	+	-	-	Fermentative	Coccus
SP08	+	+	+	+	+	-	-	Fermentative	Coccus
SP09	-	+	-	-	-	-	-	Oxidative	Coccus
SP10	-	+	-	+	+	-	-	Fermentative	Coccus

G=glucose, S=Sucrose, L=lactose

rather they only grow and collect at the middle so that they can stand upright. Pelczar and Chan (1986) added that the motility test on bacteria aims to determine whether there is cell movement or not.

The color differences in both gram-negative and gram-positive bacteria are caused by differences in cell wall structure between the two types of bacteria (Rani et al., 2017). According to Breijyeh et al., (2020), the cell wall of gram-negative bacteria consists of several layers of peptidoglycogen and an outer membrane, when gram staining is carried out it will produce a red color from the safranin color on the outer membrane, while the gram-positive cell wall consists of layers of peptidoglycan, where the outermost part is the peptidoglycan layer so that when stained it will produce a purple color from the crystal violet color in the peptidoglycan part (Garde et al., 2021). Similarly, Fitri & Yasmin (2011) explain that the cell wall structure of gram-positive bacteria produces thick peptidoglycan while gram-negative bacteria produce large amounts of lipids.

Tests and observations of river water bacteria in the upstream, middle, and downstream areas of Pelus River Purwokerto resulted in the identification of ten isolates. A total of 10 isolates were obtained, almost all in the form of coccus and one in the form of bacillus.

A bacterial isolate was tested for its ability to produce cellulase, protease, amylase and lipase using selective media. Apart from being a basis for identification, proteolytic, amylolytic, cellulolytic and lipolytic activity tests are also discussed to determine the degradation of compounds in the environment (Thatoi et al., 2013). The results showed that 7 isolates produced hydrolytic enzymes. The seven bacterial isolates are SP01, SP02, SP03, SP04, SP05,

Table 3. Enzymatic activity of bacteria

Isolate code	**Cellulolytic**	**Proteolytic**	**Amylolytic**	**Lipolytic**
SP01	-	+	-	+
SP02	-	+	+	-
SP03	-	+	-	-
SP04	-	-	-	+
SP05	-	+	-	-
SP06	-	-	-	-
SP07	-	-	-	-
SP08	-	-	-	+
SP09	-	+	-	-
SP10	-	-	-	-

Table 4. Tolerance of environmental conditions

Isolate code	pH			Temperature			Osmotic		
	4	7	9	4°C	37°C	45C	0,85%	3%	5%
SP01	-	+	+	-	+	+	+	+	+
SP02	-	+	+	-	+	+	+	+	-
SP03	-	+	+	-	+	+	+	+	+
SP04	+	+	+	-	+	+	+	+	+
SP05	-	+	+	-	+	+	+	+	+
SP06	-	+	+	-	+	+	+	+	+
SP07	+	+	+	-	+	-	+	+	+
SP08	+	+	+	-	+	+	+	+	+
SP09	+	+	+	-	+	-	+	-	-
SP10	-	+	+	-	+	+	+	-	-

SP08 and SP09. Bacteria producing hydrolytic enzymes are shown in table 3. Most of the enzyme producing bacteria are shown in proteolytic and lipolytic bacteria. The Pelus river contains protein in the form of organic matter which can be degraded by proteolytic bacteria (Nurhafid *et al.*, 2021). Additionally, lipolytic bacteria indicate that the Pelus River contains oil derived from household waste.

In Table 4, it is indicated that isolates SP01, SP02, SP03, SP05, SP06, and SP10 cannot live at pH 4, as indicated by a negative reaction or by the color of the culture media being the same as the control media. Nonetheless, the isolates were able to survive at pH 7 and pH 9, which was marked by a positive reaction. This means that these isolates are mesophilic and basophilic. Meanwhile isolates SP04, SP07, SP08 and SP09 are able to live at pH 4, pH 7 and pH 9, this is indicated by a positive reaction or a different color from the color of the control medium, meaning that SP04, SP07, SP08 and SP09 are isolates that are acidophilic, mesophilic, and basophilic. Therefore, SP01, SP02, SP03, SP05, SP06, and SP10 can be developed in areas of near neutral or alkaline acidity, while SP04, SP07, SP08 and SP09 can be developed in areas of high acidity, near neutral, or basic acidity. Lay et al. (1999) stated that bacteria are generally able to grow well at pH 7 but are still able to grow in the range of 5.0 - 8.0.

Some of the bacterial isolates that were isolated from the Pelus River had varying characteristics. According to morphological characteristics determined in the community to distinguish types of siolates obtained, there are different types of siolates. The enzymatic activity produced by bacteria shows a picture of the role of bacteria in the Pelus River aquatic environment, where bacteria can degrade compounds that enter the water so that there is balance in the environment. Further studies will be needed to determine how potential bacterial communities can be used as biocontrol agents and environmental indicators in the future.

Conclusion

Isolation of bacteria from the Pelus River resulted in 10 isolates based on morphological differences. Pelus River bacteria have a wide range of characteristics, ranging from chemical to environmental conditions, reflecting the condition of the waters in the river.

Acknowledgement

The author would like to thank Laboratorium Mikrobiologi. Faculty of Biology. Jenderal Soedirman University.

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