



Identification of Pathogenic Bacteria on Carp Commodities (*Cyprinus carpio*) at Quality Control and Fishery Product Safety Agency (BKIPM) of Bengkulu

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Abstract

Carp (*Cyprinus carpio*) is a type of freshwater fish that is widely cultivated. The increase in the amount of production and trade in freshwater fishery commodities both for consumption in Bengkulu will potentially increase the risk of entry and spread of pests and diseases in fish, which at the same time will be a threat that can endanger and damage the sustainability of fishery biological resources. Bacteria that infect fish can inhibit the expected production targets, which is an outbreak of pathogenic fish disease caused by bacteria. This study aims to identify pathogenic bacteria that infect Carp (*C. carpio*). Carp were obtained from fish traders at Panorama Market, Bengkulu City. Carp samples were selected based on clinical symptoms that were no longer healthy. Isolation of bacteria from Carp's organs using Tryptic Soy Agar (TSA) media. The isolates were screened by morphological characters and biochemical test. The results of this study showed that total of 2 bacteria were isolated. Based on biochemical tests carried out such as the Simmons Citrate test, Triple Sugar Iron Agar, Oxidative-Fermentative, Motility Indol Ornithine, Lysine Iron Agar, MR-VP, urea, catalase, oxidase, gelatin, confectionery test, and Rimmner-Shotts test, pathogenic isolates Sp 1. in the sample have a close relationship with *Plesiomonas shigelloides* while the pathogenic isolates Sp 2. and Sp 3. have a close relationship with *Aeromonas hydrophila*.

Keywords: *Cyprinus carpio*, *Aeromonas hydrophila*, *Plesiomonas shigelloides*

1. Introduction

The potential for freshwater fish cultivation is very large to be developed, one of which has the potential to cultivate freshwater fish commodities as carp. Carp (*Cyprinus carpio*) is one of the most widely cultivated types of freshwater cultivated fish because it has good prospects for development because it is popular with the community and has high economic value (Yulvizar et al., 2014). An increase in the amount of production and trade in carp commodities in Bengkulu has the potential to increase the risk of entry and spread of pests and diseases in fish, which at the same time will be a threat that can endanger and damage the sustainability of fishery biological resources.

One of the most dangerous diseases is bacterial infection or bacterial disease. Bacterial diseases that may attack freshwater fish include *Aeromonas hydrophila*, *A. salmonicida*, *Pseudomonas anguilliseptica*, *Streptococcus* (Murwantoko et al., 2013). Disease cases caused by bacteria can cause huge losses to cultivation activities, for example, mass mortality. One of the cases of the Motile Aeromonas Septicemia (MAS) disease outbreak was caused by *Aeromonas hydrophila*. Based on the description above, it is necessary to conduct research on the identification of bacteria in fisheries commodities, especially carp. This aims to determine the types of pathogenic bacteria that can attack carp and efforts to prevent them.

2. Materials and Methods

2.1. Materials

The tools and materials used in this study were Petri dishes, test tubes, Erlenmeyer, loop needles, glass objects, incubators, refrigerators, analytical scales, ovens, test tube racks, trays, spatulas, measuring cups, spray bottles, surgical instruments, beakers glass, bunsen burner, micropipette, tube, UV-Laminar flow-hood, magnetic stirrer, hotplate, autoclave, stationery, Carp (*Cyprinus carpio*), 70% alcohol, spiritus, absolute alcohol, iodine, paraffin, oxidase paper, KOH 40%, 3% KOH solution, 3% H₂SO₄ solution, aqua dest, Kovack's solution, Methyl Red solution, α -naphthol solution, paraffin, Simmons Citrate Agar media, Tryptic Soy Agar (TSA), Triple Sugar Iron Agar, Oxidative-Fermentative media, Motility Indole Ornithine, Lysine Iron Agar, MR-VP, Urease, Phenol Red Broth Base, Glucose, Maltose, Lactose, Mannitol, Sorbitol, Gelatin, RS Media, cotton, tissue, label paper, wrapping paper, matches, rubber bands, aluminum foil, and bacteria identification forms.

2.2. Carp (*Cyprinus carpio*) Sample Collection

Samples were obtained from fish traders at Panorama market, Bengkulu City. Carp samples selected that had clinical symptoms that were no longer healthy. Furthermore, in living conditions, samples were analyzed at the Testing Laboratory, Fish Quarantine Station, Quality Control, and Safety of Fishery Products (SKIPM) Bengkulu.

2.3. Isolation of Target Pathogenic Bacteria from Carp (*Cyprinus carpio*) Samples

The body surface of the fish was cleaned with iodine using a clean tissue. Then, the stomach dissected to reveal the internal organs. Furthermore, bacteria were isolated from the target organs, namely the kidneys and liver using an inoculation loop that had been glazed over the bunsen onto the TSA media with streak method and incubated in the incubator for 1x24 hours at room temperature. Isolates grown on the media were inoculated and purified.

2.4. Identification of Pathogenic Bacterial Colony Characteristics by Biochemical Test

Identification of pathogenic bacterial colonies using biochemical test, such as Potassium hydroxide Test, catalase test, oxidase test, gelatin test, Simmons Citrate test, Triple Sugar Iron Agar test, OF test, MIO test, Lysine Iron Agar test, MR-VP test, Urea test, Carbohydrate Tests including glucose, maltose, lactose, mannitol, and sorbitol as well as Rimlerr-Shottmedia test.

2.5. Identification of Pathogenic Bacterial Isolates from Target Organs, Kidney and Liver of Carp (*Cyprinus carpio*)

Identification of the type of pathogenic bacteria carried out by adjusting the test results and characteristics of the bacteria that were available on the identification sheet using the Manual for the Identification of Medical Bacteria (Cowan, & Steel, 1993) and the Standard Method of HPIK Examination, PUSKARI (Septiama et al., 2010).

3. Results and Discussion

External morphology of Carp (*Cyprinus carpio*) has wounds or red spots on the body surface of the fish and loss of scales. The symptoms of fish attacked by the bacterium *Aeromonashydrophila* show wounds (red spots) on the body surface, bleeding in the gills, and a distended stomach. Observation of Bacterial Colony Morphology in Carp can be seen in (Table 1) and (Figure 2) (Pratama et al., 2017).

Table 1. Identification of Pathogenic Bacterial Colony Morphology

Bacterial Morphology	Isolated Organs		
	Heart	Liver	Kidney
Color	Cream	Cream	Cream
Margin	Entire	Entire	Entire
Elevation	Convex	Convex	Convex
Form	Circular	Circular	Circular

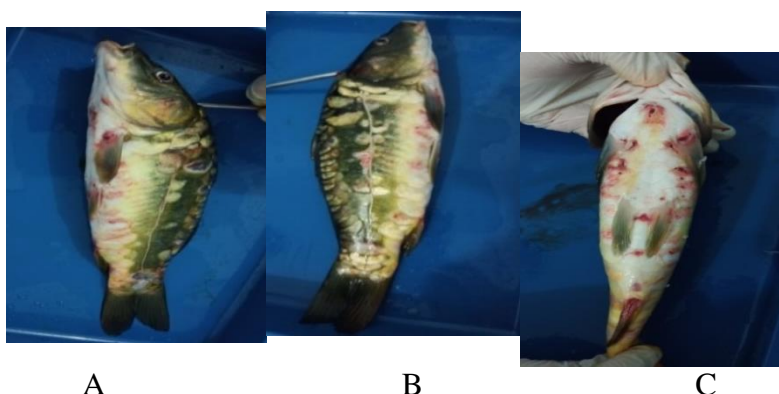


Figure 1. External morphology of the body of a Carp (*Cyprinus carpio*) on the (A) Left side 1, (B) Right side (C) Bottom side

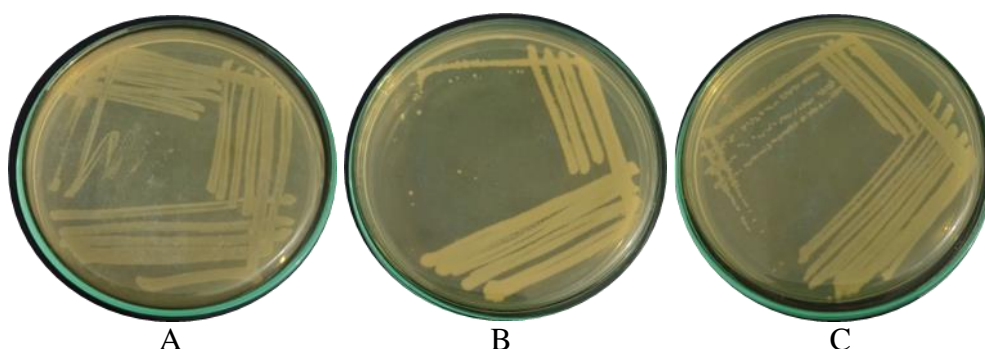


Figure 2. Photogenic Bacterial isolates from sample organs grown on Trypticase Soy Agar (TSA) media and incubated at 25 °C for 1x24 hours (A) Heart, (B) Liver (C) Kidney in Carp (*Cyprinus carpio*).

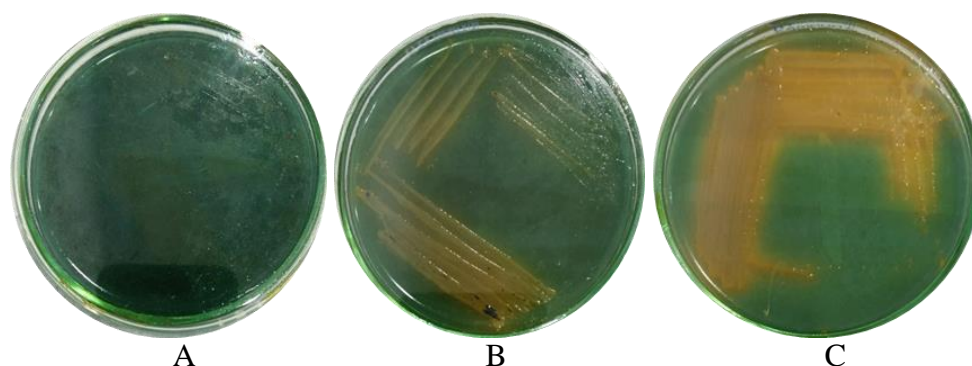


Figure 3. Photogenic Bacterial isolates from sample organs grown on Rimlerr-Shotts (RS) media and incubated at 25°C for 1x24 hours (A) Heart, (B) Liver (C) Kidney in Carp (*Cyprinus carpio*).

Table 2. Identification of phatogenic bacteria by biochemical test

Biochemical Test	Isolated Organs			Note
	Heart	Liver	Kidney	
Potassium hydroxide Test	+	+	+	Solution be viscous and form a mucoid string purple color
Oxidase Test	(Gram-negative)	(Gram-negative)	(Gram-negative)	
Catalase Test	+	+	+	Bubbles of oxygen

The three phatogenic bacterial isolates from organ samples were gram-negative bacteria, catalase-positive with the formation of gas bubbles because the bacteria could produce catalase enzymes, and able to decompose H_2O_2 (Hydrogen Peroxide), and positive oxidase to produce a purple color on oxidase paper. Gram-negative bacterial cells will break down so that they will produce mucus that comes from bacterial fat, while Gram-positive will not produce mucus.

Tabel 3. Identification of Pathogenic bacteria by biochemical test

Biochemical Test		Isolated Organs			Positive Control	
		Heart	Liver	Kidney	<i>Aeromonas hydrophila</i> (1)	<i>Plesiomonas shigelloides</i> (2)
MIO Test	Motility	+	+	+	+	+
	Indole	+	+	+	+	+
	Ornithine	+	-	-	-	+
Gelatin Hydrolysis Test		+	-	-	+	+
MR-VP Test	MR	+	-	-	No data	No data
	VP	-	+	+	Variable	-
Carbohydrate Tests	Glucose	+	+	+	+	+
	Lactose	+	-	-	Variable	+
	Maltose	+	+	+	No data	No data
	Mannitol	+	+	+	No data	+
	Sorbitol	-	-	-	Variable	-
Oxidative-Fermentative Test		F	F	F	F	F
Triple Sugar Iron Test	Slant/Butt	K/A	K/A	K/A	No data	No data
	H ₂ S	-	-	-	+	-
	Gas	-	+	+	No data	No data
Simmons Citrate Agar Test		-	+	+	+	-
Lysine Iron Agar Test	Lysine	-	+	+	+	-
	Decarboxylase	-	+	+	+	-
Lysine Deaminase Test	Lysine	+	-	-	-	+
	Deaminase	+	-	-	-	+
Urea Test		-	+	+	No data	-
Rimner-Shotts Agar		-	+	+	+	-

Control sources: 1. Pathogenic bacterial identification book from the testing laboratory, Fish Quarantine Station, Bengkulu Fishery Product Quality and Safety Control.
 2. Identification results from the research of Behera et al. (2018)

In the motility test, the three sample isolates have positive results, which means that the bacteria were motile. In the Indol test, the three isolates samples have positive results, which means that the isolate bacteria were able to produce indole from the amino acid tryptophan through the tryptophanase enzyme. Ornithine test results, bacterial isolates from heart sample organs showed positive test results, while bacterial isolates from liver and kidney sample organs showed negative test results. Bacteria can produce indole from tryptophan through the tryptophanase enzyme and have the ornithine decarboxylase enzyme MIO test (Motility, Indole, Ornithine) (Tantu et al., 2013).

In the gelatin test, bacterial isolates from heart samples showed positive results, while bacterial isolates from liver and kidney samples showed negative results. Some bacteria have the gelatinase enzyme with gelatin unable to form and will become liquid (Anggraini et al., 2016).

The results of the MR (Methyl Red) test showed that the three bacterial isolates produced different results. Bacterial isolates from liver and kidney samples showed negative results. While bacterial isolates from heart samples showed positive results. The VP test results (Voges Proskauer) on bacterial isolates from heart samples showed negative results. Meanwhile, bacterial isolates from liver and kidney samples showed positive results. The MR test is to see the ability of bacteria to oxidize glucose by producing acid at high concentrations, and the VP test to detect acetoin in bacterial cultures. A positive result will indicate a change in color to red, while a yellow-brown color indicates a negative result (Hemraj et al., 2013).

In the OF test (oxidative or fermentative), the three isolates showed a change in the color media from blue to yellow, both in the tube with paraffin or not. The principle of the oxidative/fermentative test of the media covered

with paraffin changes color from blue to yellow, so bacteria can utilize carbohydrates in anaerobic conditions and are said to be fermentative (Purnamawati, 2016).

In the TSIA (Triple Sugar Iron Agar) test, the three bacterial isolates had a color change, red on the slant (oblique part) that showed the bacteria are saline (alkaline), and yellow on the butt (stem part) that showed the bacteria are acidic (acidic). The three isolates did not produce H₂S. Bacterial isolates from liver and kidney samples showed gas, and bacterial isolates in the heart samples had no gas. TSIA media used to determine the ability of bacteria to use glucose, lactose, sucrose, and the ability of bacteria to produce gas or hydrogen peroxide (H₂O₂) (Purnamawati, 2016).

In the Lysine Iron Agar Test, the positive results of lysine deaminase in bacterial isolates from the heart organ. While positive results of lysine decarboxylase on bacterial isolates from the liver and kidneys. The results of the lysine decarboxylase test that showed positive results were purple (purple) isolates on all parts both on the bottom of the media and the slanted part of the media and able to deaminase lysine showed a faded or yellow color change (Rondonuwu et al., 2014).

In the Simmons citrate test, bacterial isolates in the heart samples showed negative results, while the bacterial isolates in liver and kidney samples showed positive results. Simmons citrate media contains the BTB indicator (BromThymol Blue), bacteria use citrate as a carbon source (Chandra and Mani, 2011).

In the urea test, bacterial isolates from liver and kidney samples showed positive results, while bacterial isolates from heart samples showed negative results. The urea test aims to determine the ability of bacteria to convert urea into ammonia (Ulfa et al., 2016).

In the carbohydrate tests, the three pathogenic bacterial isolates showed positive results in the glucose, maltose, and mannitol tests, while the three bacterial isolates showed negative results in sorbitol. In the lactose test, only bacterial isolates from the heart organs showed negative results, while bacterial isolates from the liver and kidneys showed positive results. *Aeromonas* sp. can ferment glucose and mannitol (Septiama et al., 2010).

In the RS (Rimmler-Shotts) media, the Sp 1. Isolate collected from the heart organ showed green colony color and based on the results of the biochemical test these bacteria were included in the *Plesiomonas* group. *Plesiomonas shigelloides* originated from organ samples of the heart. This is also indicated by the clinical symptoms present in Carp (Behera et al., 2018). Whereas, the Sp 2. and Sp 3. isolates were collected from the liver and kidneys showed yellow colony color, which means that the bacteria belonged to the genus *Aeromonas*. The isolates were grown on Rimmler-Shott media produced yellow without black in the middle of the growing colony, it suggested the sample was closely related with *A. hydrophila* (Saputra, 2018).

4. Conclusion

Identification of pathogenic isolates Sp 1. from heart sample have a close relationship with *Plesiomonas shigelloides* while the pathogenic isolates Sp 2. and Sp 3. collected from liver and kidneys closely relate with *Aeromonas hydrophila* based on their biochemical tests carried out such as the Simmons Citrate test, Triple Sugar Iron Agar, Oxidative-Fermentative, Motility Indol Ornithine, Lysine Iron Agar, MR-VP, urea, catalase, oxidase, gelatin, confectionery test, and Rimmler-Shotts test.

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