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Identification of *Vibrio* sp Bacteria in Intensive and Traditional Ponds of Vannamei Shrimp (*Litopenaeus vannamei*) in Teluk Pambang Village, Bengkalis Regency

Identifikasi Bakteri *Vibrio* sp. Pada Air Tambak Intensif dan Tradisonal Udang Vannamei (*Litopenaeus vannamei*) di Desa Teluk Pambang Kabupaten Bengkalis

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ABSTRACT

The identification of *Vibrio* sp. bacteria in the water of intensive and traditional ponds for vannamei shrimp in Teluk Pambang Village was conducted from March to June 2023. This research aimed to calculate the quantity of *Vibrio* sp. bacteria, understand the morphological characteristics and identify the various types of *Vibrio* sp. bacteria in the water of intensive and traditional ponds for vannamei shrimp. The sampling involved collecting water from 2 bottles, each from an intensive and traditional pond. Subsequently, *Vibrio* isolation from the water was cultured on selective TCBS media through three dilution steps. The bacterial count was then determined. Further, bacterial cultures from different colonies were streaked on TSA media and then identified through morphological observations and biochemical tests. The research results indicate that the presence of *Vibrio* bacteria in intensive ponds complies with the maximum threshold and quality standards for a favorable environment for both the pond and shrimp. However, for traditional ponds, there is an abundance of *Vibrio* bacteria exceeding the threshold, where the maximum limit or good quality standard for Vibrio in the environment and shrimp is 103 CFU/ml. The morphological characteristics include a round shape, green and yellow color, and a flat edge with emerging elevations. Furthermore, infections of *Vibrio hollisae*, *Vibrio anguillarum*, and *Vibrio damsela* were identified in the water of intensive and traditional ponds for vannamei shrimp (*Litopenaeus vannamei*) in Teluk Pambang Village, Bengkalis Regency.

Keywords: Intensive pond, Traditional pond, *Vibrio*

ABSTRAK

Identifikasi bakteri *Vibrio* sp. pada air tambak intensif dan tradisional udang vannamei di desa Teluk Pambang dilakukan pada bulan Maret sampai Juni 2023. Penelitian ini bertujuan untuk menghitung jumlah bakteri *Vibrio* sp., mengetahui karakteristik morfologi dan mengidentifikasi jenis-jenis bakteri *Vibrio* sp pada air tambak intensif dan tradisional udang vannamei. Pengambilan sampel dilakukan dengan cara mengambil 2 botol air tambak intensif dan 2 botol air tambak tradisional. Kemudian isolasi Vibrio dari air ditanam pada media selektif TCBS melalui tiga kali pengenceran. Lalu jumlah bakteri diihitung. Selanjutnya dilakukan kultur bakteri per koloni yang berbeda pada media TSA. Kemudian dilakukan identifikasi melalui rangkaian pengamatan morfologi dan uji biokimia. Hasil penelitian menunjukkan jumlah keberadaan bakteri *Vibrio* pada tambak intensif sudah sesuai dengan ambang batas maksimal dan baku mutu *Vibrio* yang baik bagi lingkungan dan udang. Sedangkan untuk tambak tradisional terjadi kelimpahan bakteri *Vibrio* yang melebihi ambang batas yang mana ambang batas maksimal atau baku mutu *Vibrio* yang baik bagi lingkungan dan udang adalah 10^3 CFU/ml. Adapun karakteristik morfologi yang ditemukan adalah memiliki bentuk yang bulat, warna hijau dan kuning, mempunyai tepian rata dana elevasi yang timbul. Kemudian didapati infeksi *Vibrio hollisae*, *Vibrio anguillarum*, dan *Vibrio damsela* pada air tambak intensif dan tradisional udang vannamei (*Litopenaeus vannamei*) di Desa Teluk Pambang Kabupaten Bengkalis.

Kata Kunci: Tambak Intensif, Tambak Tradisional, *Vibrio*

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INTRODUCTION

Every year, Indonesia continues to experience an increasing potential in shrimp production. The aquaculture sector in Indonesia currently predominantly cultivates vannamei shrimp. Vannamei shrimp is one of the fisheries sectors that can enhance the country's foreign exchange and has significant potential for further development due to several advantages, including relatively fast growth, resistance to diseases, and the ability to thrive in pond environments, allowing for high-density stocking (Mahbubah et al., 2023).

One phenomenon that often causes losses for vannamei shrimp farmers during harvest is Vibrio sp. infection. This bacterium is quite notorious in the field of aquaculture. The disease caused by Vibrio sp. bacteria is Vibriosis. One region with the most significant potential for developing vannamei shrimp cultivation is Bengkalis Regency. Vannamei shrimp cultivation has become one of the favorite fisheries industries in the Bengkalis community to improve their economic status (Suhartini et al., 2021).

The traditional pond cultivation system is standard among farmers, where aeration systems are not implemented due to cost constraints. Traditional cultivation systems' water quality management usually occurs only in the initial stages, such as when the pond water turns green and dense. Farmers then raise the water level and stock the ponds with shrimp seeds. Feeding in traditional cultivation systems typically begins when the shrimp are one month old and continues until they reach the desired harvest weight. This is in contrast to intensive pond cultivation systems, which are usually carried out by farmers with larger budgets. In intensive systems, comprehensive feed with a protein content of around 25-35% is provided, adjusted to the biomass needs of the shrimp living in the pond environment (Sipahutar et al., 2019).

The clinical symptoms observed in shrimp when affected by Vibriosis include brownish gills and hepatopancreas, while the telson, uropod, and abdomen are red. Additionally, the ability of the shrimp to swim slows down (Idami & Zuhratul, 2020). The types of Vibrio causing Vibriosis commonly found in vannamei shrimp include *V. vulnificus, V. anguillarum, V. hollisae, V. parahaemolyticus, V. fluvialis, V. damsela,* and *V. alginolyticus* (Ambat et al., 2022). Based on the issues outlined, the author is interested in isolating and identifying Vibrio sp. bacteria from the water of intensive and traditional ponds for vannamei shrimp in the Pambang Village, Bantan Subdistrict, Bengkalis Regency.

MATERIALS AND METHOD

Location and time

This study was conducted in Teluk Pambang Village, Bengkalis Regency, Riau Province (Figure 1). A sampling of vannamei shrimp pond water in intensive and traditional ponds was conducted from March to June 2023, and sample analysis was carried out at the Microbiology Laboratory at the Fish Quarantine Station for Quality Control and Safety of Fishery Products (SKIPM) Pekanbaru, Riau.

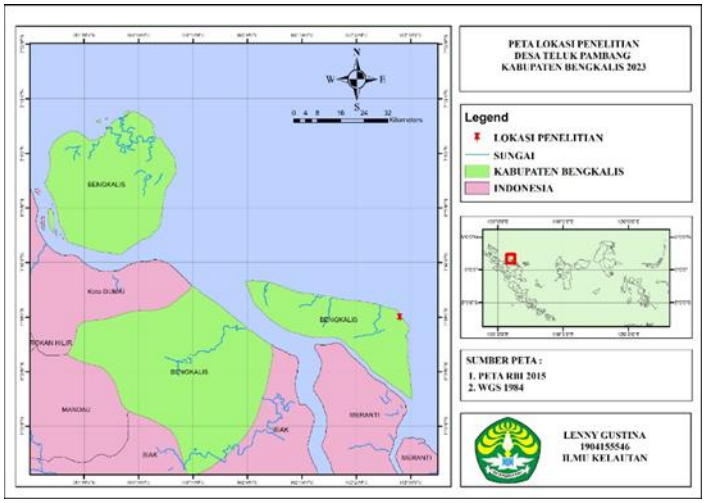

Figure 1. Map of Teluk Pambang Village as a research location

Tools and materials

The tools used in the research are a hot plate magnetic stirrer, Triangle ose, Autoclave, Petri dish, Aluminum foil, Object glass, Microscope, Micropipette, Yellow tip blue tip, Analytical balance, Erlenmeyer, Oven, Needle

ose, Camera, pH Meter, DO Meter, Thermometer, Hand Refractometer, Incubator, Bunsen. The ingredients used are α Naphtol, KOH 40%, KOVAC'S Indole Reagent, TSIA, MR-VP, Lysine Iron Agar (LIA), Citrate (SCA), Phenol-red broth, SIM, Trypton water, OF, H_2O_2, Tryptic Soy Agar (TSA), Paraffin, Crytal violet, Iodine, Safranin, TCBS, NaCl, Alcohol, Aquades.

Research methods

The method used in this research is a survey method. Primary data was collected directly in the field, while secondary data was obtained from literature studies, journals, books, and articles.

RESULT AND DISCUSSION

Research locations

The sampling location is in one of the ponds in Bengkalis Regency, precisely in Teluk Pambang Village. Geographically, Teluk Pambang Village is located at N: 01°27' - 01°28' and E 102°22' - 102°30'. This pond is owned by the Abadi Berjaya Captain group and operated by a resident named Mr. Awi. The pond business consists of 18 ponds, including nine intensive ponds covering 3200 m^2 with a stocking density of 200,000 fish/m^2 and nine traditional ponds covering 1000 m^2 with a stocking density of 150,000 fish/m^2. The influent (inflow) to this pond comes from the Kembung River, conveyed through pipes to a storage facility (tendon) before being directed to the ponds, undergoing sterilization at the Triple Point pond. Meanwhile, the effluent (outflow) is directed to the Wastewater Treatment Plant (IPAL) pond.

Water Quality Parameters

Water quality parameters measured in intensive and traditional ponds include temperature, pH, salinity, dissolved oxygen, and ammonia levels. Table 1 shows the data for pond water quality parameters.

Table. 1. Pond quality condition

Sampling Points	Temperature°C	Salinity (ppt)	pH	DO (mg/L)	Ammonia (mg/L)
Pond A1	28	19	7,6	5	1,449
Pond A2	29	19	7,6	5	1,501
Pond B1	30	18	7,7	4	1,593
Pond B2	31	18	7,7	4,6	1,565

Based on Table 1, the temperature values in intensive ponds (A1 and A2) range from 28-29°C, which is considered an optimal temperature for shrimp growth and survival (Ariadi & mujtahid, 2020); meanwhile, the temperature values in traditional ponds (B1 and B2) range from 30-31°C, which is still considered optimal for shrimp growth. The temperature increase in the ponds is attributed to hot weather, leading to increased light intensity on the water surface. The salinity in intensive and traditional ponds ranges between 18-19 ppt, within tolerance limits. This aligns with the opinion of Supriatna et al. (2020), stating that vannamei shrimp have optimal salinity tolerance ranging from 15-35 ppt. The pH values in both ponds are considered normal, according to Makmur et al. (2018), who mention that the normal pH range for vannamei shrimp growth is 7.4-8.9.

Dissolved Oxygen (DO) is the total amount of oxygen dissolved in water, essential for all marine biota as it serves respiratory, metabolic, and energy-producing functions for growth or development. The lowest Dissolved Oxygen (DO) concentration is found in traditional ponds, ranging from 4-4.6 mg/L. This is due to the presence of high concentrations of ammonia. Meanwhile, the highest DO concentration is found in intensive ponds, at 5 mg/L, which still complies with the water quality standard for DO (>5 mg/L). High ammonia in water can affect the dissolved oxygen levels (Aini & Parmi, 2022).

Ammonia in water originates from the decomposition process of organic matter containing nitrogen compounds (protein) derived from leftover feed and fertilization. The ammonia levels in both ponds are considered high and intolerable for shrimp growth. This aligns with the opinion of Scabra et al. (2021), stating that ammonia concentrations exceeding 1.0 mg/l can lead to shrimp mortality. Ammonia concentration increases with rising pH and temperature and decreasing salinity, causing ammonia poisoning in shrimp. The variation in ammonia levels in each pond is attributed to the increased stocking density (Dita et al., 2020).

Quantity of Vibrio bacteria

The quantity of Vibrio bacteria is determined by counting the bacterial colonies grown on TCBS agar media

using a Colony Counter. The obtained colony count results are then adjusted based on the Standard Plate Count (SPC) method (Hikmawati et al., 2019) With the formula below:

$$X = x \frac{1}{fp} \; x \; \frac{1}{p}$$

Information :

X = Number of colonies

FP = Dilution factor

P = Bacterial cultivation volume

The results of the Vibrio bacteria count in the water of intensive and traditional ponds for vannamei shrimp can be seen in Table 2.

Table 2. Quantity of Vibrio bacteria

Concentration	Sample	SPC (CFU/mL)	Average (CFU/mL)
10-1	A1	1,5 x 10^3	
10-2	A1	2 x 10^3	1,7 x 10^3
10-3	A1	0	
10-1	A2	8 x 10^3	
10-2	A2	6 x 10^3	8 x 10^3
10-3	A2	1 x 10^4	
10-1	B1	6 x 10^4	
10-2	B1	1 x 10^5	7,6 x 10^4
10-3	B1	7 x 10^4	
10-1	B2	6 x 10^4	
10-2	B2	7 x 10^4	6 x 10^4
10-3	B2	5 x 10^4	

Table 2 shows that in the intensive ponds (A1 and A2), the average is 1,7 x 10^3 CFU/mL and 8 x 10^3 CFU/mL, which still complies with the standard for *Vibrio* bacteria in water. Meanwhile, in the traditional ponds (B1 and B2), the average is 7,6 x 10^4 CFU/mL and 6 x 10^4 CFU/mL, exceeding the threshold for *Vibrio* bacteria in water. The maximum threshold or good quality standard for *Vibrio* in the environment and shrimp is 10^3 CFU/mL (No.75/PERMEN-KP/2016). If an abundance exceeds the maximum threshold, it can be confirmed that there will be mass mortality in cultured animals, especially shrimp commodities. One of the recommendations to reduce the abundance of pathogenic bacteria, including *Vibrio*, in shrimp cultivation is to add or utilize probiotics in cultivation activities (Anjasmara et al., 2018).

Bacterial Morphology

The results of bacterial isolation from water samples in intensive and traditional shrimp ponds after a 24-hour incubation period show the growth of bacterial colonies on the growth media. The observations of bacterial colonies include color, shape, edge, and elevation of colonies. Six different bacterial colonies were found in the intensive pond, and six were found in the traditional pond, each with morphological characteristics, as seen in Table 3.

Table 3. Morphology of the found bacteria

Sample Code	Isolate Code	Shape	Color	Edge	Elevation
A1	A1 10-1	Circular	Yellow	Flat	Arise
	A1 10^{-2}	Circular	Yellow	Flat	Arise
A2	A2 10-1	Circular	Yellow	Flat	Arise
	A2 10^{-2}	Circular	Yellow	Flat	Arise
	A2 10^{-3}	Circular	Yellow	Flat	Arise
B1	B1 10^{-1}	Circular	Yellow	Flat	Arise
	B1 10^{-2}	Circular	Yellow	Flat	Arise
	B1 10^{-3}	Circular	Green	Flat	Arise
B2	B2 10^{-1}	Circular	Green	Flat	Arise
	B2 10^{-2}	Circular	Yellow	Flat	Arise
	B2 10^{-3}	Circular	Green	Flat	Arise

Isolation of Vibrio bacteria from the intensive and traditional shrimp ponds in Teluk Pambang Village, Bengkalis Regency, yielded 11 isolates (Table 3). The isolation results showed that one bacterial isolate from the

intensive pond did not grow on TCBS media, indicating that TCBS media can inhibit unwanted bacteria, making it a selective medium. The bacterial colonies grown on TCBS medium exhibited several morphological colony characteristics, including colony color (yellow and green), round colony shape, smooth colony edges, and raised colony elevation. The green color of Vibrio bacteria colonies is attributed to their inability to ferment sucrose, while yellow indicates their ability to lower the pH of the TCBS medium (Ihsan & Retnaningrum, 2017). After observing the morphological characteristics of the colonies, including colony shape (viewed from the top), elevation (viewed from the side), colony edges (viewed from the top), and colony color, the next step is to perform gram staining to differentiate between gram-positive and gram-negative bacteria (Peter et al., 2018).

Characteristics of Bacterial Isolates

At the initial stage, 11 isolates were obtained from the water of intensive and traditional ponds on TCBS media. Subsequently, a purification step was carried out by transferring the colonies found on TCBS media to TSA media, resulting in 10 isolates because one isolate did not show bacterial growth. Then, Gram staining and a series of biochemical tests were performed on the 10 isolates, adjusting them with the determination key from Cowan (1974) for identification up to the species level. The results of Gram staining and the series of biochemical tests can be seen in Table 4.

Table 4. Biochemical characteristics of bacterial isolate

Biochemical test	Isolate Code									
	A1-1K	$A1^{-2}$ K	A2-2K	A2-3K	B1-1H	B1-3K	B1-3K	B2-2K	B2-3K	B2-3H
Grams	-	-	-	-	-	-	-	-	-	-
Motile	+	+	+	+	+	+	+	+	+	+
Catalase	-	-	-	-	+	+	+	+	+	+
Motil	-	+	+	+	+	+	+	+	+	+
Arginine	-	+	+	+	+	+	+	+	+	+
Lysin	-	-	-	-	-	-	-	-	-	-
Ornitin	-	-	-	-	-	-	-	-	-	-
Citrate	+	-	-	-	+	-	-	-	-	-
O/F	O	O	O	O	O	O	O	O	O	O
Indole	+	+	+	+	+	+	+	+	+	+
ONPG	-	-	-	-	+	+	+	+	+	+
MR	+	+	+	+	+	+	+	+	+	+
VP	-	-	-	-	-	-	-	-	-	-
Urea	-	-	-	-	-	-	-	-	-	-
TSIA	G/L	G/S	G/S	G/S	G	G/L	G/L	S/L	G/L	G/L
Species	V.h	V.h	V.a	V.a	V.d	V.d	V.a	V.a	V.d	V.a

Description: G/L/S (Glucose/Lactose/Sucrose), O/F (Oksidative/Fermentative), V.h (*Vibrio hollisae*), V.a (*V.anguillarum*, V.d (*V. damsela*).

Based on Table 4, 10 isolates have undergone Gram staining. Gram staining determines the type of bacteria based on their cell wall. Gram staining uses four types of dyes, including crystal violet (Gram A) as the primary dye; iodine (Gram B) as a color enhancer for crystal violet; acetone alcohol (Gram C) to dissolve fats; safranin (Gram D) as a secondary dye. Based on the results of Gram staining, all isolates showed the pathogenicity of *Vibrio* sp. in both ponds, indicated by the red color, which means the results are Gram-negative or may indicate the presence of Vibrio sp. pathogens in both ponds (Mahulauw et al., 2022).

Based on the positive motility test results for all isolates, as indicated by growth along the inoculation line in the SIM media. Positive motility means bacteria have a means of movement that leaves a trail resembling roots and grows outward from the inoculation line. If it is negative, they do not have a means of movement, and their growth does not spread from the inoculation line (Atiqa et al., 2016).

Based on the catalase test results, several isolates showed positive results, which are caused by bacteria using the catalase enzyme to break down hydrogen peroxide into H_2O and O_2. The presence of gas bubbles indicates a positive catalase result; for catalase-negative, there are no gas bubbles (Juliyarsi et al., 2019). In the arginine test, only isolate A1-1 K had a negative result, while the others were positive. The arginine test is one of the tests that determine a bacterium's ability to degrade arginine. In the lysine test, all isolates had negative results. The lysine test is conducted on LIA media, and it is known to be positive if the media's color changes to purple on streaks and negative if it remains yellow on stabs (Sulisyanto & Trimulyono, 2019). In the ornithine test, all isolates had negative results. The ornithine test determines a bacterium's ability to break down ornithine (amino acid) into amines. The result is positive if the media turns purple and negative if it remains yellow or yellowish (Usman, 2015).

Based on the citrate test, only isolates A1-1 K and B1-1 H had positive results, while the others were

negative. The reaction is positive if there is a color change in the SCA medium from green to blue, and the reaction is adverse if there is no color change in the medium. The citrate test is conducted to observe a bacterium's ability to utilize sodium citrate as a source of metabolism and growth (Ibrahim, 2021).

Based on the Oxidase Fermentation (OF) test, all isolates showed positive results, indicating oxidation. This test determines the oxidative or fermentative nature of bacteria in glucose. The test is performed in two tubes; one tube is supplemented with paraffin liquid, while the other is not. If both tubes are yellow, the bacteria are fermentative (F). If the tube without paraffin exhibits a green color and the other tube shows no color change, the bacteria are oxidative (O). If both tubes are green, it indicates no reaction (NR) (Sulisyanto & Trimulyono, 2019).

Based on the indole test, all isolates showed positive results in tryptone water supplemented with Kovac's reagent. The indole test is conducted to observe whether a bacterium can produce indole from tryptophan. A positive result is indicated by a red color on the surface of the media after adding Kovac's reagent, signifying indole production from tryptone (Rifai, 2021). In the ONPG (ortho-Nitrophenyl-β-galactoside) test, only four isolates yielded negative results, while the rest were positive. The ONPG test demonstrates the presence of the enzyme β-galactosidase, distinguishing lactose fermentation from non-lactose fermentation organisms using cultures from Triple Sugar Iron (TSI) media containing lactose. A positive reaction is indicated by the isolates turning yellow (Hasanah et al., 2012).

The MRVP test is conducted in two tubes. The first tube is inoculated with Methyl Red (MR) reagent up to half the total tube volume. Then, the second tube is inoculated with VP 1 reagent (∝ - naphthol), one-fourth of the total MRVP tube volume, and VP 2 reagent (KOH 40%), one-fourth of the total MRVP tube volume. The results for all isolates showed a positive reaction in the MR test and an adverse reaction in the VP test. The MR test is considered positive if there is a color change to red and negative if it remains yellow. As for the VP test, it is considered positive if there is a color change to red and negative if there is no color change or it turns yellow (Sulisyanto & Trimulyono, 2019).

Based on the urea test, all isolates yielded negative results. This test is conducted to determine a bacterium's ability to degrade urea. The test involves streaking on an inclined urea agar. A positive result is indicated by a color change in the medium to light pink (Sulisyanto & Trimulyono, 2019). Regarding the carbohydrate fermentation test using Triple Sugar Iron Agar (TSIA), TSIA contains three types of sugar: glucose, lactose, and sucrose. The TSIA test aims to determine a bacterium's ability to ferment sugars to produce acid or gas. The red coloration on the agar indicates a basic reaction, while yellow indicates an acidic reaction. Red coloration on the agar surface indicates fermentation of glucose, and yellow coloration on the tube's surface and bottom indicates lactose and sucrose fermentation (Kosasi et al., 2019).

After conducting Gram staining and a series of biochemical tests, the results identified two isolates (A1 10-1 K and A1 10-2 K) from intensive ponds that are closely related to *Vibrio hollisae*, five isolates (A2 10-2 K, A2 10-3 K, B1 10-3 K, B2 10-2 K, B2 10-3 K) from intensive and traditional ponds that are closely related to *Vibrio anguillarum*, and three isolates (B1 10-1 H, B1 10-3 H, B2 10-3 H) from traditional ponds that are identified as close to *Vibrio damsel*, following the classification based on the Cowan (1974).

Infections with *Vibrio hollisae*, *V. anguillarum*, and *V. damsela* in aquaculture animals have been widely reported. These bacteria can cause diseases in shrimp, known as Vibriosis. The infectivity of *Vibrio anguillarum* is reported to be lower than several other *Vibrio* pathogenic species. However, in populations with abundance, the infectivity of *V. anguillarum* can increase drastically, with a mortality rate of 100%. Outbreaks of Vibriosis by *V. anguillarum* are generally triggered by changes in environmental conditions (such as temperature, salinity, and others) or physiological stress. This indicates that besides being a primary pathogen causing Vibriosis, *V. anguillarum* is an opportunistic pathogen in aquaculture animals (Sanam et al., 2023).

CONCLUSION

Based on the findings of this research, it is known that the quantity of bacteria in intensive ponds is within the maximum limit and the reasonable quality threshold for Vibrio bacteria in the environment and shrimp. Meanwhile, for traditional ponds, there is an abundance of Vibrio bacteria that exceeds the maximum limit and the reasonable quality threshold, where the maximum limit or good quality threshold for Vibrio bacteria in the environment and shrimp is 103 CFU/mL. The morphological characteristics found include a round shape, green and yellow color, and a flat edge with a raised elevation. Furthermore, there were infections of *V. hollisae*, *V.anguillarum*, and *V.damsela* in the water of both intensive and traditional ponds of vannamei shrimp in Teluk

Pambang Village, Bengkalis Regency.

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