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Anti-Vibrio Activity of Bacteria Isolated from the Intestine of Mantis Shrimp (*Harpiosquilla* sp.)

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Abstract

Shrimp diseases caused by Vibrio spp. remain a major constraint in aquaculture, necessitating the exploration of alternative antibacterial agents from natural sources. This study aimed to determine the proportion and antibacterial activity of bacterial isolates derived from the intestines of mantis shrimp. Samples were collected from Jepara, Pemalang, and Cilacap waters using a purposive sampling approach. The research methods included bacterial isolation, morphological characterization, and screening of antivibrio activity. Antibacterial activity was evaluated against Vibrio parahaemolyticus, Vibrio alginolyticus, and Vibrio harveyi using dotting and disc diffusion methods. The results showed that 27% of isolates from Jepara, 44% from Pemalang, and 16% from Cilacap exhibited antivibrio activity. The inhibition zones ranged from 1.1–11.0 mm against V. parahaemolyticus, 3.3–9.0 mm against V. alginolyticus, and 2.2–11.8 mm against V. harveyi. These findings indicate that intestinal bacteria of mantis shrimp from all sampling locations possess antibacterial activity against Vibrio spp., highlighting their potential as promising candidates for the development of biocontrol agents in sustainable aquaculture systems.

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Introduction

Indonesia is a tropical archipelago country with a diverse range of plants and animals. This wealth of flora and fauna has been widely utilized in various aspects such as food science and biotechnology (Bappenas, 2024). Water is one of the riches that has been widely utilized, ranging from marine waters to freshwater with various kinds of biota in it (Darajati *et al.,* 2016). The Mantis shrimp is one of the abundant marine life, which is found in several regions, such as the north coast of Java, the Strait of Malacca, and the Pacific Ocean (Ahyong *et al.,* 2008). Research conducted by Luthfiani *et al.* (2018) on the composition of fish species from bycatch from trawls in Tambak Lorok, Semarang, found that 5.67% of the results were Ronggeng/mantis shrimp (*Harpiosquilla sp*.).

Mantis shrimp have a diverse digestive tract that is very interesting to

study. Currently, research on the types and species of bacteria in shrimp intestines is still limited. However, there is research related to isolation from other shrimp species. For example, research conducted by Widanarni *et al.* (2012) describes a bacterial isolate from the intestines of whiteleg shrimp cultivated in intensive ponds. This bacterial isolate is effective in inhibiting the attack of *Vibrio harveyi* bacteria. Furthermore, Haliza & Elviantari (2024) investigated bacterial isolation from the hepatopancreas of sand and rock lobsters collected from natural waters. Their findings revealed that the isolates exhibited antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli*.

Shrimp are inhabited by interacting microorganisms, including the pathogen Vibrio (Fu *et al.,* 2024). Certain Vibrio species can damage the shrimp's digestive tract, which then decays, forming aggregates and mixing with feces, causing white feces disease (WFD) (Zorriehzahra & Banaederakhshan, 2015). Shrimp infected with this disease will experience symptoms such as decreased appetite, the appearance of white droppings floating in controlled culture media such as ponds, and the shrimp's intestines will appear white and empty (Marbun *et al.,* 2019). A severe infection damages the mucosa lining of the intestinal tract, allowing bacteria to enter the hemolymph and cause death (Kaemudin *et al.,* 2016).

The toxin released by the *Vibrio parahaemolyticus* bacteria causes damage to gastric and hepatopancreatic tissue. Furthermore, this toxin damages hemocytes, leading to a decrease in their number (Luangtrakul *et al.,* 2021). For example, Vibrio parahaemolyticus bacteria were once reported to cause AHPND (Acute Hepatopancreatic Necrosis Disease), an outbreak in China in 2009 that resulted in up to 100% shrimp mortality (Tran *et al.,* 2013). Furthermore, a study by Pratama et al. (2014) found that shrimp infected by *Vibrio harveyi* bacteria died within 24-48 hours of infection.

Before research on antibacterials, farmers used antibiotics to fight bacteria that attacked organisms in cultivation. Continuous use of antibiotics caused bacteria to develop mechanisms to defend themselves from antibiotic exposure. As a result, the bacteria became stronger and less responsive to antibiotics given as treatment, or resistant to antibiotics (Walewangko *et al.,* 2015). Research by Fitri et al. (2020) indicates that 8 out of 32 Vibrio sp isolates tested were resistant to antibiotics. There are three known types of resistance: the first, non-genetic

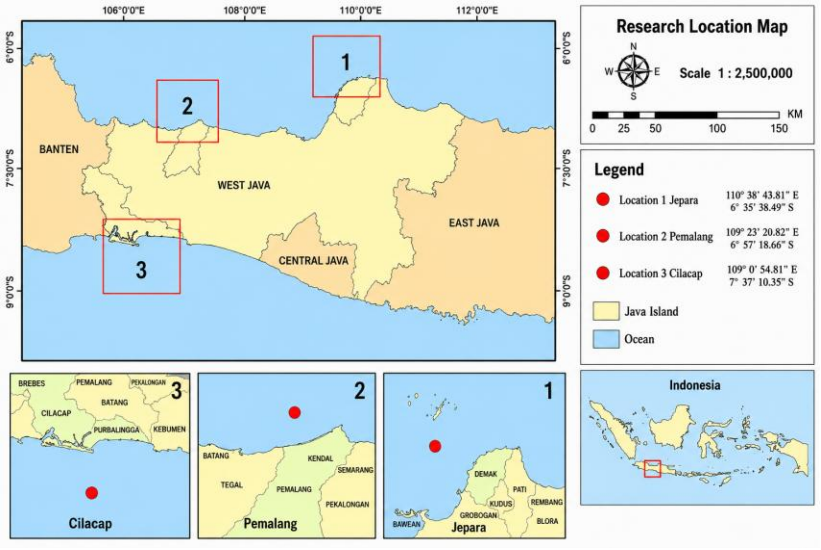

Figure 1. Mantis Shrimp Sampling Location

resistance, found in bacteria in an inactive or resting state. The second, genetic resistance, is a spontaneous gene mutation unaffected by antibacterials. Third, cross-resistance is the resistance of bacteria to a particular antibiotic and other antibiotics that have almost the same structure (Wibowo *et al.,* 2011). One alternative solution to overcome this problem is to use compounds from other organisms that can be used as antibacterials (Annisa *et al.,* 2015). Consequently, it is necessary to conduct research related to the antibacterial activity of the results of isolation from the intestinal tract of mantis shrimp against Vibrio bacteria.

Materials and methods

Time and Place of Research

The research was conducted from August 2023 to November 2024. Shrimp samples were collected at Jepara Port, Pemalang Port, and Cilacap Port (Figure 1). Preparation of tools and materials, as well as antibacterial activity testing, were carried out at the Research Laboratory of the Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University, Purwokerto.

Research Methods

The method used in this study was observation. The sampling technique used was purposive sampling. This purposive sampling method is a sampling process carried out with certain considerations. The variable in the study was the antivibrio activity index.

Research Procedures

In general, the research procedure is presented in the form of a research diagram in Figure 2. Samples were collected directly

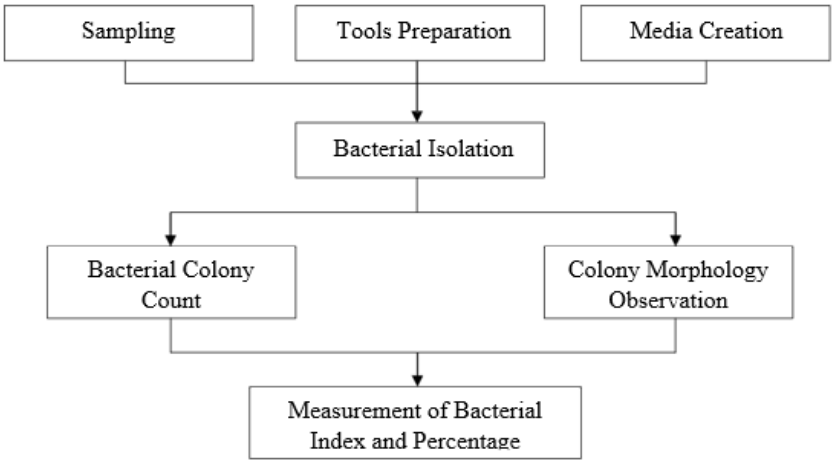

Figure 2. Research Procedures

from fishermen operating at Jepara Port, Pemalang Port, and Cilacap Port. A total of five mantis shrimp individuals were obtained from each location, resulting in 15 samples overall. The sampled individuals were selected based on healthy condition, without visible signs of disease or physical damage. The size of the shrimp varied depending on the catch, ranging from small to large individuals. All collected shrimp were then placed in a cool box and transported to the laboratory for further processing.

Tools Preparation

The tools used in the research, such as petri dishes, test tubes, measuring cups, and spatulas, were first washed and dried using an oven. Meanwhile, the tips were placed in tip boxes and the microtubes were placed in glass bottles, then closed and wrapped. Wrap the tools to be used in white paper. The tools and materials to be used were placed in an autoclave for the sterilization process. Sterilization was carried out for 20 minutes at a temperature of 121°C with a pressure of 2 atm (Ihtifazhuddin *et al*., 2016). After the autoclave was complete, the tools were removed from the autoclave and were ready for use.

Bacterial Isolation

Before removing the intestines, shrimp are first sprayed with alcohol, rubbed on their bodies, and then sterilized. The shrimp were then dissected using sterile surgical scissors from the dorsal to the anus. After that, the intestines were gently removed using sterile tweezers and placed in a dish. Gently massaging the area with sterile tweezers will remove any dirt. After that, the intestines were weighed at 1 g and then ground using a mortar and pestle until smooth and suspended in sterile physiological

solution, and homogenized using a vortex. After that, serial dilutions were carried out by adding 0.5 mL of the sample suspension into 4.5 mL of physiological solution in the first tube (10^{-1} dilution) and homogenized using a vortex. Then, 0.5 ml was taken from the first tube and homogenized in the second tube (10^{-2} dilution), and this procedure was repeated until the third tube (10^{-3} dilution). The results from the 10^{-1} to 10^{-3} dilutions were taken as much as 0.2 mL each and cultured using the spread plate method on TSA media. The bacteria must be incubated at 28ºC for 18-24 hours in order to grow.

Bacterial Abundance Calculation

The total number of colonies of bacteria grown after an incubation period of 18-24 hours was then counted on each TSA medium, from a 10^{-1} to a 10^{-5} dilution, using a colony counter. After the number of colonies on each TSA medium was obtained, bacterial abundance was calculated using the total plate count (TPC) method using the formula (Madigan *et al.,* 2006).

$$Number\ of\ Bacteria\ (CFU/g) = Numberof\ Colony \frac{1}{Dilution}\ x \frac{1}{Culture\ Volume} x \frac{1}{Sample\ Weight}$$

Morphological Observation

After incubation for 18-24 hours, 25 single colonies were selected that were visibly distinct. The bacterial colonies were then observed macroscopically and the results recorded. Morphology observed included colony shape, margins, elevation, texture, color, and size (Suryani & A'yun, 2022). The selected bacteria were then stocked using the streak plate technique on TSA media.

Preparation of Vibrio sp. Bacteria

The Vibrio sp. bacteria used include *Vibrio parahaemolyticus, Vibrio alginolyticus,* and *Vibrio harveyi*. Vibrio strains used in this study were obtained from the Research Laboratory Collection of the Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University. The Vibrio sp. bacterial strains were activated by taking 0.5 µL of the Vibrio sp. bacterial strain from the stock and then putting it into TSB (Tryptic Soy Broth) media and then incubated for 18-24 hours in an incubator shaker. The growth of bacteria in TSB media is indicated by changes in the turbidity of the media. Media that looks more turbid than before the incubation time indicates that the cultured bacteria are growing in that media (Basir *et al.,* 2023).

Antivibrio Testing of Bacterial Isolates from Mantis Shrimp Intestines

The morphologically observed bacterial isolates were tested for antivibrio

activity, including *Vibrio parahaemolyticus, Vibrio alginolyticus,* and *Vibrio harveyi*. The method used was the dotting method or by spotting bacteria on agar media using a loop needle as done by Pamungkas *et al.,* (2018). The test was carried out by dropping 100µl of Vibrio Sp. bacterial pellets with a density of 6 x 10^8 CFU/mL and spreading them using an L rod on the growth media, then waiting for 3 minutes until the Vibrio bacteria were absorbed into the media. After that, 1 loop of bacteria was taken and dotted on the media containing Vibrio sp. bacteria. The petri dish was then wrapped until the gap between the lid and the dish was tightly closed. Then the bacteria were incubated at 28ºC for 48 hours. Observe and measure the inhibition zone formed with a caliper as the value of antivibrio activity. The formula for calculating the antibacterial activity value uses the following formula (Tokasaya, 2010).

$$Bacterial\ activity\ value = Total\ zone\ diameter - disc\ paper\ diameter$$

Then, the presentation of anti-vibrio bacteria can be calculated using the following formula (Sinatryani, 2014).

$$Percentage\ of\ Anti-Vibrio\ Bacteria\ (\%) = \frac{Number\ of\ anti-vibro\ bacterial\ colonies\ obtained}{Total\ number\ of\ colonies\ observed} x100$$

Screening of Antivibrio Activity of Bacteria from Mantis Shrimp Intestine

Bacterial antivibrio activity was carried out against several Vibrio strains such as *Vibrio parahaemolyticus, Vibrio harveyi*, and *Vibrio algino*. Anti-Vibrio activity was tested using the Kirby Bauer agar diffusion method with disc paper, as in the journal written by Hitijahubessy *et al.* (2022). Test preparation began by culturing vibrio stained bacteria on TSB media and then incubating for 24 hours at 28ºC. Vibrio bacteria with a

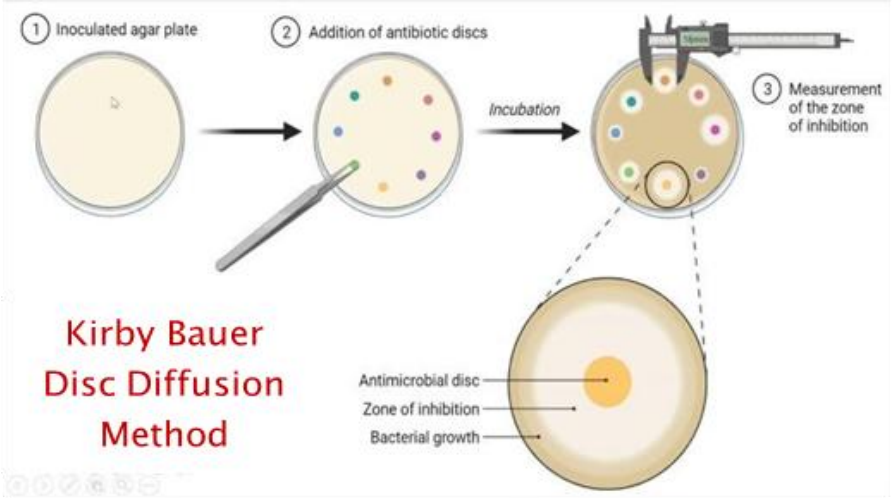

Figure 3. Illustration of Antibacterial Activity Testing with the Kirby Bauer Method (Aryal, 2022).

density of 10^6 were then swabbed evenly on the TSA media using a sterile cotton bud and left for 3-5 minutes so that the suspension could absorb into the media (Mayaserli & Shinta, 2021). Bacterial isolates with a density of 106 were inserted into a blank disk measuring 5 mm with 5 µl and left to absorb. Paper disks containing bacterial isolates were placed on TSA media containing Vibrio bacteria and then incubated at 28ºC for 48 hours (Radjasa *et al.,* 2004). The antibiotic ampicillin (30 mg) was used as a positive control. The diameter of the resulting inhibition zone was measured to determine the antivibrio activity of the bacterial isolates from the intestines of mantis shrimp (Burgess *et al.,* 2003). An illustration of the antibacterial activity test can be seen in the following figure 3.

Data Analysis

The data obtained from the research included bacterial abundance, colony morphology, and antivibrio activity index. The data was presented in figures, tables, and graphs, then analyzed descriptively and compared with the literature.

Results and Discussion

Abundance of Bacteria in the Intestines of Mantis Shrimp

The abundance of bacteria isolated from the mantis shrimp intestines varied across sampling locations. The highest bacterial abundance was found in samples collected at Jepara Port, while the lowest was found in Pemalang Port. The abundance of bacteria isolated from the mantis shrimp intestines can be seen in the

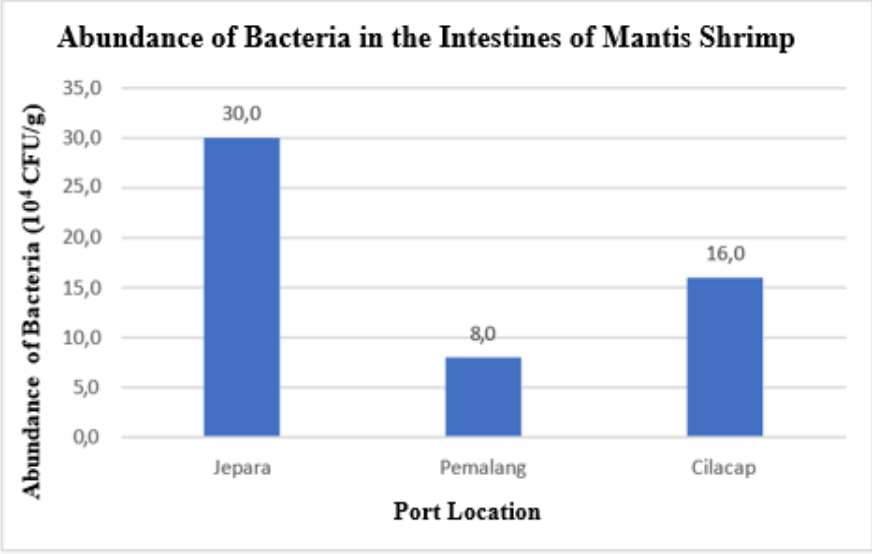

Figure 4. Abundance of Bacteria in the Intestines of Mantis Shrimp

following figure.

The abundance of bacteria in each location was within normal limits. The maximum abundance threshold for Vibrio bacteria in public waters and shrimp bodies did not exceed 10^6 CFU/ml (Taslihan *et al.,* 2015). Bacteria that reside in the shrimp intestines include lactic acid bacteria and Vibrio bacteria. Lactic acid bacteria play a role in the digestion process and maintain the balance of bacterial density in the digestive tract (Atlas & Bartha, 1993). Lactic acid bacteria can suppress the presence of pathogenic bacteria such as Vibrio by competing for space in the shrimp digestive tract. According to Widanarni *et al.,* (2012) the presence of probiotics (*Bacillus* sp.) was able to suppress the abundance of *Vibrio* sp. bacteria in the shrimp intestines. Therefore, the abundance of lactic acid bacteria and the abundance of *Vibrio* bacteria in the intestines are interrelated.

The abundance of bacteria in the intestines of mantis shrimp is thought to be influenced by the environment. Under certain temperature conditions, only a small proportion of bacteria can actively participate in the digestive tract (Lesel, 1990). Bacteria in water can attach to food and enter the shrimp's digestive tract (Wulandari, 2013). If bacteria are tolerant to alkaline or acidic environments or have the ability to adhere to the intestines, they are likely to survive in the shrimp's intestines (Wijayanto, 2009). Each type of food consumed can carry different types of bacteria. Furthermore, bacterial abundance in the shrimp intestines may be higher because the intestines are located along the dorsal abdomen and serve as a food channel leading to the anus (Ardiani, 2011).

According to various studies, environmental factors such as temperature, salinity, pH, and oxygen also influence the microbiome in the digestive tract, one of which is bacteria. Extreme water changes can lead to an increase in pathogenic bacteria such as Vibrio. Consequently, shrimp will have difficulty selecting bacteria, resulting in a change to the digestive tract microbiome's diversity and balance. According to Sukenda *et al.,* (2022), the abundance and diversity of bacteria in the intestines of normal shrimp were relatively higher than those of shrimp affected by WFD.

Morphology of Bacteria in the Intestine of Mantis Shrimp

Morphological characterization was performed by observing the color, shape, size, edges, and elevation of different bacteria. Observations of bacterial colony characteristics were based on Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 2000). The morphology of bacterial colonies obtained in this study tended to vary. Sixty-seven bacterial isolates were obtained and then categorized into 60 different types. These categories were created based on differences in the characteristics of bacterial colonies. Most of the bacteria obtained were circular and white in color. Bacterial colony sizes ranged from small to large, medium, and punctiform.

Different bacterial shapes are characteristic of a bacterial species. According to Ilyas (2001) and Waluyo (2009), age, environmental factors (abiotic and biotic), growth media, and temperature will affect the shape of bacterial colonies. The color of bacteria can vary depending on the media used. In common media such as TSA, most bacteria are white, yellowish, gray, or almost clear. The difference in color of each bacterial colony is influenced by several factors, such as temperature, pH, and free oxygen (Waluyo, 2009). The color of bacteria is produced by pigments such as anthocyanin, melanin (black, brown, and orange), carotenoids (red and yellow), tripirylmethene, and phenazine (red orange, yellow orange, and dark orange) (Safrida *et al.,* 2012). Research conducted by Wiguna et al. (2016) shows that carotenoids react with porins (transmembrane proteins), which

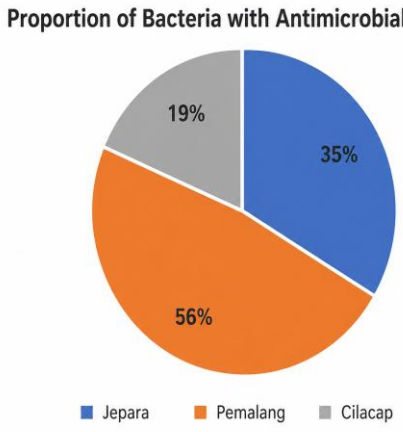

Figure 5. Percentage Diagram of Bacteria that have Antibacterial Activity

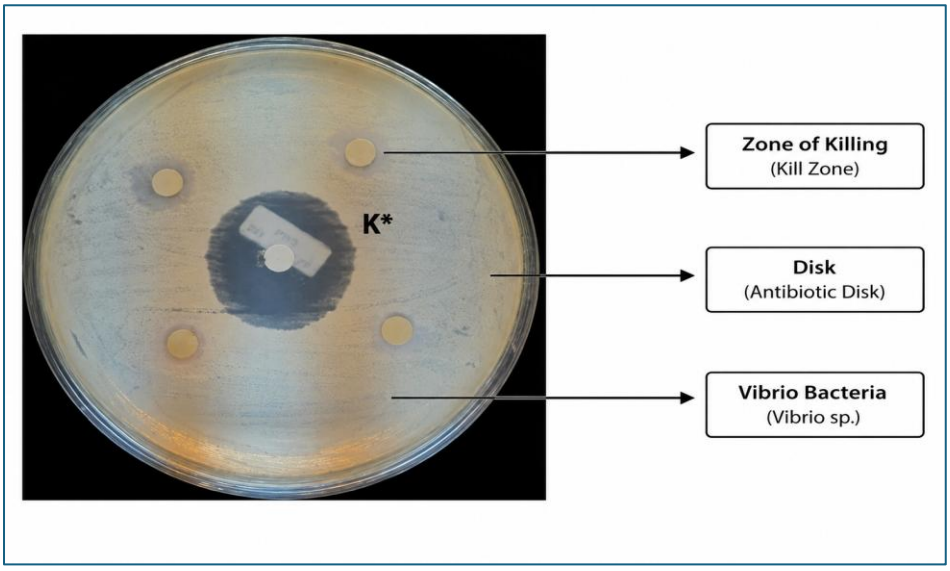

Figure 6. Zone of no growth on Antibacterial Activity

ultimately inhibits bacterial growth. High concentrations of carotenoid pigment extracts showed greater antibacterial activity.

Percentage and Antivibrio Activity of Bacterial Isolates in Shrimp Intestines

The results of the antivibrio activity test of bacteria showed that of the 67 bacterial isolates isolated from mantis shrimp, 18 of them had antivibrio activity. The number of isolates with antivibrio activity reflects the percentage of antivibrio bacteria found in the intestines of mantis shrimp. The highest percentage of antivibrio bacteria was found in shrimp caught from Pemalang waters, followed by shrimp caught from Jepara waters and from Cilacap waters. The percentage of bacteria with antivibrio activity in this study is presented in the following graph.

This percentage difference is based on the total number of colonies obtained and the weight of the shrimp obtained. Antibacterial activity is influenced by several factors, such as the concentration or density of the bacteria tested, the metabolite content, and the type of bacteria inhibited (Jawetz *et al.,* 1996). Typically, the higher the concentration or density of the bacteria used, the greater the ability to inhibit bacterial growth (Lestari *et al.,* 2016).

The antibacterial activity produced can be seen through the clear zone formed around the disc. The larger the clear zone formed, the stronger the antibacterial activity produced by the

Table 1. Antibacterial Activity Value against *Vibrio parahaemolyticus* Bacteria

Sample Code	*Vibrio parahaemolyticus*				Mean±Stadev (mm)	Category
	U1	U2	U3	U4		
UMJ 1.3	11,2	10,6	8,7	13,6	11,0±2,0	Strong
UMJ 1.6	1,9	1,1	1	1,1	1,3±0,4	weak
UMJ 1.11	1,5	2,4	1,6	2,5	2,0±0,5	weak
UMJ 1.13	4,8	3,9	5,1	8,2	5,5±1,9	moderate
UM 16	3,3	5,6	3,2	1,3	3,4±1,8	weak
UM 25	2,3	1,5	0,3	0,4	1,1±1,0	weak
UMP 16	8,2	9,2	4,5	6,1	7,0±2,1	moderate
UMP 21	1,6	4,8	5,3	3,3	3,8±1,7	weak
UMP 22	5	3,7	1	3,7	3,4±1,7	weak
UMP 25	4,3	2,7	2,2	2,5	2,9±0,9	weak

bacteria in inhibiting or killing pathogenic bacteria (Sari *et al.,* 2018). Inhibition zones can be divided into two, namely the radical inhibition zone and the irradical inhibition zone. The radical inhibition zone is a clear zone or a zone where there is no bacterial growth around the disc. The activity of the antimicrobial inhibition zone is grouped into four categories, namely: weak (<5 mm), moderate (5-10 mm), strong (>10-20 mm), and very strong (>20-30 mm) (Morales *et al.,* 2003). Meanwhile, the irradical inhibition zone is a clear zone formed around the well but still contains resistant or surviving bacterial colonies because they are not completely inhibited (Martsiningsih *et al.,* 2023). The results of antibacterial activity testing using the disc diffusion method can be seen in the following image.

The antibiotic ampicillin was used as a positive control to determine whether the tested bacteria could inhibit their growth or whether they were resistant to antibacterial agents. Ampicillin is capable of inhibiting both gram-negative and gram-positive bacteria by inhibiting the bacterial cell wall (Rakasiwi *et al.,* 2023). The tested bacterial isolates secrete bioactive compounds that can inhibit or suppress the growth of Vibrio bacteria. These bioactive compounds damage the structural components of the cell wall in Vibrio bacteria. The inhibition of bacterial growth and the formation of a clear zone around the test bacteria indicate that the

Table 2. Antibacterial Activity Value against *Vibrio alginolyticus* Bacteria

Sample Code	*Vibrio alginolyticus*				Mean±Stadev (mm)	Category
	U1	U2	U3	U4		
UMJ 1.12	3,9	6,5	2,4	2,7	3,9±1,9	weak
UMJ 2.6	9,7	12,1	5,7	8,4	9,0±2,7	moderate
UMJ 2.15	3,6	5,7	5,5	5,9	5,2±1,1	moderate
UM 8	4,3	4,4	3,2	1,1	3,3±1,5	weak
UM 20	8,4	4,3	5,1	4,9	5,7±1,8	moderate
UM 25	6,5	9,4	7,7	8,4	8,0±1,2	moderate

Table 3. Antibacterial Activity Value against *Vibrio harveyi* Bacteria

Sample Code	*Vibrio harveyi*				Mean±SD (mm)	Category
	U1	U2	U3	U4		
UMJ 1.12	12	15,3	8,6	11,1	11,8±2,8	Strong
UMJ 1.15	3,5	1,5		1,6	2,2±1,1	Weak
UMP 12	4,3	3,5	8,7	4,9	5,4±2,3	moderate
UM8	6,5	4,2	3,6	4,9	4,8±1,3	weak

test bacteria are antagonistic to Vibrio bacteria. The inhibition zones formed by each test bacterium vary in size. This is because the bioactive compounds produced by each bacterium also vary (Situmeang *et al.,* 2017).

Antibacterial compounds can be bactericidal or bacteriostatic. Bacteria are bactericidal if the antibacterial compound they produce is able to kill the pathogenic bacteria being tested. The results of this test are seen through the formation of a clear zone. Bacteriostatic bacteria inhibit bacterial growth by inhibiting their growth (Van Haren *et al.,* 2007). This is indicated by bacteria growing around the disc not being fertile or being crowded out by antagonistic bacteria (Lalamentik & Wewengkak, 2017). The type of cell wall possessed by bacteria can also vary. Data on the results of the inhibition zone and zone of no growth, measured in mm using a caliper, can be seen in the following table.

In this study, bacterial isolates were obtained that formed inhibition zones and zone of no growth. The bacterial isolates that formed the zone of no growth were isolates UMJ 1.3, UMJ 1.13, and UM 25 in

the test against Vibrio parahaemolyticus bacteria, then isolates with the code UM 8, UM 20, and UM 25 in the test against Vibrio alginolyticus bacteria, and isolates with the code UM 8 in the test against Vibrio harveyi bacteria. The best activity value was owned by the bacterial isolate with the code UM 1.3 which could kill *Vibrio parahaemolyticus* bacteria in the strong category.

Additionally, the antivibrio activity of each isolate falls into the weak to moderate category. Meanwhile, the isolates that formed inhibition zones were isolates UMJ 1.6, UMJ 1.11, UM 16, UMP 21, UMP 22, and UMP 25 in the test against *Vibrio parahaemolyticus* bacteria, then isolates with the code UMJ 1.12, UMJ 2.6, and UMJ 2.15 in the test against *Vibrio alginolyticus* bacteria, and isolates with the code UMJ 1.12, UMJ 1.15, and UMP 12 in the test against *Vibrio harveyi* bacteria. The difference in inhibition zones is influenced by several factors such as the speed of antimicrobial diffusion, the degree of bacterial sensitivity, and the speed of bacterial growth (Soleha, 2019).

Furthermore, the ability of test bacteria to inhibit pathogenic bacteria is influenced by the food or carbon source they receive during the culture period. The carbon source or nutrients in the agar medium influence bacterial growth rate and metabolism (Rinihapsari *et al.,* 2023). Bacteria can utilize the nutrients in the culture medium as growth factors or to produce bioactive compounds that inhibit the growth of pathogenic bacteria (Radji, 2019). The salinity level of the medium also plays a significant role because the bacteria tested originate from marine biota (Hamidah *et al.,* 2019).

Bacteria have cell walls composed of phospholipids and an autolayer (several types of proteins). Peptidoglycan is present between the outer and inner membranes in small amounts. Therefore, the cell wall of *Vibrio* bacteria is not easily denatured, unlike that of gram-positive bacteria, because its cell wall is composed of polysaccharides (Helmiyati & Nurrahman, 2010). Based on differences in inhibition zones formed by each test bacterium, it appears that the bacteria are capable of inhibiting *Vibrio* bacterial growth and colonization. This can be influenced by the bacteria's ability to adhere, move (motility), and chemotaxis to nutrients and organic matter (Imada *et al.,* 2007).

Every bacterial species has a different growth cycle, which includes the

log phase and the early stationary phase in producing antibacterial compounds. Typically, bacteria will produce these compounds optimally during the 24-hour and 48-hour incubation periods (Pratiwi, 2008). With longer incubation times, the bacteria's ability to produce antibacterial compounds can decrease because after that time, the bacteria can form and activate proteases or other inactivators that reduce antibacterial activity (Hoover & Steenson, 1993). Incubation temperature also affects the bacterial growth cycle on the plate. Lowering the incubation temperature below the maximum temperature and the maximum incubation time can prolong the lag phase of growth (Jiwintarum *et al.,* 2021).

Conclusion

The results of the research showed that 27% of Jepara bacteria have antivibrio activity, 44% of Pemalang bacteria have antivibrio activity, and 16% of Cilacap bacteria have antivibrio activity. The antivibrio activity values obtained from the three locations are 1.1-11.0 mm against *Vibrio parahaemolyticus* bacteria, 3.3-9.0 mm against *Vibrio alginolyticus* bacteria, and 2.2-11.8 mm against *Vibrio harveyi* bacteria. The highest antibacterial activity values against *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, and *Vibrio harveyi* bacteria were owned by isolates UMJ 1.3, UMJ 2.6, and UMJ 1.12, respectively.

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