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Identification of *Vibrio* spp. in Spiny Lobster (*Panulirus homarus*) from Natural Catch and Culture In Batu Bangka Village, Sumbawa

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Abstract

Spiny lobster (*Panulirus homarus*) is a fishery commodity with high economic value. The classic problem that has not been resolved so far is the low survival rate of around 20-30% and an average growth rate of 180-230 grams. This can be influenced by nutritional factors, environment, stress, and pathogen infection. This study aimed to identify pathogenic bacteria found in wild-caught lobsters (Labangka) and cultivated lobsters (Bungin Island). The stages of this research began with taking samples in the field, followed by isolation and purification of bacteria (tail organs, gills, and hepatopancreas), morphological characteristics, and physiological tests of bacterial isolates. From the results of the study, it was found that the isolates grown on TSA media, showed a higher diversity of bacteria in natural lobsters compared to cultivated lobsters, this is what makes natural lobsters have a high survival rate because the diversity of microflora forms a symbiotic mutualism. Meanwhile, if we look at the diversity of pathogenic bacteria (*Vibrio* spp.), namely isolates grown on TCBS media, it shows that cultivated lobsters have more diverse pathogenic bacteria, namely three types of *Vibrio (V. alginolyticus, V. harveyi,* and *V. parahaemolyticus*) are indicated, only natural lobsters identified *V. alginolyticus*.

INTRODUCTION

Spiny lobster (*Panulirus homarus*) is one of the fishery commodities with high economic value. This marine commodity is in great demand locally and for export. Currently, market demand is getting higher, which cannot be met due to insufficient production and dependence on the natural catch which is limited (Larasati *et al.,* 2018). The results of the latest survey by the Fisheries Science lecturer at the Sumbawa University of Technology show that the number of lobsters caught in the wild in Labangka District, West Nusa Tenggara Province from June to August 2022 reached 701.5 kg.

One of the most appropriate alternatives to meet market demand is production by cultivation because this process is a process of protecting against extinction and also a process of providing sufficient and sustainable stocks (Mindar *et al.,* 2017). It was recorded that the Province of West Nusa Tenggara was able to produce up to 68.01 tons, with <10% of the cultivated

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area utilized (MMAF, 2020). However, the main problem with lobster farming in West Nusa Tenggara is the low survival rate, which ranges from 20-30% and an average growth of 180-230 grams (Jones and Shanks, 2009). According to data from the Sumbawa Fisheries Extension Report, the average recovery rate for live lobsters (*P. homarus*) and (*P. ornatus*) during the period 2021 to 2022 in several cultivation areas such as Bungin Island is 14.61%, Kaung Island is 15.1%, and Batu Bangka Village is 21%.

Within the scope of lobster cultivation growth is generally influenced by nutritional, environmental, and body stress factors (Haikal *et al.,* 2017; Abdurachman *et al.*, 2020; Abdurachman, 2022). These conditions can trigger disease attacks that cause mass death and crop failure. Several types of lobster diseases that have been identified and often occur include red body disease, black gill disease, milky disease, big head syndrome, and separate head syndrome (MMAF, 2020; Sudewi *et al.,* 2018a; Sudewi *et al.,* 2018b; Sudewi *et al.,* 2020). All diseases in lobsters are caused by pathogenic bacteria which can trigger disease complications (Nur and Yusnaini, 2018). Several types of pathogenic bacteria in cultivated lobsters that are often found are *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, *Rickettsia Like Bacteria* (RLB); *Fusarium* sp.; *Vibrio damsella*, *V. mimicus, V. alginolyticus*, and *proteus*, *V. damsella, V. cholerae, Acinetobacter* sp., *V. mimicus, V. fluvialis* and *Enterobacter aerogenes* (Lasmika, 2015). Meanwhile, the results of the identification of pathogenic bacteria caught in nature include *Shewanella, Bacillus firmus, Vibrio alginolyticus, Tenacibaculum lutimaris, Lysinibacillus fusiformis*, and *Pseudomonas* sp. (Sudewi *et al.,* 2019).

The emergence of various harmful diseases in lobsters is an indication of the importance of studying diseases in lobsters. Therefore, knowledge is needed about diseases that occur in lobsters, both cultivated and caught in nature. The purpose of this study was to identify pathogenic bacteria in wild-caught and cultivated Spiny lobster (*P. homarus*) in Sumbawa Regency. This research has important implications for disease management in lobster culture, and for preventing disease translocation from one area to another in Indonesia.

METHODOLOGY

Ethical Approval

There are no animals harmed or improperly treated during this research. It was approved during the due diligence session and proposal seminar.

Place and Time

This research was conducted in September-December 2022. The research location was carried out at the Sumbawa Techno Park Laboratory (STP), Sumbawa University of Technology.

Research Materials

Tools and materials used in this research include vernier calipers (0.01g), analytical scales (0.001g), surgical tools, binocular microscope (Optika B-159ex, Italy), autoclave (GEA), petri dishes, tweezers, glass objects, cover glass, dropper pipette, ruler, oce needle, laminar flow, Bunsen, cutting drill A3, Cool box, spiritus, Erlenmeyer, spiny lobster (*P. homarus*), distilled water, alcohol 70%, NaCl (Merck KGaA, 64271 Darmstad Germany), TSA (Tryptic Soy Agar) medium (Merck KGaA, 64271 Darmstad Germany), TCBS (Thiosulfate Citrate Bile Sucrose) medium (Himedia M189-500g), iodine (Himedia K001-1KT), crystal violet (Himedia K001-1KT), ammonium oxalate (Himedia K001-1KT), ethanol 95% and safranin (Himedia K001-1KT).

Research Design

In general, this research is divided into two studies, namely wild-caught lobsters and cultivated lobsters. A sampling of lobsters caught in nature, namely the

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southern coastal area of Labangka District (-8°92'67.59" S & 117°67'56.72" E). While the location for sampling cultivated lobsters is Bungin Island (-8°47'79.03" S & 116°99'92.89" E). The total lobsters taken from each location are 3 lobsters with an average weight is 100 grams. There were three parts analyzed, namely the tail, hepatopancreas, and gills, with 1 gram of each part taken.

Work Procedure

Tool and Media Sterilization

The tools and materials to be used are first washed using water then wrapped in paper and sterilized using an autoclave at 121°C with a pressure of 2 atm for 20 minutes.

Preparation of Agar Media

Making the media serves to provide a place that supports the growth and proliferation of the microorganisms you want to grow, the media that is made is media from TSA and TCBS. Weigh the ingredients to be made first, then put them in the Erlenmeyer, after that the media is heated on a hot plate and stirred thoroughly using a magnetic stirrer. The media was sterilized using an autoclave at 121°C with a pressure of 2 atm for 20 minutes. For TCBS media, do not autoclave. Furthermore, in a warm condition (50°C), the media is poured into a sterile petri dish and allowed to solidify.

Isolation and Purification of Lobster Bacteria

Lobsters were dissected using an aseptic section set in the laminar. The hepatopancreas, tail, and gills of the lobster were taken and ground using a mortar. As much as 1 gram of each lobster organ (tail, hepatopancreas, and gills) was diluted with physiological salt up to 10^{-5} dilution and homogenized using a vortex. Then 0.1 ml of the sample was spread on TSA medium which is a common medium for bacterial culture, and incubated at 28^0C for 18 hours. Furthermore, the growing colonies were selected based on different morphological characteristics to be purified on TCBS agar media which is a selective medium for *Vibrio* spp. bacterial culture. Bacterial purification was carried out using the 3-stroke technique. After the pure culture is complete, the bacteria are incubated at 27°C–30°C for 24–48 hours in an incubator (Mindar *et al.,* 2017).

Observation of Bacterial Morphology

Morphological or physical observation of the isolated bacterial colonies was carried out by observing the morphological characters including color, edges, elevation, and shape. Then the colonies that have different morphological characters are coded.

Physiological Test of Bacterial Isolates

The physiological test carried out in this study was Gram staining. Gram staining begins with taking the bacterial culture from a petri dish and placing it on a glass slide, then fixing it over a Bunsen flame. Two drops of crystal violet were given to the bacterial isolate and allowed to stand for 1 minute, then washed with distilled water. The next step is giving 3 drops of iodine and waiting for 1 minute, then washing with distilled water. Then given 3 drops of alcohol for 10 seconds, and washed again with distilled water. Safranin dye was given to the sample and left for 1 minute, then washed again with distilled water. The preparation is dried and then dripped with immersion oil. The final stage is observing the bacterial cells under a microscope. Gram-negative bacteria are marked in pink or red, while Gram-positive bacteria are marked in blue or purple (Hamidah *et al.,* 2019; Dahlia *et al.*, 2017).

Data Analysis

The research data were analyzed descriptively and the data were presented in

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the form of pictures and tables. Data collection was carried out to determine the types of pathogenic bacteria that can attack the spiny lobster (*P. homarus*) (Sudewi *et al.,* 2019).

RESULTS AND DISCUSSION

The lobster organs used in this study were the tail, gills, and hepatopancreas (Figure 1). Selection of the tail, gills, and hepatopancreas to see the diversity of bacteria in each organ which is then compared to the location where the lobsters were collected.

Figure 1. Organ parts of lobsters used as research samples. (A) hepatopancreas, (B) gills, (C) tail.

The hepatopancreas is the combined digestive gland of the liver and pancreas. This organ has a vital role in the digestive process of lobsters, which is the center of nutrient metabolism including the secretion of digestive enzymes, absorption of nutrients, and processing of nutrients into ATP (Ihsan *et al.*, 2017). The hepatopancreas of natural and cultivated lobsters have different characteristics, namely natural lobsters have a brightly colored appearance of the hepatopancreas, compared to cultivated lobsters (Figure 2).

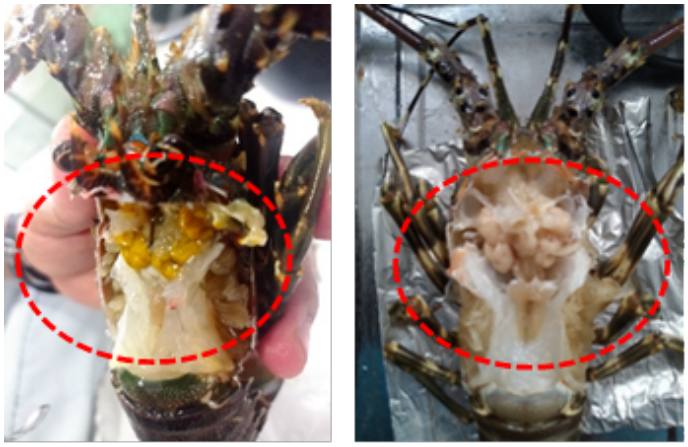

Figure 2. Condition of lobster hepatopancreas from nature and cultivation. (A) Labangka wild-caught lobster, (B) Bungin cultivated lobster.

Differences in the color of the hepatopancreas between natural and cultivated lobsters can be caused by dietary factors and immune stressors which are triggered by environmental conditions. Natural lobsters generally have high mobility to move from one place to another if there is a change in the environment. In contrast to cultivated lobsters, which have limited access to mobility, changes in the

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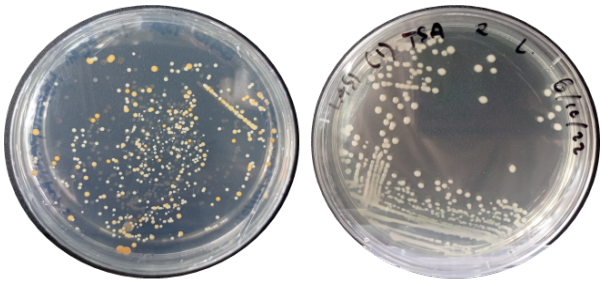
environment over a certain period will affect physiology and hemostasis which will have an impact on changes in nutritional mobility to immune responses. In addition to food factors, lobster stress is also strongly influenced by the environment. Low feed quality and worsening environmental conditions can cause disease in lobsters (Abdurachman *et al.,* 2020). In some cases, it also shows the color of the hepatopancreas greatly affects health status. Based on findings on several lobsters that identified symptoms of milky disease had the appearance of white hepatopancreas (Sudewi *et al.*, 2019). Nur and Yusnaini (2018) hepatopancreas is an organ sensitive to changes in the environment, food, or health status. The impact of several diseases on the hepatopancreas is to cause necrotic cells.

Based on research conducted by Sudewi *et al.* (2018a), total bacteria from cultivated lobsters are not much different when compared to wild catches. Bacterial cultures derived from the tail, gills, and hepatopancreas of natural and cultivated lobsters grown on TSA media, after incubation at room temperature for 18 hours, showed differences between the cultures derived from natural and cultivated lobsters. Natural lobsters tend to have a higher bacterial diversity based on color compared to cultivated lobsters (Figure 3). This can be caused by differences in environmental conditions, differences in food, and the level of lobster immunity.



A B

Figure 3. Bacterial isolates on Tryptic Soy Agar (TSA) media. (A) Bacterial isolates in natural lobsters, (B) Bacterial isolates in cultivated lobsters.

The results of the morphological characteristics showed that the bacteria derived from natural lobsters had a variety of colors, namely pink, yellow, and white. Meanwhile, cultivated lobsters only show white colonies. These results indicate that the bacteria present in natural lobsters are more diverse and help improve the standard of living of lobsters by acting as probiotics. Furthermore, all isolates owned were characterized morphologically (Table 1) and physiologically (Figure 4). Nurhasanah and Faturrahman (2019), lobster in nature has a more diverse microflora composition. Apart from being a food factor, microflora also has a role in mutualism with its host, especially in maintaining immunity, and at any time can stimulate immune function when needed.

Table 1. Morphological and physiological characterization.

Sample Code	Colony color	Colony form	Colony elevation	Gram stain
LSA (G)	white	round	convex	Positive
LSA (T)	pink	round	convex	Negative
LSA (H)	yellow	round	convex	Negative
LSBB (G)	white	round	convex	Negative
LSBB (T)	white	round	convex	Negative
LSBB (H)	white	round	convex	Negative

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Description: (LSA) natural lobster, (LSBB) lobster cultured bungin, (H) hepatopancreas, (T) tail, (G) gills.

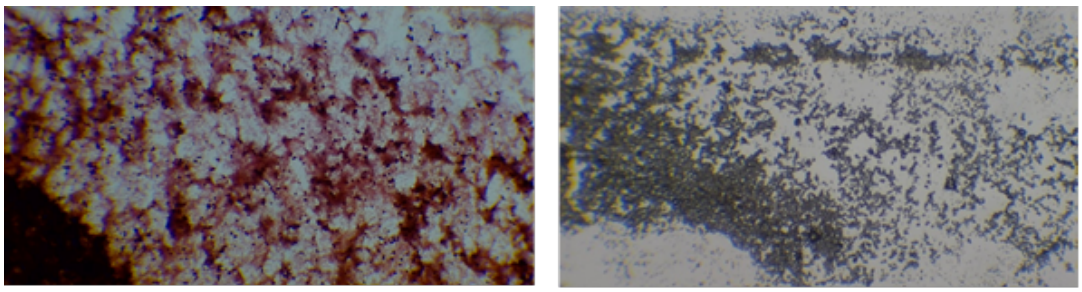

Figure 4. Physiological testing (gram staining) with a microscope magnification of 100x. (A) LSAG gram-negative bacteria, (B) LSBBG gram-positive bacteria.

Based on the above data, of the 6 isolate codes obtained, only one was a gram-positive bacteria. Valente and Wan (2021) gram positive or negative bacteria in the gills are greatly influenced by environmental conditions. Generally, gram-positive bacteria have a lower resistance level than gram-negative bacteria. Apart from the fact that gram-negative bacteria have a layer of lipopolysaccharide in a more complex peptigolic, gram-positive bacteria have a lower structure. Based on some cases, gills act as the earliest and simplest defense system, but not more complex and selective in certain bacteria (Mahulauw *et al*., 2022). Gram staining is a method for determining the type of bacteria from its cell. Gram-positive bacteria will produce a purple color because it has a thick peptidoglycan, while gram-negative bacteria will produce a red color because it has a thin peptidoglycan (Sudewi *et al*., 2018a). *Vibrio* sp. are gram-negative bacteria. Furthermore, the isolates were grown in TCBS media to determine the type of *Vibrio* bacteria (Figure 5 and Table 2).ls through the process of photosynthesis.

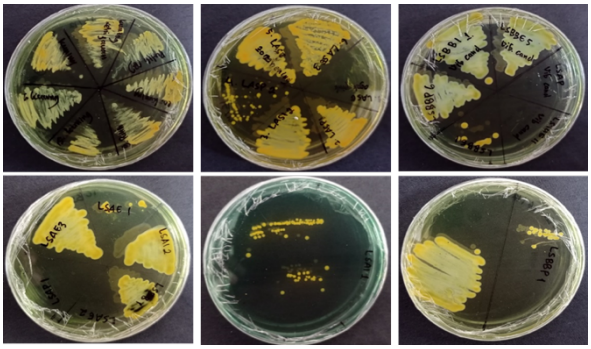

Figure 5. Pure isolates of natural and cultivated lobster bacteria on TCBS media.

Table 2. Types of pathogenic bacteria in wild-caught and cultivated lobsters.

Sample Code	Colony color	Identification of Bacteria
LSA (G)	yellow	*V. alginolyticus*
LSA (T)	yellow	*V. alginolyticus*
LSA (H)	yellow	*V. alginolyticus*
LSBB (G)	yellow and green	*V. alginolyticus, V. harveyi, V. parahaemolyticus*
LSBB (T)	yellow and green	*V. alginolyticus, V. harveyi, V. parahaemolyticus*
LSBB (H)	yellow and green	*V. alginolyticus, V. harveyi, V. parahaemolyticus*

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In general, from the results of this temporary study, 3 bacterial strains were obtained, which were dominated by *Vibrio* spp. It can be concluded that cultivated lobsters have more potential for pathogenic bacteria such as *V. alginolyticus, V. harveyi,* and *V. parahaemolyticus* which are more diverse compared to natural lobsters. This can be caused by various factors including the environment. Marjanto and Syaifuddin (2013), Bungin Island has open characteristics and is not far from Kaung Island which is on the north side of Sumbawa Island, NTB. Cultivation activities have so far been carried out on several fishery commodities, while the conversion of mangrove land and community settlements are several factors that influence the success of cultivation on Bungin Island.

Sudewi *et al*. (2019) apart from the injection method, exposure to bacteria can also be through maintenance media, although the potential infection rate is low. Meanwhile, direct contact through cannibalism can increase the risk of infection. According to Sudewi *et al.* (2020), water is a transfer medium for lobster pathogenic bacteria. There are similarities in the types and symptoms of disease between lobsters and shrimp, apart from their close kinship, the presence of similar types of pathogens certainly causes similar diseases. In addition, the thing that affects it is the quality of the feed which then affects the immunity to stress on lobsters, when lobsters are very easy to be infected by pathogens.

CONCLUSION

From the results of the study, it was found that the isolates grown on TSA media, showed a higher diversity of bacteria in natural lobsters compared to cultivated lobsters, this is what makes natural lobsters have a high survival rate because the diversity of microflora forms a symbiotic mutualism. Meanwhile, if we look at the diversity of pathogenic bacteria (*Vibrio* spp.), namely isolates grown on TCBS media, it shows that cultivated lobsters have more diverse pathogenic bacteria, namely three types of *Vibrio* (*V. alginolyticus, V. harveyi,* and *V. parahaemolyticus*) are indicated, only natural lobsters identified *V. alginolyticus*.

CONFLICT OF INTEREST

There is no conflict of interest in this manuscript between all authors upon writing and publishing this manuscript.

AUTHOR CONTRIBUTION

The contributions of each author are as follows; Muhammad Haikal Abdurachman collected and analyzed data, Adelia Elviantari followed the conception, design experiments, drafting and drafting of the manuscript, and Adi Suriyadin drafted the manuscript and revised it.

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