**Identification of Vibrio sp. Causes Vibriosis on White Snapper (Lates calcarifer) in Ponds of Balai Besar Perikanan Budidaya Laut (BBPBL) Lampung**

[Bakteri *vibrio* sp. Penyebab Vibriosis pada Ikan Kakap Putih (*Lates calcarifer*) di Tambak Balai Besar Perikanan Budidaya Laut (BBPBL) Lampung ]

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**ABSTRACT**

 *Vibrio* is a group of bacteria that causes biota of water that is cultivated in ponds death. This research aims to determine the type of bacteria *Vibrio* sp. which causes the disease in white snapper fish in the ponds BBPBL Lampung. The research method is exploration method by isolating *Vibrio* bacteria from the organ in white snapper, mud, and water and identifying bacterial isolate characteristics with biochemical test. Besides, water quality examination of pond to temperature, pH, salinity, DO, BOD and ammonia and calculation of ALT (*Total Plate Number*) *Vibrio* sp. on the pond. Based on the results of the research, three types of isolates are suspected as the cause of disease in white snapper fish in Lampung BBPBL pond, namely *Vibrio vulnificus*, *Vibrio alginolyticus* and *Vibrio parahaemolyticus*. The three isolates obtained were known to be α-hemolysis. The results of pond water quality inspection showed the temperature of 29.8 oC, pH 7.8, salinity 33 psu, DO 4.4 mg / L, BOD 2.2 mg / L and ammonia 2.2 mg / L. Calculation of ALT Bacteria *Vibrio* sp. shows inlet of pond <25 CFU / ml, pond water 7.6x104 CFU / ml, and pond water outlet 2.1x103 CFU / ml. Levels of ammonia and calculation of ALT Bacteria *Vibrio* sp. exceeds pond water quality standards for fish farming.

Keywords: Identification, Vibriosis and white snapper

**ABSTRAK**

*Vibrio* merupakan golongan bakteri penyebab kematian biota air yang dibudidaya di tambak. Penelitian ini bertujuan untuk untuk mengetahui jenis bakteri *Vibrio* sp. yang menyebabkan penyakit pada ikan kakap putih di tambak BBPBL Lampung. Metode penelitian yang dilakukan adalah metode eksplorasi dengan mengisolasi bakteri *Vibrio* dari organ dalam ikan kakap putih, lumpur, dan air serta mendidentifikasi karakteristik isolate bakteri dengan uji biokimia. Selain itu dilakukan pemeriksaan kualitas air tambak terhadap suhu, pH, salinitas, DO, BOD dan ammonia dan dilakukan perhitungan ALT (Angka Lempeng Total) *Vibrio* sp. pada tambak. Berdasarkan hasil penelitian diketahui tiga jenis isolat yang diduga sebagai penyebab penyakit pada ikan kakap putih di tambak BBPBL Lampung, yaitu *Vibrio vulnificus*, *Vibrio alginolyticus* dan *Vibrio parahaemolyticus*. Ketiga isolat yang diperoleh diketahui bersifat α-hemolysis. Hasil pemeriksaan kualitas air tambak menunjukkan suhu 29,8 oC, pH 7,8 , salinitas 33 psu, DO 4,4 mg/L, BOD 2,2 mg/L dan ammonia 2,2 mg/L. Perhitungan ALT Bakteri *Vibrio* sp. menunjukkan air masuk (*inlet*) tambak <25 CFU/ml, air tambak 7,6x104 CFU/ml, dan air keluar (*outlet*) tambak 2,1x103 CFU/ml. Kadar ammonia dan perhitungan ALT Bakteri *Vibrio* sp. melebihi baku mutu air tambak untuk budidaya ikan.

Kata penting: Identifikasi, *Vibriosis* dan Kakap Putih

**Introduction**

Usually, microorganisms that cause disease in fish and shrimp are bacteria, fungi, and viruses. Pathogen bacteria often attack fish and it can cause mass deaths in fish and shrimp by *Vibrio* sp. [1, 2, 3]. The pathogen *Vibrio* bacteria can cause *Vibriosis* as a disease and it can result in deaths of more than 80% in fish in floating nets [4].

According to Taslihan et al. [5] *Vibrio alginolyticus* is the type of bacteria that it most often infects white snapper, causing mass death. These bacteria are very harmful both in seawater and brackish water fish farming. They can cause primary and secondary infections.

The environment is very important to support the success of fish farming. Unsuitable conditions can lead to disease infection in white snapper especially from *Vibrio* group bacteria. At present there is a mass death of white snapper in the pond of the Balai Besar Perikanan Budidaya Laut (BBPBL) Lampung. Clinical symptoms of fish in the ponds indicate vibriosis infection in these fish such as anorexia, abnormal swimming behavior, loss of balance, the color of the body of the fish is red or black, and scales are peeling. Based on the description, it is necessary to research for the identification of *Vibrio* sp. causes of vibriosis in white snapper in ponds of Balai Besar Perikanan Budidaya Laut (BBPBL) Lampung. This study aims to determine the type of *Vibrio* sp. which causes disease in white snapper in pond of BBPBL- Lampung.

**Material and Methods**

**Isolation and Characterization of *Vibrio* sp.**

*Vibrio* sp. was isolated from internal organs (spleen, liver, kidneys), fish wounds, mud and water of pond. Isolation was carried out from internal organs and wounds of fish by the streak method. Isolation from pond mud is done by pour plate method. Samples of 1 gram of sludge were put into 9 ml Alkaline Peptone Water (APW), then it was shaken until homogeneous. Then, the suspension is grown on TCBSA media with the spread method. Isolation from pond water is done by put in 1 ml sample to 9 ml APW media and shake until homogeneous. After that, the suspension is grown on the Thiosulfate Citrate Bile Sucrose (TCBS) media (Yeast Extract, Bacto Peptone, Sodium Thiosulfate, Sodium Citrate, BTB, Agar, OX Bile, Sucrose) with the spread method. All samples were then incubated at 30-35oC for 24 hours. After incubation for 24 hours, the bacteria were observed for colony characteristics, Gram staining, catalase testing, oxidase testing and motility testing in each isolate.

**Hemolysis assay**

*Vibrio* sp. was grown on the media of Blood Agar Plates (BAP) to determinate hemolytic or non-hemolytic bacteria. BAP media is made by adding 5% sheep blood to the total volume of media to be used (NA media). The bacteria were grown by the streak method, then it was incubated at a temperature of 30-35oC for 24 hours. The hemolysis test results refer to Nelce and Setha [6] stating that there are 3 types of hemolysis: β-hemolysis (there is no blood around the colony), α-hemolysis (some blood cells found at the zone of hemolysis or some blood have a greenish color change around the colony) and γ-hemolysis (non hemolysis). The γ-hemolysis shows that these microorganisms can not have the potential as pathogenic bacteria [6].

***Identification of Vibrio sp.***

Identification of *Vibrio* sp. performed on 27 isolates. Then, a series of biochemical tests conducted i.e. test of catalase, oxidase, motility, nitrate, lysine, ornithine, H2S, glucose, Mannitol, Xylose, ONPG, indole, Urease, VP, citrate, TDA, Gelatin, Malonate, Inositol, Sorbitol, Rhamnose, Sukrose, Lactose, Arabinose, Adonitol, Raffinose, Salicin and Arginine.

**Pond Water Quality assay**

Water quality was observed by enumeration of pathogenic bacteria (*Vibrio* sp.) from pond environments. Physical parameters which observed namely pond salinity and water temperature. Chemical parameters which observed namely pH, DO, BOD, and ammonia levels in pond water.

**Results**

**Isolation and Characterization of Pathogenic Bacteria**

Choosing fish samples is done by observing the clinical symptoms that appear in fish such as injury to the body, swimming abnormally, aloof, wrinkling on the fin decreases to stimulation, scales peeling, there are wounds on the body, exoptalmis (eyes protruding), tail fin wheeled backs, pale internal organs, excess slimy gills, liver experiencing multifocal necrosis, kidney swelling, swelling of the spleen and decreased appetite. Symptoms of fish affected by vibriosis (Figure 1).

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| --- | --- |
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| Figure 1. (a). Clinical Symptoms of White Snapper Fish Infected with *Vibrio* sp. bacteria;  (b) Healthy White Snapper Fish |
|  |

Isolation and characterization of bacterial colonies were taken from white snapper, mud and pond water as many as 30 isolates. However, only four isolates with strong characteristics were suspected to be pathogenic and it cause disease in the white snapper (Table 1 and Table 2).

Table 1. Isolation of *Vibrio* sp.

|  |  |
| --- | --- |
| Source | Characterization |
| Quantity of Isolate | Hemolysis Test | Biochemical Test(Identification) |
| Pathogen | Non-Pathogen  |
| Water  | 2 | 257721 | 0 | 0 |
| Kidney  | 6 | 1 | 1 |
| Heart  | 9 | 2 | 1 |
| Spleen  | 9 | 2 | 2 |
| Wound  | 3 | 1 | 0 |
| Mud  | 1 | 0 | 0 |
| Total  | 30 | 24 | 6 | 4 |

Table 2. Characteristics of *Vibrio* sp. colonies.

|  |  |  |
| --- | --- | --- |
| Isolate | Source | Characteristics of *Vibrio* sp. colonies on TCBSA medium |
| Size  | Color  | Morphology of colony | Border  | Elevasi |
| 14 | Kidney  | medium | Green | Irreguler | Undulate | Convex |
| 16 | Heart  | medium | Green | Irreguler | Undulate | Convex |
| 24 | Spleen  | medium | Yellow  | Circular | Entire | Raised |
| 25 | Spleen  | medium | Yellow  | Irreguler | Undulate | Raised |

The Vibrio isolate was grown on TCBSA media. The colonies are green and yellow (Figure 2).

|  |
| --- |
| E:\Berkas\bidik misi nandi\koloni.jpg**cb****d****a****b** |
| Figure 2. Isolate of *Vibrio* on TCBSA media; (a) Isolate-14; (b) Isolate-16; (c) Isolat-25;  (d) Isolat-24 |

The results of Gram staining show that 30 isolates are Gram of negative and form of cocobasil or short stem. The motility test results showed that 30 isolates were motile which was characterized by active movement (Figure 3).

|  |
| --- |
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| Figur 3. Gram staining |

Then, the observation results proved that 30 isolates produce enzyme of the oxidase and catalase. It can show that 30 isolates are group of *Vibrio* sp. (Figure 4).

|  |  |
| --- | --- |
| E:\katalase.jpg**a** | E:\oksidase.jpg**b** |

Figure 4. (a) Catalase activities; (b) Oxidase activities

Based on observations on the 30 isolates of bacteria, they were 24 isolates as α-hemolysis i.e: 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 , 14, 15, 16, 17, 18, 19, 22, 24, 25, 28, 29 and 30 isolates. Whereas the other 6 isolates were γ-hemolysis (non hemolytic) i.e: 3, 20, 21, 23, 26 and 27 isolates (Figure 5).

|  |
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Figure 5. Hemolysis activities

**Biochemical assays**

It was found that isolates 14th from spleen organs identified as *Vibrio vulnificus*. The bacteria were determinated by yellow colonies on TCBSA media because they were able to utilize sucrose compounds in the media. The bacteria created positively reaction to the indole, ONPG, glucose, and mannitol assay. However, the bacteria created reaction negatively to the hydrolysis of urea, lactose, arginine and ornitine assays (Table 3).

Table 3. Data of biochemical assay

|  |  |
| --- | --- |
| biochemical assay  | Isolate code |
| 14 | 16 | 24 | 25 |
| Swarming | - | - | + | - |
| Growth at 37 oC | + | + | + | + |
| Growth with 0 % NaCl | - | - | - | - |
| Gram | - | - | - | - |
| Motility | + | + | + | + |
| Oksidase | + | + | + | + |
| Catalase | + | + | + | + |
| Moller’s decarboxylases: |  |  |  |  |
|  Arginine | - | - | - | - |
|  Lysine | + | + | + | + |
|  Ornithine | - | - | - | - |
| Nitrate Reduction | + | + | + | + |
| Gas from glucose | - | - | - | - |
| Indole | + | + | + | + |
| ONPG | + | + | + | + |
| Voges Proskauer  | - | - | - | - |
| Acid from: |  |  |  |  |
|  Glucose | + | - | - | + |
|  Sorbitol | + | - | - | + |
|  Lactose | - | - | - | - |
|  Raffinose | - | - | - | - |
|  Mannitol | + | + | - | + |
|  Rhamnose | - | - | - | - |
|  Arabinose | - | - | - | - |
|  Inositol | - | - | - | - |
|  Sucrose | + | - | + | - |
|  Adonitol | - | - | - | - |
|  Xylose | - | - | - | - |
|  Salicin | + | - | - | + |
| Gelatin Liquefaction | - | - | - | - |
| Citrate Utilization | + | + | + | + |
| Malonate Inhibition | - | - | - | - |
| H2S Production | - | - | - | - |
| Urea Hydrolysis | - | + | + | + |
| Tryptophan Deaminase | + | + | + | + |
| Hemolysis | α | α | α | α |
|  | 84,00 % | 95,34 % | 87,33 % | 97,15 % |
| *Species*  | *V. vulnificus* | *V.* *Parahaemolyticus* | *V.**alginolyticus* | *V.**Vulnificus* |

Isolate 16th origin from the liver was identified as *Vibrio parahaemolyticus* which is characterized i.e: its colonies are bluish green on TCBSA media. The bacteria can not utilize sucrose compounds on the media. The bacteria created positively reaction test of lysine, indole, ONPG, mannitol, and hydrolyze urea. The bacteria created negatively reaction test for arginine, ornitine, glucose, sorbitol and lactose (Table 3).

Isolate 24th origin from the kidney was identified as *Vibrio alginolyticus* which is characterized i.e: its colonies are yellow on TCBSA media. The bacteria can utilize sucrose compounds in the media, react positively to the test of lysine, indole, and ONPG. The bacteria created negatively reaction to the arginine test, ornitin, glucose, lactose and mannitol (Table 3).

Isolate 25th origin from the kidney was identified as *Vibrio vulnificus* which is characterized i.e: its colonies are bluish green on TCBSA media. The bacteria can not utilize the sucrose compounds present in the media, react positively to the test of lysine, indole, ONPG, glucose, sorbitol, and able to hydrolyze urea. The bacteria created negatively reaction to the test for arginine, ornitine, mannitol, and lactose (Table 3).

**Pond Water Quality Measurement**

Quantity of *Vibrio* sp. is obtained from the pond's abiotic environment. The other environmental data are pH, temperature, salinity, BOD, COD, and ammonia. The environments data are obtained from inlet water, pond water and outlet water (Table 4).

Table 4. Result of Pond Water Quality Measurement

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| No | Parameter | Unit  | Yield  | Standart\* |
| I | BIOLOGICAL |  |  |  |
|  | Pathogen bacteria (*Vibrio* sp.) |  |  |  |
|  | Water run in pond (*inlet*) | CFU/ml | <25 | <25 |
|  | Water of pond  | CFU/ml | 7.7x104 | 1x102 **(A)** |
|  | Water run out pond (*outlet*) | CFU/ml | 2.2x103 | 1x102 **(A)** |
| II | PHYSICAL  |  |  |  |
|  | Salinity | Psu | 33 | 30-34 |
|  | Temperathure | oC | 29.8 | Normal  |
| III | CHEMICAL |  |  |  |
|  | pH | - | 7.89 | 7-8.5 |
|  | DO | mg/L | 4.4 | >4  |
|  | BOD | mg/L | 2.26 | <3  |
|  | Amonia | mg/L | 2.24 | <0.4 (A) |

Note:

 \*: Based on Sea Water Quality Standards for Marine Biota from Declaration of the Minister of Forestry and

 Environment No.51 in 2004

 A: Abnormal

**Discussion**

Green colonies of bacteria were caused by the bacteria can not ferment sucrose such as *Vibrio vulnificus* and *Vibrio parahaemolyticus*. The yellow colonies of bacteria were caused by the bacteria can ferment sucrose contained in TCBSA media.

This condition makes decrease of the pH of the media which eventually results in a yellow bacterial colony such as *Vibrio alginolyticus* and *Vibrio fluvialis*. The Vibrio bacterial colonies that grow on TCBSA media are usually sticky like glue [6]. Another researcher, Felix et al. [1] states that the *Vibrio* group bacteria have gram negative properties, a short trunked cell shape with a size range of 2-3 µm. The bacteria of *Vibrio* sp. motile and very active when in liquid culture. According to Bergey and Boone [7] *Vibrio* sp. has fermentative properties, produce oxidase and catalase enzymes, and facultative of anaerobes. The results were Gram staining, motility test, oxidase test and catalase test showed that 30 isolates as *Vibrio* sp. bacteria. Color changes became purple by the formation of cytochrome oxidase [8].

*Vibrio* bacteria are known to produce hemolysin enzymes. Haemolysis and leukosidine activities produced by bacterial Extracellular products (ECPs). ECPs as bacterial defense factors were used against host blood defenses because they can lyse blood cells. The bacteria that are able to survive will enter the bloodstream so that it spreads to all cells of the host's body as well as to the target organ [9].

Based on the ability to produce hemolysin, bacteria can be grouped into two positive Kanagawa Phenomena (KP) which will show β-hemolysis reactions, which the bacteria are characterized by colonies with clear areas around it, and negative Kanagawa Phenomena (KP) that the bacteria will show reaction-hemolysis shows by colonies which show signs of blanching. According to Nelce and Setha [6] the *Vibrio parahaemolyticus* bacteria were classified into positive kanagawa by showing clear areas around the colonies on agar media with high salinity to test Vibrio hemolytic activity. The bacteria *Vibrio parahaemolyticus* isolated from the feces of patients with gastroenteritis showed a positive Kanagawa Phenomenon (KP) reaction, while those isolated from fish, crabs, shrimp, shells, sand and mud on the edge of the rice were strongly suspected to be negative of Kanagawa Phenomenon (KP) reactions .

The density of *Vibrio* bacteria in ponds is very influential on fish farming. Total bacteria *Vibrio* sp. in pond water 7.65x104 CFU / ml. This amount has exceeded the threshold, where the total Vibrio threshold recommended for the level of ponds / aquaculture ponds is 1x102CFU / ml [10]. Total bacteria that exceeds the threshold will potentially increase infection. The emergence of disease infections, especially Vibriosis, it is one of the problems in the effort of fish and shrimp cultivation because it can lead to death [11].

Vibrio bacteria are classified as opportunistic bacteria [12]. Vibrio bacteria can be pathogenic if the cells were the sufficient amount (quorum). Then, virulence factors expressed lead to death in fish. Quorum sensing is a mechanism in which bacteria coordinate the expression of certaingenes in response to their population density by producing, releasing and detecting small signal molecules [13].

The pH value is 7.89, the salinity level shows a value of 33 ppt, the water temperature shows a value of 29.8oC, the DO value shows a value of 4.4 mg / L, the BOD measurement shows the value of 2.26 mg / L indicates that it is still within the threshold suitable for conducting cultivation [14].

The results of ammonia measurements on white snapper ponds at BBPBL showed a value of 2.24 mg / L. According to the Declaration of the Minister of Forestry and Environment No.51 in 2004, ammonia levels for aquaculture activities must be <0.4 mg / L. That it shows that the ammonia value in the pond has exceeded the quality standard threshold because it shows a value of 2.24 mg / L. Ammonia levels that have overdone the threshold can cause pollution in these waters. The high amount of ammonia is assumed with the high percentage of feeding in the area of ​​fish farming in the pond. The mode caused the waste remnants of metabolic products by fish, feces increase, thus affecting the high amount of ammonia in the pond.

 The high ammonia levels also indicate indirectly that the levels of organic matter in these waters are high. The condition means that the higher the organic matter in a water, the higher the population of microorganisms in the waters. This situation will have an impact on the total *Vibrio* sp. in pond water which is 1.8 x 105 CFU / ml.

**Conclusion**

Based on the research was concluded that it was known three types of isolates pathogen cause of disease in white snapper at the pond of the Balai Besar Perikanan Budidaya Laut (BBPBL). The three pathogen bacteria are *Vibrio vulnificus, Vibrio alginolyticus* and *Vibrio parahaemolyticus*.

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