IDENTIFICATION OF BACTERIA AND PARASITES ON SEMI-INTENSIVE CULTURE OF CATFISH *Pangasius sp*.

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Abstract. Catfish (*Pangasius sp*.) is a freshwater fish that has a high economic value and widely cultivated. Catfish culture can be done in semi-intensive ponds. The problem often occured in semi-intensive culture is the disease infection which is causes large losses. The disease infection can cause high mortality in catfish cultivation. It's resulting to decrease in catfish productivity. Sample of fish were taken from farmers, in semi-intensive ponds, with target organs were muscle, gill, and kidney. The identification of disease agent conducted to biochemical test and Polymerase Chain Reaction (PCR). The result shown that Catfish infected positively by bacterium *Aeromonas hydrophila*, and ectoparasites were *Dactylogyrus sp*. Positive *Aeromonas hydrophila* means that there are scattering in the test sample that is aligned with the band in the positive control (597 bp), while *Aeomonas hydrophila* negative has no scattering in a test sample to the band in the positive control.

Keywords: Ectoparasite, Fish Disease, Molecular, PCR

1. Introduction

Catfish (*Pangasius* sp.) is a freshwater fish that has high potential for developing cultivation due to its adaptation ability to environmental changes and fast growth. Catfish can tolerate low dissolved oxygen (DO) levels in the water and can be cultured in ponds, concrete tanks or fish cages (Vaishnav *et al*., 2017). Catfish is widely cultivated in several countries such as Thailand, Vietnam, Indonesia, Cambodia and Myanmar.

Catfish culture can be done in semi-intensive ponds. Semi-intensive cultivation is a cultivation activity that can be carried out with a fairly high stocking density. Semi intensive cultivation with high density makes the water quality decreases. Declining water quality causes fish to become infected with disease and die. Need to identify the causative agent of the disease in semi-intensive cultivation of *Pangasius* sp.

2. Materials and Methods

2.1 Research Material

This research was carried out ex-situ in Balai KIPM Surabaya I. Samples of *Pangasius sp.* were taken from farmers. The tools used in this study are thermal bicycle riders, electric balance, hot plates, centrifuges, dry shower incubators, vortex mixers, micropipets, power supplies, and UV gel docks. The materials used in this study were pangasius sp, a magic genomic purification device, reagents, positive KHV samples, DNA markers, and agarose gels.

2.2 Research Methods

- **PCR test**

DNA Extraction

DNA extraction is the process of separating DNA from other cell components. In the Laboratory of Molecular Biology Balai KIPM Surabaya I, Work Instructions for Development of DNA extraction materials using the Wizard Purification Genomic Kit. Samples to be extracted are gills and digestive organs.

Amplification

Amplification aims to increase the target DNA to be analyzed using electrophoresis. One cycle in the amplification process includes Pre-denaturation, Denaturation, Annealing, Extension and Final Extension. The amplification process occurs 30-40 cycles which can produce millions of DNA.

Electrophoresis

Electrophoresis is the transfer of charged molecules in response to an electric field. This is the process of transferring negatively charged molecules to positive poles. The media used in the molecular transfer process is agarose gel. This electric current makes the DNA molecule move from the negative pole to the positive pole according to the size of the base pair. The speed of movement is determined by the length of the DNA band.

- **Ectoparasites**

Ectoparasites were performed by visual examination. Visual inspection, seen from the external morphology that includes operculum, gills, skin, and fins. Then placed on preparations that have been prepared, observed under a microscope.

- **Water quality**

Water quality observations are carried out every day during the maintenance period. The observed water quality is temperature, pH, Dissolved oxygen (DO), nitrate (NO_3-N), nitrite (NO_2-N), and phospate (PO_4).

3. Results

- **PCR test**

Catfish in semi-intensive ponds positively attacked by *A. hydrophilla* bacteria. The results of PCR testing showed a band of DNA bands that ran parallel to the positive control of *A. hydrophilla* measuring 597 bp.

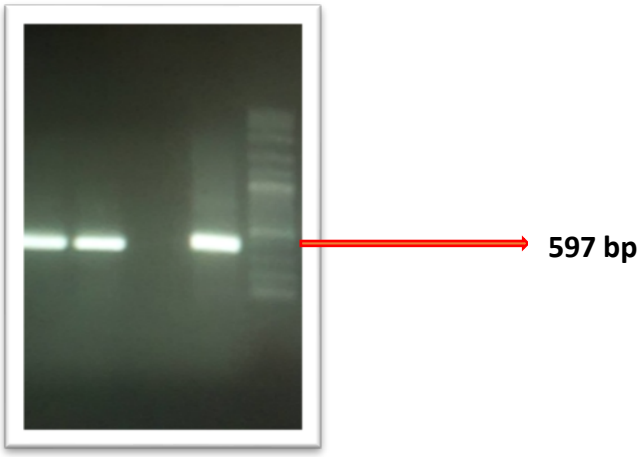

Figure 1. Bacterial PCR Results (Lane 1: Marker; Lane 2: positif control Aeromonas hydrophila; lane 4-5: positif sample)

Staining method was also carried out on aeromonas hydrophila isolates to determine gram-positive or gram-negative. Staining is done using a violet crystal solution, iodine lugol, 90% alcohol and safranine. after coloring it is next observed under a microscope with a magnification of 100x. the results of the staining found that A. hydrophila belong to gram negative bacteria (figure 2).

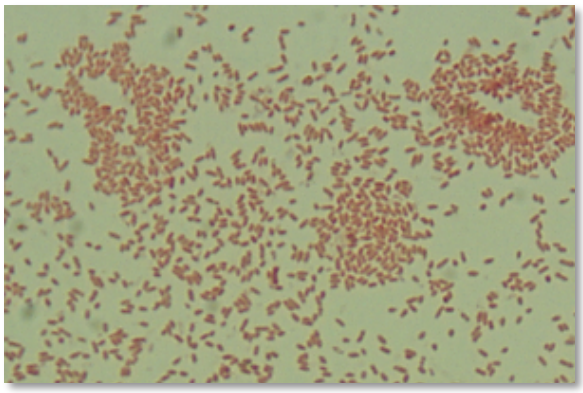

Figure 2. Bacterial Staining

- **Ectoparasites**

Morphologically observed parasites were then identified. Parasit identification can use identification book. The results obtained from the identification of parasites in catfish (*Pangasius* sp.) are *Dactylogyru*s sp.

Dactylogyrus sp. is a parasite that often infects freshwater ornamental fish and freshwater fish consumption. This parasite often attacks catfish, tilapia and koi. This parasite is found in the gills of fish. Gills taken in fish affected by *Dactylogyrus* sp. looks pale. Fish infected with *Dactylogyrus* sp. experiencing a change in behavior.

Figure 3. The ectoparasite of *Dactylogyrus sp.* in *Pangasius sp.*

- **Water quality**

Table 1. Water quality test results

Parameters	Unit	Test results	Test Method
• Temperature	^oC	27	2550 B
• Ph	-	7.1	4500-H^+-B
• Nitrate (NO_3-N)	mg/L	0.05	4500-NO_3^-E
• Nitrite (NO_2-N)	mg/L	< 0.003	4500-NO_2^-B
• BOD, 5 hari 20^oC	mg/L	108	5210 B
• COD $K_2Cr_2O_7$	mg/L	377	5220 B
• Dissolved Oxygen (DO)	mg/L	< 0.35	4500-O B
• Phosphate (PO_4)	mg/L	7	4500 P C

Data from the measurement of water quality parameters are used to analyze the relationship between environmental factors and catfish maintenance.

4. Discussion

- **PCR Test**

Identification of fish pathogenic bacteria is important in the diagnosis of disease. However, conventional methods of isolation and identification are cumbersome and time consuming. Testing methods using PCR with specific DNA sequence amplification provide very specific and sensitive tools for detecting microorganisms, one of which is aeromonas

bacteria. PCR test results obtained a DNA fragment of about 597 bp amplified only in A. hydrophila strains (figure 1).

The pathogenic and virulence characteristic of *A. hydrophila* are associated with the range of different exotoxins and exoenzymes. PCR test was done for detecting haemolysin as well as aerolysin gennes as genetic markers for virulence determinants. The final product from PCR testing was completed in 1.5% agarose gel in a TBE buffer and the gel was stained with EtBr and viewed under UV light. The amplification results obtained through this study also showed a band in the virulent gene which was 597 bp.

Bacterial diseases are the most common infectious problem of commercial fish farms and ornamental fishes. Some pathogenic bacteria are responsible for fish diseases in captivity and responsible for kidney disease, dropsy, enteric red-mouth, tuberculosis, vibriosis, motile aeromonad septicemia, bacterial gill disease, mouth fungus, tail and fin rot, and columnaris diseases. The major bacterial pathogen *Aeromonas* spp. is responsible for hemorrhagic septicemia, a disease affecting a wide variety of freshwater and marine fish [25]. *Aeromonas hydrophila* is the causative agent of MAS (*motile Aeromonas septicemia*) in both farmed and wild fishes. The disease is characterized by swollen abdomen, red mouth, hemorrhage in external surface and surrounding of the anus (Nahar, *et al.,* 2016).

Aeromonas hydrophila is a ubiquitous gram-negative bacterial inhabitant of freshwater environments and is a wellknown pathogen of many species of cultured and wild fish, including channel catfish (*Ictalurus punctatus*). It was often associated with disease outbreaks secondary to stress or infection with a primary pathogen. *Motile aeromonas septicemia* (MAS) is caused by infection with aeromonad bacteria, most commonly applied to *A. hydrophila*, but it may include species such as *Aeromonas sobria* and *Aeromonas caviae*. A diverse pattern of lesions can be elicited in diseased fish, depending on the species affected, the strain of bacteria, and the method of infection. Dermal necrosis, ulcerations, petechiation or hyperemia, and ocular disease are frequent external manifestations. Hepatic and renal necrosis are common, often with hemorrhage and necrosis in the intestine; splenic necrosis is variable (Baumgartner, *et al.,* 2017).

- **Ectoparasite**

Dactylogyrus sp. often infects parts of the gills of freshwater fish in brackish water and sea. Adult worms are up to 0.2 - 2 mm. They have two pairs of eye spots on the anterior end. They have a sucker located near to their anterior end. At the posterior end of the body, there is a sticking device which consists of 2 large hooks surrounded by 14 smaller hooks called opisthaptor (Fitriani, *et al.,* 2019).

The oviparous dactylogyrids are primarily gill parasites of freshwater fish but they may be seen in skin of freshwater fish. The fish infected with dactylogyrus showed clinical symptoms including the lethargy, unilateral swimming and erosion on gill filament and scale loss (Mohammadi, *et al.,* 2012).

- **Water quality**

Water quality is the most important factor to know the state of the pond culture environment. Poor water quality can cause a disease to appear. Success of aquaculture depends almost completely on the quality of different water parameters. Water quality for aquaculturists refers to the quality of water that enables successful propagation of the desired organisms. The maintenance of good quality of water is essential for both survival and optimum growth of culture organisms. Good water quality is characterized by adequate oxygen and limited levels of metabolites. This is because large quantities of feed is loaded in ponds and excess feed, fecal matter and then metabolites can cause drastic changes in water quality parameters and sediment chemistry which may affect the growth (Begum, *et al.,* 2014).

The results of water quality testing in table 1 shows that optimal water quality is very important for the *Pangassius* sp.The growth of different fish species is also influenced by a different range of factors, among them water quality parameters. Fish growth is generally greater in ponds with optimal levels of DO, temperature among other parameters, though different fish species have ideal levels of water quality parameters within which they grow optimally. Water temperature that is not optimal is one of the factors that influence fish growth. The optimal temperature for raising catfish is 28-32^{o}C. High temperatures can affect pH, if the temperature rises eating pH will also rise which can produce toxins (ammonia) that come from the rest of metabolism and feed that is not consumed. The pH value is lethal to fish, which is less than 4 and more than 11. Dissolved oxygen becomes an important factor in determining fish life, breathing will be disrupted if oxygen levels are lacking in aquaculture waters. Some fish are able to survive in waters with dissolved oxygen concentration of 3 mg / L, but the concentration of dissolved oxygen that is good for aquaculture fish is 5 mg / L (Makori, *et al.,* 2017).

5. Conclussion

Catfish samples were identified conducted PCR and biochemical test. Identified disease agents from groups of parasites and bacteria. The result of the PCR test showed the bacterium *Aeromonas hydrophilla* positively infected in the catfish. The parasitic test by visually showed the fish attacked by the ectoparasite *Dactylogyrus* sp..

6. Acknowledgement

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