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Characterization of *Aeromonas hydrophila* bacteria on dumbo catfish (*Clarias gariepinus*) from Bungo Jambi Province, Indonesia

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ABSTRACT

This study aims to isolate, characterize and detect virulent genes of *A. hydrophila* bacteria that cause MAS disease in African catfish in the Bungo regency of Jambi Province on a molecular basis. The results of the study are expected to provide information on the microorganisms that contains in *Aeromonas hydrophila* cause MAS in catfish with a biofloc system in Bungo. The samples of research was 35 catfishs that had symptoms of MAS in a biofloc from Bungo. The sample used is in the form of liver, kidney, spleen and intestine swabs, and identification based on the Indonesian National Standard (SNI 7303.1-2015). Gene detection use PCR method to targeting the *16S rRNA* gene and the *aerA* gene. *aerA* gene is an aerolysin virulence gene that causes *Motile Aeromonas Septicemia* (MAS). Based on the results of biochemical testing A. microscopic *Aeromonas hydrophila* is a bacteria Gram negative with a short rod shape, and confirm with specific media show 17 positive *Aeromonas hydrophila* isolates. The results of the detection of *16S rRNA* genes of *Aeromonas hydrophila* from 17 tested show that 6 isolates are positive for *16S rRNA* genes at 1500 bp. Detection of the *aerA* gene show 6 isolates of *Aeromonas hydrophila* have the virulent factor aerolysin at 309 bp.

Key words : *Aeromonas hydrophila, Biofloc, Bungo, Clarias gariepinus, aerAgene*

Introduction

Clarias gariepinus belong is the air breathing catfish are freshwater fish that are widely cultivated in Indonesia, one of which is the Bungo district of Jambi Province. Based on data obtained from the Ministry of Maritime Affairs and Fisheries (KKP) demand for consumption and production of catfish continue to increase in Indonesia. In 2017 it was recorded that catfish production reached 1.77 million tons or increased by 131 percent from 2016 which reached 764,797 tons (KKP, 2017). The farm catfish usually carried out in concrete ponds, ponds and tarpaulin ponds (Satyani and Priono, 2012). KKP contributes to provided biofloc cultivation system programs in each region, especially in Jambi Province in 2018. In 2013, catfish production in Jambi Province was recorded at 543,461 tons, then decreased to 463,221 tons in 2014 (DJPB, 2014).

Biofloc system is a fisheries cultivation technology that is based on the principle of inorganic nitrogen assimilation or environmental engineering to overcome conventional water quality problems (KKP, 2017). According to Rusherlistyani *et al.*,

(2017) biofloc systems help reduce the incidence of diseases caused by microorganisms. During the maintenance of catfish using the biofloc system is more ffective because the number of deaths in catfish is very low Sudaryati *et al.,* (2017); Rachmani, (2011) states that the application of biofloc ponds is more effective to maintain water quality, but the conditions in the field of farmers more apply stocking-dense systems. According to (Sheperd and Bromage, (1988) the spread of fish with high density can cause a decrease in water quality, thus triggering the emergence of various diseases. Cultivation of fish using stocking-dense methods can reduce water quality and lead to disease infection, one of MAS diseases caused by *A. hydrophila* bacteria (Sukenda *et al.,* 2008; Agus, 2004). Bacterial infection of *A. hydrophila* can reduce the quality of fish meat and cause high economic losses for fish farmers (Supriyadi and Taufik, 1981). Infection due to *A. hydrophila* can cause symptoms including sores on the surface of the body, enlarged abdomen, swelling of the liver, kidneys and lymph, bleeding fins, gills and anus and protruding eyes (Austin and Austin, 1993; Austin and Adams, 1996).

Problems regarding the bacterial infection of *A. hydrophila* in Indonesia are still quite high. This bacterium is able to infect seawater or freshwater fish. Isolation and characterization of *A. hydrophila* bacteria need to be done to determine its characteristics as a cause of MAS disease, especially in African catfish by detecting its virulent genes using the Polymerase Chain Reaction (PCR) method. This study aims to isolate, characterize and detect virulent genes of *A. hydrophila* bacteria that cause MAS disease in African catfish in the Bungo regency of Jambi Province on a molecular basis.

Materials and Methods

Time and Place

The research was conducted at the Integrated Laboratory of Medical Microbiology Division of the Department of Veterinary Disease and Veterinary Public Health, Faculty of Veterinary Medicine, IPB. Confirmation tests with Polymerase Chain Reaction (PCR) were carried out at the Fish Quarantine Standard Testing Office for Quality Control and Safety of Fisheries in East Jakarta. Sampling was conducted in the Bungo district in Jambi province. The sample used was catfish using Random sampling method as many as 35 catfish and used liver, kidney, spleen and intestine.

Bacterial isolates

Organ samples were isolated using blood agar and incubated for 18 24 hours at 25°C – 28°C. The suspected bacterial colony was then purified by Trypticase Soy Agar (TSA). Identification was carried out macroscopically and microscopically. Biochemical tests were carried out using catalase, Indol, Triple Sugar Iron Agar (TSIA), Methyl-Red, Voges proskauer (MR-VP), urease, citrate, Fermentative Oxidative (O/F) and Rimler-Shotts (RS) tests (SNI, 2015). Positive isolates of *A. hydrophila* were re-cultured on TSA media and incubated for 24 hours at 37°C. The Rimler-Shotts media test was then evaluated as selective media.

Genomic DNA extraction

A. hydrophila positive samples were subsequently carried out DNA extraction using boiling method. The 1-2 ose Bacterial colony was added 100 µL PBS and homogenized using a vortex, then heated at a temperature of 100 °C for 10 minutes. The heated suspension is cooled for 4 minutes, in centrifugation for 2 minutes at a speed of 12500 rpm to precipitate bacterial cells. Further part of the the supernatant which is DNA was taken as much as 50 µL and inserted into the micro tube 1.5 mL. This DNA will be used for gene detection with PCR (Qabajah *et al.,* 2014; Wulandari *et al.,* 2019).

Molecular identification

The gene sequence universal primers 16 *Srna* amplification using one pair of primer 27F5'–AGAGTTTGATCCTGGCTCAG3' and reverse 1492R 5'–GGT TAC CTT GTT ACG ACT T3', the expected PCR product size is 1500 bp (Hill *et al.,* 2002). The gene sequence pecific *aerA* gene amplification using one pair of primer F 5'–CAAGAACAAGTTCAAGTGGCCA3' and reverse 1492R 5'–GGTTACCTTGTTACGACTT3', the expected PCR product size is 309 bp (Sarkar *et al.,* 2012). PCR reaction to detect genes by using GoTaq® Green DNA polymerase (Promega). The Master mix PCR for each of the target genes is 12.5 ìl PCR buffer, 2.5 µL MgCl2 (25 Mm), 0.5 µL Dntp Mix (200 Ìm), 2.5 µL primary forward (12 Mm), 2.5 primary reverse µL (12 Mm), 12.5 Mm GoTaq® Green DNA polymerase, 3 µL DNA template. The amplification gene 16 *Srna* condition were the dena-

turation stage at a temperature of 95 °C in 3 minutes, followed by 35 cycles that consist of a temperature denaturation phase of 94 °C for 1 minute, annealing temperature of 55 °C for 1 minute, extension 72 °C for 2 minutes and terminated Final extension 72 °C for 3 minutes. *AerA* gene amplification process, preceded by a denaturation stage at 95 °C in 5 minutes, followed by 50 cycles that take place with a denaturation stage at 95 °C for 30 seconds, annealing 59 °C for 30 seconds, and extensions at 72 °C for 30 seconds. Final elongation at 72 °C for 7 minutes. Amplification result is visualized with electrophoresis agarose gel 1.5% The buffer of Tris-acetate-EDTA (TAE), SYBR safe, loading dye and DNA ladder/marker 100 bp.

Data analysis

Data analysis use in this research is descriptive analysis by presenting data in the front of drawing and tables to describe bacterial resistance pattern and molecular interpretation.

Results and Discussion

Based on the isolation and identification of 35 Dumbo catfish allegedly suffering from MAS disease, macroscopic in order to allow the blood to exhibit a creamy, circular-shaped colony, convex elevation, smooth edges and forming a hemolysis zone α hemolysis. That *A. hydropila* in the media so that the blood is formed α-hemolysis zone in the incubation 28 hours because *A. hydropila* is able to circulate red blood cells and hemoglobin. Hemolysine is a protein that is capable of damaging cell membranes and the use of red blood cells (Wang *et al.,* 2003) the microscopic observation using Gram staining suggests the morphological of the rod-shaped and red-colored bacteria that Gram-negative bacteria. According to (Buckley and Howard, 1999). The morphology of the colony of *A. hydrophila* is beige, convex elevation, and the edges are smooth, while the cell macroscopic morphology of the trunk and is Gram-negative.

Biochemical test Results Table 1, test indol after added reagent Kovac's, indicates the presence of a red ring on the surface. Methyl-Red (MR) and Voges Proskauer (VP) positive isolates on the media change color to red. This signifies that there is no mixed fermentation in bacterial isolates (Wahjuningrum *et al.,* 2013). Citrate testing is characterized by the ability of bacteria to utilize citrate as a source of carbon and energy for metabolism. Another test conducted is testing using TSIA media and obtained the results that the bacteria are able to ferment 3 kinds of sugar with changes in the section slant and Butt terbut not able to produce gas or H2S. Oxidative/fermentative tests demonstrate the ability of bacteria in the use of carbohydrates in anaerobic conditions through the fermentation process into oxidation and fermentative (Anggraini, 2016). The testing of specific media Rimler-Shotts (RS) shows A typical colony of bacteria *A. hydrophila* as many as 17 samples are marked with yellow without green spots (Tulung *et al.,* 2014). *A. hydrophila* bacteria in RS media testing there is the growth of bacteria with a yellow colony characteristic that states the positive *A. hydrophila* especially on the scratches done (Clarridge, 2004).

Table 1. Results of biochemical testing of 28 isolates suspected bacteria *A. hydrophila*

Test Type	Bioclinical test	
	Positive (+)	Negative (-)
Indol	28	28
Triple Sugar Iron Agar	28	28
Citrat	28	0
Methyl Red	28	0
Voges Proskauer	19	9
Oksidatif/fermentatif	22	6
Rimler Shotts	17	10

The DNA amplification of gene *16S rRNA* of 17 confirmed samples using RS media then carried out advanced testing using PCR and obtained 6 isolates had a gene *16S rRNA*. The visualization process is performed on agarose electrophoresis 1.5% using DNA Penada Ladder 1 KB. The profile of 17 samples contained clear and thick Amplikon bands with the Amplikon length of 1500 bp (Fig. 1). Gen *16S rRNA* is a conserved gene of all bacteria, which

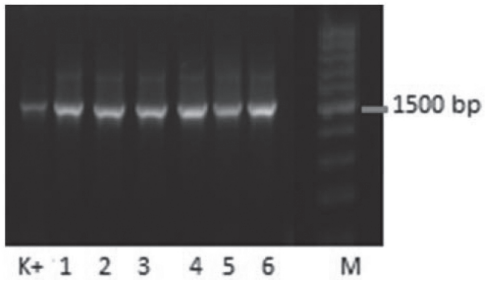

Fig. 1. The results of the positive amplification *16S rRNA* (1500 bp) of *A. hydrophila* M: The Thermo scientific Marker 1kb.

is a gene that plays an important role for the survival of bacteria in protein synthesis Suryani *et al.* (2009). This is in line with the research of Ruhil *et al.* (2015), that reagent-reagent composition on the ampification process in optimum condition determines the existence of gene-encoding DNA microorganisms.

Positive results of the gene detection *16S rRNA*, in addition to the testing of the existence of virulent gene *aerA* with a long Amplikon 309 bp using a bioline marker 100 bp. Molecular detection of virulent gene *aerA* conducted against isolates *A. hydrophila* using a primer specifically designed to detect the virulent gene *aerA*. Gene detection *aerA* Bungo District shows 6 positive isolates *A. hydrophila* with 309 bp of 17 isolates (35%) (Fig. 2). The virulent gene research of *aerA* was conducted by the Samira *et al.,* (2012) on freshwater fish and obtained 76% of the gene *aerA*. Gen *aerA* also found in indigo fish with a percentage of 78.95% [26]. Gene *aerA* is an aerolysin virulence gene causes *Motile Aeromonas Septicemia* (MAS). According to Heuzenroeder *et al.,* (1999). The presence of *aerA* is a strong indication of the pathogenic virulence of *A. hydrophila*.

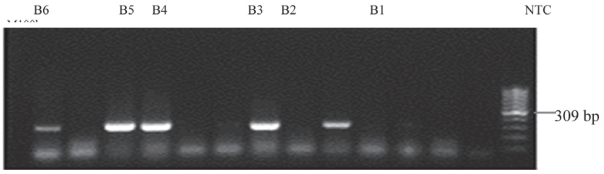

Fig. 2. Result of *aerA* gene amplification (309 bp) on *A. hydrophila* (A) Bungo district, Non template control; NTC: Marker (Bioline) 100 bp.

Conclusion

Based on the isolation and identification of 35 Dumbo catfish allegedly suffering from MAS disease, macroscopic in order to allow the blood to exhibit a creamy, circular-shaped colony, convex elevation, smooth edges and forming a hemolysis zone á hemolysis. That A. hydropila in the media so that the blood is formed α-hemolysis zone in the incubation 28 hours because A. hydropila is able to circulate red blood cells and hemoglobin. The DNA amplification of gene 16S rRNA of 17 confirmed samples using RS media then carried out advanced testing using PCR and obtained 6 isolates had a gene 16S rRNA. Detection of the *aerA* gene show 6 isolates of *Aeromonas hydrophila* have the virulent factor aerolysin at 309 bp.

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