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Isolation and Identification of *Aeromonas hydrophila* and *Saprolegnia* sp. on Catfish (*Clarias gariepinus*) in Floating cages in Bozem Moro Krembangan Surabaya

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Abstract. Catfish (*Clarias gariepinus*) is one of the familiar freshwater fish cultured in Indonesia farmer. One of the reason is the high mortality of the catfish infected by *Aeromonas hydrophila* and *Saprolegnia* sp. *Motile Aeromonas septicemia (*MAS) is a common bacterial disease, caused by *Aeromonas hydrophila*, which affects freshwater fish. In Southeast Asia, the outbreak of this disease was firstly reported from West Java in 1980, when a total of 82.5 tons a month of catfish were lost, while in Central Java in 1984, the total loss was 1.6 tons. Saprolegniosis can cau economic loss due to high mortality from its case reaching 10% to 50% . This research aimed to identify and determine the percentage of *A. hydrophila* and *Saprolegnia* sp. the catfish farmed in bozem Moro Krembangan, Surabaya, East Java. Meanwhile, a supporting parameter in this research is the value of water quality parameter including pH, temperature, ammonia and dissolved oxygen that were measured during sampling. The results showed that of the 20 samples taken from the two cages, 19 fish were positively infected by *A. drophila*. percentage of infections of *A. hydrophila* that infect umbo catfish in Moro Krembangan, was 95%, while the percentage *Saprolegnia* sp. was 90%.

1. Introduction

Surabaya has a system of outdoor water reservoirs that have the same functionality with reservoirs namely Bozem. These Bozems include: Bozem Moro Krembangan, Kalidami, Bratang, Rungkut industrial, Wonorejo, Kedurus and Broken Gorge. Ecosystem of Bozem is built as Surabaya is a city in flood-prone with lack of water catchment areas. This is due to the development in the city of Surabaya that develops rapidly. In General Bozem is built without cutting off rivers. In general Bozem serves as a controller and water storage, and the society also utilizes it as a place to fish farming and irrigation of field (planning and developm agency of the city of Surabaya, 2010). One of the farmed fish in water of Bozem was catfish. Catfish is one type of fish consumption that are quite popular in Indonesia. Some advantages of the fish are to have meat that are thick and tasty as well as easy maintenance, its relatively quick growth, and high selling prices, so that catfish arelargley farmed in to involves several factors: the surroundings (environment), the farmed fish (host), and the disease-causing organisms (pathogens). *A. hydrophila* can affect fish farming and often lead to outbreaks of disease with mortalities occasionally approaching 80-100 percent in a short period of 1-2 weeks [1]. While from the fungus genus, many *Saprolegnia* sp. infect eggs

and freshwater fish which can lead to massive death of fish in fish farms [2]. *Saprolegnia* sp. quickly transmitted to other catfish that were in one pool. Thus, it spread more quickly and potentially inflicts considerable losses for farmers [3].

Fish farming in Bozem Moro Krembangan having some problems such as the water environmental problems that is less support, because the waters such a reservoir of water from various sources and disposal of waste. Based on the research the reason they will do. The purpose of this research is to determine The presence of infection Aeromonas hydrophila and Saprolegnia sp. on catfish (*Clarias gariepinus*) cultured in Bozem Moro Krembangan Surabaya.

2. Research Methodology

2.1. Research Materials

Research materials that are used in this research are *Clarias gariepinus* obtained from Bozem Moro Krembangan, Surabaya, aquades sterile, spirtus, alcohol 70%, selective medium Triptic Soy Agar (TSA), Saboraud Dextrose Agar (SDA) and safranin. The instruments used are a petri dish, needle ose, mikropipet, ring, light microscopy, object glass, hot plate stirer, thermometer, pH meters, DO the test kits and ammonia test kit.

2.2. Research Methods

This study used a survey method. The determination of the sampling method used purpossive sampling [4]. Sampling is to retrieve data of only a portion of the population who are expected to describe the nature of the population from the object of research.

2.3. Work Procedures

2.3.1. Sampling

Catfish taken for the sample are consuming catfish with a size of 10-15 cm age 2-3 months with a weight of 100-110 grams. The number of cages used for sampling amounted to two cages, the total population is 400 catfish, in one population cage is 200 catfish, and taken a sample of 5% of the population that is as many as 20 catfish out of a total population or 10 for each cage.

2.3.2. Sterilization of Tools

Equipment such as needles of ose before used first were sprayed alcohol 70% then conducted burning directly. Petri dish was sterilized by autoclave at a temperature of 121 ° C with pressure 1 atm for 10-15 minutes.

2.3.3. Isolation of Aeromonas hydrophila and Saprolegnia sp.

The medium used for the isolation of *A. hydrophila* was Trypticase Soy Agar (TSA), while the medium for isolation of *Saprolegnia* sp. was Saboraud Dextrose Agar (SDA). The isolation was carried out using streak methods at medium of isolation. Furthermore, purification was done by moving the isolates that grew into new media until pure isolate was obtained.

2.3.4. Identification of Aeromonas hydrophila and Saprolegnia sp.

Bacterial identification was conducted with the macroscopic test, Gram staining and biochemistry test. Observations of macroscopic samples test of catfish included size, shape, and pigmentation seen from the inside, beside and top. Microscopic tests were done by staining Gram by observing the color and shape of bacteria as well as biochemical test was done. Biochemical test involved Motile test, indol test, O/F test, and H2S test.

Identification of the fungus *Saprolegnia* sp. with macroscopic test and microscopic test. Observations of macroscopic samples test catfish includde size, pigmentation (the colour of the colony) and

shape seen from the inside, beside and top. While the observations of the microscopic test were conducted by observing the color, the shape of the hypha, forms spores, Spore and form layout cells of fungi by using inverted microscope [5].

2.3.5. Calculating the percentage of Fish infected by Aeromonas hydrophila and Saprolegnia sp.

The percentage of infections was the number of fish infected by the bacteria/fungi that are divided with many samples taken multiplied by one hundred. Resulting in the percentage of fish infected with the disease.

$$\text{The percentage of catfish infected bacteria/fungi (\%)} = \frac{\text{Number of infected fish} \times 100}{\text{The number of fish samples}}$$

3. Results and Discussion

3.1 Identification and Isolation of Aeromonas hydrophila and Saprolegnia sp.

Isolation of *A. hydrophila* and *Saprolegnia* sp. was conducted on 20 samples of fish that were taken that showed pathologic conditions including hemorrhagic septicemia and tail or fin rot. Isolation of a fungus was conducted by taking body parts catfish that showed clinical symptoms of fungus infection. The fish part isolated was part of the surface of the body, fins, gills, and tail, because the parts that would change as result of infection of fungus *Saprolegnia* sp. On the skin of infected catfish there was smooth yarn resembling cotton and spreading to the surface of the skin to another. This is in accordance with statement [6], stating that fish infected with *Saprolegnia* sp. Have objects that resemble cotton on the surface of their body, tail, fins or gills.

The results of the identification of the bacterial isolate showed negative staining Gram, motile, form rod, while biochemical test results are fermentative, positive oxides, positive glucose and negative indol.

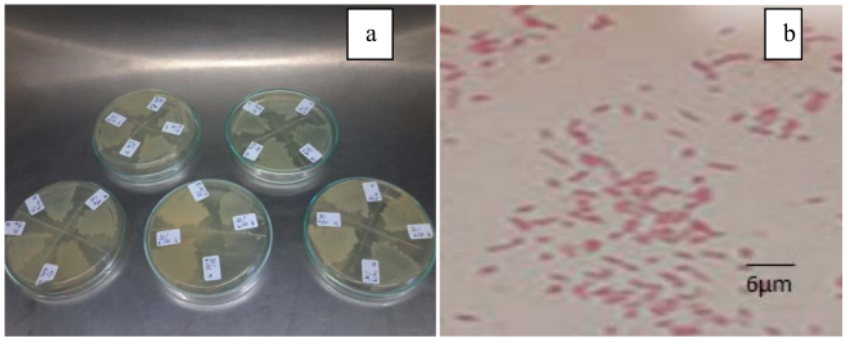

Figure 1. Bacterial isolates (a), (b) bacterial Morphology

Identification of fungus isolate in macroscopic colony characteristics can be seen with a yellowish white with forms such as cotton. This is in accordance with [6], who states that the genus *Saprolegnia* have characterized the colony resembling cotton, colored white and the colony can be found on the surface of the skin, fins and gills.

Microscopically *Saprolegnia* sp. morphology appear to have long Hypha and aseptat (not (not septad), tube-shaped, and has the zoospora on its corner. Sporangia widens and the short as well as at the end of the sporangia looks a bit dark. The zoospora *Saprolegnia* sp was resulted from the tip of the long Hypha . Zoosporangia Saprolegnia SP.that are long and cylindrical has a length

of 180-350 µm in width 20-24 µm [6]. Macroscopic and microscopically Observation can be seen in Figure 2.

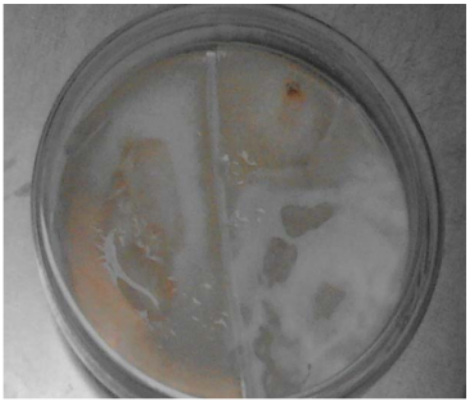

Figure 2. a colony of *Saprolegnia* sp.

One of the types of diseases that are often found in cultivated organisms is bacterial disease caused by the bacterium *Aeromonas hydrophilla*, which is the pathogenic bacteria cause disease "Motile Aeromonas Septicemia" (MAS), especially for freshwater fish species in tropical waters [7]. The bacterium *Aeromonas hydrophila* is one of the dangerous disease-causing bacteria in freshwater fish farming. The bacteria attack the Catfish that is one of the leading commodities in freshwater and can infect fish in all sizes which can lead to death until it reaches 80%, resulting in huge losses in the freshwater fish farming ventures [8].

Aeromonas hydrophila is a bacteria that can grow in the waters of the optimum operating temperature of 20-30 ° C [9]. *Aeromonas hydrophila* is able to grow at temperatures up to 37 ° C [10]. *Aeromonas hydrophila* extracellular products of consist of the α and β hemolisin, protease, elastase, lipase, cytotoksin, enterotoxin, gelatinase, caseinase, lecithinase, and leucocidin. The liver is the organ target of Aeromonas hydrophila and disruption can have an effect on metabolic processes [11]. *Aeromonas hydrophila* is pathogens because it only can cause disease in fish populations that are weak or as secondary infections when fish are infected with other diseases [12].

The process of when *Aeromonas hydrophila* bacteria infects into the body of the fish is preceded by that bacteria stick on the skin surface by utilizing the flagella to move towards the fish and then use hooks to attach to strong on the outermost layer of a body of fish scales. Scales are covered by the substance chitin. During the process of *Aeromonas hydrophila* bacteria takes plac, it produces enzymes that play a role in kitinase layer of chitin degrades, so that bacteria can easily fit into the body of the fish. In addition to utilizing the *Aeromonas hydrophila* kitinase, the bacteria also secrete enzymes such as lesitinase in an attempt to get into the blood stream and go directly to the kidney to breed [13].

Saprolegnia sp. are fungi with yarn shape, resembling cotton, white to gray and Brown. *Saprolegnia* sp. fungi belongs to the class of Oomycetes, also known as fungi with algae because its nature is similar to that of algae but do not contain chlorophyll [14]. Compiled by the threads of a Hypha, it has no bulkhead separators (aseptat), but the branched into misselium. The walls of *Saprolegnia* sp. Hypha are composed of glycogen and cellulose [9]. Hypha branches *Saprolegnia* sp. form woven wool like yarn. *Saprolegnia* sp. has a Hypha which is larger, tapered shape and able to spread faster than the other fungi.

3.2 The percentage of Clarias gariepinus Infected by Aeromonas hydrophila and Saprolegnia sp.

The data results of the counting of the *Aeromonas hydrophila* percentage of fungi farmed in floating net cage in Bozem Moro Krembangan Surabaya can be seen in table 1.

Table 1. The results of the counting of the percentage of the Catfish infected *Aeromonas hydrophila.*

Cages	The number of Fish Samples (fish)	The number of fish infected by *Aeromonas hydrophila* (fish)		The percentage (%)
		+	-	
A	10	9	1	90
B	10	10	0	100
The total number of	20	19	1	95

Results data of counting of percentage of fungi *Saprolegnia* sp. observed in cages floating in Bozem Moro Krembangan Surabaya (seen in table 2)

Table 2. The results of the counting of the percentage of the Catfish infected with *Saprolegnia* sp.

Cages of floating	The number of fish sample (fish)	The The number of fish infected by *Aeromonas hydrophila* (fish)		The percentage (%)
		+	-	
A	10	10	0	100
B	10	8	2	80
Total	20	18	2	90

On this study, the estimated value of water quality is obtained : temperature 30^{o}C, DO 4 ppm, pH 6 - 7 and ammonia 1.0 ppm.

Of the 20 fish samples taken, all were experiencing clinical symptoms such as wounds to the skin also damaged fins and tail. Then at 19 fish's kidney organ were suffered swelling and only 1 fish had not experienced swelling in his kidney. The high percentage of fish infected by *Aeromonas hydrophila* and *Saprolegnia* sp. was 95% and 90% indicating that the waters in Morokrembangan Surabaya Bozem were less support for fish farming activities. Remember that waters had the function as shelter of water, such as rain water and waste water, both household waste as well as industrial waste, with the aim to control the occurrence of floods.

According to [15] *Aeromonas hydrophila* bacteria infection can happen in 4 levels, namely Acute septicemia which is fatal, infection quickly with marked swelling of the internal organs. Sub acute, can be seen with symptoms such as wounds and bleeding on the scales. Chronic can be seen with symptoms such as ulcers and sores of the skin damage whose development lasts a long time. Latent, it can happen with not showed symptoms of the disease, but on the internal organs contained disease-causing bacteria. While infection of *Saprolegnia* sp. on fish generally are secondary infections that preced the onset of bacterial infection, the aquatic environment are not heeded, the parasitic infestations, post-harvest handling, dense stocking high causing easy fish infected with *Saprolegnia* sp. [16]. Bad water quality, low dissolved oxygen, high organic matter content and the presence of bacteria will create an environment that is less good for fish and cause stress. The low oxygen levels in the waters can cause fish become stressed out so that the immune system can decline [17] . At that time, the pathogen will easily fit into the body of the fish, either in the form of bacteria, fungi or parasites. While according to [18], pH changes can cause fish stressed

so that they are easily affected by desease, and automatically law pH can cause the damage on skin, so pathogen can easily infect it. the value of optimal water quality for fish growth, namely: water temperature 28-32°C. In addition to temperature, DO and pH, the content of ammonia is a factor to consider. The content of DO is in the water so that fish growth is ideal not less than 2 ppm, pH 6.5 to 8.5 and an ammonia content of less than 1 ppm.

4 Conclusion

Catfish (Clarias gariepinus) cultured in Moro Krembangan Surabaya, East Java, are mostly infected with Aeromonas hydrophila and *Saprolegnia* sp., It is caused by the environmental conditions that are unfavorable. The percentage of catfish (Clarias gariepinus) infected with *Aeromonas hydrophila* is 95% and infected with *Saprolegnia* sp. is 90%.

References

[1] Lukistyowati, I. dan Kurniasih. 2012. Pelacakan Gen Aerolysin dari *Aeromonas hydrophila* pada Ikan Mas yang diberi Pakan Ekstrak Bawang Putih. Jurnal Veteriner 13 (1) : 43-50.

[2] Akbar, J. 2008. Pemanfaatan Ekstrak Bunga Kecombrang (*Nicolaia speciosa horan*) Terhadap Penyembuhan Infeksi Jamur *Saprolegnia* sp. Pada Ikan Nila Merah. Fakultas Perikanan Universitas Lambung Mangkurat.

[3] Khairyah U., Kismiyati., dan Kusdarwati R. 2013. Identifikasi dan Prevalensi Jamur pada Ikan Gurami (*Osphronemus gouramy*) di Desa Ngrajek, Kecamatan Mungkid, Kabupaten Mungkid, Jawa Tengah.

[4] Singarimbun dan Effendi. 1995. *Metode Penelitian Survei*. Jakarta : PT Pustaka LP3ES Indonesia.

[5] Yuasa K., Novita P., Meliya P., dan Edy B.K, 2003. Teknik Diagnosis Penyakit Ikan Budidaya Air Tawar di Indonesia. Japan International Cooperation Agency dan Panduan Diagnosis Penyakit Ikan. Balai Budidaya Air Tawar Jambi. Jambi.Stoskopf, M. K. 1993. Fish Medicine. WB Saunders Company. Mexico.

[6] Rahmaningsih, S. 2012. Penagruh Ekstrak Sidawayah dengan Konsentrasi yang Berbeda untuk Mengatasi Infeksi Bakteri *Aeromonas hydrophyla* pada Ikan Nila (*Oreochromis niloticus*). Jurnal Ilmu Perikanan dan Sumberdaya Perairan.

[7] Sanoesi, E. 2008. Penggunaan Ekstrak Daun Pepaya (*Carica papaya Linn*) terhadap Jumlah Sel Makrofag pada Ikan Mas (*Cyprinus carpio* L) yang Terinfeksi Bakteri *Aeromonas hydrophila*. Jurnal Penelitian Perikanan, Vol 11, No. 2.

[8] Indrawati, I. 2007. Potensi Ekstrak Rimpang Lengkuas (*Alpinia galanga*) terhadap jamur *Saprolegnia* sp. dari telur ikan mas (*Cyprinus carpio*) Secara in Vitro. Skripsi. Fakultas Kedokteran Hewan. Universitas Airlangga. Surabaya.

[9] Austin, B. and D. Austin. 1987. Bacterial Fish Pathogen. Ellis Hoorwood. London. pp. 250-262.

[10] Cipriano RC, GL Bullock and SW Pyle. 1984. *Aeromonas hydrophila* and *Motile Aeromonad Septicemia* of Fish. Fish Diseases Leaflet 68, US. Fish and Wildlife Service. West Virginia.

[11] Kabata, Z. 1985. Parasites and Disease of Fish Cultureed in Tropics. Taylor and Francis, London.

[12] Mangunwardoyo, W., R. Ismayasari., E. Riani. 2010. Uji Patogenisitas dan Virulensi *Aeromonas hydrophila* Stanier pada Ikan Nila (*Oreochromis niloticus* Lin.) melalui Postulat Koch. J. Ris. Akuakultur Vol. 5 Tahun 2010: 245-255.

[13] Nuryati, S., F.B.P. Sari., dan Taukhid. 2009. Identifikasi dan Uji Postulat Koch Cendawan Penyebab Penyakit pada Ikan Gurami. Program Studi Budidaya Perairan. Fakultas Perikanan dan Ilmu Kelautan. Institute Pertanian Bogor. Bogor.

[14] Sniezko SF and HR Axelrod. 1971. Diseases of Fishese. TFH Publication Ltd., Hongkong.

[15] Bruno D. W and Woo, P. T. K.. 1999, Fish Disease and Disorders vol. 3 (Viral, Bacterial and Fungal Infection). CAB International Publishing. Wallingford, UK. Pp 366-432.

[16] Shafrudin, D., Yuniarti dan Setiawati, M., 2006. Pengaruh Kepadatan Benih Ikan Lele Dumbo (Clarias sp.) Terhadap Produksi Pada Sistem Budidaya Dengan Pengendalian Nitrogen Melalui Penambahan Tepung Terigu. Jurnal Akuakultur Indonesia 5(2) : 137-147.

[17] Taufik,P. 1984. Faktor Kualitas Air Dapat Mempengaruhi Timbulnya Suatu Penyakit Pada Ikan. Majalah Pertanian No 3. Departemen Pertanian. Jakarta.

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