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Identifikasi Mikroba (Parasit dan Bakteri) yang Berpotensi sebagai Patogen pada Sidat (*Anguilla* spp.) pada Lokasi Penangkapan Sidat di Sukabumi, Jawa Barat.

Identification of Micro-organisms (Parasites and Bacteria) which are Potential as Pathogenic Agent in Glass Eel of *Anguilla* spp. at the Eel Capture Location, Sukabumi, West Java

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Abstrak

Ikan sidat (*Anguilla* sp.) merupakan salah satu ikan komersial yang penting di beberapa negara khususnya di negara-negara Asia Timur seperti Jepang, Korea Selatan, China, dan Taiwan. Kebutuhan benih ikan sidat atau sering disebut *glass eel* masih mengandalkan hasil tangkapan alam. Masalah ikan tangkapan alam adalah tingginya tingkat infeksi bakteri dan penyakit. Ketergantungan *glass eel* dari alam memberikan indikasi adanya infeksi parasit dan bakteri pada *glass eel*. Keberadaan parasit dan bakteri sebagai mikroorganisme yang berpotensi sebagai agen patogen dalam siklus budidaya sidat dapat menjadi kendala serius, sehingga penanganan *glass eel* perlu mendapat perhatian khusus sejak proses penangkapan dari alam. Oleh karena itu, identifikasi parasit dan bakteri di lokasi penangkapan *glass eel* perlu dilakukan di Teluk Pelabuhan Ratu, Sukabumi, Jawa Barat. Pengambilan sampel glass eel dilakukan pada bulan Mei-Juni 2019, di 3 lokasi muara dengan hasil tangkapan tertinggi yaitu Sungai Cimandiri, Sungai Cikaso dan Sungai Cibuni. Jumlah sampel ikan sidat yang diambil sebanyak 30 ekor pada setiap lokasi sungai dengan 2 kali ulangan, sehingga total sampel ikan sidat yang diambil sebanyak 180 ekor. Identifikasi parasit dilakukan dengan pemeriksaan ektoparasit dan endoparasit dan identifikasi bakteri dilakukan dengan metode konvensional (metode biokimia). Berdasarkan hasil identifikasi tidak ditemukan parasit pada sampel glass eel, sedangkan bakteri ditemukan sebanyak 12 spesies dan 3 spesies dominan yaitu Listeria sp. ditemukan pada 40 sampel sidat dengan prevalensi 22,2%, *Aeromonas hydrophila* ditemukan pada 28 sampel sidat dengan prevalensi 15,6%, dan *Staphylococcus* spp. ditemukan pada 22 sampel sidat dengan prevalensi 12,2%.
Kata Kunci : Mikroba, Patogen, Sidat, *Anguilla* spp.

Abstract

Eel (*Anguilla* sp.) is one of the important commercial fish in some countries particularly in East Asian countries such as Japan, South Korea, China, and Taiwan. The need of eel fry or frequently called as glass eel still relies on the natural capture. The issue of natural captured fish is the high level of bacterial infections and diseases. The dependence of glass eel from the nature provides an indication of infections of parasites and bacteria in glass eel. The existence of parasites and bacteria as microorganisms which are potential as pathogenic agents in the cycle of eel culture can be a serious obstacle, so that the glass eel handling needs a particular attention since the capturing proses from the nature. Therefore, identification of parasites and bacteria in the capture location of glass eel was necessarily conducted in Pelabuhan Ratu Bay, Sukabumi, West Java. The glass eel sampling was conducted in May-June 2019, in 3 locations of estuary with the highest capture yield namely Cimandiri River, Cikaso River and Cibuni River. The number of samples taken were 30 eels at each river location with 2 replicates, so that the total eel samples which were taken were 180 eels. Identification of parasites was conducted by examination of ectoparasite and endoparasite and dentification of bacteria was conducted by conventional method (biochemical method). Based on the result of identification, there was no parasite found in the glass eel

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samples, while bacteria found as many as 12 species and 3 dominant species namely *Listeria* sp. found in 40 eel samples with prevalence of 22.2%, *Aeromonas hydrophila* found in 28 eel samples with prevalence of 15.6%, and *Staphylococcus* spp. found in 22 eel samples with prevalence of 12.2%.
Keyword : Micro-organism, Pathogen, Glass Eel, *Anguilla* spp.

INTRODUCTION

Eel (*Anguilla* spp.) becomes one of the fishery commodities which has high potential to be expanded in Indonesia. Eel is very popular in some countries such as Japan, South Korea, China and Taiwan (Wahjuningrum et al., 2018) as a protein source having high economic value. Eel has high nutritional value particularly vitamin A, E, and unsaturated fatty acids (EPA dan DHA) (Seo et al., 2013).

Indonesia has a great potential regarding the eel expansion, since the species diversity of eel is high namely 9 species (*Anguilla bicolor bicolor, A. nebulosa nebulosa, A. bicolor pacifica, A. interioris, A. borneensis, A. celebesensis, A. marmorata, A. obscura and A. megastoma*) from the global number of eel species as many as 18 species (Sugeha et al., 2008). The eel species which are mostly cultured in Indonesia are namely *Anguilla bicolor* and *Anguilla marmorata*, however the most popular and the most dominant species is *A. bicolor.*

The activities of eel culture in Indonesia are mostly found in some regions such as Banyuwangi, Cilacap, Cirebon and Bogor. The eel culture is expected to be able to comply the eel export need for the world. Data showed that the global need for eel is 250,000 ton and the largest consumer is Japan consuming 150,000 ton (Wahjuningrum et al., 2018). The high need of eel for export provides opportunities for Indonesia to improve the yield of eel culture.

Currently the challenge of eel culture is survival rate (SR) in the phase of glass eel and elver. Based on the direct observation results in some eel culture locations, the survival rate (SR) in eel culture process from the early phase (glass eel) to the final phase (consumption size) was about 16-25%. This case is very important to be observed, considering that the source of glass eel still relies on the natural capture, in which the artificial hatching activity is not able to be performed yet until now. Therefore, the low SR will have a large impact on the availability of glass eel in the nature.

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Fish commodity produced from the natural capture process is vulnerable to the diseases since the condition of natural environment is very fluctuating. The stability of environment particularly physical and chemical parameters of water at a media where the fish lives determine the fish health. The fluctuation of temperature, pH, salinity or dissolved oxygen which exceeds the optimum limit can induce stress and finally will induce the diseases.

Disease is the cause of economic loss in aquaculture industry. Disease caused by parasites and bacteria is the main problem which has an impact on eel population in the nature and culture pond (Joh et al., 2013). Parasites, bacteria, and other pathogens are very influential to health, reproduction, and survival of glass eel. Particularly the impact on the body weight reduction, growth inhibition, and mortality increase. These cases have an impact on the reduction of quantity and quality of culture yield (Elgendy et al., 2015). However, understanding of initial identification of pathogen onset in glass eel is still limited. So far, valid data of disease source in glass eel has not been obtained yet.

Assumption based on the field observation stated that the disease emerges when glass eel has started to be processed in the culture pond. However, the other assumption stated that disease originates from the freshly caught glass eel or from the common waters. So that, initial screening for disease identification in glass eel is important to be conducted to obtain data and information regarding the cause of disease. The study of identification of disease particularly parasites and bacteria attacking glass eel in the capture location of eel in Sukabumi was conducted for further to determine control measures and handling method.

METHODS

Glass Eel Sampling

The study was conducted in May – June 2019. The sampling of water and glass eel was conducted in Pelabuhan Ratu Bay, Sukabumi since it was a location having high potential of glass eel capture. Sampling was conducted in the 3 locations, with the highest capture quantity namely Cimandiri River (11,318,744 eels), Cikaso River (1,897,944 eels) and Cibuni River (1,114,830 eels) (DKP Kab. Sukabumi, 2019).

The number of samples taken was 30 eels for each location with 2 replicates. The glass eel samples from the 3 capture

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locations have diverse weight in the first location, the second location, and the third location which were namely 0.15 ± 0.03 gram; 0.14 ± 0.04 gram; and 0.13 ± 0.02 gram, consecutively. The glass eel samples were subsequently placed in the plastic bag and provided with oxygen then transported to the Laboratory.

Identification and characterization of parasites and bacteria were conducted in Laboratory of Research Institute for Freshwater Aquaculture and Fisheries Counseling (LU-BRPBATPP), Ministry of Maritime Affairs and Fisheries (KKP).

Identification of Parasites

The examined organs consisted of the external body part (ectoparasites) and the internal body part (endoparasites). The examined external parts were namely: the surface of the body (mucus, fins and skins), gills and eyes.

Examination of ectoparasites was conducted as the following:

a. The whole body examination was observed visually, the macro ectoparasites which were found were transferred into a petri dish containing physiological solution.
b. Furthermore, the mucus on the body surface and fins was scraped using a scalpel and a test preparation was made on an object glass which was then observed under a microscope with a magnification of 40 - 1000x.
c. Operculum was opened and all parts of the gills were removed and transferred to an object glass that had been given physiological solution then observed under a microscope with a magnification of 40 - 1000x.
d. Each of metazoan parasites found was immediately transferred into a petri dish containing physiological solution before fixation was conducted.

The parasite species found was identified by using guideline of Hoffman (1967), Kabata (1985) and Lom (1995).

Analysis was performed regarding prevalence value of the infection and the parasite density calculated based on the definition developed by Margolis et. al. (1982):

$$Prevalence = \frac{Total\ number\ of\ fish\ infected\ by\ parasites}{Number\ of\ the\ observed\ fish}\ x\ 100\%$$

$$Parasite\ density = \frac{Number\ of\ a\ parasite\ species}{Microscopic\ field\ of\ view\ (100X)}$$

Identification of Bacteria

Bacteria was tested by conventional method (biochemical method). The tested characteristics consisted of: gram coloration, motility, catalase-oxidase, Indol, Ornithin, L arginin, Aesculin, OF, methylred (MR), Voges Proskauer (VP), L arabinose, glucose, TCBS, and RS agar. The obtained results were subsequently identified based on the guideline of Buller

(2004). The test result data was analyzed descriptively by calculating the prevalence of bacteria in the eel (*Anguilla* sp.) (Syafitrianto, Aqmal, and Lande, 2016).

Water Quality Measurement

At each location of eel sampling, water quality measurement process such as dissolved oxygen (DO), pH, salinity and temperature was also conducted. Water quality measurement was conducted simultaneously with glass eel sampling. The locations of water quality measurement were namely in the capture location and in the water sources which were utilized as a shelter media. The aim of water quality measurement was as supporting data for the study. While the method used was a direct measurement by using the prepared devices such as DO meter (DO, pH and temperature) and refractometer (water salinity).

RESULT AND DISCUSSION

Based on the test results of parasites and bacteria in Laboratory of Research Institute for Freshwater Aquaculture and Fisheries Counseling (BRPBATPP), of 180 samples no parasite was found. While in the process of bacteria isolation, 12 bacteria species were found. Bacteria species of *Listeria* sp. was the most bacteria found namely in 40 eel samples with prevalence of 22.2%, then species of *Aeromonas hydrophila* found in 28 eel samples with prevalence of 15.6% and *Staphylococcus* spp. found in 22 eel samples with prevalence of 12.2% (Table 1).

Table 1. Prevalence of bacteria in glass eel in the capture location, Kab. Sukabumi.

No	Bacteria	Prevalence (%)
1	*Aeromonas hydrophila*	15.6
2	*Citrobacter freundii*	1.1
3	*Corynebacterium spp.*	2.8
4	*Planococcus* spp.	3.3
5	*Staphylococcus* spp.	12.2
6	*Streptococcus spp.*	0.6
7	*Lactobacillus* spp.	1.7
8	*Listeria spp.*	22.2
9	*Neisseria* spp.	1.1
10	*Pseudomonas aeruginosa*	1.7
11	*Lactobacillus* spp.	3.3
12	*Kurthia* spp.	3.3

The results of this study found 3 dominant bacteria species infecting eel namely *Listeria* spp., *Aeromonas hydrophila* dan *Staphylococcus* spp. Those 3 bacteria species were the bacteria species with the highest prevalence among the other bacteria species. *Aeromonas* sp., is the most common bacteria species found in fish. The similar results of a study conducted by Kusen et al., (2015) also

found *Aeromonas hydrophila* in glass eel of *Anguilla marmorata* cultured in Tatelu Freshwater Aquaculture Center. The other study mentioned the existence of *Aeromonas* spp. in eel transported via Mutiara Palu Airport. The result of the study showed that of 396 eels tested, 128 among them were identified Aeromonas (32.32%). Species of Aeromonas which were identified were namely *Aeromonas sobria*, *Aeromonas hydrophila*, and *Aeromonas caviae* (Syafitrianto et al., 2016).

Ideally, the study was continued by conducting LD 50 test, however in this study we compared to the results of a study which had been conducted by Wahjuningrum et al., (2018). The study mentioned that pathogenic bacteria infecting *A. bicolor* were namely *Aeromonas hydrophila*, *Streptococcus agalactiae*, and *Listeria grayi*. Those 3 bacteria species were the dominant bacteria which were found in eel.

Based on the acute toxicity test LD 50 to those 3 bacteria, the results of LD 50 value of *Aeromonas hydrophila* and *Streptococcus agalactiae* were 10^4 CFU/mL and 10^5 CFU/mL consecutively. While *Listeria grayi* was not lethal or caused death of 50% eels which were used as the experimental material (Wahjuningrum et al., 2018). This study was conducted by Koch's postulate method, in which bacterial isolates were injected into the body of the eels. The size of eels used was namely average length of 15.00 ± 0.65 cm and average weight of 3.00 ± 0.75 g. Eels were reared in aquarium with size of 30 x 28 x 30 cm^3. The other study mentioned that *A. hydrophila* could be detected by using PCR and DNA amplification of 685 bp. *A. hydrophila* was also confirmed to contain the gene of aerolysin 290 bp DNA which could be an indicator of virulence. Pathogenicity test showed that the estimated LC50 value was 10.9 x $10^{6.33}$ CFU/mL (Dewi and Koesharyani, 2017).

Listeria sp. was the bacteria species which was mostly found in this study. This bacteria species is normally found in skin, gills, and mucus of glass eel and is pathogenic bacteria which is zoonotic (Kwantes and Isaac, 1975; Suhendi, 2009). One of this bacteria species is namely *Listeria monocytogenes* and reported that it had been found in 37 species of mammals both wild animals and pet animals, 17 species of birds, some were found in fish and bivalves (Esteban et al., 2009; Sutherland, 1998; Ariyanti, 2010).

Listeria monocytogenes has ability to survive and grow particularly in ready-to-eat seafood and in partially-cooked seafood affected by temperature, duration of storage, and characteristic of the seafood (McCarthy, 1997). *Listeria monocytogenes* is able to survive and to grow at the temperature range of $(-1)^0$C - 45^0C and in

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pH range of 4.4-9.4 (FDA, 2011b; Mou, 2013). *Listeria monocytogenes* was found in a rainbow trout pond (Gray and Killinger, 1966; Dillon, 1992) and also in shrimp (Motes, 1991; Dillon, 1992). The species of Listeria was found in freshwater and seawater (Colburn et al., 1990; Dillon, 1992). *Listeria monocytogenes* can cause food contamination inducing Listeriosis, a bacterial infection in human (Todd & Notermans, 2011; Mou, 2013).

Fish or processed fish products can be contaminated with waste pollution either in the living environment of the fish or in the fish processing process (Abdelgadir et al., 2009; Nadal et al., 2007; Rivoal et al., 2010; Ariyanti, 2010). The bacteria normally live in an environment contaminated with pollution and waste (Suhendi, 2009). A species from *Listeria* spp., namely *Listeria welshimeri* was found in boiled green mussels without shells when they were sold. Contamination could occur due to the less hygienic peeling process of green mussels, the less hygienic environment, or the high initial microorganism quantity. However, *Listeria welshimeri* does not have ability of hemolysis (Volokhov et al., 2006; Rahayu et al., 2016). *Listeria welshimeri* is included as non-pathogenic bacteria (Hain et al., 2008; Rahayu et al., 2016). Location of glass eel sampling in Cimandiri River, was an estuary area which was contaminated with household waste and also waste of a steam-electric power station (PLTU). This case provided high opportunity for bacteria contamination by *Listeria* sp. in the captured glass eels.

The next bacteria species with the second highest prevalence level was *Aeromonas hydrophila*. Aeromonas is a type of bacteria which is able to cause various pathological conditions such as: acute, chronic, and invisible infection. Motile Aeromonas has different pathogenicity level, malignancy of disease caused by Aeromonas is affected by various factors which are interconnected such as virulence, type of virulence, stress level in fish population, physiological condition of the host, and genetic (Syafitrianto et al., 2016).

Symptoms caused by a bacterial infection of *Aeromonas* sp. are namely dark body color, lesion in the fish body, hemorrhage, and slow movement (fish starts to move slowly) (Suhendi 2009). The transmission of the bacteria is very quick through contact intermediary with body parts of fish or contamination of aquaculture equipment. Aeromonas is pathogenic and is able to cause mass death in *Garra rufa* fish (Majtán et al., 2012). This bacterial infection can be caused by environmental condition, stress, temperature change and contaminated water (Dooley et al., 1985; Haryani et al., 2012). Besides the stress level, high density condition, mishandling, poor transportation

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system and poor feeding were also intermediaries of this bacterial infection (Dewi and Koesharyani, 2017). *Aeromonas hydrophila* was able to cause an increase in the number of leukocytes and decrease in the number of erythrocytes, hemoglobin, and hematocrit in the concentration of 10^5 CFU/mL which was injected (intramuscular) to *Anguilla bicolor* as much as 0.1 mL/eel (Endang and Alfi, 2015).

Bacteria species with the third highest prevalence was *Staphylococcus* sp. This bacteria species has a large distribution on the human skin and the other vertebrates and is also an opportunist pathogen (can infect a host in appropriate condition). *Staphylococcus aureus* was found in fish, mussel and shrimp with incidence of 3.8%, 8.3%, and 20% consecutively, of the total seafood samples (100) namely 78 fish, 12 mussels, and 10 shrimps (Mus et al., 2014). Species of Staphylococcus which were isolated and identified from carp fish (*Cyprinus carpio*) and cat fish (*Silurus glanis*) were namely *S. saprophyticus, S. epidermidis, S. hyicus, S.aureus,* and *S.intermedius.*

Those five species of Staphylococcus were found in skins, muscles, livers, and intestines of *Cyprinus carpio* and *Silurus glanis* (Ali, 2014). The range of temperature (^{0}C) and pH of *Staphylococcus aureus* to grow and to form toxin are 7^0C-50^0C and 4-10 consecutively (FDA, 2011b; Mou, 2013).

This bacteria species is easy to grow and to evolve in an aquatic environment (Greenwood *et al*., 1995; Suhendi, 2009). The condition of injured body is an entry point for *Staphylacoccus* sp. and is able to cause an infection (Hadiotomo et al., 1998; Suhendi, 2009). In fish, this bacteria species can be seen if the fish has red spots. The result of a study conducted at a seawater fish farming in Turkey stated that the fish samples which were infected naturally showed darker skin, missing scales, hemorrhage on base of the fin, hemorrhagic ulcers on the skin, hemorrhage and clouding on the external eye, and anemia in the liver, hemorrhage in the visceral organs and internal splenomegaly (Canak and Timur, 2018).

In general, bacteria which were found in this study were opportunistic bacteria which were able to play a role as disease agent in glass eel. Decreasing environmental quality condition, increasing number of populations or pathogen concentration in environment and low immune system of fish can be an interaction of death cause in fish in an aquatic environment as well as in culture place. Therefore, an initial investigation in minimizing the environmental effect and disease agent and handling enhancement of glass eel in culture media is necessary. Investigation process will be synergized with effort of handling improvement of glass eel in capture location and right

monitoring during transportation process from the capture location to the culture location.

CONCLUSION

Based on the result of this study from the 3 study locations, there were 3 bacteria species which were mostly found in eel samples namely *Listeria* sp., *Aeromonas hydrophila*, and *Staphylococcus* spp., with prevalence of 22.2%, 15.6%, and 12.2% consecutively.

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