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Microbial isolation from gastrointestinal tract of catfish (*Clarias gariepinus*)

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Abstract

The purpose of this research was to describe the characteristics and number of microbes that grow in the gastrointestinal tract of catfish and to determine the potential of microbes as probiotics. The type of this research was observational conducted on 30 September-15October 2018 at the Microbiology Laboratory, Faculty of Science and Technology, Universitas Airlangga, Surabaya. Microbial isolation using Nutrient Agar (NA), Mann de Roosa Shrape agar (MRSA) and potato dextrose agar (PDA) media. The fish that used is catfish that are bred in ponds at Desa Tlasih, Kecamatan Tulangan, Kabupaten Sidoarjo, East Java. The identification includes microscopic and macroscopic characteristics. Based on the research that has been done, it can be concluded that there are 8 kinds of bacterial colonies in NA media, on PDA media there are 8 kinds of fungi, and on MRSA media there are 2 bacterial colonies and each has different macroscopic and microscopic characteristics. The total number of bacteria growing in NA and MRSA media respectively were 8.7 x 10^4 CFU/g and 1.2 x 10^5 CFU/g. It is suspected that there are potential bacteria as beneficial probiotics for catfish which still need further research.

Keywords: bacteria, catfish, gastrointestinal tract, fungi, microbes

Introduction

Indonesia is a maritime country with a fisheries cultivation sector with high potential to be developed, both through extensification and intensification, especially for fish with high economic value. One of the freshwater fish that has the highest economic value is catfish (Soebjakto 2016). Catfish cultivation at the level of hatchery and enlargement has a bright prospect so that this fish is included in the commodity that is spurred on by developing its cultivation (Riyanto *et al.* 2010).

Catfish farming is mostly done by the community because catfish is always in demand by the community. Evidenced by the production of catfish which increased 21.31% per year in the last five years (2011-2015). From 337,557 tons in 2011, to 722,623 tons in 2015 (Soebjakto 2016). Catfish cultivation has many advantages including an easy cultivation system, growth of catfish is relatively fast, adaptable, efficient to the feed provided (Nasrudin 2010), can be cultivated on land and limited water sources (Ferdian *et al.* 2012) and fish are able to survive and grow well in waters that have poor water quality (Dewi *et al.* 2013).

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Polluted aquatic environments as habitat for catfish cause fish to be easily attacked by diseases that develop in ponds. In addition, Ferdian *et al.* (2012) stated that catfish used to live in ponds with high stocking densities. In aquaculture, high stocking densities as well as excessive use of feed cause the maintenance water environment to be a suitable medium for heterotrophic or opportunistic bacterial growth (Chinabut and Puttinaowarat 2005). This bacterium will cause fish to be easily attacked by diseases that will inhibit the growth process. This is the reason why catfish farmers often add antibiotics and other chemical compounds to maintain the quality of catfish pond waters.

The use of chemical compounds such as disinfectants and antibiotics is not very effective for the prevention or treatment of fish diseases (Olafsen 2001). In addition, the presence of antibiotic residues causes fisheries products not to meet the standards of several countries that are export destinations (Lestari *et al.* 2016). Excessive use of antimicrobial compounds also select resistant strains and then bacteria can exchange resistance coding genes. Ultimately this causes resistance coding genes to spread to human pathogens and then reach humans (Heuer *et al.* 2009).

The bacterial community (microbiota) in aquatic animals is thought to have an influence on growth and host survival. Microbiota can affect a large number of gene expression in its host, especially genes that play a role in immunity and nutrition (Nayak 2010). Bacteria can help host nutrition with the production of enzymes or essential compounds (Verschuere *et al.* 2000) and can prevent opportunistic pathogenic bacteria from proliferating and colonizing the host body, especially at the larval stage where the immune system is not fully developed (Hansen and Olafsen 1999).

According to Naim and Ahmed (2012), catfish are organisms with intestinal microflora composition that are able to increase the host's immune response and are able to assimilate organic carbon from the environment so that catfish are able to live in extreme environments. Catfish live in waters and share the same ecosystem with the bacterial community associated with it, so the bacterial community always changes dynamically to follow the bacterial community in its environment (Cahill 1990). This provides an opportunity to introduce beneficial bacteria into the bacterial community that has formed at the host. Thus, modulation of the microorganism community associated with fish is one way to prevent disease and increase production more safely. These bacteria are opportunistic and their existence is transitory depending on environmental conditions (Gatesoupe 2005).

Another advantage of using probiotic bacteria is that probiotics have been proven to help the digestive process by secreting extracellular enzymes such as proteases and lipases. Macromolecules that have been overhauled by extracellular enzymes can increase the digestibility of fish, so fish more easily get the nutrients contained in feed. Nutrients that are easily obtained will reduce the amount of energy needed to overhaul the feed, so that energy tends to be used by fish for growth (Balcazar *et al.* 2006).

Research related to the isolation and identification of microbes contained in the digestive tract of catfish some have been reported before, but because certain types of microbes grow to adapt to the environment of their host waters, it is necessary to conduct research to find out the microbial communities that live in the intestines of catfish that are cultivated in tarpaulin waters. The results of isolation will be continued to find out candidate bacteria that can act as probiotics.

In a previous study conducted by Lusiastuti *et al.* (2013) successfully analyzed the effect of Bacillus firmus on increased phagocytosis and differential leukocyte indices by challenging tests using *Aeromonas hydrophila* on Dumbo catfish. In Hastuti's study (2015), the number of normal erythrocytes was 2 x 10^6 cells/µL, the amount of normal hemoglobin was 9 g/dl. Based on the background above, the purpose of this study is to determine the characteristics and number of microbes that grow in the intestines of catfish to

simultaneously determine the potential of microbes isolated from catfish intestines as probiotics.

Materials and methods

Research to determine the type of bacteria found in the intestines of catfish, including in observational research. The research was conducted on 30 September-23 October 2018 at the Microbiology Laboratory, Faculty of Science and Technology, Univ. Airlangga Surabaya. The tools used for this research are an incubator, laminar air flow, autoclave, Erlenmeyer tube, vortex, bunsen, Petri dish, test tube, tube rack, needle, dropper, microscope, glass cover, digital scale, mortar pestle, mortar, beaker glasses, micropipettes, knives, rulers and digital cameras. While the materials used in this study were catfish, Mann de Roosa Shrape agar (MRSA) sterile media, sterile nutrient agar (NA) media, sterile potato dextrose agar (PDA) media, aluminum foil, cotton, label paper, plastic wrap, 70% alcohol, distilled water, physiological solution, sterile blue tip and markers.

The work procedure in this study was started by taking 1 catfish from a catfish breeding pond in Tlasih Village, Tulangan District, Sidoarjo Regency, Indonesia. Catfish dissected and removed the internal organs, then separated the intestine. The fish intestine is then measured in length and weight and then washed with sterile physiological salt (0.85% NaCl) to remove bacteria that are attached to the digestive tract. The fish intestine is then dried. The next step is isolation and enumeration. Catfish digestive tract weighed as much as 1.5 g and homogenized in 14.5 mL of 0.85% sterile physiological solution then made serial dilutions between 10^{-1} to 10^{-4} dilutions. 0.1 mL of 10^{-2}, 10^{-3} and 10^{-4} dilution series were planted on sterile NA, MRSA and PDA media, respectively, by the spread plate method. To plant fish intestine samples in PDA media, one mL of chloramphenicol 2000 ppm should be added. The culture results were then incubated at 28 °C for 24-48 hours. For PDA media, observations will be made after 72 hours.

After the incubation period, NA and MRSA media were observed to grow colonies and count the number of colonies using a colony counter. Observation and settlement of the colony were carried out on each cup with the number of each type of different bacteria according to the appearance of the bacterial colony morphology. Counting the number of bacteria using the total plate count (TPC) method in which the cup that produced the number of colonies of 30-300 pieces was selected and counted. The number of bacteria is stated as colony forming unit (CFU). Colonies with different morphological appearances are then taken and purified on new media using the scratch plate method. Each isolate was stored in media so that it tilted according to the media used at the isolation stage and stored at 4 ^{o}C.

Bacterial isolates from pure recultation were then identified based on morphological characters including colony morphology, cell morphology, and gram staining tests. Observation of colony morphology seen from the shape, elevation, size, color and edges of bacterial colonies. Observation of cell morphology includes cell shape and results of gram staining. For macroscopic observation of fungi are the shape and color, while microscopic observation is the form of fungi and spores. To observe mushrooms, it is done by making a mushroom culture slide first.

Results

Research into microbial isolation in the digestive tract of catfish gives results that show that in the digestive tract catfish contain microbes derived from groups of bacteria and fungi. One type of bacteria that has been successfully isolated is from the type of lactic acid bacteria which generally have a role as probiotics. The following are the results of macroscopic and microscopic identification of catfish intestines.

Figure 1. Catfish digestive channel (*Clarias gariepinus*)

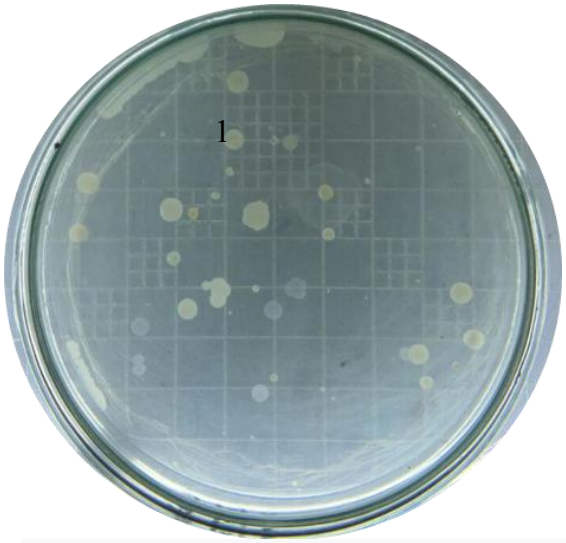

Figure 2. Appearance of bacterial colonies on NA growth media

In NA media macroscopic observations were started from optical opaque characters in colonies 1,2,3,4,5,7,8 and optical translucent characters in colonies 6. Colony surface characteristics 1,2,4,5,6,7,8 fine while colony 3 is rough, the color of the colonies 1,4,5,7,8 white while the colonies 2 and 3 are yellowish-white and the colonies are 6 clear. Form 1,2,3 irregular colonies, 4,6,7,8 circular colonies. The edges of 1,2,3 undulate colonies, 5,6,8 flat colonies while 4 convex colonies and 7 raised colonies. This shows that differences in macroscopic appearance or cultural characteristics are used as a basis to separate microorganisms into taxonomic groups.

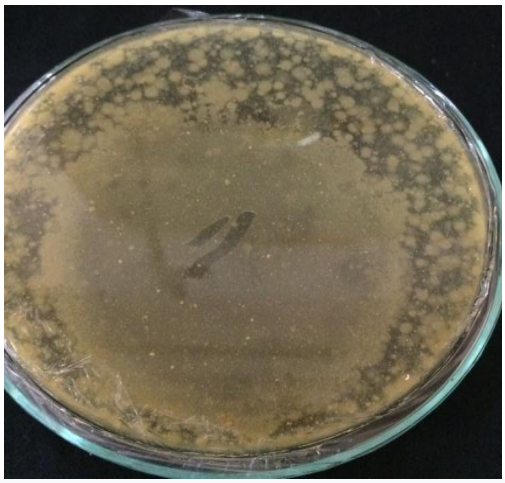

Figure 3. Appearance of bacterial colonies on MRSA growth media

On MRSA media, it is known that there are 2 bacterial colonies which have almost the same similarity, namely optical opaque characteristics, smooth colony surface, yellow color, round shape, elevation of the entire flat or flat edge shape, only in terms of different colony sizes, ie size 0.4 in colonies 1 and 0.2 in colony 2, both incubated for 24 hours.

Figure 4. Appearance of fungi on PDA growth media

On PDA media there are 8 growing fungi colonies that have a white topside and reverse color 1 colony, a velvety colony texture, there is an exudate point that does not have radial furrow and has no zoning. Colony 2 has the character of beige top color, reverse white, velvety colony texture does not have an exudate point, radial furrow and zoning. In the 3 character colonies, the colors are white and reverse black, the texture of the colony is cottony, has no exudate points, has radial furrow and zoning. Colony 4 has a white reverse brown top character, velvety colony texture, has an exudate point, has no radial furrow and zoning. Colony 5 has white top and bottom brown velvety colony texture, has no exudate point, radial furrow and zoning. Colony 6 has the character of white top color under brown, velvety colony texture, has no exudate point, radial furrow, zoning. Colony 7 has the upper white and orange color, velvety colony texture, has no exudate point, has radial furrow and zoning. Colony 8 has the characters of black top, black bottom, powdery colony texture, has an exudate point, radial furrow, zoning.

Table 1. Macroscopic identification of bacterial colonies on NA growth media

Colony	Optics Characteristic	Surface Characteristics	Color	Size (µm)	Form	Elevation	Edge shape	Gram
1	Opaque	Smooth	White	0.6	Irregular	Raised	Undulate	Positive
2	Opaque	Smooth	Cream ++	0.2	Irregular	Umbonate	Undulate	Positive
3	Opaque	Rough	Cream +	0.5	Irregular	Convex	Undulate	Positive
4	Opaque	Smooth	White	0.3	Circular	Entire	Convex	Positive
5	Opaque	Smooth	White	0.1	Spindle	Entire	Flat	Positive
6	Translucent	Smooth	Transparent	0.3	Circular	Entire	Flat	Positive
7	Opaque	Smooth	White	0.5	Circular	Entire	Raised	Positive
8	Opaque	Smooth	White	0.3	Circular	Entire	Flat	Positive

Table 2. Macroscopic identification of bacterial colonies on MRSA growth media

Colony	Optics Characteristic	Surface Characteristics	Color	Size (µm)	Form	Elevation	Edge shape	Gram
1	Opaque	Smooth	Yellow	0.4	Circle	Entire	Flat	Positive
2	Opaque	Smooth	Yellow	0.2	Circle	Entire	Flat	Positive

Table 3. Identification of fungi on PDA growth media

Fungal	Characteristics					
	Color		Colony texture	Fungal Exudate	Radial furrow	Zonation
	Top side	Reverse				
1	White	White	Velvety	+	-	-
2	Cream	White	Velvety	-	-	-
3	White	Black	Cottony	-	+	+
4	White	Coklat	Velvety	+	-	-
5	White	Coklat	Velvety	-	-	-
6	White	Coklat	Velvety	-	-	-
7	White	Orange	Velvety	-	+	+
8	Black	Black	Powdery	+	+	+

Discussion

The use of the digestive tract of catfish is used to determine the intestinal microbiota has a protective function to suppress pathogenic bacteria by mechanisms of attachment (receptors), increased production of intestinal mucus or mucosa, and nutritional competition (Salminen and Wright 1993). The existence of a balanced bacterial community of bacteria can also prevent pathogenic bacteria from proliferating and colonizing, and this is an important body defense mechanism at the larval stage where the immune system is not fully developed (Skjermo *et al.* 1997).

In the digestive tract of catfish, which consists of proximal intestine, middle intestine, and distal intestine is a microbial habitat because microbes enter through feed and water as a substrate to enter the digestive tract. Conditions in the gastrointestinal tract including anaerobes and microbes (normal flora) tend to be pathogenic if the condition of the substrate or host is malnourished resulting in commensalism and parasitism.In the isolation stage, the digestive tract is grown on NA, PDA, and MRSA media. In the NA media used for bacterial isolation in general, which can be used for the growth media of some bacteria, while in the MRSA media it is used for lactic acid bacterial growth media as a selective medium. PDA media is used for fungi growth media.

The number of microbes in the gastrointestinal tract was identified in each medium ie in NA media, colony 1 there were 13 x 10^3 CFU/mL, colony 2 there were 9 x 10^3 CFU/mL, colony 3 there were 7 x 10^3 CFU/mL, colony 4 there were 23 x 10^3 CFU/mL, colony 5 has 14 x 10^3 CFU/mL, colony 6 has 8 x 10^3 CFU/mL, colony 7 has 6 x 10^3 CFU/mL, colony 8 has 7 x 10^3 CFU/mL. On MRSA media, it cannot be counted because the number of colonies is

<300 colonies where the incubation time is more than 24 hours. On PDA media, 1,2,3,4,6,7 and 8 colonies have 1 same species, in 5 colonies there are 2 same colonies.

In NA media there are 8 kinds of bacterial colonies, on PDA media there are 8 kinds of fungi, and on MRSA media there are 2 growing bacterial colonies. After growing several kinds of isolates, morphological identification (macroscopic and microscopic) was carried out.On PDA media there are 8 growing fungi colonies that have a white topside and reverse color 1 colony, a velvety colony texture, there is an exudate point that does not have radial furrow and has no zoning. Colony 2 has the character of beige top color, reverse white, velvety colony texture does not have an exudate point, radial furrow and zoning. In the 3 character colonies, the colors are white and reverse black, the texture of the colony is cottony, has no exudate points, has radial furrow and zoning. Colony 4 has a white reverse brown top character, velvety colony texture, has an exudate point, has no radial furrow and zoning. Colony 5 has white top and bottom brown velvety colony texture, has no exudate point, radial furrow and zoning. Colony 6 has the character of white top color under brown, velvety colony texture, has no exudate point, radial furrow, zoning. Colony 7 has the upper white and orange color, velvety colony texture, has no exudate point, has radial furrow and zoning. Colony 8 has the characters of black top, black bottom, powdery colony texture, has an exudate point, radial furrow, zoning.

After macroscopic characterization, microscopic characterization of colonies that grew in the three media was carried out. In NA and MRSA media, the colonies that appeared were Gram-negative and rod-shaped. In colonies 1, 2, 4, 5, 6, 7, 9 the colonies are rod-shaped and include Gram-negative while in colonies 8, 10 are Gram-positive rods. In colony 3 it is Gram negative comma. Based on the results of research that has been done it can be seen that in the gut of catfish there are a variety of microbes in the form of bacteria and fungi. Microbes in the digestive tract of catfish are thought to help the digestion of the host by producing hydrolytic enzymes to digest food. In addition, the presence of microbes in the digestive tract of catfish is suspected to play a role in the body's defense system of catfish so that even though catfish live in an environment that is not good enough, they still survive.

According to Naim and Ahmed (2012), catfish are organisms with intestinal microflora composition that are able to increase the host's immune response and are able to assimilate organic carbon from the environment so that catfish are able to live in extreme environments. Catfish live in the waters and share the same ecosystem with the bacterial community associated with it, so the bacterial community always changes dynamically to follow the bacterial community in its environment (Cahill 1990). This provides an opportunity to introduce beneficial bacteria into the bacterial community that has formed at the host. Thus, modulation of the microorganism community associated with fish is one way to prevent disease and increase production more safely. These bacteria are opportunistic and their existence is transitory depending on environmental conditions (Gatesoupe 2005).

Conclusion

Based on the research that has been done, it can be concluded that there are 8 kinds of bacterial colonies in NA media, on PDA media there are 8 kinds of fungi, and on MRSA media there are 2 growing bacterial colonies and each has different macroscopic and microscopic characteristics. other. The total number of bacteria growing in NA and MRSA media were 8.7×10^4 CFU/g and 1.2×10^5 CFU/g respectively. It is suspected that there are potential bacteria as beneficial probiotics for catfish which still need further research

Conflict of interest

The authors whose names are listed immediately below certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such

as honoraria; educational grants; participation in speakers bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript (Nurul 'Aini and Muhammad Bachruddin).

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Author contributions

All authors have reviewed the final version of the manuscript and approved it for publication. NA and MB designed the study, performed research, collected and analysedthe data; NA, IT and MB wrote and reviewed the paper. NA is the main contributor of this manuscript.

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