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METAGENOMIC ANALYSIS OF CATFISH (*CLARIAS GARIEPINUS*) INTESTINE BACTERIA

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ABSTRACT

Metagenomic analysis of intestinal bacteria is one of the strategies that can be done to improve aquaculture in Indonesia. Metagenomic analysis is to analyze the diversity of intestinal bacteria have been successfully carried out in various countries. Nevertheless, analysis of the main trade diversity of fisheries in Indonesia still needs to be done. This research suggests looking at the metagenomic analysis of Catfish (*Clarias gariepinus*) intestine bacteria. Samples of catfish came from the Ciparanje FPIK Unpad Wet Laboratory. This research was conducted FPIK Unpad Microbiology and Biotechnology Laboratory and subsequently sequenced by the HiSeq NGS in Novogene, Singapore. The results of the study obtained showed Fusobacteria (79.96%), Bacteroidetes (15.24), Proteobacteria (3.71%), Firmicutes (0.94%) and other phylum (0.10%). It was identified that the highest number of taxon in each phylum is generally in the proteobacteria phylum. There are several genera that can be pathogenic in fish, one of which is *Enterovibrio* and *Vibrio* and Several other detected bacteria, among others, from the genus Lactobacillus. These bacteria are known to be used as probiotics.

KeyWords
Bacteria, Catfish, Metagenomic, Intestine

1. INTRODUCTION

The method of reading all the DNA from a complete ecosystem (not just one organism) is known as the metagenome approach [1]. This metagenome can determine the composition of the microbial community which is then described in phylogenetic form, which is based on the diversity of one gene, for example the 16S rRNA gene. This metagenome can also provide genetic information about biocatalysts, enzymes, function and structure of the organ-ismen community [2]. Currently, research on the metagenome has been done a lot, one of which is the metagenome research in fish intestines. The digestive tract is a very complex ecosystem that is divided into several sections extending from the lips to the anus including the stomach, small intestine and large intestine [3].

This digestive tract represents between the environment and the fish's body. The main function of the digestive tract is to convert food into components that can be digested and absorbed by the body, and in the metabolic process symbiosis with bacteria [3]. Several types of microflora found in the digestive tract of animals have an important role in improving feed utilization, fish health and improving the quality of the environment and microorganisms [4]. The microflora present in the gut performs a number of functions such as fermenting unused energy substrates, exercising the immune system, preventing the growth of harmful species, regulating intestinal development and producing vitamins for the host. However, under certain conditions some species can cause disease by causing infection to the host [5].

One of the most prospective fishery commodities to be cultivated on an industrial and household scale is catfish (*Clarias gariepinus*). Catfish is one type of freshwater fish that belongs to the order Siluriformes and is classified as true bony fish. One of the strains of catfish is *Clarias garieoinus*. The productivity at the rearing stage of Pearl catfish is 20-70% higher than the seeds of other strains [6]. The use of this seeds in cultivation activities can result in higher productivity, so the demand for seeds is increasing. Information about the metagenomic analysis of catfish intestine bacteria is important data for the development of the productivity aquaculture industry in Indonesia, so research on it is needed. The research aims to describe the diversity of bacteria in the gut of catfish through the metagenomic analysis.

2. MATERIAL AND METHOD

2.1 Sampling of Fish Gut

This research was conducted in the Laboratory of Microbiology and Biotechnology, Faculty of Fisheries and Marine Sciences Unpad. Samples of catfish (*Clarias gariepinus*) from the Ciparanje FPIK Wet Laboratory. The first step used to take a sample of the gut is killed the fish with piercing the brain using a *sonde*. And then, fish are weighed using digital scales. After that, makes an incision on the belly of the fish until the internal organs are visible and take the intestine. The contents of the gut are taken as much as 250 mg by splitting the gut of fish using scissors then scraped off the contents of the gut. The results contents of the gut are then stored in a sterile microtube to be used as a bacterial DNA isolation metagenome material.

2.2 Isolation of intestinal bacterial DNA metagenome

Metagenomic DNA from fish intestinal bacteria was isolated and extracted according to procedures are using

the Quick-DNA ™ Fecal / Soil Microbe Miniprep Kit (Zymo Research). The fish gut sample was added into the ZR BashingBead ™ Lysis Tube and then added ZR BashingBead ™ Lysis Tube, then the next step is performed according to the procedure from the kit used.

2.3 Visualization and Measurement of Concentrations, Purity of DNA

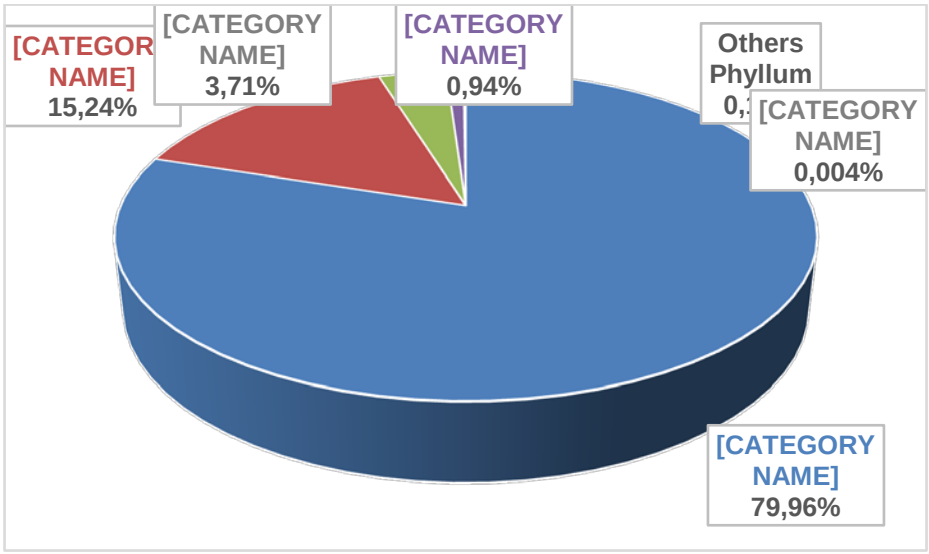
The first step is to make 1% agarose gel weighed with 1 gram agarose powder and add 100 ml of TAE 1 × to the Erlenmeyer tube and mix them well. Then 10 ml of the red gel was added and the gel was printed. Then 2 µl Bench Top DNA Ladder 1 kb and 2 µl loading dye were put into the first gel well. A total of 4 µl of 2 µl loading dye insulation product was put into the second gel well. The electrophoresis tool is run with an electric current of 80 volts for 55 minutes. After the running process is complete, agarose gel is taken and observed on a UV transilluminator. While the measurement of DNA concentration and purity with a spectrophotometer and absorbance are adjusted at wavelengths (λ) 260 and 280 nm.

2.4 Sequencing and Data Analysis

This research uses the Illumina HiSeq Next Generation Sequencing (NGS) method. DNA samples that have been tested for results by visualizing and measuring the concentration and purity of the DNA are sent to Novogene, Singapore, for sequencing.

3. RESULTS AND DISCUSSION

Samples catfish (*Clarias* gariepinus) obtained from the Ciparanje FPIK Unpad Wet Laboratory. Ciparanje Wet Laboratory FPIK Unpad is located in Cileles, Jatinangor District, Sumedang Regency, West Java. Based on the results of body measurements, the body length is 31 cm with a weight of 668.33 grams. While the length of the intestine obtained is 25 cm. Catfish which are classified as carnivorous fish [7]. has a length of intestine that is shorter than its body. As with the type of food that will be eaten by fish, it depends on the availability of the type of food in nature and also the physiological adaptation of the fish, for example the length of the intestine, the nature and physiological conditions of digestion, the shape of the teeth and pharyngeal bones, the shape of the body and its behavior.



Others Filum:

Filum	Total Bacteria	Abundance (%)
Actinobacteria	17	0,0230
Cyanobacteria	67	0,0908
Tenericutes	0	0
Verrucomicrobia	9	0,0122
Acidobacteria	0	0
Saccharibacteria	0	0
Chloroflexi	0	0
Spirochaetes	0	0
Latescibacteria	0	0
Elusimicrobia	0	0
Parcubacteria	0	0
Total Others Phylum	93	0,103

Figure 1. Absolute Abundance at the Phylum Level

The results of the abundance of bacteria in catfish (Figure 1) showed Fusobacteria (79.96%), Bacteroidetes (15.24%), Proteobacteria (3.71%), Firmicutes (0.94%) and other phylum (0.10%). This is in accordance with the research of Li [8] who examined 8 species of fish with differences in carnivores, herbivores, omnivores, and filter feeding, found that the phylum that dominates each fish is the phylum fusobacteria. This phylum fusobacteria in the intestinal bacteria of Channel Catfish in America, the abundance is dominated by the phylum firmicutes (38%), proteobacteria (37%), fusobacteria (11%), and cyanobacteria (6%) [5].

In addition, the species most commonly found is Cetobacterium somerae from the phylum Fusobacteria [9]. This genus is found to be abundant in several freshwater fish species [10,11]. This species is known to produce high amounts of vitamin B12 [12], can ferment peptides and carbohydrates [13] and can inhibit the growth of potential pathogens [14]. This is supported by the results obtained by Sullam [15] stated that the diversity and community of bacteria in the fish gut is influenced by differences in water salinity.

In the results of the abundance at the phylum level, it can be seen that there are many unidentified phyla. Apart from environmental and habitat factors, one of the factors that causes this to happen is that there are bacteria that are not classified. These unclassified bacteria is due to a lack of references in the database regarding bacterial samples so that the taxonomy of these bacteria is incomplete [16].

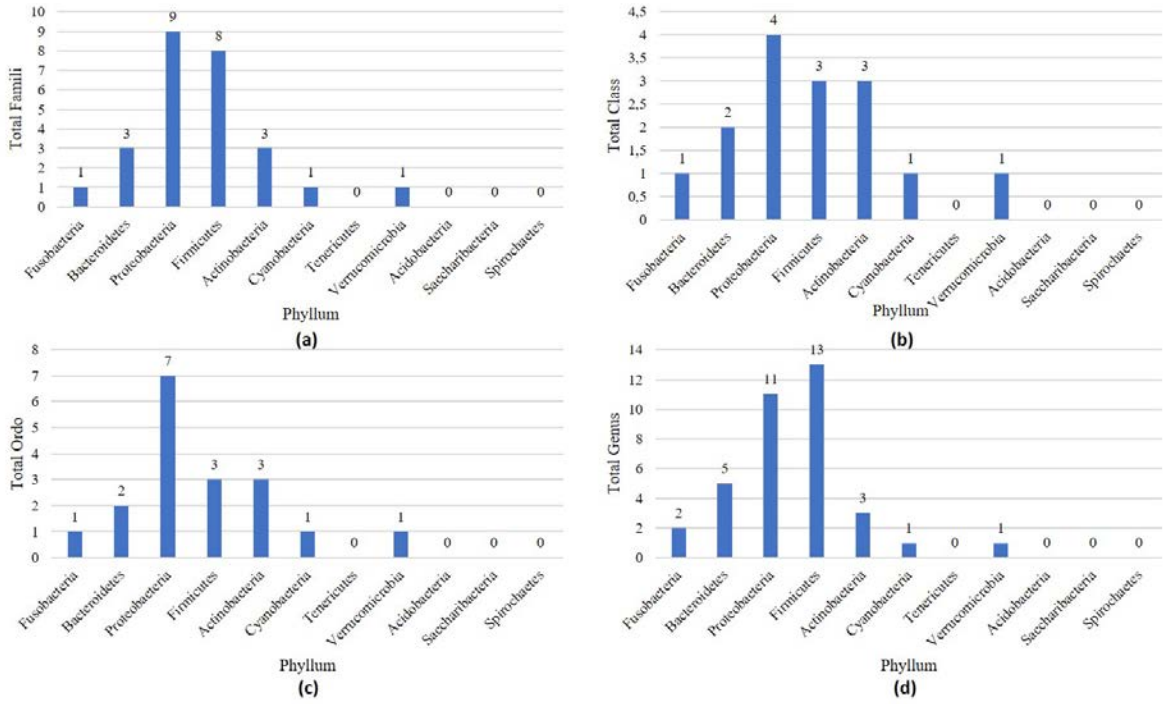


Figure 2. Number of Taxons in Each Identified Phylum
(a = Famili; b = Class; c = Ordo; d = Genus)

Based on Figure 2. It was identified that the highest number of taxon in each phylum is generally in the proteobacteria phylum. Where the total identified family was 9, the total identified class was 4, the total order identified was 7, and the total identified genus was 11. While the highest number of genera was found in the phylum firmicutes. Other phyla such as bacteroides, fusobacteria, cyanobacteria, tenericutes, verrumicrobia, acidobacteria, saccharibacteria, spirochaetes have a small number of genera compared to the phylum firmicutes, proteobacteria and actinobacteria. This is in line with previous studies which stated that the highest diversity in catfish species was generally in proteobacteria with the genus Cetobacterium. The several genera found to have various functions can be seen in Table 1.

Table 1. Table of Functions of Genus / Family Level Bacteria in Catfish

Genus / Family	Phylum	Function	Reference
Bacteroides	Bacteroidetes	The fermentation of carbohydrates results in a collection of volatile fatty acids which are reabsorbed through the large intestine and utilized by the host as an energy source, providing a significant proportion of the host's daily energy requirements.	[17]
Cetobacterium	Fusobacteria	It is known to produce vitamin B12 and is found in plant food in the intestines of fish.	[12, 14, 18]
Clostridium_sensu_stricto_1	Firmicutes	It is an anaerobic obligate gram-positive bacteria with many pathogenic species. These bacteria have been shown to contribute to host nutrition, especially by supplying fatty acids and vitamins.	[19]
Desulfovibrio	Proteobacteria	Sulfate reducing bacteria and in large numbers can become pathogens.	[20]
Enterovibrio	Proteobacteria	Producing indole acetic acid which can be harmful to lactic acid bacteria in the intestines if in excess amounts.	[21, 22]
Flavobacterium	Bacteroidetes	Causes fry syndrome and cold water bacterial disease, which causes high mortality rates in young fish.	[23, 24]
Lactobacillus	Firmicutes	Produces extracellular degradative enzymes and is dependent on other microorganisms to provide certain nutrients.	[25]
Pseudomonas	Proteobacteria	Anti-pathogenic bacteria in aquaculture	[26]
Turicibacter	Firmicutes	It contains butyric acid, an important short chain fatty acid with antimicrobial properties	[27]
Vibrio	Proteobacteria	Fermentation, pathogens and found in fish	[18, 28]

In catfish, there are several genera that can be pathogenic in fish, one of which is *Enterovibrio* and *Vibrio*. *Enterovibrio* functions to produce indole acetic acid which can be harmful to lactic acid bacteria in the intestine if in excess amounts [21,22]. The amount of enterovibrio as much as 2% in the intestines of catfish will not be bad for the lactic acid bacteria in the catfish intestines as well as vibrio bacteria which are present in the intestines in small amounts.

Several other detected bacteria, among others, from the genus Lactobacillus. These bacteria are known to be used as probiotics. The enzymes produced by these bacteria can be used as probiotics for fish feed, especially in the larval stage. According to Feliatra [29] several types of probiotic candidate bacteria include *Lactococcus sp., Carnoacterium sp., Bacillus sp., Eubacterium sp., Pseudomonas sp., Lactobacillus sp., Micrococcus sp. and Bifidobacterium sp.*

3. Conclusion

The results of the abundance of bacteria in catfish showed Fusobacteria (79.96%), Bacteroidetes (15.24%), Proteobacteria (3.71%), Firmicutes (0.94%) and other phylum (0.10%). It was identified that the highest number of taxon in each phylum is generally in the proteobacteria phylum. There are several genera that can be pathogenic in fish, one of which is *Enterovibrio* and *Vibrio* and Several other detected bacteria, among others, from the genus Lactobacillus. These bacteria are known to be used as probiotics.

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