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COMPARATIVE METAGENOMICS STUDY ON SEVERAL ORGAN FISH : A REVIEW

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ABSTRACT

New metagenome technology has been widely used in molecular medicine, especially in the field of fish biology. The metagenome technique allows direct extraction of DNA from the environment for analysis of species diversity and function. This is necessary to determine the abundance of community and diversity in fish. The metagenome has proven researchers can obtain large amounts of raw data in a short amount of time. The combination of DNA sequencing and bioinformatics has enhanced our ability to design custom microarrays and study genomes and transcriptomes extensively in various organisms especially in fish. One of the parts of the fish body observed were the intestines, meat and gills. There are differences between these three organs, namely that the intestine is a place for microorganisms to live in relation to immunity and fish metabolism. Whereas the gills are the entry and exit points for fish excrement, and the meat where there is a potential for disease transmission or putrefaction in fish products. This is supported by the main objectives of each study. Where the observed bacteria will differ according to the surrounding environment.

KeyWords
Diversity, Flesh, Fish, Gill, Gut Metagenomic

1. INTRODUCTION

DNA-based studies produce illustrative large-scale data sets microbial composition of a particular sample. Understand microbial diversity very important because you can know the composition, function, and dynamics microbiota related to health and disease sample [1]. There is a new step in genomic analysis by isolating genomic DNA directly from the environment called the metagenome [2]. The obtained genomic DNA from the environment was then cloned, the genome map was constructed, then further research was carried out to look for new enzymes [3]. This technique is a technique that combines several methods and fields of science, such as genetics, microbiology and bioinformatics. Unlike the general bacterial genome analysis technique, the metagenome technique is carried out by directly extracting the genomic DNA from the environment and does not require a bacterial culture process on an artificial medium [4].

Most of the research conducted to determine the structure and function of microbiomes comes from research on mammals [5]. Although covering nearly half of vertebrate species [6] few studies have examined the gut, meat and gill microbiome of fish [7,8,9]. At present, it is well accepted that microbial communities that inhabit the gastrointestinal vertebrates (gut microbiome) play an important role in body development, physiology and health [7].

Currently, there are many studies on the gut microbiota of fish. According to Yan [10] stated that the abundance and diversity of gut bacteria is different at each stage of fish development. This is due to differences in the selectivity of food at each stage of development.[11] stated that environmental factors affect the microbiota composition of Mudsucker (*Gillichthys mirabilis*) fish in California. In addition, Wu [12] also stated that the gut microorganisms of Grass Carp fish in China correlate with various factors, such as feed, pond water and sediment. The effect of the gut microbiota on fish innate immunity has also been extensively studied [13]. According to Li [14] eating habits and genotypes affect the microbiota community in 8 different fish intestines in China and the research of Liu [15] gave results that differences in trophic levels in fish originating from Lake Liangzi affect the gut microbiota community, metabolism and enzyme activity.

In addition, the gills are the entry and exit points of substances in fish, and in the flesh part, we can find out the potential for microbes that carry foodborne diseases and putrefaction in fish products. There is no journal that discusses the comparison of several fish organs used in metagenome analysis. This journal review is expected to explain the diversity of bacteria observed in each organ of the fish, the purpose of each organ being observed and what are the benefits for aquaculture activities.

2. ETYMOLOGY OF ‘METAGENOMIC’ AND ITS DEFINITION

Metagenomics is a very precise way to find out the microbial community in a certain environment that is uncultured / unculturable. The principle of metagenomic diversity analysis is based on DNA analysis taken directly from a community. DNA originating from a community and used for direct DNA analysis is related to total DNA diversity where the genome isolated from the community can be identified using phylogenetic markers such as the 16s rRNA gene (ribosome-RiboNucleic Acid) [16].

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 the biocatalyst, enzymes, function and community structure of the organism [17]. The steps contained in the metagenome technique are sample processing, sorting, assembly, binning, annotation, experimental design, statistical analysis, data storage and data sharing [18].

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2. 1 IMPORTANT METAGENOMIC OF AQUACULTURE

Aquaculture or better known as aquaculture has now become the backbone of the world in supplying world food, especially from the fisheries sector. Aquaculture production can be increased faster, causing aquaculture to be expected by the world and Indonesia. Aquaculture is a sub-sector that can meet healthy food for the world community as their daily consumption. In the future, world aquaculture production will continue to advance and of course become a world fish producer compared to world capture fisheries whose production increase has generally been optimal. This is certainly a big opportunity for Indonesia as a country with enormous aquaculture potential to contribute more to world aquaculture as a world fish producer.

Wang [20] suggested that various types of aquatic bacterial communities are dominated by strong habitat associations. This implies that the relative contribution of deterministic and stochastic processes to microbial community assembly must vary across ecosystems. The importance of this process can also change significantly in certain ecosystems, especially when serious ecological disturbances occur [21,22]. The gut microbiota community is a complex community compared to other common communities because the gut microbiota is not only influenced by the environment but also significantly influenced by the ecology and physiology of the host [23].

2. 2 CHARACTERISTIC OF METAGENOMIC IN ORGAN FISH

Aquatic organisms are different from terrestrial organisms. The microbiota that lives in association with its body is always in contact with water from the environment in which it lives, while the terrestrial microbiota is relatively more stable. The microbiota in aquatic organisms is unstable and only temporary because the water as a place to live continues to wash and the water that enters the digestive tract will flush and introduce bacterial species from outside. Therefore, the composition of the microbiota that lives in association with aquatic organisms can reflect the composition of the microbiota in the surrounding water [24].

Bacteria are very sensitive to environmental changes, small changes in the environment can cause differences in the number of bacteria present [25]. One of the environments that is a habitat for bacteria is the digestive tract of fish. In the digestive organs of fish, especially the intestine, there is a micro-environment where there are many microbes that interact with each other and also interact with their hosts. The effect of giving probiotics to fish needs to be supported by knowledge about the diversity of gut bacteria in it, so that it can be seen the effect on metabolism, growth, immunity, and maintenance of fish health [26].

Over the last decade, numerous studies have found high levels of microbial diversity in the intestinal ecosystem of vertebrates, which are of great importance to the host. nutrition, immunity, health, disease prevention, development [27,28,29]. Borsodia [30] stated that the bacterial community in the front and hind gut of fish has a significant difference. The bacteria that dominate the front and hind gut are *Cetobacterium* (Fusobacteria) as cellulolytic and decomposing bacteria.

Fish breathe and excrete waste through their gills. The gills are also a potential site of pathogenic invasion and colonization by other microbes. Based on the results of research by Pratte [8] showing that it shows unique taxonomic signs, the gill microbiome is influenced by several factors that also affect the gut microbiome. These factors include the specific identity of the individual host. These results suggest basic principles that explain how the fish interact with the structure of the microbial community composition.

At the same time, consumer demand for high quality fish products has been reported globally. However, fresh fish is a food product that is highly perishable due to its composition and spoilage, especially for microbial activity. Cleanliness and temperature during handling, transportation and storage are the most important factors determining the safety and quality of fresh fish down to the consumer level. So it is important to know the quality of the fish flesh. One of them is a metagenome analysis by Tsironi [9] to find out how the diversity of bacteria in fish flesh is so that fish is known to stay fresh.

2. 3 METAGENOMIC IN SEVERAL ORGAN FISH

Metagenomes in various fish organs have been widely used. This is compared to several fish species and the purpose of the research is carried out. The results obtained show differences in OTU as in Table 1. This is influenced by morphological, developmental and biochemical parameters that vary with environmental conditions [31].

Table 1. Comparison of Metagenome in Several Fish Organs

Fish	Organ	Dominant OTUs	Studies Outcome	Reference
Atlantic salmon (*Salmo salar*)	Fish Flesh	Blastococcus, Kosuria, Propionibacterium, Brochothrix streptococcus Janthinobacterium Shewanella Acinetobacter Pseudomonas morganella morganii	Determine the microbes that carry foodborne diseases and putrefaction in fish products. Judging from the formation of histamine correlated the number of concentrations and the number of bacteria in the identified microorganisms.	[9]
Albacore tuna (*Thunus alalunga*)	Fish Flesh	Chryseobacterium, Flavobacterium, Soonwooa, Sphingobacterium, Paracoccus, Comamonas, Acinetobacter, Enhydrobacter, Moraxella, Psychrobacter, Pseudomonas, Stenotrophomonas, Xanthomonas morganella morganii	Determine the microbes that carry foodborne diseases and putrefaction in fish products. Judging from the formation of histamine correlated the number of concentrations and the number of bacteria in the identified microorganisms.	[9]
European anchovy (*Engraulis encrasicolus*)	Fish Flesh	Corynebacterium, Microbacterium, Propionibacterium, Staphylococcus, Lactobacillus, Streptococcus, Aeromonas, Shewanella, Acinetobacter, Enhydrobacter, Psychrobacter, Pseudomonas, Aliivibrio, Photobacterium, Vibrio, morganella morgan	Determine the microbes that carry foodborne diseases and putrefaction in fish products. Judging from the formation of histamine correlated the number of concentrations and the number of bacteria in the identified microorganisms.	[9]
Channel catfish *(Ictalurus punctatus),* large-mouth bass *(Micropterus salmoides)* dan bluegill (*Lepomis)*	Fish Gut	Intestinal bacteria are dominated by the phylum Proteobacteria. The species that dominates it the most is *Cetobacterium somerae*	Knowing which bacteria have the potential to be used as probiotics.	[32]
Grass carp (*Ctenopharyngodon idellus*)	Fish Gut	*Anoxybacillus, Leuconostoc, Clostridium, Actinomyces,* and *Citrobacter.*	Factors affecting the composition of the microbial community in digestive tract and the environment related to fish farming is carried out.	[33]
Grass carp (*Ctenopharyngodon idellus*	Fish Gut	Dominated by *Aeromonas, Enterobacter, Enterococcus, Citrobacter, Bacillus, Roultella, Klebsiella, Hydrotaela, Psedomonas, Brevibacillus* and some bacteria are not classified	To know diversity and activity of cellulolytic bacteria, isolated from the gut contents of grass carp (*Ctenopharyngodon idellus*) (Valenciennes) fed on Sudan grass (*Sorghum udanense*) or artificial feedstuffs	[34]
Tilapia (*Oreochromis niloticus*)	Fish Gut	*Enterococcus, Bacillus* and *Streptophyta*	To determine the bacteria that have the potential to increase growth, immunological status, intestinal morphology and microbiology of tilapia, were observed after giving AquaStar commercial probiotic diet.	[35]
Bighead carp (*Hypophthalmichthys no-*	Fish Gut	Phylum Proteobacteria, Firmicutes, and	To find out the appropriate method for laboratory	[36]

bilis), silver carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio*), and freshwater drum (*Aplodinotus grun*)		Fusobacteria dominate the gut of carp.	studies on goldfish and other potentially *cyprinidae.*	
Channel Catfish (*Ictalurus punctatus*)	Fish Gut	Phyllum Proteobacteria, Firmicutes, Fusobacteria and Cyanobacteria	To characterize how the gut microbiome developed during the early stages of Channel Catfish life and to identify i) which bacteria are the main constituents of the microbiome in the gut at different stages of ontogenesis, and ii) at which point in time the gut microbiome is stable	[37]
Reef Fish	Fish Gill	Genus *gammaproteobacterial, Shewanella* and family Endozoicimonaceae	To know the basic principles that explain how the relationship with fish is the structure of the composition of the microbial community.	[8]
Tilapia (*Oreochromis niloticus)*	Fish Gut	Isophaeraceae, Peptostreptococcaceae, Bradyrhizobiaceace, genus *Arthrobacter* and *Rhodococcus*, species *Mycobacterium llatzerense*	This study explores the influence of the maintenance environment on aquatic and bacterial communities association with those in the intestines of the larvae of tilapia (Oreochromis niloticus, Linnaeus) that grow on either a recirculating or active suspension system	[38]
Tilapia (*Oreochromis niloticus)*	Fish Gut	*Cetobacterium, Clostridium sensu stricto 1, Bacteroides, Enterovibrio, Plesiomonas, Lactococcus, Romboutsia, Stenotrophomonas, Turicibacter, Edwardsiella*	This research suggests looking at the community structure in the gut that has different feeding (carp, tilapia, and catfish) habits using the Next Generation Sequencing (NGS) method.	[39]

Based on the Table 1, it is found that there are various studies on the metagenome that have been carried out on several fish species. The main objective of conducting research on metagnome is to determine the diversity of bacteria that live in an environment, including the intestines, gills, and fish meat. Furthermore, knowledge about the diversity of bacteria can be developed by turning the microbiota into probitic, prebiotic or probiotic effectors as new additives to improve fish health [40]. Probiotics and prebiotics are beneficial in increased activity of the intestinal microbiota and increased immune status, disease resistance, survival, feed utilization and growth. This has the potential to increase and the efficiency and sustainability of aquaculture production [41].

The various microbiota observed are influenced by several factors. The composition of the gut microbiota depends on genetic, nutritional and environmental factors. The microbiota is not always completely washed into environmental water. Certain microbes that are able to colonize will remain alive in the host of aquatic organisms because these microbiota have become part of their host body and have certain mechanisms in their host bodies [42]. Some of the functions between bacteria and their host are (1) as commensal bacteria [43] which are neutral or beneficial to their host [44]; (2) mutually beneficial [45]; (3) bacteria that can become pathogenic under certain conditions; and (4) pathogenic bacteria that cause disease [46].

3. CONCLUSIONS

Currently, there are many metagenome studies on the analysis of community and bacterial diversity in various fish organs. It was found that the organs studied included gills, intestines, and fish flesg with the aim of different studies. In general, the diversity analysis was mostly carried out on the intestinal organs because it is the digestive tract where the most microorganisms are found and is the most effective according to the objectives. want to achieve. Where information about community abundance and gut diversity can be applied for further research.

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