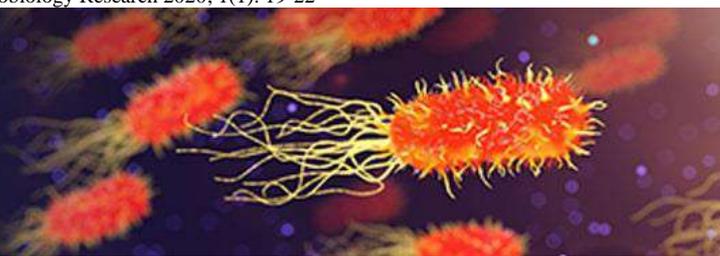


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The fish gut microbiota, current approaches and perspectives: A review

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Abstract

Fishes are poikilothermic aquatic animals that include a wide variety of vertebrates and invertebrates from the animal world. Fish is high in omega-3 fatty acids, vitamins B2 and D, and essential minerals, calcium, phosphorus, iodine, magnesium, zinc, and potassium. Since the mid-twentieth century, global population growth has increased fish consumption. Teleosts continue to draw significant scientific attention as the most diverse vertebrate group and a key component of a growing global aquaculture industry. Global aquaculture growth, driven by declines in wild stock, has created considerable empirical demand, and therefore opportunities, to investigate teleost diversity. The recent increase in microbiome exploration, enabled by breakthroughs in high-throughput sequencing techniques, is one of the most significant recent developments. The gut flora not only supports the digestive and immune systems of fish, but it is also influenced by a variety of host-associated factors. Unfortunately, the majority of studies have concentrated on the structure of the fish gut microbiome, leaving little knowledge of the distinct effects of these factors and the enormous functional potential of the gut microbiome. We highlighted recent discoveries about the diversity and functions of the fish gut microbiome in this review. The current approaches used to study the fish gut microbiome in order to compile all of the knowledge gained and draw accurate conclusions for their application-based perspectives. According to the reviewed literature, future research should focus on functional microbiomics to improve maximum sustainable yield in aquaculture.

Keywords: Fish, microbiome and gut microbes

Introduction

Ever since inception in the 1980s to describe soil ecology, the term microbiome has managed to evolve into a heavily researched area of study. In recent years, this field has shifted from an anthropocentric and medically influenced field to a taxonomically broad field examining research questions in non-model species ranging from trees to frogs and, increasingly, fish. Increased access to next generation sequencing (NGS), a tool that is not reliant on culture-based techniques, which frequently require previous information of target microbes, has fueled the diversification of microbiome studies [1-3]. Presently, gut bacteria in over 145 species of teleosts from 111 genera have been evaluated, indicating a wide variety of physiology and ecology, with commonalities in microbial phyla content between species of fish, dominated by Bacteroidetes and Firmicutes. Non-model teleost gut microbiome research ranges from studies that demonstrate rapid gut microbiome reconfiguration after feeding in clownfish (*Premnas biaculeatus*) to the effect of various climatic conditions, including dissolved oxygen concentration, on the gut microbial diversity of blind cave fish (*Astyanax mexicanus*) [4-7]. Fish have bacterial flora on and in their skin and other organs. In general, the microbes isolated are associated to the fish's environment and vary with a variety of factors including salinity and the load of microbial flora in the water. The bacteria recovered from the fish's skin and gills may be transient rather than resident on the fish's surface. Fish gut microbiota appear to change in response to digestive system complications [8].

The gut microbiota is a depiction of those in the local environment or even the diet fed that can sustain and keep increasing within the intestinal tract; however, there is indications for a different intestinal flora in certain species. Fish gut microbiota can influence development, life span, physiology, immunity, and pathogen defences. As a result, the constituents that direct the invasion of microbes in the fish gut will provide an important foundation for anticipating and treating fish diseases [9-11]. Numerous factors can influence the fish intestinal flora. Changes in the external factors such as diet, environment, and trophic levels can all

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influence the fish's gut microbiota. Various methods are being used to examine the confederation of microorganisms in the gut microbiomes of fish. Previously, culture dependent methods, such as denaturing gradient gel electrophoresis and temporal temperature gradient gel electrophoresis, revealed only a tiny proportion of the considerable microorganisms. However, there are many

culture-independent techniques available today for analysing fish microbiota. We can manipulate microbial communities in fish bodies to bring out possible roles to be expressed in their bodies for the beneficial of fish production by knowing the significance of bacterial diversity in fish bodies [12-17].

Table 1: Factors that are affecting the microbial communities in fish

Factors	Findings
Age	Microbial diversity increases with host-age.
	Age has a significant influence on the intestinal microbiota; water microbiota strongly influence gut microbiota at early life stages.
Diet	Gut microbial diversity decreases as the source of nutrient derivation in the diet are restricted.
	Gut microbiome composition as well as metabolite profiles are significantly altered by host species and feeding behaviour.
	Colonisation with significantly different adherent and non-adherent communities; non-adherent microbiome are much diverse and diet-dependent than adherent microbiome.
	Gut microbiome differs by diet treatment but communities in biofilters remain stable independent of diet; gut communities less diverse than those of water and biofilters.
Environment	Gut microbial diversity increases as the fish develop and is less affected by the surrounding environment than by host diet and development.
	Gut environment and other host development processes shape the microbiome.
	Water microbial communities strongly shape those in the gut thereby resulting in a correlation between water and gut microbial community dynamics.
	Environmental factors more strongly influence microbiome at early life stages.
	The microbiome is significantly altered even at a low level of environmental changes but has strong resilience power.
Host factors	Presence of core gut microbial flora regardless of the habitat type will indicate the operational host selective forces.

Composition of Fish Gut Microbiome

The colonisation of the fish gut begins early in the larval stage and continues until a complicated assemblage of gut affiliated microbes is achieved. It has been reported that approximately 10^8 bacterial cells from over 500 distinct species populate the fish GI tract, which is influenced by aerobes or facultative anaerobes, though strict aerobes were also identified. The diversity of a metagenomics sample is defined in terms of the number of OTUs. OTUs are groups of similar sequences that define a taxonomic unit based on divergence [18-20]. To date, sequencing data analyses have demonstrated a distinctively low phylogeny diversity, with Proteobacteria, Firmicutes, and Bacteroidetes accounting for up to 90% of the fish intestinal flora across various species, and Fusobacteria, Actinobacteria, and Verrucomicrobia among some of the represented phyla. It is not surprising given our insight into the challenges imposed by the intestinal environment on microorganisms in order to reduce diversity in the niche. The variety of fish generally increases as their diet shifts from carnivorous to omnivorous to herbivorous. The content also varies due to environmental factors. Freshwater species' guts are dominated by the bacteria *Acinetobacter*, *Aeromonas*, *Flavobacterium*, *Lactococcus*, and *Pseudomonas*, as well as the obligate anaerobes *Bacteroides*, *Clostridium*, and *Fusobacterium* and members of the Enterobacteriaceae family. The guts of marine fish are influenced by *Aeromonas*, *Alcaligenes*, *Alteromonas*, *Carnobacterium*, *Flavobacterium*, *Micrococcus*, *Moraxella*, *Pseudomonas* and *Vibrio* [21-26].

Profiling the Fish Gut Microbiome

The selection of amplicon or shotgun sequencing is determined by the study's objectives. To date, research on the fish gut microbiome has been limited to determining its composition. Shotgun assessments of the gut microbial genetic repertoire, on the other hand, are required to provide

critical insights in to their functional potential, and it can be aided even more by genomic sequencing and assessment of cultured isolates, as has already been investigated in other niches. The gut tends to produce and continues to receive an arsenal of enzyme secretions while conducting functions like mechanically and chemically grinding food and extracting and absorbing nutrients, posing a serious challenge in deriving quality community DNA from bacterial diversity. Bile salts and complex polysaccharides found in the gut, for example, inhibit the downstream processes of PCR amplification [27-34].

It is essential using an ideal technique of bacterial communities DNA extraction, which serves as the basis for exact structural and functional characterization of the gut microbiota. Before relying on the results of DNA extraction method comparison studies, it is important to emphasise the sample source and type because not all results are comparable due to sample variability.

Effects of the microbiota on fish health

Pathogenic organisms are an essential element of fish microbiomes, but they do not always cause disease. Pathogens, like mammals and plants, could become more prevailing and lead infection and disease whenever the fish commensal microbial community balance is disrupted, a process known as dysbiosis. Pathogenic *Aeromonas* species, for example, were found in the guts of healthy largemouth bronze gudgeons (*Coreius guichenoti*), but in greater abundance in furunculosis-infected fish, indicating that pathogen virulence and predominance are suppressed in healthy individuals. Changes in the environment, such as water conditions, temperature, seasonal changes, climate change, antibiotic use, or changes in rearing conditions, can cause imbalances in the protective commensal microbial community. Another source of disruption could be infection by a primary pathogen, which causes a microbial imbalance

and allows a secondary pathogen to enter. This was demonstrated in a large study in which bacterial community shifts occurred in the skin mucus of Atlantic salmon post-smolts during salmon lice infection, and a greater abundance of genera containing possible secondary pathogenic bacteria (*Pseudomonas* and *Flavobacterium*) was noticed in fish with elevated lice infestation^[35].

Manipulation of the Fish Gut Microbiota

Humans and other animals have recognised the innate link between a host's microbial community and its health status, and much study is now directed forward into methods to modify these microbial communities human health and wellbeing. Fish have still not been excluded from this area of nutrition, and as the aquaculture industry grows, there's been a great deal of interest in manipulating fish gut microbiota to improve wellbeing and nutrition. The primary methods of gut microbes manipulation now include dietary protein and lipid modification, and the addition of probiotics and prebiotics to the diet.

Probiotics

Probiotics are defined as "live microorganisms that provide a health benefit to the host when administered in adequate amounts". Their use in fisheries as an alternative to antibiotics increased significantly as legislation limiting the widely use of chemicals in animal rearing was introduced. As potential probiotics in fish, Gram-positive and Gram-negative bacteria, bacteriophages, microalgae, and yeasts have all been tested. Probiotics from the *LAB Bacillus*, *Lactococcus*, *Shewanella*, and *Aeromonas* genera are among the most frequently studied. However, administering probiotics successfully in aquaculture can be difficult. Low bacteria viability during storage and processing, loss from leaching in the water during feeding, and problems with feed handling and preparation have all been reported. Amidst this, when used correctly, probiotics have been shown to decrease the price of fish farming by improving fish wellbeing and nutrition^[36-39].

Conclusion

A number of important topics in this area would benefit from additional future research. To begin, gathering baseline information on the gut microbiota of wild species, including domains other than bacteria, should be prioritised. Research studies into the possible impact of upcoming climate change on fish gut microbiota, including changes in water salinity, acidity, and temperature, will be critical. Diet manipulation is another area that will continue to be prioritised. Finding sustainable diets that also advantage the fish in aspects of nutrition and health is critical for the aquaculture sector. The role of the gut microbiota will need to be considered throughout these studies. Despite the fact that researchers in this field have markedly enhanced our knowledge on the subject, there still is a lot of scope for more research. Data gathering from wild species, experiments, and aquaculture work will all make significant contributions.

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