

CHAPTER 4

Glimpses Into the Probiotic Potential of Fish Gut Microflora

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Abstract: The gut microbiota of fish represents a dynamic and complex microbial community that maintains a pivotal role in host nutrition, immunity, and overall health. Recently, increasing attention has been directed toward understanding the probiotic potential of fish gut microflora, particularly in the context of sustainable aquaculture and disease management. This chapter explores the composition, functional attributes, and diversity of the fish gut microbiome, emphasizing its capacity to act as a reservoir of beneficial microorganisms. Specific focus is placed on lactic acid bacteria, *Bacillus* spp., and other autochthonous microbes that exhibit antagonistic activity against pathogens, enhance digestive efficiency, and modulate immune responses in fish. By providing an overview of recent advances and research trends, this chapter aims to shed light on the untapped potential of fish gut microflora as natural probiotics, paving the way for eco-friendly and effective strategies in fish health management and sustainable aquaculture practices.

Keywords: Fish gut, probiotic, antagonistic, lactic acid bacteria, freshwater, marine

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Introduction

The host gut microbiome is a different world which cannot be actively seen but its role is unknowingly carried out throughout the life span of the vertebrates. The gut microbiome makes a different ecosystem which houses different types of microorganisms of commensal, symbiotic, and pathogenic nature. The beneficial gut microbiota plays many positive roles to host such as mineral and nutrient absorption. The synthesis of vitamins, enzymes, and amino acids are also the major roles of the gut microbiome, apart from the generation of short-chain fatty acids. But the shifts in gut microbiota due to an imbalance or due to various factors can be detrimental for the host.

Fish retain varied bacterial diversity in organs especially in the gastrointestinal tract and skin (Austin, 2002). Bacteria found in fishes are generally related to environment and varies depending on factors like alkalinity, etc. It may change with the obstacles of the digestive system.

Microbiota found on the fish gut were found similar to that of the environment or may be entirely different. In fishes, these gut microbiota can affect their life span, development, physiology, barriers against the pathogen, and immunity (Yan et al., 2016). Studies have revealed that variations in external features like environment, diet, and tropical level will alter the gut microbiota (Wang et al., 2018). Previously, denaturing gradient gel electrophoresis, culture-dependent methods, and temporal temperature gradient gel electrophoresis techniques show a minor fraction of important microorganisms. Nonetheless, nowadays there are varied diversities of the culture-independent method present for the estimation of fish microbiota (Tarnecki et al., 2017).

Gut microbiota of freshwater fish

The gut microbiota of freshwater fish and marine water fish are diverse. Environment also has a part in determining the microbiota of fresh water fish (Uma et al., 2020). **Table 1** shows that *Acinetobacter*, *Aeromonas*, *Flavobacterium*, *Pseudomonas*, *Lactococcus*, obligate anaerobes (*Clostridium*, *Bacteroides*, *Fusobacterium*), and members of the family Enterobacteriaceae control the gut of freshwater fish species. GI tract of the herbivorous and omnivorous fish shows difference in the total length in the gut as well as diversified gut microbiome. Different levels of short-chain fatty acids (SCFA) are produced by the gut microbiota, for example, the posterior intestine of *Oreochromis niloticus* has the maximum level of SCFA reported (Clements et al., 2014).

Table 1 Tabulation of few freshwater fish containing beneficial microbiota

S. No.	Host fish	Dominant gut microbiota	Reference
1	Nile tilapia (<i>Tilapia nilotic</i>), ayu (<i>Plecoglossus altivelis</i>), goldfish (<i>Carassius auratus</i>)	Obligate anaerobes (<i>Bacteroidaceae</i>) were predominant over facultative anaerobes (<i>Vibrionaceae</i> , <i>Enterobacteriaceae</i>)	Sakata et al., 1980
2	Carp (<i>Cyprinus carpio</i>), Japanese eel (<i>Anguilla japonica</i>), goldfish (<i>Carassius auratus</i>), tilapia (<i>Tilapia nilotica</i>), ayu (<i>Plecoglossus altivelis</i>), and channel catfish (<i>Ictalurus punctatus</i>)	<i>Bacteroides</i> , capable of producing vitamin B ₁₂	Sugita et al., 1991
3	Carp (<i>Cyprinus carpio</i>), ayu (<i>Plecoglossus altivelis</i>), channel catfish (<i>Ictalurus punctatus</i>), tilapia (<i>Tilapia nilotica</i>), and Japanese eel (<i>Anguilla japonica</i>)	<i>Bacteroidaceae</i> , <i>Aeromonas</i> , and <i>Clostridium</i> strains secreting amylase that play a vital role in starch digestion.	Sugita et al., 1997
4	Common carp (<i>Cyprinus carpio</i>), silver carp (<i>Hypophthalmichthys molitrix</i>), deepbodied crucian carp (<i>Carassius cuvieri</i>), and channel catfish (<i>Ictalurus punctatus</i>) and	<i>Lactococcus raffinolactis</i> , a prominent intestinal lactic acid bacteria (LAB)	Hagi et al., 2004
5	Common carp (<i>Cyprinus carpio</i>), goldfish (<i>Carassius auratus</i>), and Mozambique tilapia (<i>Oreochromis mossambicus</i>)	Vancomycin-resistant bacteria such as <i>Cetobacterium somerae</i> <i>Bacteroides</i> and strains with extraordinary vitamin B ₁₂ secreting ability	Tsuchiya et al., 2008
6	Rohu (<i>Labeo rohita</i>) and catla (<i>Catla catla</i>)	Lactic acid bacteria <i>Pediococcus acidilactici</i> and <i>Enterococcus faecalis</i> and	Rai et al., 2011
7	Zebrafish (<i>Danio rerio</i>)	<i>Pseudomonas spp.</i> , <i>Aeromonas spp.</i> , <i>Plesiomonas spp.</i> , <i>Vibrio spp.</i> , <i>Shewanella spp.</i> and <i>Cetobacterium spp.</i>	Roeselers et al., 2011
8	Mrigal (<i>Cyrrhinus mirgala</i>), rohu (<i>Labeo rohita</i>), and tilapia (<i>Oreochromis mossambicus</i>)	<i>Pichia kudriavzevii</i> , <i>Enterobacter asbura</i> , <i>Candida parapsilosis</i> , and <i>C. tropicalis</i> can secrete tannase to overcome the anti-nutritional elements in the feedstuffs.	Mandal and Ghosh, 2013
9	Largemouth bass (<i>Micropterus salmoides</i>), channel catfish (<i>Ictalurus punctatus</i>), and bluegill (<i>Lepomis macrochirus</i>)	<i>Plesiomonas shigelloides</i> , <i>Cetobacterium somerae</i> , <i>Aeromonas sp.</i> , <i>Fusobacterium mortiferum</i>	Larsen et al., 2014
10	Mrigal (<i>Cyrrhinus mirgala</i> and tilapia (<i>Oreochromis niloticus</i>)	<i>Candida rugosa</i> , and <i>Pichia kudriavzevii</i> , extracellular enzyme synthesizing yeasts	Banerjee and Ghosh, 2014

Gut microbiota of marine fish

Marine fish have unique features to interact with the environment (**Table 2**). The major bacterial genera inhabiting the gut of marine fishes are *Alcaligenes*, *Aeromonas*, *Alteromonas*, *Flavobacterium*, *Moraxella*, *Carnobacterium*, *Pseudomonas*, *Micrococcus*, and *Vibrio*. The intestinal microflora of marine and freshwater fish larvae revealed the predominance of bacteria belonging to *Pseudomonas*, *Vibrio*, *Flavobacterium*, *Cytophaga*, and the family *Enterobacteriaceae*. With a variation in fish habitat, the microbial community of the fish also changes, and within the first fifty days for many species, a relatively stable gut microbiota will be formed (Larsen et al., 2014). Both the freshwater and marine water fish gut contain minor quantities of lactic acid bacteria (mainly *Lactobacillus* sp.) (Egerton et al., 2018).

Table 2 Tabulation of few marine fish containing beneficial microbiota

S. No.	Host	Dominant gut microbiota	Reference
1	Long jawed mudsucker (<i>Gillichythis mirabilis</i>), Atlantic salmon (<i>Salmo salar</i>)	Tenericutes (<i>Mycoplasma</i> sp.)	Llewellyn et al., 2016
2	Neritic sharks	<i>Photobacterium damsela</i>	Grimes et al., 1985
3	Pacific white shrimp (<i>Penaeus vannamei</i>)	Gamma <i>Proteobacteria</i>	Rungrassamee et al., 2016
4	Bony fish and sharks	<i>Proteobacteria</i> , <i>Firmicutes</i>	Givens et al., 2015
5	Damselfish (<i>Pomacentridae</i>) and cardinal fish (<i>Apogonidae</i>)	<i>Shewanellaceae</i> , <i>Endozoicomonaceae</i> , <i>Fusobacteriaceae</i> , <i>Pasteurellaceae</i> , and <i>Vibrionaceae</i>	Parris et al., 2016
6	Rabbit fish (<i>Siganus muscescens</i>)	<i>Firmicutes</i> , <i>Bacteroidetes</i> and <i>delta-Proteobacteria</i>	Nielsen et al., 2017
7	Pacific white shrimp (<i>Penaeus vannamei</i>)	<i>Alpha Proteobacteria</i> alongside <i>Planctomycetales</i>	Chen et al., 2017
8	Atlantic cod (<i>Gadus morhua</i>)	<i>Proteobacteria</i> , <i>Firmicutes</i> , <i>Bacteroidetes</i> , and <i>Fusobacteria</i> act as biomarker for oil contamination	Walter et al., 2019

Importance of gut microbiota in disease resistance

Fish gut microbiota comprises of microorganisms that colonizes the digestive tract and envelops the complete gamut of the biochemical cycle in the fish. Numerous useful strains have been proven to harbour these unique strategies. Studies have demonstrated that some beneficial microorganisms will inhibit or destroy the inhibitory compounds so generated in the host (Teplitzki et al., 2009). For example, *Lactococcus lactis* isolated from marine fish has been used to treat fish pathogens. This microorganism produces an adequate amount of nisin Z, which can detain the growth of the fish pathogen *Lactococcus*

garvier, thereby rendering a good option for the prevention of lactococcosis (Sequeiros et al., 2015). One more study by Bindiya et al. (2015) have reported that *Centroscyllium fabricii* isolated from the deep sea shark possess antagonistic property in the fish gut. **Table 3** summarizes a handful of beneficial microorganisms present in the fish gut and their associated probiotic activities with special emphasis on inhibition of microorganisms capable of causing disease in fish.

Table 3 Fish gut bacteria and associated probiotic activity against pathogens

S. No.	Host	Habitat	Bacteria in fish GI tract	Active against	Reference
1	Repang (<i>Puntioplites waandersi</i>)	Mahakam river, a fresh water	<i>Enterococcus sp.</i> , <i>Lactobacillus sp.</i> , and <i>Lactococcus sp.</i>	<i>Aeromonas hydrophila</i> , <i>Pseudomonas sp.</i> and <i>Edwardsiella ictaluri</i>	Agustina et al., 2024
2	Atlantic salmon (<i>Salmo salar</i>)	Aquaculture Centre, Institute of Marine and Antarctic Studies (IMAS) Launceston, University of Tasmania	<i>Pediococcus acidilactici</i> , <i>P. pentosaceus</i> , and <i>Lactobacillus farraginis</i>	<i>Vibrio alginolyticus</i> , <i>V.</i> <i>anguillarum</i> , <i>V. harveyi</i> , <i>V.</i> <i>proteolyticus</i> , <i>Aeromonas</i> <i>hydrophila</i> and <i>Yersinia</i> <i>ruckeri</i>	Amin et al., 2017
3	<i>Nile tilapia</i>	Fresh water	<i>Enterococcus durans</i> , <i>E.</i> <i>faecium</i> , <i>Leuconostoc sp.</i>	<i>Streptococcus sp.</i>	Lara-Flores and Olvera-Novoa, 2013
4	Tunisian freshwater fishes	Freshwater fishes	<i>Enterococcus faecium</i> and <i>Leuconostoc</i> <i>mesenteroides</i>	<i>Listeria innocua</i> , <i>L.</i> <i>monocytogenes</i> , <i>Candida</i> <i>albicans</i> , and <i>Aeromonas</i> <i>hydrophila</i>	El-Jeni et al., 2016
5	<i>Labeo rohita</i>	Fresh water	<i>Lactobacillus plantarum</i> , <i>Pseudomonas</i> <i>aeruginosa</i> , and <i>Bacillus</i> <i>subtilis</i>	<i>Aeromonas sp.</i>	Giri et al., 2012
6	Hari fish (<i>Anguilla sp.</i>), cat fish (<i>Clarias orientalis</i>), Rohu fish (<i>Labeo rohita</i>), Gende fish (<i>Punitus carnaticus</i>) and Jillabe fish (<i>Oreochromis spp.</i>)	Fresh water	<i>Aeromonas salmonicida</i> and <i>Vibrio</i> <i>parahaemolyticus</i>	<i>Vibrio</i> and <i>Aeromonas</i>	Kuhn et al., 2010

7	Hari fish (<i>Anguilla</i> sp.), cat fish (<i>Clarias orientalis</i>), Rohu fish (<i>Labeo rohita</i>), Gende fish (<i>Punitus carnaticus</i>) and Jillabe fish (<i>Oreochromis</i> spp.)	Freshwater	<i>Lactobacillus</i> sp.	<i>Aeromonas salmonicida</i> and <i>Vibrio parahaemolyticus</i>	Dhanasekaran et al., 2010
8	<i>Labeo calbasu</i>	Freshwater	<i>Bacillus subtilis</i> , <i>B. cereus</i> and <i>B. amyloliquefaciens</i>	<i>Aeromonas veronii</i> , <i>A. hydrophila</i> , <i>A. tandoii</i> , <i>A. junii</i> , and <i>Pseudomonas stutzeri</i>	Kavitha et al., 2018
9	<i>Labeo rohita</i> (Ham.)	Fresh water	<i>Lactobacillus plantarum</i>	<i>Aeromonas hydrophila</i>	Kumar et al., 2019
10	<i>Labeo rohita</i> , <i>Catla catla</i> , <i>Cyprinus carpio</i> , <i>Cirrhinus mirigala</i>	Fresh water	<i>Lactobacillus</i> sp.	<i>Aeromonas hydrophila</i> , and <i>A. salmonicida</i>	Muthukumar and Kandeepan, 2015
11	<i>Tor putitora</i> (Ham.)	Fresh water	<i>Chloroflexi</i> , <i>Bacteroidetes</i> , and <i>Actinobacteria</i>	<i>Proteobacteria</i> , <i>Aeromonas</i> , <i>Caulobacter</i> , <i>Klebsiella pneumoniae</i> , and <i>Escherichia coli</i>	Khurana et al., 2020
12	Tilapia and common carp	Fresh water	<i>Lactococcus lactis</i>	<i>Vibrio</i> spp., <i>Salmonella</i> spp., <i>Escherichia coli</i> , and <i>Staphylococcus</i> spp.	Marie et al., 2017
13	<i>Labeo rohita</i> (Ham.)	Fresh water	<i>Lactobacillus plantarum</i>	<i>Aeromonas hydrophila</i>	Kumar et al., 2019
14	Common carp <i>Cyprinus carpio</i> L.	Fresh water	Lactobacilli	<i>Escherichia coli</i> , <i>Aeromonas hydrophila</i> , <i>Salmonella enterica</i> subsp. <i>enterica</i> , <i>Listeria innocua</i> , L. <i>monocytogenes</i>	Al-Noor et al., 2023

Fish gut microbiota at various life stages of fish

Right from the larval stage, microorganisms are colonized and become consequently more complex when it gets associated with the gut (Nayak, 2010). In the larval stage, colonization of the microorganisms on the gut occur from the environment, and first feed. Before hatching, microorganisms in the surrounding environment dominate, and allow colonization. After hatching, the chorion-associated bacteria are taken up by the sterile larvae. These are the first bacteria that colonizes the GI tract (Egerton et al., 2018). They first drink water after hatching to regulate osmoregulation and henceforth the diversity of microbes increases by feeding. The fish gut contains more than 500 distinct species of bacteria having around 10^8 bacterial cells, which are predominated by facultative anaerobes and aerobes. When a fish's diet alters from predation to omnivorous, or from omnivorous to herbivorous, its diversification of the microbes also increases (Liu et al., 2016). The gut colonization can be driven by deterministic (non-neutral assembly) or stochastic (neutral assembly) model. The GI tract of the fish microbial community will be changed by numerous factors that affect the host, which includes the varying environmental situation such as salinity and temperature, growing stage, feeding tactics, and digestive physiology (Uchii et al., 2006). Few gut microflora are observed as temporary while others are permanent (Kim et al., 2007).

Conclusion

The fish gut microbiota represents a rich and largely untapped reservoir of probiotic candidates with immense potential for application in aquaculture and beyond. As demonstrated throughout this chapter, various bacterial genera isolated from fish intestines, including *Lactobacillus*, *Bacillus*, *Pseudomonas*, and *Shewanella*, exhibit promising probiotic attributes such as pathogen inhibition, immunomodulation, nutrient enhancement, and stress tolerance. These microbial communities not only contribute to host health and growth but also play a pivotal role in maintaining environmental balance within aquaculture systems.

Harnessing the probiotic potential of fish gut microflora aligns with the growing emphasis on antibiotic-free aquaculture practices. However, realizing their full potential requires a deeper understanding of host-microbe interactions and microbial stability under farming conditions. Future research integrating omics tools, *in vivo* trials, and biotechnological advancements will be crucial in characterizing, and commercializing fish-derived probiotics. In summary, fish gut microflora offer a promising frontier in the search for next-generation probiotics.

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