Modulation of Fish Gut Microbiota through Prebiotic and Probiotic Interventions

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Abstract

Several beneficial microbes inhabit the gastrointestinal tract of fish. Prebiotics and probiotics are commonly used in fish diets to enhance the growth of these microorganisms. Prebiotics are non-digestible food ingredients such as fiber that work by selectively feeding beneficial gut microbes, encouraging their growth and activity, which in turn enhances the overall microbial community structure. Probiotics are live microorganisms that, when administered, improve fish health by directly introducing beneficial microbes that can compete with pathogens for space and nutrients, or by producing antimicrobial compounds. Both prebiotics and probiotics improve growth, gut microbial diversity, feed efficiency, digestive enzyme function, immune response, disease resistance and overall fish health for sustainable aquaculture production. However, a more advantageous approach known as synbiotics is the combination and administration of prebiotics and probiotics simultaneously. These interventions provide an alternative to antibiotics in overcoming the challenge of emerging antimicrobial resistance in the aquatic environment and also substitute the costly prophylactic measures such as fish vaccines for disease prevention. However, using these interventions in aquafeeds requires further research on long-term effects on fish health and regulatory considerations for enhancing aquaculture productivity.

Keywords: Gut microbiota, Prebiotics, Probiotics, Synbiotics, Immune response

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Introduction

The microbiota is the complex population of microorganisms that live in bodily cavities and surfaces that are exposed to the environment. Additionally, a wide range of microorganisms, referred to as microbiota, colonize fish and vertebrates' epithelial surfaces from birth and establish commensal or mutual relationships with their hosts (Spor et al., 2011). The microbial composition, especially bacterial, may be influenced by the impact of bacteriophages, which can also be found in the fish digestive tract. It has been suggested that bacteriophage lysis is a significant selective pressure to regulate the populations of bacteria in the animal microbiome (Backhed et al., 2005).

It is commonly recognized that fish lack certain of the necessary enzymes to cope with the dietary needs of aquaculture production. The use of probiotics in the diet, however, may offer the opportunity to utilize carbohydrate sources as animal energy sources since the gut microbiota having probiotic potential secretes different degradation and digestive enzymes to break down various nutritional substrates (Sun et al., 2022). Finding strategies to alter the gut microbiota of fish to improve fish health, productivity and sustainable aquaculture has drawn more attention recently (Luna et al., 2022). This chapter will enhance our understanding of current improvements in fish nutrition using prebiotics and probiotics.

Fish Gut and its Modulation through Dietary Manipulations

The following are the primary regions of the fish GIT:

- Hindgut: The region where a buccal and pharyngeal cavity can be distinguished;
- Foregut: The region that starts at the posterior end of the gills and comprises the stomach, pylorus and esophagus;
- Midgut: The anterior intestine, which has various numbers of pyloric caeca that are helpful to increase the surface area and maximizing nutritional absorption;
- Hindgut: The region that includes the distal intestine and the anus (De Marco et al., 2023).

Microbiota in fish gut may aid in host nutrition by offering enzymatic activities that complement those of the host (Ray et al., 2012). A wide variety of microbiota that are enzyme-producing have been found in the GI tract of fish and isolated, suggesting that the gut microbiota of fish may improve the digestive processes. Besides Bacillus, yeast, unidentified anaerobes, Mycobacterium and Micrococcus are also considered potential contributors (Romero et al., 2014). Nonetheless, dietary manipulations, seasonal variations, individual variations, stress, different GI tract regions, daily variations, male versus female, cultured versus wild, starvation, developmental stages, microbial aspects of live feed, triploid versus diploid, hierarchy formation, fast versus slow growing, migration between freshwater and seawater, water quality, ecological and environmental factors and host environment and ecology all influence the gut microbiota (Ringo et al., 2016).

1) Prebiotics in Fish Nutrition

Prebiotics are short-chain length saccharides that are categorized as mono, oligo, or polysaccharides based on their molecular size or degree of polymerization (Gibson et al., 2015). Beneficial bacteria use these substances as food, which promotes their multiplication and enhances their advantageous effects on the host fish. Prebiotics help fish have a more resilient and stable gut microbiota by encouraging the growth of beneficial bacteria. The overall health of fish and resistance to disease may then improve as a result (Assan et al., 2022). Certain saccharides trigger an immune response by interacting with innate immune cells' pattern recognition receptors, such as dectin-1 receptors (macrophages) or β-glucan receptors (Meena et al., 2013). The immune system may get activated as a result of additional interactions, such as signal transduction (Song et al., 2014).

Case Studies and Experimental Findings Related to Prebiotics

i. Plant-Based Materials

Fish can benefit from prebiotics that are composed of plant-based materials like cellulose and hemicellulose, as these ingredients are abundant in dietary fiber. Fiber enhances the diversity of microorganisms in the gut by promoting the growth of bacteria that degrade fiber (Di Gioia & Biavati, 2018). These substances inhibit the upper gastrointestinal tract's digestion and enter the lower intestine, where they specifically promote the growth of beneficial bacteria (Amenyogbe et al., 2024).

ii. Inulin

The addition of the dietary prebiotic inulin to Nile Tilapia (*Oreochromis niloticus*) alleviated the dysbiosis induced by stress from salinity. In this instance, even in the presence of hypersaline stress, inulin enabled to shift of the GIT microbiota to a regular pattern (Zhou et al., 2020).

iii. Arabinoxylan-Oligosaccharides and Polysaccharides

The gut microbiota of the Siberian Sturgeon (*Acipenser baerii*) is significantly modulated by using the arabinoxylan-oligosaccharides as prebiotics, promoting the presence of various families of bacteria, such as Lactobacillaceae (Geraylou et al., 2012). Prebiotic polysaccharides may be found in yeast, fungi, bacteria and plants. In this instance, polysaccharides work as prebiotics and are used as dietary components to improve growth and health (Mohan et al., 2019).

iv. Chitin

Chitin, the key component of arthropod exoskeletons, is believed to be the second most prevalent biomass in the world, after cellulose (Rinaudo, 2006). A study was carried out to assess the impact on the composition of fish gut microbiota and growth as a result of replacing fishmeal in Rainbow Trout (*Oncorhynchus mykiss*) feed with insect meal (*Hermetia illucens*). Fish gut microbiota was positively modulated by insect meal, which increases its diversity and richness particularly an increased number of beneficial bacteria that produce butyrate and lactic acid, ultimately improving the host's overall health. Furthermore, it is also considered that fermentable chitin is primarily responsible for the prebiotic action of insect meal (Terova et al., 2019).

Since the majority of fish do not break down chitin, it can be regarded as an insoluble fiber having possible prebiotic characteristics that could support the maintenance of a healthy and balanced microbiota in the gut. In turn, the gut microbiota contributes significantly to the metabolism of its host by aiding in the breakdown of otherwise indigestible components of feed, which results in short-chain fatty acids (SCFAs) synthesis, that serve as the primary source of energy for epithelial cells of the intestine (Ghanbari et al., 2015).

2) Probiotics in Fish Nutrition

Probiotics are “live microorganisms that when administered in adequate amounts confer a health benefit on the host” (Schepper et al., 2017) (Figure 1). Probiotics are characterized as dead (whole or in part) or live microorganisms that have positive effects on farmed fish by enhancing intestinal balance, which improves feed consumption and growth as well as stress and disease resistance (Truong Giang Huynh et al., 2017). In addition, its application in aquaculture as a sustainable substitute for chemicals and medications has drawn a lot of interest recently (Jahangiri & Esteban, 2018).

Mechanisms of Probiotic Action in Fish Gut Modulation

It has been documented that probiotic populations have established themselves in the stomach, intestine and pyloric caeca. These probiotic populations can affect the levels and composition of the native microbiota. Fish maturation does not seem to limit the GI microbiota's sensitivity to probiotic modulation, as multiple studies have shown how probiotics affect fish gut microbiota at various life stages (larval, fry, fingerlings, juvenile and adult stages) (Merrifield & Carnevali, 2014).

Aquatic animals' digestive tracts, especially the gastrointestinal mucosal epithelium, are colonized by probiotics. By establishing a physical barrier in the intestinal mucosa of the host, probiotics use competitive exclusion to inhibit pathogenic bacteria from growing on the surface of the gut. Probiotics can limit the amount of nutrients that harmful bacteria can utilize, which limits their ability to survive in the host, in addition to competing for space. Disrupting the pathogens' quorum sensing system has also been suggested as a novel aquaculture anti-infective strategy

(Figure 2). Lastly, through the production of substances with antibacterial properties and organic acids that reduce the pH of the stomach and stop the growth of pathogen bacteria, the pathogen can be excluded (Hoseinifar et al., 2024).

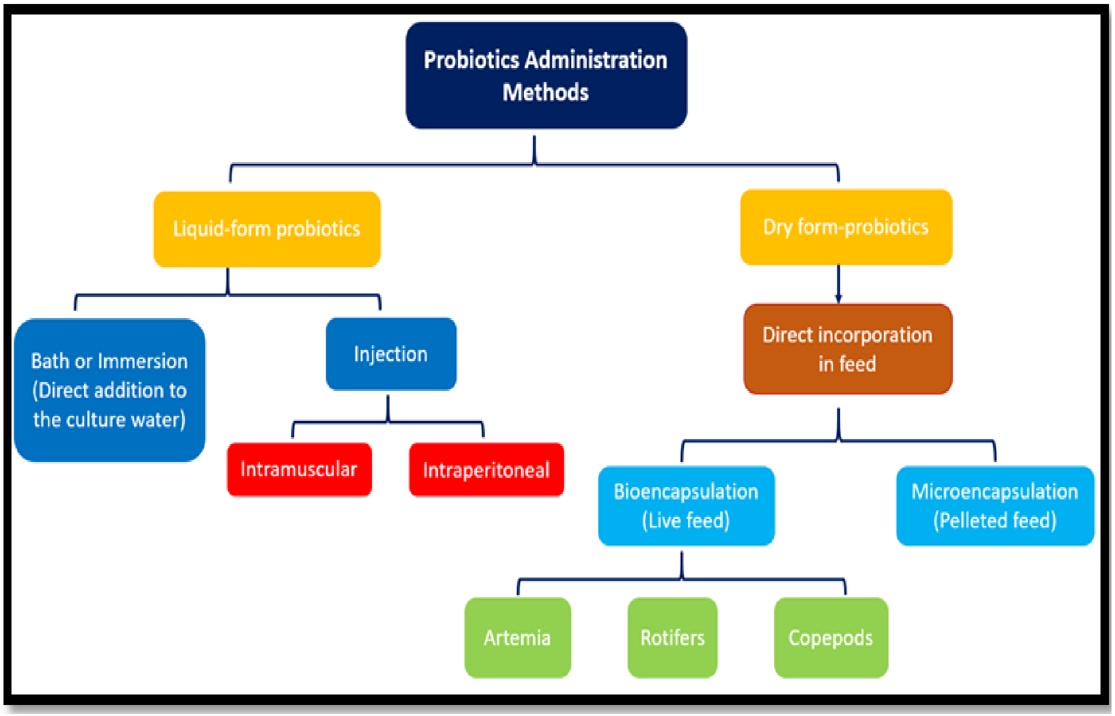

Fig. 1: Flow Chart Representing the Commonly Used Methods to Administer Probiotics in Fish.

~~1.~~ **Case Studies and Experimental Findings Related to Probiotics**

i. ***Bacillus spp.***

Two of the most extensively researched probiotic species in fish are *Bacillus licheniformis* and *Bacillus subtilis*. Nevertheless, investigations have also been conducted on *Bacillus pumilus*, *Bacillus cereus*, *Bacillus toyoi*, *Bacillus circulans*, *Bacillus amyloliquefaciens*, *Bacillus clausii* and unidentified Bacillus species. However, only a small number of Bacillus probiotic uses have included thorough evaluations of the effects on GI microecology. Applications of probiotic Bacillus can change the composition of the indigenous bacterial population, increase fish GI Bacillus levels and modify total or indigenous bacterial levels (Merrifield & Carnevali, 2014).

ii. **Lactic Acid Bacteria (lab)**

This group of bacteria often produces bacteriocins and other chemicals that may prevent disease-causing bacteria from colonizing the gastrointestinal tract (Ringo, 2008). *Lactococcus*, *Carnobacterium*, *Lactobacillus*, *Streptococcus/Enterococcus*, *Leuconostoc* and *Pediococcus* genera are currently the most often used LAB probiotics for applications to fish; however, there is also some information available on *Vagococcus fluvialis* (Roman et al., 2012). In one study, juvenile Turbot (*Scophthalmus maximus*) were given a bath with the *Leuconostoc mesenteroides* HY2 strain that was screened from wide-caught fish. A total of 42 phyla were found, with the dominant groups being *Actinobacteria*, *Firmicutes*, *Bacteroidetes* and *Proteobacteria* (Guo et al., 2020).

iii. **Yeast**

A study examined how the health performance of Rainbow Trout (*Oncorhynchus mykiss*) was affected by Aqualase®, a commercial probiotic consisting of *Saccharomyces elipsoedas* and *Saccharomyces cerevisiae*. The findings showed that Aqualase® is a potential yeast-based probiotic that can alter the growth, immunity and intestinal microbiota of Rainbow Trout. After four weeks of feeding, the average total viable aerobic bacterial count (TVAC) in the intestine of the fish group that received 2% in-feed probiotics increased significantly by around 10% compared to the control. Groups with lower inclusion levels of probiotics did not exhibit this change. There were no notable variations in the LAB population across all probiotic-fed groups in the intestine throughout this period, compared to the TVAC profile at week 4 (Adel et al., 2017).

iv. **Other Probiotics**

Other bacterial genera, such as *Pseudomonas*, *Psychrobacter*, *Shewanella* and *Phaeobacter*, have also been studied for potential use as probiotics in finfish. Furthermore, some early research studied members of the *Vibrio* and *Aeromonas* genera as potential probiotics; using these strains may not be an effective strategy and even if efficacy is high, their regulatory authorization and safety are the concern (Figure 3) (Merrifield & Carnevali, 2014). The gut and gonads of the Nile Tilapia (*Oreochromis niloticus*), were used to isolate *Pseudomonas* species and *Micrococcus luteus*. *Aeromonas hydrophila* was inhibited by *Pseudomonas* species and *Micrococcus luteus*, with inhibition zones measuring 9cm and 4cm in diameter, respectively. The artificial basal diet was supplemented with both microorganisms. Eleven isolates of *Pseudomonas* sp. (Gram-negative bacilli) were isolated from the intestine and stomach of fish, while 5 isolates of *M. luteus* (Gram-positive cocci) were obtained from the gonads and intestine (Abd El-Rhman et al., 2009).

When administered encapsulated, a study examined how a probiotic affected the gut microbiota and immunological state of Gilthead Seabream (*Sparus aurata* L.) specimens. Before being encapsulated in calcium alginate beads, the commercial feed was supplemented with *Shewanella putrefaciens* Pdp11 (SpPdp11) at a concentration of 10^8cfug^{-1}. The administration of alginate-encapsulated SpPdp11 resulted in immunostimulant effects on humoral parameters (serum peroxidase activity and IgM level) and also significant alterations in the Denaturing Gradient Gel Electrophoresis (DGGE) patterns corresponding to the intestinal microbiota. Predominant bands associated with lactic acid bacteria, including strains of *Lactobacillus* and *Lactococcus*, in their DGGE patterns were sequenced from fish having a probiotic diet while fish with the control diet did not show these bands (Cordero et al., 2015).

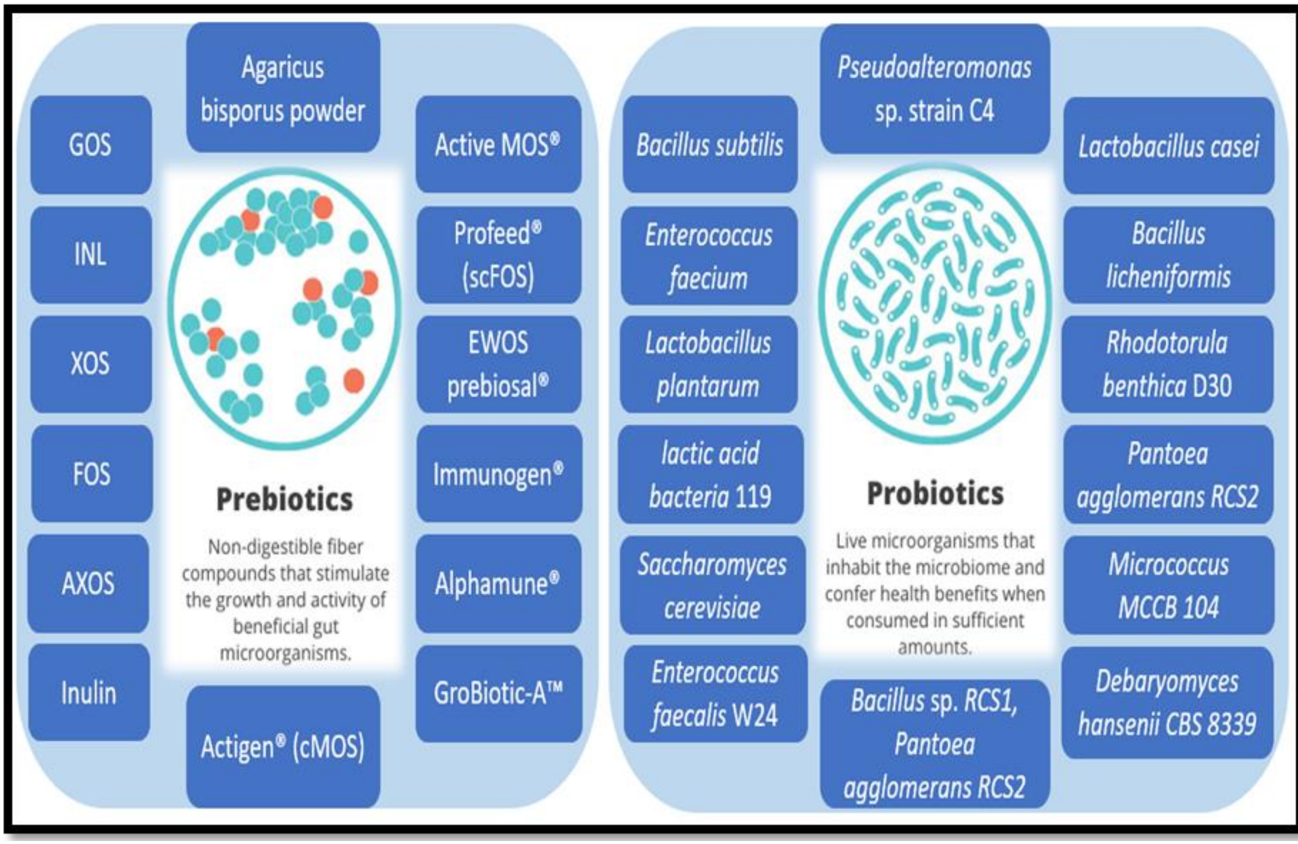

Fig. 2: Common Prebiotics and Probiotic Strains Used in Aquaculture.

3) Synbiotics: Combined Prebiotic and Probiotic Approaches

Synbiotics are the combination and administration of prebiotics and probiotics in the same mixture (Kolida & Gibson, 2011). Aquaculture has made successful use of this combination. Providing the probiotic bacterium with enough substrates to support its gut colonization and survival while creating a synergistic interaction is one of the principles of this approach (Cerezuela et al., 2011) (Table 1). For instance, the fish probiotic *Lactococcus lactis* spp. lactis ST G45 works best when combined with arabinoxylan-oligosaccharides prebiotic because the polysaccharide preserves and colonizes the bacteria, modulating the innate and humoral immunological responses of fish (Geraylou et al., 2013).

Research indicates that the symbiotic approach is more likely to yield favorable outcomes than the separate use of probiotics and prebiotics. Nonetheless, specific and adequate combinations are needed. Since other species can also metabolize prebiotics, using probiotic species that have already adapted to particular prebiotics may be advantageous for the effectiveness of the therapeutic approach, regardless of whether the purpose of the prebiotics is to affect the probiotic species (Vargas-Albores et al., 2021).

Advantages of Synbiotics Over Standalone Prebiotics or Probiotics

By establishing an appropriate nutritional environment with prebiotics, the use of synbiotics in fish farming is aimed at maximizing the activity and colonization of probiotics in the gut. Probiotics and prebiotics combination can have many benefits, including prolonged activity of beneficial microorganisms in the fish GIT, increased probiotic adherence to the gut lining and higher probiotic survival rates during transportation and storage (Amenyogbe et al., 2024). Phytogenics have been proven to function as sedatives, immunostimulants, antimicrobials, anti-inflammatory agents and antioxidants in farmed fish. In addition to stimulating appetite and growth, they may also have an impact on the secretion of bile and several associated digestive enzymes (Caipang et al., 2021).

Table 1: Impacts of Prebiotic and Probiotic Interventions on Fish Health.

Sr. No.	Fish Parameters	Prebiotics	Probiotics
1.	Effects on gut microbial diversity and nutrient absorption	Prebiotics increase intestinal villi height and improve intestinal mucosa integrity, villi density and caliciform cell count. This increases the surface area available for bacterial adhesion and nutrient absorption, which in turn affects the composition of the gut microbiota (Ringo et al., 2010).	Applications of probiotics may produce populations that may reside in the digestive tract, intestinal mucus and epithelium (Merrifield & Carnevali, 2014). Probiotic bacteria improve nutrient absorption and facilitate more effective food digestion (Amenyogbe et al., 2024).
2.	Modulation of immune responses	Prebiotics can directly interact with host immune components to activate the immune system, or they may promote the growth or reinforcement of commensal microbiota (Song et al., 2014).	The establishment of *B. subtilis* colonies in Rainbow Trout's intestinal tract triggered cellular and humoral immune responses (e.g., phagocytic killing, serum and gut lysozyme, peroxidase and respiratory burst) (Newaj-Fyzul et al., 2007).
3.	Enhancing the antioxidant defense system	By encouraging the growth of advantageous gut bacteria, which can result in the formation of bioactive molecules with antioxidant characteristics, prebiotics indirectly enhance antioxidant defenses. They may reduce oxidative stress as they usually have anti-inflammatory properties (Amenyogbe et al., 2024).	Fish's antioxidant defense system can benefit from probiotics. These microorganisms can increase the activity of antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) (Mounir et al., 2022).
4.	Improvement of digestive enzymes	Prebiotic dietary supplements can increase digestive tract lipase and protease activity (Amenyogbe et al., 2024).	Probiotic strains may affect fish amylase activity, produce protease and enhance lactase activity (Amenyogbe et al., 2024).
5.	Enhancement of growth performance	Prebiotics added to fish diets can enhance overall performance, feed conversion efficiency and growth rates (Amenyogbe et al., 2024).	Probiotic bacteria boost the activity of digestive enzymes, which promotes Humpback Grouper (*Altivelis Cromileptes*) growth (Amenyogbe et al., 2024).
6.	Reduction in disease incidence and dysbiosis	Prebiotics have been used to reset the gut microbiota or attenuate dysbiotic influences in fish (Zhou et al., 2020).	Use of *Carnobacterium* sp. decreased the infection by *Yersinia ruckeri*, *Aeromonas salmonicida* and *Vibrio ordalii* in Rainbow Trout (*O. mykiss*) and Atlantic salmon (*Salmon salar*). In Tilapia, a combination of *Lactobacillus acidophilus* and *Bacillus subtilis* improved defense against harmful pathogens (Hoseinifar et al., 2024).

4) Environmental and Economic Benefits of Prebiotic and Probiotic Interventions

a) Role in Reducing Antibiotic Use and Cost in Aquaculture

Antimicrobials are frequently used in aquaculture for promoting growth, preventative purposes, or treatment therapy. Subtherapeutic antibiotic doses, however, have accelerated antimicrobial resistance and a negative impact on humans, aquatic ecosystems and fish (Cabello et al., 2016). Due to the development of antibiotic-resistant bacteria, disturbance of microbial ecosystems in aquaculture environments, antibiotic residues in aquatic food and possible immune system suppression in aquatic animals, the traditional use of antibiotics in aquaculture has been questioned during the past three decades (Amenyogbe et al., 2024). By mutation or horizontal transfer of resistant genes by conjugation, transduction and transformation, bacteria may develop antibiotic resistance. Among these, conjugation appears to be the most frequent (Hoseinifar et al., 2024). Prebiotics have gained a lot of attention in the aquaculture sector as a substitute for antibiotics (Amenyogbe et al., 2024).

Vaccination is an effective approach for fish disease prevention. Nonetheless, aside from their high cost, vaccinations only offer immunity against specific pathogens as they act specifically. Therefore, the limited information on fish immunology, the limited economic feasibility, the stress of handling during injection and the need for approval are the factors that limit the development of fish vaccines. However, fish need a larger dosage of antigens than terrestrial animals (Hoseinifar et al., 2024). Therefore, it is necessary to find highly effective and environmentally friendly alternative disease prevention methods. Thus, throughout the past ten years, there has been an increase in research interest in the use of bioactive feeds, such as probiotics, prebiotics and sometimes a combination of them called synbiotics, to improve the welfare status and health of fish (Cabello et al., 2016).

b) Sustainability Implications for Aquaculture Practices

It has previously been suggested that using synbiotics to treat dysbiosis in fish could be a potential strategy to transition to more sustainable aquaculture (Infante-Villamil et al., 2021). Prebiotics that are frequently used in aquaculture to supplement probiotics include mannan oligosaccharide, chitosan, fructooligosaccharide, polyhydroxy butyrate acid and others (Cerezuela et al., 2011).

5) Challenges and Limitations in Prebiotic and Probiotic Applications

a. Lack of Standardized Protocols for Evaluating Efficacy

Diet, fish species and environmental parameters in aquaculture systems, including pH, water quality, pH, dosage and temperature all affect how effective prebiotics, probiotics and synbiotics are in aquaculture. It can be difficult to determine the optimal duration and dosage of probiotic supplementation. While too little amount can be ineffective, too much can be wasteful resulting in unexpected consequences

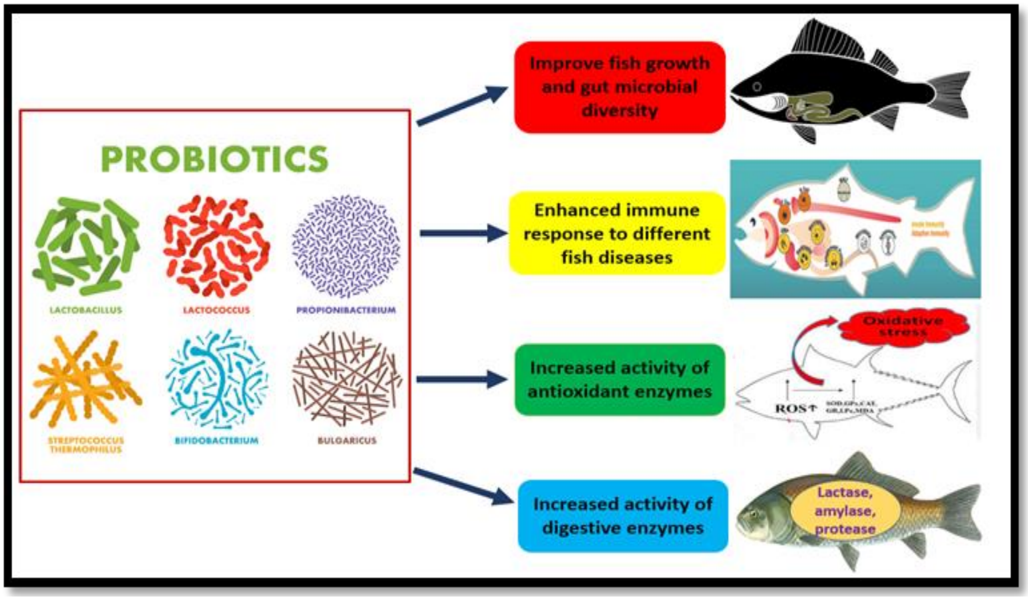

Fig. 3: Beneficial Effects of Probiotics on Fish Health. (Cavalcante et al., 2020).

b. Variation in microbiota Response Based on Host

It can be challenging to find probiotic strains that work well for a variety of species as the dietary needs and gut microbiomes of various fish species vary. For optimal performance, probiotic formulations must be specific for certain fish species (Amenyogbe, 2023). Probiotics need to remain viable during the process of manufacturing, fish administration and storage. Unfavorable environmental circumstances could lessen their effectiveness (Hoseinifar et al., 2018).

c. Ecological Dynamics Disturbance and Regulatory Concerns

The administration of high probiotic dosages in aquaculture environment has raised some concerns. The main concerns are the potential alteration of the natural populations of microorganisms in aquaculture systems as well as the potential disturbance of the ecological dynamics and existing microorganism balance in the aquaculture environment. Overuse of probiotics in aquaculture, however, can lead to a surplus of biomass or the growth of specific microbial species. The aquaculture waste management system and nutrient utilization processes may suffer as a result of this disruption of the microbial community's equilibrium (Hasan & Banerjee, 2020). In consideration of these issues, it is critical to select appropriate probiotic strains that are native to the specific aquaculture environment, determine the appropriate amount of inclusion and perform routine ecosystem monitoring to evaluate any possible effects of probiotics (Hoseinifar et al., 2024).

6) Future Perspectives

Improved encapsulation methods and storage conditions are necessary to increase probiotic viability (Hoseinifar et al., 2018). To comprehend the long-term effects of probiotics on fish growth and health, more research is needed (Amenyogbe, 2023). For efficient probiotic administration, research into the application of nanotechnology and other cutting-edge delivery techniques is necessary. Dose-response studies must be carried out to ascertain the ideal probiotic concentration for different fish species and life stages. Investigating the possibility of generating time-release formulations for sustained probiotic delivery is essential. A systematic examination of the interactions between probiotics and other feed additives is required in order to enhance feed formulations. To improve the efficacy of probiotics, researchers can concentrate on identifying feed additives that complement probiotics effectiveness (Amenyogbe et al., 2024).

Research into creating resilient probiotic strains that can withstand a range of environmental circumstances is required. The need for standard regulations governing the use of probiotics in aquaculture is one challenge (Amenyogbe et al., 2020). Before probiotics may be utilized extensively, they must receive regulatory approval. International standards and recommendations for probiotics in aquaculture must be defined in order to ensure the efficacy and safety of the product. Understanding the host-microbiome interactions is crucial for effective species-specific probiotic approaches (Kuebutornye et al., 2019). Probiotics' ecological effects on the aquatic environment must be assessed, taking into consideration non-target species and microbial communities as well. Investigating the potential to develop environmentally friendly probiotics is one strategy. Long-term research is needed to determine how probiotic supplementation affects fish health, resistance to diseases and overall performance (Amenyogbe et al., 2024).

Conclusion

This chapter focused on sustainable aquaculture approaches in order to enhance fish growth, gut health, nutritional absorption, antioxidant defense system and disease resistance. Prebiotics, probiotics and synbiotics can be added to fish diets to alleviate dysbiosis, which is defined as an imbalance in the usual composition of the gut microbiota or a substitution of beneficial microorganisms by pathogenic ones. Since they lessen reliance on antibiotics and other chemical interventions, these methods are sustainable aquaculture practices. These methods do, however, present certain challenges, such as the absence of standard protocols and the disturbance of the aquatic environment's ecological dynamics and environmental conditions. To make these interventions more environmentally friendly, research is needed to create resilient probiotic strains, standardize probiotic usage restrictions and better understanding of host-microbiome interactions and ecological effects.

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