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A comprehensive review of gut-associated bacteria in aquaculture and their roles in fish health

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Abstract

An industry that is growing quickly and is essential to the world's food security is aquaculture. However, the hunt for sustainable alternatives has been fueled by issues including antibiotic misuse, environmental concerns, and disease outbreaks. A viable remedy is probiotics, which are good bacteria that support fish health.

The gut microbiome is critical in maintaining fish health and productivity in aquaculture. This review provides a comprehensive analysis of gut-associated bacteria in various aquaculture species, focusing on their diversity, functional roles, and interactions with the host. Beneficial bacteria contribute to nutrient metabolism, immune modulation, and disease resistance, while pathogenic bacteria can disrupt gut homeostasis, leading to infections and reduced growth performance. Diet, water quality, antibiotics, and probiotics significantly influence gut microbial composition. Understanding these microbial communities is essential for developing sustainable aquaculture practices, including probiotics and prebiotics for disease prevention and growth enhancement. This review highlights recent advancements in microbial research, emerging technologies for microbiome analysis, and potential strategies for improving fish health through targeted microbiome management.

Keywords: Aquaculture, bacterial communities, fish gut microbiota, fish health, nanoencapsulation, probiotics

1. Introduction

Enhancing food security and boosting fish farmers' incomes are two chief goals of aquaculture, particularly in underdeveloped nations. Furthermore, there is an urgent need to improve aquaculture due to the fast-growing human population, the growing need for affordable protein sources, and the loss of fish captures from inland natural lakes. The food industry with the fastest rate of growth is aquaculture, which makes a substantial contribution to global food security. However, problems like antibiotic resistance, environmental deterioration, and disease outbreaks are brought on by intensive agricultural methods (El-Saadony *et al.*, 2021) ^[11]. A significant source of protein for human consumption, aquaculture has emerged as one of the global food production industries with the quickest rate of growth (FAO, 2022) ^[13]. However, several issues that have a substantial influence on fish health and production, such as disease outbreaks, poor water quality, and inadequate nutrition, pose a threat to aquaculture's sustainability (Hai, 2015) ^[19]. Antibiotic overuse damages gut microbiota and increases antibiotic resistance, which is harmful to human and aquatic health. Among them, the gut microbiota is crucial to host health, impacting immunological responses, digestion, and general physiological processes (Ringø *et al.*, 2016) ^[35]. To optimize aquaculture procedures and reduce disease risks, essential to understand the makeup and role of gut-associated bacteria in fish.

According to Eckerton, the gut microbiota of fish is a dynamic and varied community that is impacted by several variables, such as host species, environmental circumstances, food, and microbial interactions. "Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" is how the FAO/WHO (2001), defines probiotics, which present a prospective substitute. They improve digestion, boost immunity, stop illness, and preserve the balance of gut microbes. *Lactobacillus*, *Bacillus*, and *Pseudomonas* species are specimens of beneficial gut bacteria that help with better digestion, higher resistance to infections, and improved nutritional absorption (Nayak, 2010) ^[30, 31]. On the other hand,

pathogenic and opportunistic bacteria such as *Aeromonas*, *Pseudomonas*, and *Vibrio* species can upset the balance of the gut, resulting in infections and poor development (Merrifield & Rodiles, 2015) [28]. For fish health and aquaculture sustainability, it is therefore essential to maintain a healthy gut microbiome.

Probiotics, prebiotics, and synbiotics are microbiome-based therapies that have gained popularity in the last ten years to boost fish immunity and beneficial bacterial populations (Gómez & Balcázar, 2008) [17]. Researchers may now create focused strategies for aquaculture microbiome management because of developments in high-throughput sequencing technology, which have given them new insights into the composition and function of fish gut microbiomes (Tarnecki *et al.*, 2017) [39]. Nonetheless, there are still unanswered questions about the long-term consequences of microbiome changes, environmental factors, and host-microbiome interactions. Probiotic bacterial applications have generated a lot of attention and provide an alternative approach to aquaculture disease management. Furthermore, because beneficial probiotic bacteria are thought to be well-adapted to the targeted host gut environment and play a crucial role in boosting resistance against pathogenic bacteria, attention has been paid to host-associated microorganisms for the study of these bacteria (Mukherjee *et al.* 2016; Van Doan *et al.* 2018) [29, 41].

A thorough examination of gut-associated bacteria in aquaculture species is the goal of this review, emphasizing the species diversity, functional roles, and relationships to fish health. We also examine how diet, water quality, antibiotic use, and probiotics affect the composition of gut microbes, highlighting current developments and potential avenues for future microbiome research in support of sustainable aquaculture methods.

2. Isolation and Characterization of Gut-Derived Bacteria

There is a strong correlation between the bacterial community of the gut and the health, and immune status of fish. The gastric tracts of fish contain intricate microbial communities essential for feeding and host immunity. Assessing the microbiota in fish digestive tracts aids in locating potentially beneficial probiotics and dangerous bacteria, improving our knowledge of the microbial makeup of the fish gut and health care (Asaduzzaman *et al.*, 2018) [3]. Species, diet, and environment influence the diverse microbial ecosystem. Advanced techniques like 16S rRNA sequencing and metagenomics have expanded our understanding of gut microbiota composition. Probiotics isolation involves collecting gut samples, cultivating bacteria on selective media, and characterizing strains through morphological, biochemical, and molecular identification. *In vitro*, screening tests assess probiotic properties, including acid and bile tolerance, intestinal adhesion, and antimicrobial activity. Safety evaluations ensure the absence of virulence factors and antibiotic resistance. Three Indian major carp (IMC) species rohu, catla, and mrigal obtained from a polyculture pond under the same feeding regime when subjected to a study that examined the microbial communities of their gastrointestinal tracts (GITs) revealed unique insights. Phylum and genera showed wildly disparate relative abundances and diversities, and several microbial communities were discovered (Foyosal *et al.*, 2020) [14]. *Aeromonas*, *Enterobacter*, and *Serratia* are common bacteria found in the gut of Mrigal, Catla, and Rohu, as well

as *Cetobacterium*, *Shewanella*, and *Plesiomonas* in overwhelming abundance (Foyosal *et al.*, 2020) [14].

Thirty-seven bacterial isolates were isolated from the digestive tracts of Malaysian mahseer (*Tor tambroides*). All three nutritional enzyme activities (lipolytic, proteolytic, and cellulolytic activity) were present in nine out of 37 isolates (Hossain *et al.*, 2022) [20]. Wild stinging catfish (*Heteropneustes fossilis*), an important aquaculture species when studied for isolation and identification of their probiotic bacteria, *Acinetobacter lwoffii*, *Macrococcus caseolyticus*, *Lysinibacillus sphaericus*, *Bacillus cereus*, *Glutamicibacter mysorens*, *Kocuria subflava*, *Staphylococcus arlettae*, *Bacillus subtilis*, *Staphylococcus succinus*, and *Bacillus velezensis* were among the isolates found in this investigation. They showed significant growth at all tested pH levels (pH 2, 3, and 4) and bile salt concentrations (0.3%, 0.5%, and 1.0%), with *B. subtilis*, *S. succinus*, *B. velezensis*, *M. caseolyticus*, *G. mysorens*, and *B. cereus* showing the most promise as probiotic bacteria. Furthermore, *S. arlettae* showed encouraging development, except for pH 2. Although they could withstand bile salt concentrations, *L. sphaericus* and *K. subflava* showed restricted growth at low pH. *A. lwoffii* grew very little at lower bile salt concentrations but not at all at pH testing (Jilani *et al.*, 2024) [23].

3. Selection Criteria and Mechanisms of Action

Researchers can learn more about the variety and function of bacteria by investigating the gut microbiota. Investigating fish gut microbiota raises the possibility of discovering novel strains. The effectiveness of probiotics depends on host specificity, colonization ability, immunomodulatory effects, and pathogen inhibition. Key mechanisms include competitive exclusion of pathogens, production of antimicrobial compounds, gut barrier enhancement, and immune modulation. Probiotics also boost immune responses by stimulating immunoglobulin production and activating immune cells (Sufiara and Rahul, 2023) [38]. An investigation on marine fish gut-associated actinobacteria with antibacterial and anti-quorum sensing properties was conducted. Kuster's agar medium and starch casein agar were used to isolate 25 actinobacterial strains from fish gut samples. The antimicrobial activity of approximately 12 morphologically distinct strains that were recovered from the Indian mackerel (*Rastrelliger kanagurta*), squid (*Decapodiformes*), red snapper (*Lutjanus campechanus*), bluefin trevally (*Caranx melampygus*), and *Staphylococcus aureus* (MTCC96), *Escherichia coli* (MTCC739), *Candida albicans*, and quorum sensing inhibition (QSI) against *Chromobacterium violaceum* were tested. The *Decapodiformes* (squid) actinobacterial strain SQA4 exhibited both QSI and antibacterial activity (Vadivel *et al.*, 2021) [40].

An investigation of the digestive system of an adult *Channa striatus* gathered from Punjab's Doaba district was conducted. 34 isolates were separated and tested for antibiotic activity against *Pseudomonas aeruginosa* and *Aeromonas hydrophila*, two fish infections. These isolates included four Gram-positive ones. Only PMS1, one of these four isolates, exhibited stout antibacterial activity against *Aeromonas hydrophila*. The isolate demonstrated a significant capacity to adhere and a high resistance to low pH. All tested antibiotics were shown to be effective against the isolate. The strain was identified as *Lactococcus garvieae* based on biochemical, 16srRNA, and *in vitro* evaluation (Sufiara and Rahul, 2023). [38]

In addition to aiding in the host's growth and development, gut microbial characterization also reduces the vulnerability to illness. An important fish species in the Himalayan River system's Chenab and Ravi rivers is *Cirrhinus mrigala*. Fish gut bacterial diversity was revealed by a study on the core gut microbiota of *C. mrigala* using Illumina Miseq Sequencing. The genera *Acinetobacter*, *Lactobacillus*, *Methanosaeta*, *Prevotella*, and *Pseudomonas* make up the core microbiome found in fish gut samples. Additionally, the results indicate that the Ravi River has a higher relative abundance of *Lactobacillus* and *Bacteroides* than Chenab. In fish, three genera—*Bacteroides*, *Oceanobacillus*, and *Lactococcus* display considerable variance (Sharma *et al.*, 2024) [36].

4. Probiotic Feed Formulation and Delivery

Probiotic foods and nutraceuticals have grown in popularity due to the rise of bacteria resistant to antibiotics and natural methods inhibiting the growth of infections. Probiotic bacteria have several positive health impacts in addition to competing with and suppressing "unhealthy fermentation" in the gut. A nutraceutical product for enhancing the natural gut flora is a probiotic delivery method. As per Melo *et al.*, (2020) [26], the most common method of administering probiotic mixtures is to incorporate them into the feed (92.8%), followed by direct incorporation into water (4.8%) and through live food (1.6%), showed in Figure.1. The efficacy of these delivery methods in improving health varies widely. Probiotic delivery techniques fall into two categories: non-traditional, commonly commercial food-based products, and conventional, pharmaceutical formulations. The degree of health benefits offered by these probiotic formulations differs depending on their viability (the ability to survive formulation processes), effectiveness (the ability to deliver viable, functional bacteria in sufficient quantities), and *in vivo* protection (the ability to protect against the harsh effects of the intestinal bile and gastric environment). For many industrial sectors, the feasibility of probiotics has presented both a technological and commercial issue. Two crucial factors in the health benefits that probiotics provide are viability over the product's shelf life and survival in the GI tract to fill the fish's gut. Successful probiotic incorporation into fish feed requires maintaining viability during processing and storage. Encapsulation enhances stability and ensures targeted delivery. Probiotics are primarily administered *via* feed, but immersion and water treatment are alternatives for certain species (Chomová *et al.*, 2023) [6].

Using locally accessible agricultural waste as a primary feed material in solid-state fermentation promotes the development of probiotics, making it suitable for use as functional feed. A safe way to avoid disease and manage aquaculture is to formulate, distribute, and administer probiotic-based functional feed regularly. Probiotic usage in aquaculture also promotes growth, inhibits infections, and improves immunity, water quality, and nutrient digestion, among other benefits. Apart from probiotics, the use of prebiotics, synbiotics, and cobiotics as dietary supplements in aquaculture improves the feed's functional qualities and encourages environmentally friendly farming (Raghavan *et al.*, 2019) [33]. Probiotic bacteria-loaded nanofibers provide a novel mode of preserving fish fillets against microbial growth (Ceylan *et al.*, 2018) [5]. In food and digestive system models, it has been demonstrated that the binary application of biopolymers increases probiotic survival rates and improves nanoencapsulation effectiveness (Durazzo *et al.*, 2020) [8]. Due to its desirable structural and biological qualities, alginate, a polysaccharide derived from marine weed, is one of the most widely used natural polymers and is thought to be a suitable component in the encapsulation of bacterial strains (Liu *et al.*, 2018) [25]. Additionally, starch is a very accessible and inexpensive biopolymer that has been utilized to create materials with a starch-based nanostructure for encasing food preservatives and nutraceutical ingredients (Atraki and Azizkhani, 2021) [4].

The most suitable method for all fish ages, from larvae to marketable size, is the delivery of probiotics in rearing water. Probiotics used as water and/or feed additives in aquaculture have a wide range of commercial goods worldwide. There are significant differences in their effectiveness, price ranges, and fraud rates among some of these items. However, one crucial element influencing the probiotics' acceptable function in aquaculture is the method of administration (Jahangiri and Esteban, 2018) [22]. While some commercial probiotics are frequently worthless, others are beneficial. Probiotics can be administered orally, through water, or as feed additives, the latter of which is frequently utilized in aquaculture. Applications for probiotics might involve single or several strains, living or dead forms, immunostimulants like synbiotics and synbiotism, or even prebiotics. One effective way to give probiotics to aquatic animals is to encapsulate them in live feed. To get the intended benefits, dosage and timing are important considerations. Probiotics are known to work in several ways, some of which are unclear.

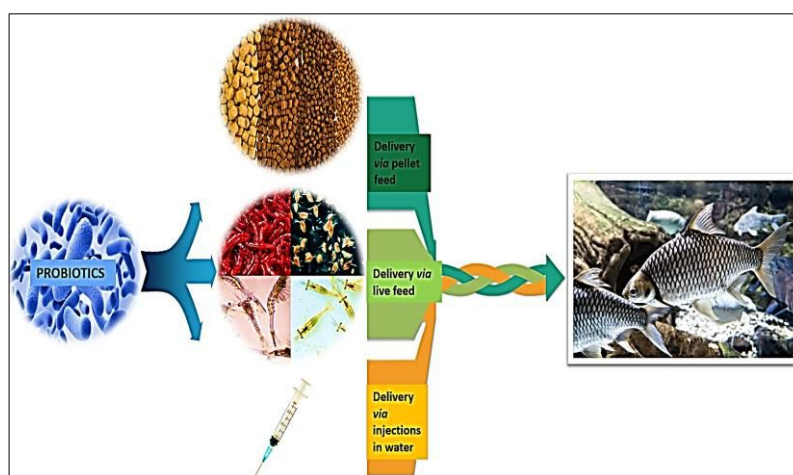


Fig 1: An illustration of the administration of probiotics *via* injections in water, pellets, and live feeding

5. Impacts on Fish Growth and Performance

It has been demonstrated that probiotics positively affect the development, survival, and health of aquatic life. Probiotics, which have been identified as both Gram-positive and Gram-negative bacteria and nonbacterial (bacteriophages, microalgae, and yeasts), can be obtained in aquaculture from intestines, gills, the skin mucus of aquatic animals, habitats, or even culture collections and commercial products. A probiotic's species specificity is largely determined by their screening procedures. A bacterium harmful to one species of fish may be beneficial to another (Subedi and Shrestha, 2020) [37]. Probiotics' impacts on fish growth performance and other positive actions are well-established and in general, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Carnobacterium*, *Shewanella*, *Bacillus*, *Aeromonas*, *Vibrio*, *Enterobacter*, *Pseudomonas*, *Bifidobacteria*, *Clostridium*, and *Saccharomyces* are the primary probiotics utilized for aquatic animals. Probiotics improve fish growth by enhancing feed conversion ratio, increasing weight gain, and improving nutrient absorption. They strengthen gut morphology, enhance digestive enzyme activity, and mitigate stress responses by reducing cortisol levels (Allameh et al., 2017; Elsabagh et al., 2018; Rahman et al., 2021) [1, 12, 34].

Water quality can be improved by using *Bacillus* strains to reduce ammonia toxicity, nitrite toxicity, H⁺ ions, and toxic algal blooms. Greater use of probiotic *Bacillus* in place of dangerous synthetic chemicals will encourage environmentally friendly, low-input, sustainable aquaculture for the growing global population's food and nutritional security (Rahman et al., 2021) [34]. A study was conducted to evaluate the effects of probiotics on the expression of the genes for insulin-like growth factor 1 (IGF-1), growth hormone receptor (GHR), tumor necrosis factor α (TNF- α), growth hormone receptor (GHR), and interleukin 1 beta (IL-1 β) in juvenile Nile tilapia, *Oreochromis niloticus*, as well as on water quality, growth, feed utilization, carcass composition, blood biochemical parameters, and histology. Compared to the control, probiotic-treated groups showed a substantial increase in growth performance, fish biomass, and feed consumption. Probiotic groups showed a notable increase in blood biochemistry and immunological measures (phagocytic activity and lysozyme). Comparing the liver and spleen to control groups, there was a substantial rise in the relative expression of the genes for IGF-1, GHR, TNF- α , and IL-1B. Histological analysis of the gut, liver, spleen, and gills showed that the probiotic-fed animals had better organs than the control groups (El-Kady et al., 2022) [10].

6. Applications of Probiotics in Aquaculture

Because farmed fish and shellfish are constantly in contact with and consume the water, they are significantly impacted by the microorganisms present in the surrounding water. In the aquaculture ecology, commensal bacteria and eukaryotes coexist, and opportunistic diseases can grow to large densities. While certain bacteria are detrimental to a single aquatic animal, they can benefit other species when used as probiotics. For example, *P. aeruginosa* is widely recognized for being a part of the human and animal skin pathogenic microbiota (Andonova and Urumova 2013) [2], however, they function as a beneficial probiotic for western king prawns. Furthermore, the lion's paw scallop, scientifically known as *Nodipecten subnodosus*, grew and survived better when *P. aeruginosa* and *Burkholderia cepacia* were co-

cultured (Granados-Amores et al. 2012) [18]. In Rohu, dietary *P. aeruginosa* supplementation enhanced innate immunity and disease resistance (Giri et al. 2012) [16].

Aquaculture uses probiotics for infection control, water treatment, and feed augmentation. *Lactobacillus* species are among the probiotic genera that are often employed. generates antimicrobials and lactic acid. *Bacillus* species. improves digestion by producing enzymes. *Pseudomonas* species. offers pathogen-repelling biocontrol. *Enterococcus* species. boosts immunity and intestinal health (Subedi and Shrestha, 2020) [37]. Bacteriocins, siderophores, lysozymes, proteases, and hydrogen peroxides are produced by probiotic bacteria, which prevent the growth of dangerous pathogens. *Aeromonas* species, *Bacillus subtilis*, *Bacteridaceae*, *Clostridium* species, *Lactobacillus plantarum*, and *Staphylococcus* all produce amylase, while *B. subtilis*, *L. plantarum*, and *Staphylococcus* species produce protease and cellulase enzymes. Probiotics may work through a variety of mechanisms, such as (i) competitive exclusion via the synthesis of inhibitory compounds, (ii) competition for nutrients, chemicals, or energy, (iii) adhesion site competition, (iv) aiding in digestion, (v) aiding in the production of macro and micronutrients, (vi) boosting the immune system, and (vii) reducing virulence through manipulation of quorum sensing (QS) (El-Saadony et al., 2021) [11]. Microencapsulation of probiotics is crucial to determining how probiotics affect *in vivo* systems.

7. Challenges and Future Research Directions

Probiotics provide many advantages in aquaculture and are crucial for enhancing growth performance, immunity, disease resistance, intestinal epithelial barrier integrity, gut microbiota, and water quality. Almost half of the seafood consumed globally comes from aquaculture, which has made a substantial contribution to the animal protein production sector in practically every country over the past few decades (Jahangiri and Esteban, 2018) [22]. Despite their benefits, probiotic use in aquaculture faces challenges such as strain viability, stability, and regulatory approval. Future research should explore metagenomics for understanding microbial interactions, genetically engineered probiotics to enhance disease resistance, and optimized formulations for large-scale applications. Research should be conducted on how to choose the right probiotic strain or strains for fish in the future to provide the best possible outcome. A key consideration should be to determine their precise mechanism of action when it comes to aquaculture probiotics.

8. Conclusion

Aquaculture, which accounts for almost half of global seafood consumption, has made substantial contributions to animal protein production in practically every country over the past few decades. Numerous disease outbreaks, environmental degradation, particularly eutrophication, fishery failures, and a reduction in biodiversity were all brought on by the global expansion of intensive aquafarms and their toxic emissions of organic and inorganic metabolites. Probiotics are living, healthy bacteria ingested through food or drink to improve the internal microbial balance in the gastrointestinal system. They provide several advantages in aquaculture and are crucial for enhancing growth performance, immunity, disease resistance, intestinal

epithelial barrier integrity, gut microbiota, and water quality. Furthermore, including probiotics in aquaculture diets might reduce the adverse effects of antibiotics. Promoting these fish feed additives will increase fish productivity and protect human health by enhancing their productive performance and feed consumption.

Probiotics have demonstrated significant potential in improving fish health, immunity, and sustainability in aquaculture. Gut-derived bacteria such as *Lactobacillus*, *Bacillus*, and *Pseudomonas* exhibit strong probiotic properties. The development of sustainable fish farming strategies based on probiotics will be further optimized with the help of research in the fields of microbial biology and biotechnology. As feed additives, probiotics help ensure that feed is digested and release nutrients for increased production and quicker growth. The addition of probiotics to fish diets can also enhance immune responses. Including probiotics in fish meals can help prevent many fish illnesses. Future studies should be conducted to choose the probiotic strain or strains required to provide fish with the best possible outcomes. Furthermore, further research should be done to determine the precise mechanisms of action of the probiotics employed in aquaculture.

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