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Immunomodulatory roles of probiotics in aquaculture: mechanisms and applications for fish disease control

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Abstract

The rapid intensification of global aquaculture has increased reliance on antibiotics, contributing to the emergence of antimicrobial resistance (AMR) and environmental degradation. Consequently, there is growing interest in sustainable alternatives such as probiotics, which function as “immunobiotics” to enhance host health and disease resistance. This review synthesizes current knowledge on the multifaceted roles of probiotics in aquaculture, with particular emphasis on the integrated “gut–mucus–immune–redox axis”. Probiotic taxa, notably *Bacillus* and *Lactobacillus* species, promote growth and feed efficiency through the secretion of digestive enzymes and the improvement of nutrient utilization, while also maintaining intestinal integrity via modulation of mucosal architecture, including goblet cell proliferation. At the molecular level, probiotics exert immunomodulatory effects by regulating the expression of immune-related genes, including Toll-like receptors (TLRs), cytokines, and immunoglobulins, thereby enhancing both innate and adaptive immune responses. Additionally, probiotics contribute to disease control through quorum quenching mechanisms that interfere with pathogen communication and virulence. By integrating insights into strain-specific functionality and host–microbe interactions, this review highlights the potential of probiotics to replace conventional chemotherapeutics and supports a transition toward biologically driven, sustainable disease management strategies in aquaculture.

Keywords: Probiotics, immunomodulation, gut microbiota, innate immunity, antimicrobial resistance (AMR)

Introduction

The global population is increasing continuously, leading to challenges such as food shortages and the depletion of natural resources (Boyd *et al.* 2022). Aquaculture has become an important sustainable source of high-quality protein, to contribute to global food security and rapid population growth (Obirikorang *et al.* 2024, Alam *et al.* 2023). According to (FAO 2024), the aquaculture reached a rapid production milestone of 130.8 million tons in 2022, officially becoming the world’s largest food production sector. The bloom in the production has led to several challenges including deteriorating water quality, increased disease outbreaks, overdose of antibiotics. However, the rapid intensification of aquaculture practices has introduced several challenges, including deteriorating water quality, increased disease outbreaks, over dependence on antibiotics, and impaired physiological well-being of fish. These combined stressors elevate mortality rates and reduced growth performance, ultimately resulting in significant production deficits and compromising the economic viability of commercial aquaculture operations (Stentiford *et al.* 2017). For disease management antibiotics were the primary tool in fish farming; however excessive use of antibiotics resulted in antimicrobial resistance (AMR) and the accumulation of chemical residues in aquatic habitats (Elsegeny *et al*. 2025a). Supporting sustainable practices is therefore essential to guaranteeing a steady supply and securing the industry’s long-term survival.

In order to address this problem, biological control methods like probiotics and prebiotics are being adapted by aquaculture industries over antibiotics. The most recent definition of probiotics is “a live microorganism which when administered in adequate amounts confer a health benefit on the host” (Hill *et al.* 2014). Probiotics have been widely applied in both freshwater and marine aquaculture systems to enhance growth, immunity and disease resistance though their effectiveness may vary depending on their environmental conditions as well as host species (Hasan & Banerjee 2020). Probiotics used in aquaculture application include microalgae such as microalgae (*Tetraselmis*), yeast (*Debaryomyces, Phaffia, Saccharomyces*), Gram-positive bacteria (*Bacillus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus*, *Micrococcus, Streprococcus,* and *Weisssella*) and Gram negative bacteria (*Aeromonas, Alteromonas*), administered either through feed or by bath exposure (Balcazar *et al.* 2006). Probiotics fight for nutrients and space and by creating antimicrobial substances that stop the growth of pathogens (El-Saadony *et al.* 2021). They provide protection against several bacterial diseases in aquaculture including edwardsiellosis, enteric red-mouth disease, furunculosis, lactococcosis, streptococcosis, and several other diseases in aquaculture, while enhancing the immune response of the host (Kuebutornye e*t al.* 2019). The application of probiotics can reduce reliance on antibiotics in aquaculture, thereby contributing to the mitigation of global antimicrobial resistance (Defoirdt *et al.* 2011). In recent years, especially for the purpose of immune response and disease resistance stimulation in aquaculture, use of probiotic instead of the antibiotic is concern one of the best steps proposed by many experts. In case of any fish species proper knowledge on use of probiotics instead of antibiotic is a must for successful disease resistance. The review paper is therefore written to consolidate the already available information with pointing out the information lacking which will be helpful for its overall fisheries health and disease management along with documentation of some possible strategies which can be considered for its availability in coming days.

The mechanism of mode of action of probiotics in aquaculture

Competitive inclusion

Probiotics exert their benefits in aquaculture through multiple interrelated processes, among them competitive inclusion serves as the main defense against pathogenic invasion (Balcazar *et al.* 2006). This process involves the ability of probiotics to colonies the intestinal mucosa

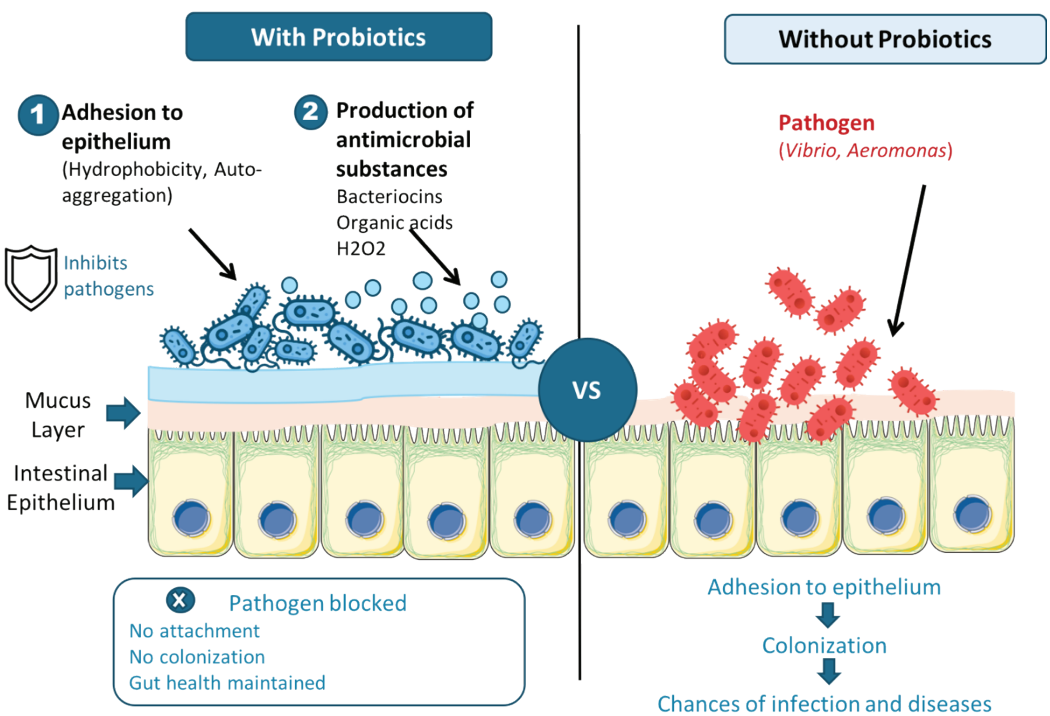

Figure 1. Probiotic protection against intestinal pathogens.

thereby occupying available adhesion sites resulting in limiting pathogen access to epithelial receptors (Hoseinifar *et al.* 2018). By promoting humoral and cellular immune responses and activating antigen-presenting cells probiotic strengthens the host immunity, increasing the production of antibodies and improving disease resistance.

Antimicrobial activity

The antimicrobial activity of probiotics represents the principle mechanism by which they confer protection against pathogenic microorganisms in aquaculture systems (Defoirdt *et al.* 2011). A wide range of bioactive substances directly inhibit the growth and proliferation of pathogens produced by probiotic strains. In addition, probiotics produce organic acids including lactic and acetic acid that lower the intestinal pH and inhibit pathogenic bacteria (Miquel *et al.* 2004). Moreover, some probiotics secret enzymes and secondary metabolites that may degrade the toxins and reduce pathogen virulence and biofilm formation. This multifaceted antimicrobial strategy not only limits pathogen colonization but also enhances the stability of gut microbiota. In aquaculture these microbial effects are effective against pathogens like *Vibrio spp* and *Aeromonas spp*, which cause major economic losses (Smith & Ritar 2006). By inhibiting these pathogens, probiotics improve host health, enhance disease resistance and reduce antibiotic use that supports the sustainable aquaculture.

Immunomodulation of immune-related genes by probiotics

Probiotics modulates the host immune system at the molecular level by regulating the expression of immune related genes. Immune related genes are those genes that encode the proteins responsible for pathogen recognition, activation and regulation of immune responses and maintenance of immune homeostasis (Llewellyn & Foey 2017). These genes participate in key processes such as inflammatory signaling (cytokines), pathogen detection toll-like receptors, antimicrobial defense (lysozyme and complement proteins) and adaptive immunity (immunoglobulin), thereby playing a central role in host defense mechanisms. Probiotics modulate host immunity at the molecular level by regulating the immune-related gene expression. They enhance pro-inflammatory cytokines such as IL-1β, TNFα, IL-6 and IFN-γ during

Table 1. Effects of probiotics on immune-related genes and biomarkers in fish

Gene/Marker	Types	Effect of probiotics	Functions	Fish species (Example)	Key references
IL-1β	Pro-inflammatory	Unregulated	Initiates	Nile tilapia, Zebra fish	(Nayak 2010)
TNFα	Pro-inflammatory	Unregulated	Activates immune cells and inflammation	Rainbow trout	(Panigrahi *et al.* 2007)
IL-6	Pro-inflammatory	Unregulated	Mediate acute immune response	Common carp	(Han *et al.* 2021)
IL-10	Anti-inflammatory	Upregulated	Suppresses excessive inflammation	Zebra fish, Tilapia	(Kim & Austin 2006)
TGF- β	Anti-inflammatory	Unregulated	Maintains immune tolerance	Atlantic salmon	(Wang *et al.* 2023)
Lysozyme	Innate immunity	Increased expression or activity	Break bacteria cell walls	Carp, Tilapia	(El-Son *et al.* 2022)
Complement (C3)	Innate immunity	Upregulated	Opsonization and pathogen lysis	Salmon, Carp	(Løvoll *et al.* 2007)
Toll like receptors (TLRs)	Innate immunity	Modulated	Pathogen recognition	Zebra fish, Salmon	(Chen *et al.* 2021)
IgM	Adaptive immunity	Increased	Antibody production	Tilapia, Carp	(Chan *et al.* 2024)
Mucin	Mucosal immunity	Upregulated	Enhances gut barrier protection	Zebra fish, Salmon	(Benktander *et al.* 2021)
Tight junction proteins	Barrier function	Strengthened	Prevent pathogen entry	Tilapia, Carp	(Krug & Fromm 2024)

early immune responses to promote pathogen recognition and clearance, while also inducing anti inflammatory cytokines including IL-10 and TGF- β (Llewellyn & Foey 2017). This gene-level regulation ultimately leads to improved disease resistance and immune homeostasis in aquaculture species.

Gut microbiota modulation and gut-mucus-immune-redox axis

Probiotic plays a vital role in modulating the gut microbiota of fish, promoting intestinal homeostasis and overall host health. They colonize in the gastrointestinal tract thereby competitively exclude opportunistic pathogens such as *Vibrio* and *Aeromonas spp* (Verschuere *et al.* 2000) Through adhesion, nutrient competition and production of antimicrobial substances. This results in a stable microbial community that supports digestion, nutrient absorption and disease resistance (Balcazar *et al.* 2006). Probiotic mediated microbiota is closely related to improved intestinal structure and barrier function. Increased villus height, length and goblet cell density enhance epithelial integrity and mucus secretion, forming a protective layer that prevents pathogen attachment while supporting commensal microbiota (Abarra *et al.* 2025) These interconnected processes collectively form the gut-mucus-immune-redox axis.

However, these interconnected processes form the gut-mucus-immune-redox axis, through which probiotics enhance intestinal integrity and fish health.

Morphological and secretary enhancement

The structural improvement of the gut architecture is a key feature of probiotic-mediated regulation. As illustrated in figure 2 probiotic colonization correlates with an increased villus length that effectively expands the absorptive surface area and facilitates nutrient utilization. Besides structural changes, probiotics also stimulate secretary functions. An increased number of goblet cells have been consistently reported in probiotic fed fish resulting in elevated mucus production. The mucus layer forms protective "gelatinous shield" that reinforces the intestinal barrier and limits pathogen adhesion (Ramos *et al.* 2015). These morphological and secretory enhancements contribute to improved intestinal

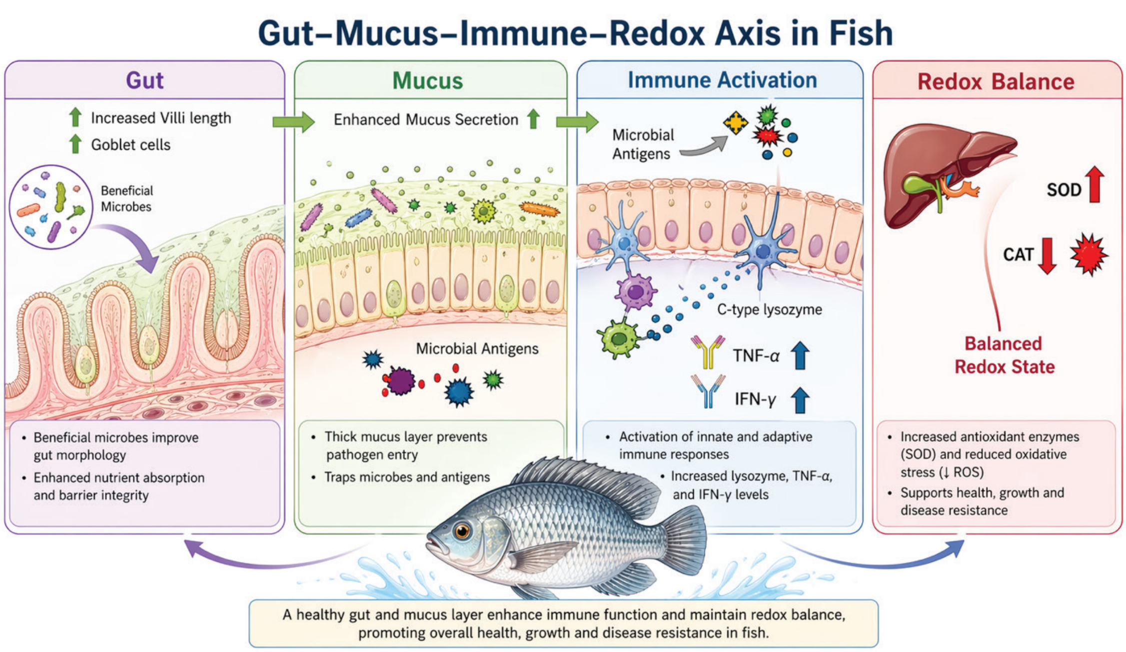

Figure 2. Schematic representation of the gut-mucus-immune-redox axis in fish. Probiotics enhance intestinal morphology and mucus secretion, stimulate immune responses and regulate antioxidant defenses collectively improving the gut health and disease resistance.

integrity, nutrient absorption and overall health status in cultured fish and shellfish species.

Immunological priming

Beyond strengthening of physical barriers, modulation of gut microbes has been increasingly recognized as a central regulator of innate immune activation in fish that contribute to host disease resistance. The microbial-associated molecular patterns (MAMPS) interact with intestinal epithelial receptors to activate regulated immune signaling pathways, establishing a primed immune state (Mohammed *et al.* 2025). This state is reflected by elevated C-type lysozyme activity and modulation of pro-inflammatory cytokines, including TNF-α and IFN-γ, indicating enhanced immune responsiveness. Collectively, these responses strengthen systemic immune readiness and improve disease resistance in cultured aquatic species (Egerton *et al.* 2018).

Systematic redox homeostasis

Gut microbiota modulation extends to the hepatic system, contribution to improved systematic oxidative balance in fish. This is evidence by enhanced antioxidant defenses, including increased superoxide dismutase activity and regulated catalase response (CAT), which together facilitate efficient neutralization of reactive oxygen species (ROS) (Egerton *et al.* 2018). In parallel, reduced HSP70 expression indicates alleviation of cellular stress and improved protein stability under adverse physiological conditions (Roberts *et al.* 2010). Collectively these responses reflect improved systemic redox homeostasis an enhanced host physiological resilience in cultured aquatic species.

Quorum quenching: the silent defense mechanism

Beyond direct bacterial actions, a sophisticated ecological strategy employed by immunobiotics is Quorum Quenching (QQ), which disrupts the Quorum sensing (QS) pathways utilized by devastating aquatic pathogens such as *Vibrio* and *Aeromonas spp.* to synchronize virulence (Egerton *et al.* 2018). The *Bacillus* strain interferes with this process by secreting enzymes like AHL-lactonases, which cleave the amide bond of the signal molecule. By silencing these signals, probiotic neutralize pathogenicity without threatening bacterial survival, thereby imposing significantly less selective pressure mitigating the risk of Antimicrobial Resistance (AMR) a critical shift from pathogen eradication to the management of microbial behavior (Defoirdt *et al.* 2011).

Current challenges and future perspective

Though the application of probiotics for boosting immunity in fish is promising, there remain some challenges. One of the major constrains include the inconsistent efficacy of probiotic strains across different host species, life stages and environmental conditions resulting in complexity in host-microbe interactions. Also, there is lack of standardized protocols on specific strain selection, doses and delivery methods often leads to variable outcomes. The high temperature and prolonged storage can easily reduce their functional efficacy. In addition, regulatory ambiguity and biosafety concerns cause the risk of horizontal gene transfer and disruption of native microbial communities continue to hinder large-scale adaptation and commercialization (Imperial & Ibana 2016). To overcome these limitations, future research is increasingly focused on innovative strategies. The integration of advanced omics tools, including metagenomics and transcriptomics, is enabling the identification of host-specific core microbiomes, facilitating the development of "designer probiotics" with improved specificity and efficacy (Al-Fakhrany & Elekhnawy 2024). Postbiotics and parabiotics are emerging as stable and safe alternatives to live microbes, retaining immunostimulatory effects while overcoming viability and storage issues (Tao *et al.* 2024). For climate changing issues probiotics are being investigated for their potential to improve host tolerance to thermal stress and hypoxia by modulating immune and antioxidant responses (Mahfuj *et al.* 2026). Collectively these approaches are expected to improve prebiotics-based disease management to establish a sustainable aquaculture system.

Strategic approaches to probiotic delivery

The effect of probiotic administration in aquaculture is governed by the "Entry-to-colonization" pathway, where the choice between dietary inclusion, water immersion,

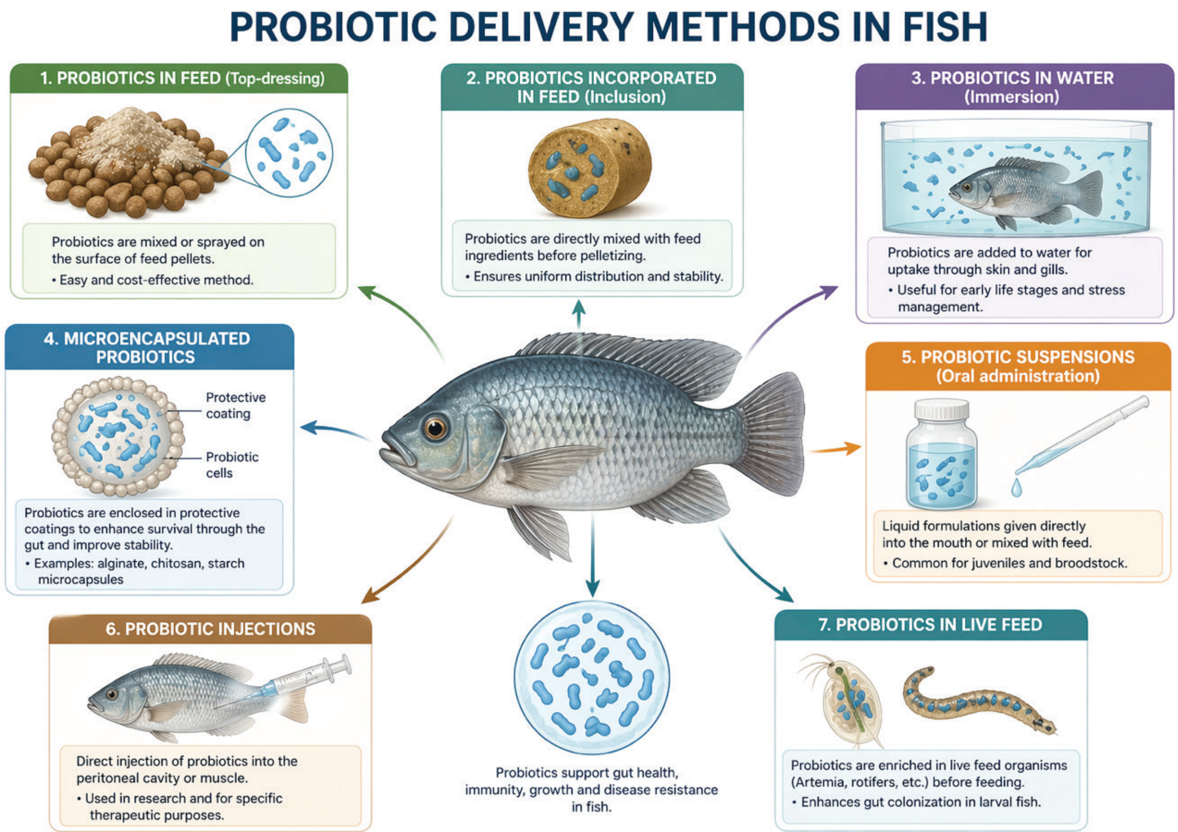

Figure 3. **Methods of probiotic administration in aquaculture based on species, life stage and culture system.**

or direct delivery determines the primary site of host-microbe interaction (Verschuere *et al.* 2000) Dietary supplementation targets the posterior intestine and gut-associated lymphoid tissue to enhance systematic immunity and nutrient absorption, necessitating the use of thermo-tolerant strains like *Bacillus spp.*, lactic acid bacteria and yeast to survive feed processing (Merrifield *et al.* 2010). Alternatively water based application methods, including immersion or bath treatment facilitates the uptake through skin, gill and ingestion and thereby contributing to environmental modulation, nutrient recycling and pathogen exclusion (Verschuere *et al.* 2000)

In intensive settings direct delivery methods as gavage or injection are employed for research or high value brood-stock, allowing precise dosing and facilitating the study of host-microbe interactions as well as potential transfer of immune genes to offspring (Zorriehzahra *et al.* 2016). These administration methods ensure the importance of probiotic delivery system to maximize their functional benefits in disease resistance and health management in aquaculture.

Conclusion

In conclusion, probiotics have emerged as a promising and sustainable alternative to antibiotics in modern aquaculture, playing a crucial role in enhancing fish health through immunomodulation. By interacting with host immune systems at molecular and cellular levels, probiotics stimulate both innate and adaptive immune responses, improve gut microbiota balance, and strengthen mucosal barrier integrity. They also contribute to disease resistance by inhibiting pathogenic microorganisms through competitive exclusion, production of antimicrobial compounds, and modulation of host signaling pathways. Despite these advantages, the efficacy of probiotics is highly dependent on factors such as strain specificity, dosage, host species, and environmental conditions. Therefore, further research is needed to better understand host–microbe interactions, optimize probiotic formulations, and develop standardized application strategies. Overall, integrating probiotics into aquaculture practices offers a viable pathway toward improved fish health management, reduced antibiotic reliance, and enhanced sustainability of the industry.

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Conflicts of Interest

The authors declare no conflict of interest.

Author's Contributions

MSR, SI and MSA designed and wrote the review; MNH and TA reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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