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Review

Microecological Preparations as Antibiotic Alternatives in Cyprinid Aquaculture

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Abstract: Microecological preparations (MPs), encompassing probiotics, prebiotics, synbiotics, and postbiotics, are microbial feed supplements that enhance host health through gut microbiota modulation. Unlike the narrow definition of probiotics (viable microorganisms), MPs constitute a broader category including non-viable microbial derivatives and selectively fermented substrates. Their application in aquaculture significantly reduces antibiotic dependence. Given the industry's intensification challenges, while meeting global protein demands, high-density aquaculture elevates disease risks, driving prophylactic antibiotic overuse. This practice accelerates antimicrobial resistance (AMR) development, compromising treatment efficacy and causing residual antibiotics in aquatic products. Such residues violate international food safety standards, triggering trade disputes. As sustainable alternatives, MPs operate through multiple mechanisms: the competitive exclusion of pathogens, immune stimulation, and nutrient absorption enhancement. This review examines the patterns of antibiotic abuse and the emergence of AMR in carp aquaculture, evaluates MP-based mitigation strategies from the perspective of antibiotic alternatives, and analyzes the advantages, disadvantages, and application progress of MPs. Based on existing evidence, we propose targeted research priorities for MP optimization, advocating for scientifically guided implementation in commercial cyprinid aquaculture.

Keywords: cyprinid aquaculture; microecological preparations; AMR; antibiotic alternatives

Key Contribution: Cyprinid production leads global freshwater aquaculture. However, challenges such as high feed costs, antibiotic residues, and drug resistance raise significant concerns. Antibiotic-free aquaculture presents a promising solution by prioritizing aquatic animal health, protecting human health; and ensuring the production of safe, nutritious, and residue-free aquatic products. Feed additives serve as effective alternatives to antibiotics, offering multiple benefits. This article highlights the importance, current status, and challenges of microecological agents, antibiotic residues, and resistance. It aims to provide valuable insights for fish farmers and regulatory bodies to deepen their understanding and promote the research, development, application, and management of feed additives in aquaculture.

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1. Introduction

Cypriniformes represents the most speciose order of freshwater teleosts, encompassing 321 genera and 3268 documented species [1]. Notably, Cyprinidae exhibits the most extensive geographical distribution and taxonomic diversity within this order. Economically significant species within Cyprinidae encompass *Mylopharyngodon piceus*, *Ctenopharyngodon idellus*, *Hypophthalmichthys molitrix*, *Aristichthys nobilis*, *Cyprinus carpio*, *Carassius auratus*,

Megalobrama terminalis, and *Parabramis pekinensis*, which collectively constitute the principal species in commercial aquaculture. Since the 1980s, the aquaculture sector has experienced substantial production growth, concurrently facing escalating challenges from disease outbreaks that correlate with intensive cultivation practices [2,3].

Aquaculture is one of the fastest-growing industries in the world, helping to address food shortages and promote economic development. The Food and Agriculture Organization of the United Nations (FAO) predicts that by 2030, fish production in aquaculture may reach 109 million tons, with Asia being the dominant sector, accounting for 89% of total production [3]. In 2020, antibiotic consumption in global aquaculture reached 99,502 metric tons, with pronounced regional disparities: Asia accounted for 67% of total usage, while Africa represented a negligible share (<1%). Current models project an 8.0% increase in global antibiotic use by 2030, escalating to 107,472 metric tons. These trends underscore urgent challenges, including the proliferation of antimicrobial resistance (AMR) and the ecological risks posed by antibiotic residues in aquatic ecosystems [2]. The escalating dependence on antibiotics in aquaculture poses profound threats to the sector's sustainable development. Rampant misuse and overuse of these agents have precipitated a critical surge in antimicrobial resistance (AMR), severely undermining the efficacy of therapeutic interventions against bacterial infections in cyprinid species. Key pathogens complicating cyprinid aquaculture include *Aeromonas punctata*, *Corynebacterium columnum*, *Aeromonas hydrophila*, and *Aeromonas thermophila*, among others, with AMR exacerbating disease management challenges across global aquaculture systems [4,5]. Most of these pathogens are aerobic, Gram-negative bacteria, leading to the widespread use of antibiotics targeting such bacteria. However, the issue of antibiotic residues remains severe [4,6] (Table 1). Globally, the most commonly used antibiotics in aquaculture include tetracyclines, oxalic acid, fluoroquinolones (e.g., sarafloxacin, enrofloxacin), beta-lactams (e.g., amoxicillin), macrolides (e.g., erythromycin), sulfonamides (e.g., sulfamethoxazole), diaminopyrimidines (e.g., omeprazole), and bisphenols (e.g., florfenicol) [7,8]. The types and levels of antibiotic residues in cyprinid fish are similar to the overall residue situation in fish farming. Currently, there is still a lack of systematic analyses and reports on antibiotic residues in cyprinid fish that are accurate to different regions of each country.

On the one hand, antibiotic residues pose significant toxic risks. The primary public health impacts include the development of antimicrobial resistance (AMR) (Figure 1), allergic reactions (e.g., to penicillin), carcinogenicity (e.g., sulfamethoxazole, oxytetracycline, and furazolidone), anaphylactic shock, nephropathy (e.g., gentamicin), mutagenicity, teratogenicity, bone marrow suppression, and disruptions to the normal gut microbiota [9,10].

Table 1. Adverse effects of antibiotic residue on human health.

Side Effect	Description	Reference
AMR	The residues in aquaculture products can lead to the emergence of antibiotic-resistant bacteria. This will reduce the effectiveness of the antibiotics used to treat human infections, leading to prolonged disease progression and increased healthcare costs.	[11]
Residue accumulation	The continued consumption of residues in fish products can lead to biological accumulation in the human body, which, over time, may increase the risk of chronic health problems, such as organ damage and endocrine disorders.	[12]
Allergic reactions	Some people may experience allergic reactions to antibiotic residues, ranging from mild symptoms, such as rash or urticaria, to more severe reactions, such as allergic reactions. Specific antibiotics, such as penicillin, are more likely to trigger allergic reactions.	[13,14]

Table 1. *Cont.*

Side Effect	Description	Reference
Toxicity	Long-term exposure to antibiotic residues, especially those that have not been fully metabolized, may have toxic effects on human organs and systems. For example, certain antibiotics, such as tetracyclines and sulfonamides, can cause kidney or liver damage if consumed in large quantities.	[15,16]
Disruption of gut microbiota	Antibiotic residues can alter the natural balance of gut bacteria, potentially leading to digestive problems, weakened immune responses, and even increased susceptibility to infections. This kind of destruction is particularly concerning in children, who heavily rely on healthy gut microbiota for normal development.	[17]
Potential carcinogenicity	Some antibiotics used in aquaculture, such as nitrofurans and quinolones, have been shown to have carcinogenic properties. These antibiotics may cause DNA damage or mutations, thereby disrupting normal cell growth. For example, nitrofuran can form reactive intermediates that bind to cellular macromolecules, leading to potential cancer development. Similarly, it has been found that certain quinolone drugs increase oxidative stress and interact with enzymes involved in DNA replication, which may lead to chromosomal damage.	[18–22]
Endocrine disruption	Some antibiotics, such as fluoroquinolones and tetracyclines, can act as endocrine disruptors. Their residues can interfere with hormone regulation and may affect reproductive health, growth, and development. This kind of destruction may have a significant impact on human fertility and development.	[23]
Drug interactions	Antibiotic residues in food can interact with drugs taken by humans, reducing their effectiveness or causing adverse reactions. For example, residual tetracycline may reduce the efficacy of certain anticoagulant drugs, thereby increasing the risk of blood clotting.	[24]

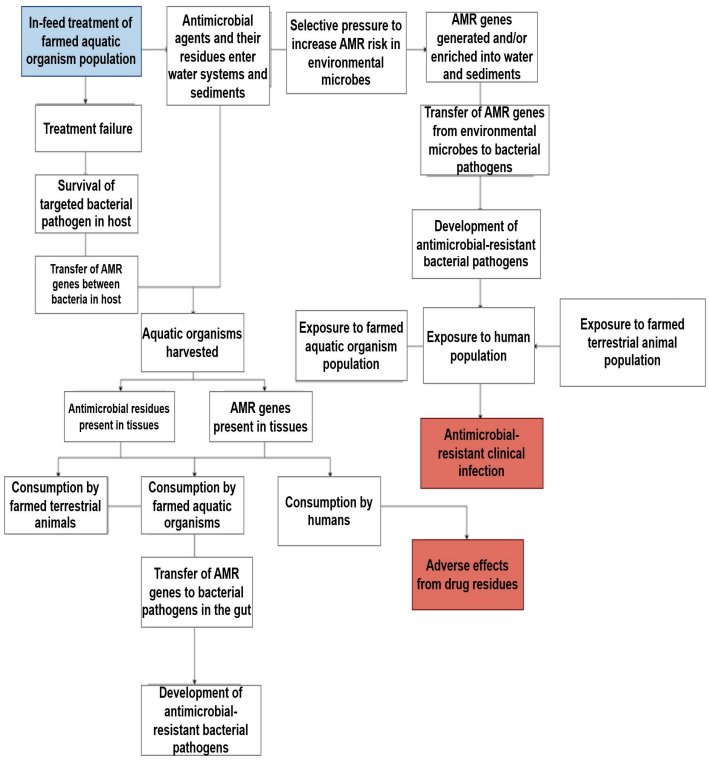


Figure 1. Potential negative impact of pharmaceutical feed treatment in aquaculture on antimicrobial resistance (AMR) in aquaculture (all figures in this review were created by Figdraw 2.0).

Once bacteria develop AMR, they can transfer the resistance to other species and strains through horizontal gene transfer. For example, zoonotic pathogens, such as *Streptococcus suis*, *Pseudomonas aeruginosa*, and *Vibrio vulnificus*, carry extended-spectrum beta-lactamases (ESBLs) and other antimicrobial resistance genes (ARGs), which can spread through the food chain [25]. Humans can become infected with these zoonotic bacteria through contact with aquatic animals, highlighting the transmission of aquaculture-associated bacteria to humans. This greatly complicates the treatment of human diseases such as pneumonia, tuberculosis, septicemia, gonorrhea, and salmonellosis, which are becoming increasingly resistant to common antibiotics [26–28]. Unfortunately, cyprinid fish also serve as hosts for zoonotic diseases like salmonellosis, underscoring the urgent need for alternative strategies to control Gram-negative bacterial infections [29].

On the other hand, challenges persist in the detection and regulation of antibiotic residues in food. The detection process typically involves two steps: sample pretreatment and analysis. Pretreatment techniques include solid-phase extraction (SPE) [30], QuEChERS technology [31], matrix solid-phase dispersion extraction (MSPD) [32], Pressure Liquid Extraction (PLE) [33], and microwave-assisted extraction (MAE) [34–36]. Common analytical methods include thin-layer chromatography (TLC) [37], high-performance liquid chromatography (HPLC), capillary electrophoresis (CE) [38], biosensors, microbial assays, and immunoassays. However, traditional detection methods face several limitations, including difficulty in identifying diverse antibiotics, limited sensitivity for trace detection, complexity and potential errors in sample pretreatment, and high costs associated with advanced detection equipment. Although techniques like fluorescence polarization and enzyme-linked immunosorbent assay (ELISA) are reliable and sensitive, they are seldom used by testing institutions and local authorities [35]. Moreover, high-resolution mass spectrometry and nuclear magnetic resonance instruments, while offering precise results, are prohibitively expensive, with equipment costs often reaching millions of USD—posing a major barrier to widespread adoption (Figure 2).

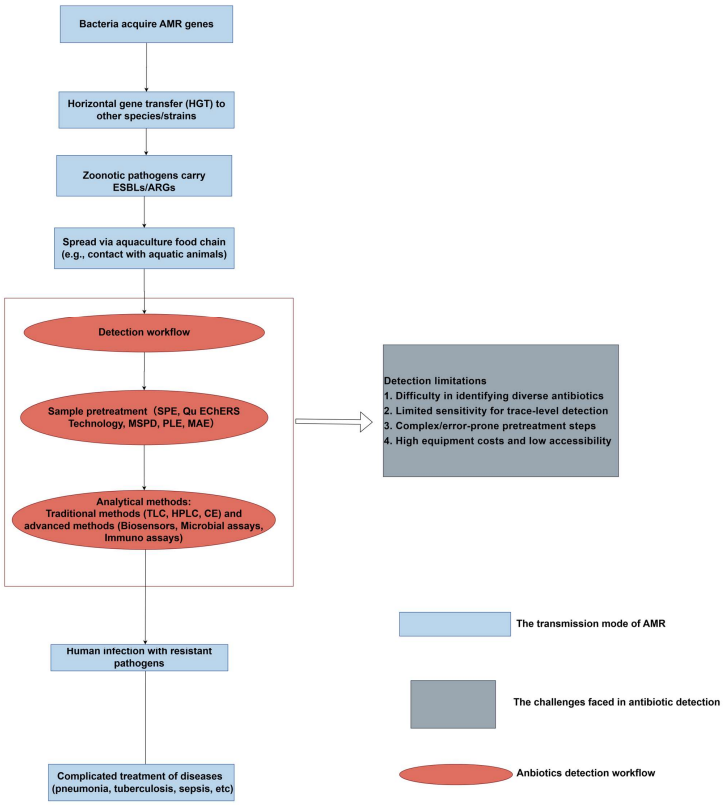


Figure 2. The transmission mode of AMR and the challenges faced in antibiotic detection.

Antibiotics at therapeutic levels, for example, are commonly given to groups of fish that share tanks or cages via the oral route for brief periods in aquaculture. Antibiotics have played an essential role in the aquaculture sector in terms of treatment and growth promotion [37]. While antibiotics are administered via parenteral and oral routes in terrestrial animals, the most common method for delivering antibiotics to fish is to mix the antibiotic with the prepared feed. Antibiotics, on the other hand, are not properly metabolized by fish, and they are passed largely unused back into the environment in feces. Of the antibiotics supplied to fish, 75% are excreted into the water [37], and this fact has prompted most countries with a significant aquaculture business to take some control measures through government organizations. For example, since 2006, the European Union (EU) has banned the use of antibiotics for growth promotion to reduce the risk of AMR and ensure food safety [3]. Similarly, the U.S. Food and Drug Administration (FDA) has issued guidelines limiting antibiotic use for growth promotion and mandates veterinary oversight for antibiotic use in aquaculture [1–3].

Modern aquaculture best practices now emphasize the therapeutic use of antibiotics strictly under veterinary supervision and only when necessary to treat specific infections. Additionally, comprehensive management strategies are encouraged, such as improving feeding practices, enhancing disease prevention, and adopting alternatives like vaccines and probiotics to minimize antibiotic reliance and lower the risk of drug resistance [3]. This article aims to review recent advancements in the use of feed additives as alternatives to antibiotics in cyprinid fish farming. It seeks to provide valuable insights to help fish farmers and regulatory authorities deepen their understanding and promote the research, development, application, and regulation of feed additives in sustainable aquaculture.

2. The Advantages and Disadvantages of Different Antibiotic Alternatives

Current alternatives to antibiotics in aquaculture include gene editing, vaccination strategies, phage therapy, probiotics, prebiotics, chicken egg yolk antibodies (IgYs), medicinal plants, and the use of “clean seeds” or specific pathogen-free (SPF) stocks as essential components of biosafety strategies [39]. However, the development and application of SPF systems face opposition due to high investment and maintenance costs, requiring skilled personnel, specialized knowledge, and advanced facilities [40]. Moreover, SPF systems have not fundamentally addressed antibiotic dependency, especially in developing countries.

Medicinal plants have long been recognized as natural immunostimulants due to their bioactive properties. As eco-friendly and cost-effective alternatives to antibiotics and synthetic immunoprophylactics in aquaculture, these plants are gaining global attention for their advantages: simple preparation, low cost, and minimal adverse effects on aquatic species and ecosystems. Extensive studies have investigated diverse plant-derived materials in aquatic animals, including herbs, spices, seaweeds, herbal extracts, traditional Chinese medicine formulations, and commercial phytogenic products. These materials can be utilized either as whole plants or specific parts (e.g., roots, leaves, seeds, flowers) or as purified bioactive compounds.

For compound extraction, ethanol and methanol are commonly employed solvents owing to their efficiency in isolating target molecules. However, the choice of extraction solvents and conditions may influence the biological activity of the final product and potentially affect aquatic organisms. Application methods vary flexibly: medicinal plants can be administered individually or synergistically combined with other immunostimulants, either through water immersion, feed additives, or bioenrichment protocols. Both single-dose and combinatorial approaches have demonstrated efficacy, though optimal dosages and treatment durations require further species-specific validation.

The primary roles of medicinal plants in aquaculture include growth promotion, immune enhancement, and antimicrobial/antiviral activity. Despite their potential, the mechanistic pathways underlying these effects remain poorly elucidated. Consequently, most studies emphasize the need for further research before direct industrial application. Critical knowledge gaps include long-term safety assessments, interactions with aquatic microbiomes, and synergistic effects with probiotics. The exploration of plant–microecological agent combinations represents a promising frontier for sustainable aquaculture development [41].

CRISPR-Cas genome editing has emerged as a transformative tool for advancing fish genetics and disease management yet faces critical challenges in aquatic applications. Three fundamental limitations impede progress: (1) Off-target effects from imperfect guide RNA (gRNA) binding, which is particularly impactful in early developmental stages where genetic mosaicism propagates through rapidly dividing cells. (2) Delivery constraints, where conventional mammalian methods require adaptation for aquatic species, with microinjection dominating embryonic stages but lacking the tissue-specificity for adult applications. (3) Validation complexities involving labor-intensive detection methods (WGS, GUIDE-seq) to ensure edit accuracy and safety. Key mitigation strategies include bioengineered solutions like high-fidelity Cas9 variants (SpCas9-HF1/eSpCas9) and advanced delivery systems such as magnetite nanoparticles [42].

Vaccine development shows great promise; however, vaccines are typically suited for large-scale commercial operations and high-value finfish species [43], primarily addressing bacterial or viral infections. Significant challenges remain in developing vaccines for key parasites such as protozoa, crustaceans, amoebas, monogeneans, and worms [43,44] (the detailed discussion is shown in Table 2) [6,45–58]. Although vaccination technology looks promising, its practical application is limited due to serious side effects, such as growth damage, inflammation, visceral fiber adhesion, scar formation, and pigment deposition [43].

In addition, vaccinating fish is labor-intensive, time-consuming, and often results in inconsistent outcomes [18]. Phage therapy offers a targeted approach without harming gut microbiota or surrounding microbial communities. Bacteriophages have been used to control bacterial infections in aquaculture. Numerous studies have shown that bacteriophages are effective against the most destructive bacteria in aquaculture, such as Aeromonas, Edwardsiella, Flavobacterium, Lactococcus, Pseudomonas, Streptococcus, and Vibrio [59–85].

Previously, the application of bacteriophages in aquatic animals has been extensively reviewed, demonstrating their potential as biological control agents against bacterial infections (Table 3). Although bacteriophages are considered alternatives to traditional antibiotics for treating aquatic animal diseases, their practical application in aquaculture should consider key points:

1. The efficacy of bacteriophages must be supported by in vivo experiments.
2. Ensuring the safety of bacteriophages for humans and aquatic animals is non-negotiable.
3. Phage management should be economically feasible.

In theory, phage therapy could disrupt quorum sensing (QS) and biofilm formation by pathogens on solid surfaces, but practical application without impacting beneficial microbes remains uncertain. Furthermore, bacterial hosts may harbor virulence factors encoded by bacteriophages, potentially altering their pathogenicity [39]. Bacteriophages exhibit genetic diversity that enables them to overcome bacterial defenses through mechanisms such as adsorption inhibition, restriction-modification systems, and CRISPR-Cas systems, which can enhance infectivity and expand host ranges [41]. However, bacterial resistance to one phage can lead to susceptibility to others, and some phages have broad host ranges. In some cases, bacteriophages and their bacterial hosts may form mutualistic relationships, meaning

phage resistance could promote the emergence of resistant bacterial strains [86]. Moreover, phage therapy's reliance on live organisms makes it susceptible to point mutations and genetic drift, reducing treatment efficacy [87]. Despite these challenges, bacteriophage-based products—especially for Vibrio species—are already commercially available. However, more research is necessary before phage therapy can be widely adopted in aquaculture.

In contrast, feed additives, such as probiotics and prebiotics, offer broad-spectrum benefits. These additives can combat bacterial diseases and have shown efficacy against viruses, parasites, and protozoa.

Table 2. Overview of types of aquatic vaccines and their implications for aquatic disease resistance.

Vaccine Type	Administration Method	Advantages	Disadvantages	Application Examples (Pathogens/Diseases)	Host Fish	Significant Effect	Reference
Inactivated vaccine	Injection, oral administration.	High safety. Easy production and storage.	Multiple vaccinations are required. Short duration of immunity.	Salmon Aeromonas and Streptococcus infections.	Salmon and tilapia.	++	[45–47]
Attenuated live vaccine	Injection and soaking.	Long lasting immunity. Single vaccination is effective.	Potential risk of toxicity reversal. Poor thermal stability.	Koi herpesvirus, Edwardsiella late-onset.	Carp, sea bream.	+++	[48–50]
Subunit vaccine	Oral administration and injection.	High purity and safety. Targeted key antigens.	Adjuvant is needed to enhance the effect. Weak immunogenicity.	Fish respiratory enterovirus (PRV), rhabdovirus.	Atlantic salmon.	++	[51,52]
DNA vaccine	Intamuscular injection.	Inducing cellular immunity. Rapid production.	Potential risks of genome integration. Local absorption limitation.	IHNV, SVCV Bullet virus.	Salmonidae fish.	+++	[53–55]
Vector vaccine	Injection, oral administration.	Multivalent antigen delivery. Mucosal immune activation.	Risk of carrier autoimmune response.	Recombinant Delayed Edwardsiella (RAEV).	Zebrafish, salmon.	++	[6,56]
Nanoparticle vaccine	Oral administration, soaking.	Enhance antigen stability. Targeted delivery.	Long-term toxicity unknown. High cost.	Virus-like particles (VLPs), ISCOM.	Various freshwater fish.	++	[57,58]

(++) represents significance; (+++) represents highly significant.

Table 3. Phage trials of pathogens in aquatic animals and seafood.

Pathogen	Disease	Animal or Seafood Species	Name of Phage (Morphology) [1]	Method of Application [2]	Outcome	Reference
Aeromonas hydrophila	Septicemia	Catfish (*Pangasianodon hypophthalmus*)	φ2 and φ5 (*Myoviridae*)	I.P. injection	Improved survival rates from 18.3% to 100%.	[59]
		Loach (*Misgurnus anguillicaudatus*)	Akh-2 (*Siphoviridae*)	Immersion	Reduced cumulative mortality rates from 100% to 56.67%.	[60]
		Loach (*Misgurnus anguillicaudatu*)	AH1 (n.d.)	Infection with phage before injection in fish	Pathogenicity eliminated following phage infection. Reduced cumulative mortality rates.	[61]
		Loach (*Misgurnus anguillicaudatu*)	pAh1-C, pAh6-C (*Myoviridae*)	I.P. injection and oral administration via feeding	I.P. injection: from 100% to 43.33% using pAh1-C and to 16.67% using pAh6-C. Oral administration: from 95.83% to 46.67% with pAh1-C and to 26.67% with pAh6-C.	[62]
A. salmonicida	Furunculosis	Rainbow trout (*Oncorhynchus mykiss*)	PAS-1 (*Myoviridae*)	I.M. injection	Increase in survival rates from 0% to 26.7%.	[62]
		Senegalese sole (*Solea senegalensis*)	AS-A (*Myoviridae*)	Immersion	Decrease in cumulative mortality rates from 36% to 0%.	[63]
		Brook trout (*Salvelinus fontinalis*)	HER 110 (*Myoviridae*)	Immersion	Decrease in total mortality rates from 100% to 10%.	[64]
Edwardsiella tarda	Edwardsiellosis	Zebrafish (*Danio rerio*)	ETP-1 (*Podoviridae*)	Immersion prior to bacterial challenge	Survival rates improved from 18% to 68%.	[65]
Flavobacterium columnare	Columnaris disease	Rainbow trout and zebrafish	FCL-2 (*Myoviridae*)	Immersion	Improved survival rates. Rainbow trout: increased from 8.3% to 50%. Zebrafish: increased from 0% to 60%.	[66]
F. psychrophilum	Rainbow trout fry syndrome and cold water disease	Rainbow trout and Atlantic salmon (*Salmo salar*)	1H, 6H (*Siphoviridae*)	I.P. injection	Reduced cumulative mortality rates. Trout: decreased from 80% to 67% (1H), 47% (6H). Salmon: decreased from 13% to 0% (1H), 6% (6H).	[67]
Lactococcus garvieae	Lactococcosis	Yellowtail (*Seriola quinqueradiata*)	PLgY-16 (*Siphoviridae*)	I.P. injection and oral administration (feeding)	I.P. injection: improved survival rates from 45% to 90%. Oral administration: reduced cumulative mortality from 65% to 10%.	[68]
Pseudomonas plecoglossicida	Bacterial hemorrhagic ascites disease	Ayu (*Plecoglossus altivelis*)	PPpW-3 (*Myoviridae*), PPpW-4 (*Podoviridae*)	Oral administration (feeding)	Reduced cumulative mortality rates from 93.3% to 53.3% for PPpW-3, 40.0% for PPpW-4, and 20.0% for PPpW-3/W-4.	[69]
Streptococcus agalactiae	Streptococcosis	Nile tilapia (*Oreochromis niloticus*)	HN48 (n.d.)	I.P. injection	Improved survival rates from 0% to 60%.	[55]
S. iniae	Streptococcosis	Japanese flounder (*Paralichthys olivaceus*)	PSiJ31, 32, 41, 42 (*Siphoviridae*)	I.P. injection	Improved survival rates from 0% to 28 or 33% (combined usage of PSiJ31 and 32); from 0% to 48, 70, or 90% (combined usage of PSiJ31, 32, 41, and 42).	[70]
Vibrio anguillarum	Hemorrhagic septicemia	Atlantic salmon	CHOED (n.d.)	Immersion	Survival rates improved from less than 10% to 100%.	[71]
	Vibriosis	Zebrafish larvae	VP-2 (n.d.)	Immersion	Cumulative larval mortality rates reduced from 17% to 2%.	[72]

Table 3. *Cont.*

Pathogen	Disease	Animal or Seafood Species	Name of Phage (Morphology) [1]	Method of Application [2]	Outcome	Reference
V. harveyi	Luminescent vibriosis	Shrimp (*Penaeus monodon*) larvae	A (*Siphoviridae*)	Immersion	Larval survival rates improved from 17% to 86%.	[73]
		Shrimp larvae	VHM1, VHM2 (*Myoviridae*) VHS1 (*Siphoviridae*)	Immersion	Larval survival rates improved from 26.6% to 86.6%.	[74]
		Abalone (*Haliotis laevigata*)	vB_VhaS-tm (*Siphoviridae*)	Immersion	Larval survival rates improved from 0% to 70%.	[75]
		Black tiger shrimp (*Litopenaeus monodon*) larvae	VHP6b (*Siphoviridae*)	Immersion	Cumulative larval mortality rates reduced from 70% to 20%.	[76]
		Shrimp larvae	Viha10, Viha8 (*Siphoviridae*)	Immersion	Larval survival rates improved from 65% to 88%.	[77]
V. parahaemolyticus		Blue mussel (*Mytilus edulus*)	VP10 (n.d.)	Immersion	Reduction in bacterial growth to undetectable levels.	[59]
		Oyster (*Crassostrea gigas*)	pVp-1 (*Siphoviridae*)	Immersion, surface inoculation	Reduction in bacterial growth from 10^6 CFU/g to 10 CFU/g.	[78]
	Acute hepatopancreatic necrosis disease	Marine shrimp (*P. vannamei*)	pVp-1 (*Siphoviridae*)	Immersion, oral administration	Reduced cumulative mortality rates from 100% to 0%.	[79]
V. splendidus	Vibriosis	Sea cucumber (*Apostichopus japonicus*)	PVS-1, PVS-2 (*Myoviridae*) PVS-3 (*Siphoviridae*)	Oral administration (feeding) and coelomic injection	Survival rates increased from 18% to 82% through feeding and from 20% to 80% via coelomic injection.	[60]

[1] n.d. (not determined); [2] I.P. (intraperitoneal); I.M. (intramuscular).

3. Microecological Agents

3.1. Probiotics, Prebiotic, Synbiotics, and Postbiotics

3.1.1. Probiotics

The term probiotics is derived from the Latin word pro ("for") and the Greek word biotic ("life"). Lilly and Stillwell first introduced this term in 1965 to describe substances that stimulate microbial growth. The FAO/WHO (2001) defines probiotics as live beneficial microorganisms that, when administered in adequate amounts, confer health benefits to the host by altering its associated microbial population [88]. Lazado and Caipang (2014) demonstrated the practical advantages of probiotics in aquaculture, building upon studies dating back to 1986 [89]. It is essential to understand the full context of this definition. Microbial cultures can only be classified as probiotics if their beneficial effects on the host have been confirmed through clinical studies or in vivo animal experiments. If evidence is limited to in vitro studies without in vivo confirmation, the microorganism is classified as a potential probiotic. Probiotics colonize animal intestines by maintaining or increasing favorable gut microbiota and restore the stability of gut microbiota disrupted by antibiotic treatment.

Probiotics benefit aquatic animals primarily in two ways:

1. Improving Water Quality: they aid in nitrification, denitrification, water quality management, and pathogen control.
2. Enhancing the Intestinal Microenvironment.

Compared to adult fish, juvenile fish possess immature immune systems and primarily depend on innate immunity for pathogen defense [90].

The mechanisms of probiotic action are multifactorial and context-dependent, presenting challenges for comprehensive elucidation (Table 4). Proposed mechanisms include the following:

1. The synthesis of antimicrobial metabolites.
2. Competitive exclusion (nutrient/attachment site competition).
3. The suppression of virulence-related gene expression.
4. Quorum-sensing interference.
5. Water quality modulation.
6. Immune function enhancement.
7. The provision of essential nutrients.
8. The facilitation of digestive enzyme activity [91].

To ensure efficacy and safety, probiotic strains must satisfy rigorous selection criteria:

1. Host species specificity.
2. Non-pathogenicity and genomic stability.

3. Antimicrobial compound production capacity.
4. Immunomodulatory competence.

Merrifield et al. (2010) proposed expanding these criteria to prioritize strains with validated probiotic functionalities, enabling optimized strain selection [92]. Nevertheless, identifying candidates fulfilling all requirements remains challenging, leading to the prevalent use of multi-strain probiotics [93] or synergistic formulations with prebiotics [94]. Combination strategies (e.g., probiotic consortia or synbiotics) generally demonstrate superior efficacy compared to single-strain applications. Critically, the introduction of probiotics harboring mobile antibiotic resistance genes into aquaculture systems is strongly discouraged due to horizontal gene transfer risks [95].

Before using probiotics in aquaculture, it is critical to screen for the presence of antibiotic resistance or virulence genes in mobile genetic elements [96].

The U.S. Food and Drug Administration (FDA) has approved several microorganisms for use in fish feed and aquatic environments, confirming their safety for direct application. In China, probiotics have been used in aquaculture since the 1980s, and their popularity has grown substantially. In recent years, over 100 companies have participated in the probiotic market, producing more than 50,000 tons of commercial probiotic products annually by Qi et al. (2009) [97] and Wang et al. [98]. In 2020, the European Commission authorized the use of Lactococcus lactis CNCM I-4622 (MA 18/5 M), developed by Lallemand Animal Nutrition. This probiotic, approved for aquaculture in the European Union, is widely recognized for improving the digestion of fish and shrimp. Other probiotic microorganisms approved by the European Union (EU) are internationally recognized for enhancing digestive efficiency in aquatic species through probiotic supplementation.

Lactic acid bacteria (LAB), exemplified by strains such as Lactobacillus acidophilus and Lactococcus lactis, are widely employed in aquaculture to enhance intestinal health and immune function in crucian carp (*Carassius auratus*). Studies indicate that LAB supplementation elevates serum immune biomarkers, including immunoglobulin M (IgM), lysozyme (LYZ), alkaline phosphatase (AKP), and superoxide dismutase (SOD) [99]. These markers correlate with three key immunological benefits:

(1) IgM-mediated pathogen recognition;
(2) LYZ-driven nonspecific immunity;
(3) AKP-SOD antioxidant synergy against oxidative stress.

LAB exert dual antimicrobial mechanisms: the biofilm-mediated competitive exclusion of pathogens and the secretory modulation of mucosal immunity via intestinal epithelial interactions. The transcriptional regulation of inflammatory cytokines (IL-1β↑/IL-10↑) and antiviral factors (IFN-γ/TNF-α) further highlights LAB's immunomodulatory potential. Recent screening identified LABy11 and y78 strains with enhanced efficacy [100], while interspecies variability in LAB responses necessitates further investigation into gut microbiota dynamics and bioactive compound mechanisms.

Similar findings have been reported for other probiotic strains (Table 5). Li et al. [101] studied the impact of adding Clostridium species to the diets of juvenile carp (*Cyprinus carpio*). The results showed that fish fed with this supplement experienced greater weight gain and specific growth rates. Furthermore, dietary Clostridium enhanced the mRNA expression of growth-related genes (PepT-1, PepT-2, and IGF-1) and genes associated with the TOR signaling pathway (TOR, 4E-BP2, and S6K1) [102]. In another study, different doses of LAB were added to *Carassius auratus* diets (5×10^3, 5×10^4, and 5×10^5 CFU/mL) over eight weeks. The results revealed improvements in water quality, reduced nitrogen concentration, better growth performance, increased serum antioxidant capacity, and the heightened activity of protease, digestive enzymes, and amylase.

Escherichia coli, a Gram-negative, rod-shaped bacterium within the family Enterobacteriaceae, is ubiquitously distributed across terrestrial, aquatic, and intestinal ecosystems. Despite its historical notoriety as an opportunistic pathogen (e.g., serotype O157:H7), emerging evidence highlights the probiotic potential of avirulent *E. coli* variants engineered through virulence gene deletion [103]. The Nissle 1917 strain (EcN), a prototypical non-pathogenic variant, demonstrates two key attributes for aquaculture applications: exceptional thermo-stability in lyophilized feed matrices and broad-spectrum antimicrobial activity mediated by microcin B17 synthesis, showing 82.4% growth inhibition against *Pseudomonas aeruginosa* ATCC 27,853 [103]. These findings underscore that targeted strain selection can repurpose *E. coli* from conditional pathogen to a precision biocontrol agent.

This paradigm is further exemplified by *Enterobacter asburiae* E7, a candidate probiotic isolated from the intestinal microbiota of *Cyprinus carpio*. Following rigorous safety screening (the absence of *hly*, *stx1*, and *stx2* virulence factors), dietary supplementation with E7 at 10^7 CFU/g for 28 days induced significant immunomodulatory effects: the fold upregulation of *IL-1β* and *TNF-α* mRNA expression in head kidney leukocytes (qPCR, $p < 0.01$). A 57% reduction in mortality during *Aeromonas veronii* challenge trials compared to controls was identified [104].

Notably, while no significant growth promotion was observed, these results position *E. asburiae* E7 as a viable immunostimulant for sustainable cyprinid aquaculture [105].

Table 4. Mechanisms employed by probiotics to confer benefits.

	Description	Reference
1.	Probiotics compete for resources and receptor binding sites, making it difficult for harmful microorganisms to survive in the intestine. They produce peptides, including anti-microbial peptides, viz., bacteriocins and β-defensins, to limit the pathogenic growth within the host species.	[106,107]
2.	Probiotics synthesize anti-bacterial substances, such as short chain fatty acids (SCFAs), e.g., propionate, butyrate, acetate, and H_2O_2. They also produce organic acids that lower the pH of the GIT, preventing harmful microorganisms from growing and supporting the propagation of probiotics.	[107]
3.	Probiotics improve intestinal barrier function by regulating the expressivity of tight junction proteins, including occludin, zonula occludens, and claudin, promoting defensin and mucin formation and regulating the immune function in the intestine.	[108]
4.	They affect both innate and adaptive immunity by modulating B- and T-cell, lysozyme, dendritic cell, complement and macrophage activities. Probiotics interact with intestinal epithelial cells, attracting macrophages and mononuclear cells, leading to the increased production of anti-inflammatory cytokines. They also inhibit pro-inhibitory markers by regulating the cytokines.	[91]
5.	Probiotics have the ability to produce neurotransmitters in the intestine through the gut–brain axis. Some strains can change the amounts of GABA, serotonin, and dopamine, which can have a positive effect on gastrointestinal motility and stress, as well as mood- and behavior-related pathways	[109]
6.	Probiotics alter the microbiome of the GIT through boosting the diversity of healthy microbes and decreasing the number of harmful ones.	[110]

Table 5. More types of probiotics are being used in cyprinid fish.

Empty Cell	Probiotics	Species	References
Aeromonas	*Aeromonas veronii*	Grass carp (*Ctenopharyngodon idellus*)	[111]
		Common carp (*Cyprinus carpio*)	[112]
Bacillus	*Bacillus amyloliquefaciens*	Common carp (*Cyprinus carpio*)	[113]
	Bacillus coagulans SCC-19	Common carp (*Cyprinus carpio*)	[114]
	Bacillus subtilis	Grass carp (*Ctenopharyngodon idellus*)	[115]
	B. velezensis R-71003	Common carp (*Cyprinus carpio*)	[116]
Carnobacterium	*C. divergens*	Common carp (*Cyprinus carpio*)	[117]
Enterococcus	*Enterococcus faecium* L6	Grass carp (*Ctenopharyngodon idellus*)	[118]
Flavobacterium	*Flavobacterium sasangense* BA-3	Common carp (*Cyprinus carpio*)	[112]
	Lactobacillus acidophilus	Goldfish (*Carassius auratus gibelio*)	[119]
	Lactobacillus delbrueckii	*Cyprinus carpio* Huanghe	[120]
Lactobacillus	*Lactobacillus casei*	Common carp (*Cyprinus carpio*)	[121]
	Lactobacillus sakei	Common carp (*Cyprinus carpio*)	[122]
		Common carp (*Cyprinus carpio*)	[123]
Pediococcus	*Pediococcus acidilactici MA18/5M*	Common carp (*Cyprinus carpio*)	[124]
Pseudomonas	*Pseudomonas aeruginosa VSG2*	Common carp (*Cyprinus carpio*)	[125]
Saccharomyces	*Saccharomyces cerevisiae*	Common carp (*Cyprinus carpio*)	[126]
	Shewanella xiamenensis	Grass carp (*Ctenopharyngodon idellus*)	[127]
Vibrio	*Vibrio lentus* BA-2	Common carp (*Cyprinus carpio*)	[112]

Probiotic application in aquaculture faces multifaceted challenges. Technical constraints in mass-producing aquatic-derived strains, compounded by practitioners' limited knowledge of administration protocols, have led to the predominant use of terrestrial alternatives [128]. Critical research gaps persist regarding

1. Ecological impacts on aquatic microbiomes.
2. Host-specific isolation hurdles from mucosal tissues [104].
3. Underexplored Bacillus-mediated mucosal immunity mechanisms. Environmental parameters (pH, temperature, dissolved oxygen) crucially influence probiotic viability, with alkalinity fluctuations altering metabolic rates [129–131].

Metabolic engineering breakthroughs, particularly CRISPR/Cas9 systems [132], enable targeted probiotic optimization through

1. Transporter engineering for enhanced substrate uptake.
2. Pathway refinement via toxic byproduct elimination.
3. Heterologous enzyme integration (cellulase/pectinase for SCFA production [133]).
4. Feedback-resistant overexpression strategies [134].

While probiotics demonstrate safety and eco-compatibility, they remain supplementary to pharmaceutical additives. Urgent priorities include the standardized validation of indigenous strains for disease prevention [105] and rigorous ecological risk assessments [128].

Probiotics like *lactic acid bacteria* and *bifidobacteria* lack the enzymes necessary to break down dietary fiber and complex polysaccharides. By introducing genes that encode enzymes such as cellulase, pectinase, and xylanase, these probiotics could metabolize complex substrates more effectively. This enhancement would improve their competitiveness and survival while promoting the production of short-chain fatty acids (SCFAs) [133].

Currently, while probiotics are regarded as safe and environmentally friendly feed additives, they cannot fully replace pharmaceutical additives in aquaculture.

3.1.2. Prebiotic

Prebiotics are non-digestible compounds that can be absorbed by the gut microbiota. They serve as essential nutrients for probiotic growth, selectively stimulating the proliferation and activity of specific probiotic strains. This promotes the growth of beneficial gut bacteria, enhances the gut microenvironment, strengthens host immunity, and reduces the need for antibiotics in aquaculture practices [135]. Common prebiotics primarily include oligosaccharides (composed of 2–10 monosaccharides) and polysaccharides. In aquaculture, widely used prebiotics include inulin and various oligosaccharides. Other prebiotics, such as bifidogenic factors and isomaltose, act as "nutrient media" for beneficial gut bacteria in aquatic animals. These compounds stimulate beneficial bacterial growth, increase their activity, and suppress harmful bacterial populations. Prebiotics can also enhance the activity of various enzymes in aquatic species.

Inulin, extracted from plants like lilies and chicory, is widely used due to its abundance and low cost. However, as of 2022, the application of oligosaccharides in fish farming remains limited.

The mechanisms by which prebiotics enhance the immune system in animals can be categorized into three primary aspects (Figure 3):

1. Regulating the gut microbiota.
2. Strengthening the intestinal mucosal barrier.
3. Directly activating the immune system.

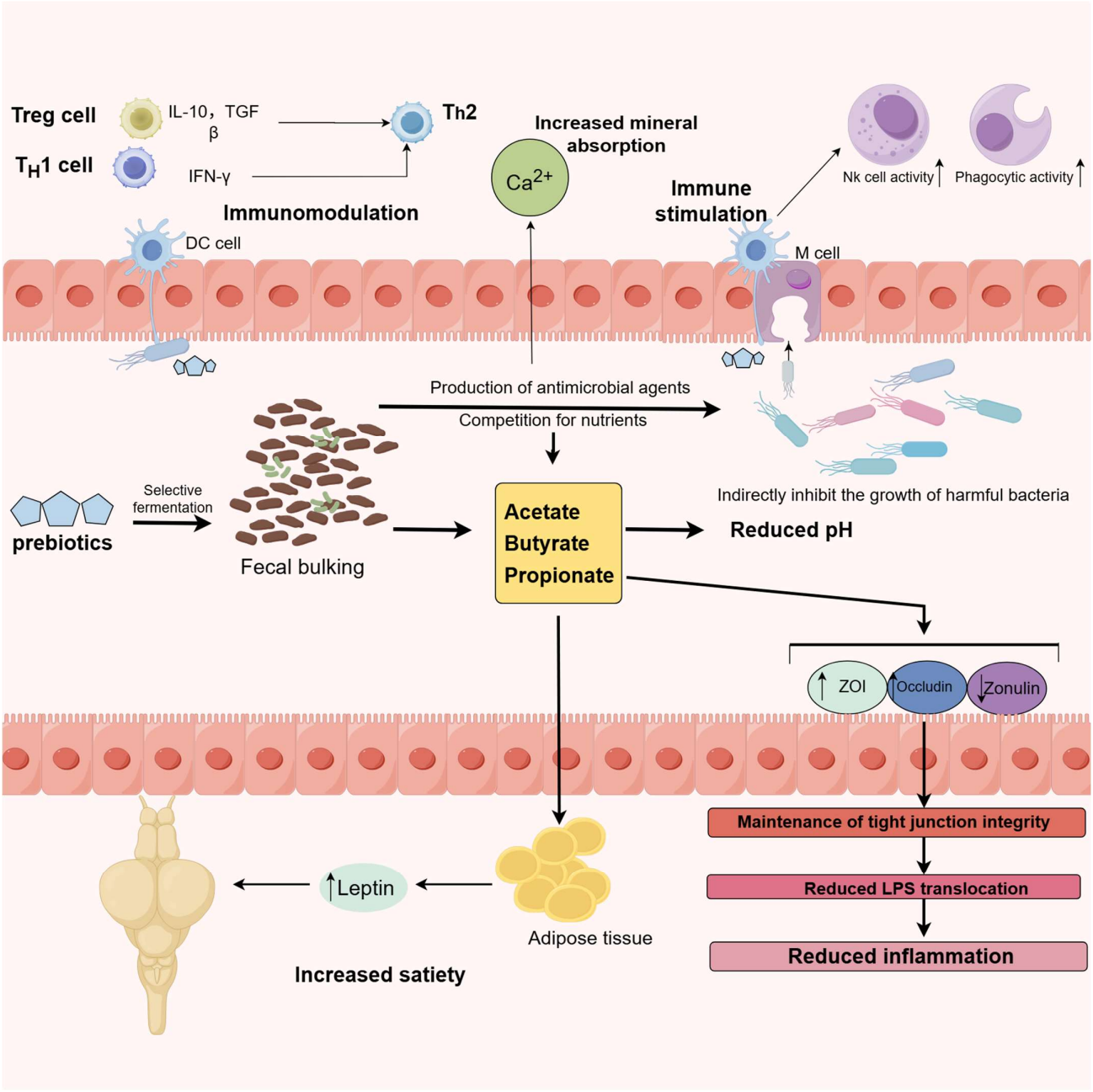


Figure 3. The mechanism of prebiotics on intestinal immunity in fish.

Cells research shows that certain strains of lactic acid bacteria can regulate the maturation of dendritic (DC) cells, enhance NK cell and macrophage activity, and facilitate the differentiation of T-cells into Th1, Th2, or regulatory T-cells (T-reg), thereby improving immune responses. Additionally, prebiotics increase the populations of T and B lymphocytes.

Oligofructose, a water-soluble dietary fiber, has undergone extensive experimentation and has shown no evidence of genetic toxicity [136]. Hoseinifar S.H. (2017) demonstrated that adding short-chain oligofructose to carp feed significantly increased probiotic abundance in the fish's gut microbiota [137]. Similarly, Dimitroglou A. (2009) reported that fish fed prebiotic-supplemented diets had significantly higher levels of short-chain fatty acids (SCFAs) in their intestines [138]. SCFAs, including acetic acid, propionic acid, and butyric acid, are key regulators of intestinal health. Butyric acid, in particular, nourishes intestinal epithelial cells, serves as a primary energy source for intestinal tissues, and supports immune cell development. Additionally, SCFA metabolites help lower intestinal pH, creating

an unfavorable environment for pathogenic bacteria [139]. Therefore, prebiotics play a crucial role in enhancing fish gut health.

3.1.3. Synbiotics

The concept of synbiotics was first proposed by Gibson and Roberfroid in 1995 [140], with practical applications emerging in aquaculture by 1998. Synbiotics are defined as synergistic formulations combining probiotics and prebiotics, designed to selectively enhance the proliferation and metabolic activity of beneficial gut microbiota. This dual-action mechanism not only promotes the intestinal microbial dominance of probiotics but also augments host immunity and pathogen resistance through microbiome modulation [141]. While probiotics and prebiotics have been extensively utilized in aquaculture independently, synbiotics represent a paradigm shift by integrating their complementary functions. Their efficacy stems from the strategic pairing of specific probiotic strains with compatible prebiotic substrates, thereby optimizing microbial colonization and host–microbe crosstalk [142]. This synergy facilitates three key outcomes: enhanced niche competition against pathogens, the selective amplification of endogenous beneficial species, and systemic immunoregulation [143].

Competitive exclusion constitutes a cornerstone of probiotic functionality in gut ecosystems, operating through spatial and nutritional antagonism. Upon intestinal colonization, probiotics adhere to mucosal epithelia, creating a physicochemical microenvironment unfavorable to pathogens [144]. Vine et al. (2004) demonstrated this phenomenon through in vitro models comparing five probiotic strains (e.g., *Lactobacillus* spp.) against enteropathogens like *Aeromonas hydrophila* [145]. Furthermore, under nutrient-limiting conditions, probiotics exhibit metabolic flexibility to outcompete pathogens for critical substrates (e.g., iron-siderophores, oligosaccharides) [145,146].

Beyond direct microbial antagonism, synbiotics modulate immune homeostasis through microbiota-derived signals. Probiotic metabolites (e.g., short-chain fatty acids) are prime innate immune effectors—notably enhancing natural killer (NK) cell cytotoxicity by folding and upregulating interleukin-10 (IL-10) production in lymphocytes [147]. These immunostimulatory effects are amplified when combined with prebiotics, which act as vaccine adjuvants by prolonging antigen-presenting cell (APC) activation and antibody titer persistence [148,149]. However, three critical considerations must be addressed in application:

1. Compatibility: prebiotic physicochemical properties (e.g., molecular weight, solubility) must align with antigen delivery systems to prevent epitope masking [148].
2. Dose Optimization: immunomodulatory effects follow U-shaped dose-response curves, requiring empirical determination to avoid immunosuppression (low dose) or inflammatory cascades (high dose) [147].
3. Safety Profiling: chronic exposure risks include microbiota dysbiosis and hepatic xenobiotic metabolism alterations, necessitating longitudinal toxicity assessments [149].

Oral administration (feed addition) is the main approach in the large-scale aquaculture of cyprinid fish, but the survival rate and intestinal colonization efficiency of prebiotics in feed processing need to be considered. The feasibility of injection or immersion vaccination is low, and the production cost of prebiotics needs to be balanced with the degree of vaccine protection efficacy enhancement, especially in low-value-added fish species; this needs to be carefully evaluated. Establish quality control standards for the purity of prebiotics and the concentrations of active ingredients (such as SCFAs) to ensure consistency between batches. Different pathogens (such as bacterial vs. viral) may need to be matched with specific prebiotics (such as bacteriocins targeting bacterial vaccines), and the immune system differences of host species also need to be considered. Finally, long-term use may alter the microbial community structure of aquaculture water bodies or wild populations,

and its potential impact on environmental microbial resistance and ecological balance needs to be evaluated. Oligosaccharides, a type of prebiotic, can stimulate the liver to secrete specific proteins that bind with oligosaccharides to form glycoproteins, which subsequently influence the immune system [150]. Dietary supplementation with *Bacillus subtilis* and/or oligosaccharides has been shown to significantly increase the levels of alkaline phosphatase (AKP), acid phosphatase (ACP), and lysozyme (LZM) in various fish species, including crucian carp [150], carp [151], and grass carp [152]. Moreover, the supplement effectively mitigated the upregulation of inflammatory cytokines (IL-6, IL-8, IL-1β, and TNF-α) and the downregulation of anti-inflammatory cytokines (IL-10 and TGF-β) caused by $NaHCO_3$ exposure. This indicates that $NaHCO_3$ stress disrupts immune homeostasis in crucian carp, leading to intestinal inflammation, whereas synbiotics can enhance immune function and alleviate this inflammation [153]. In this study, the combination addition had the best effect. Research indicates that xylooligosaccharides (XOSs), fermented by gut microbiota, enhance intestinal epithelial barrier integrity and stimulate enterocyte proliferation, thereby promoting gut structural development and nutrient absorption [151]. XOS fermentation by probiotics generates small organic acids (e.g., SCFAs), lowering the intestinal pH and suppressing pathogenic bacterial growth [152]. Notably, SCFAs—classified as postbiotics—play dual roles in immune regulation:

1. Innate Immunity: activate GPR43 to induce neutrophil chemotaxis, functional activation, and the modulation of innate immune cells [152,154].
2. Adaptive Immunity: enhance T-cell and B-cell responses, bolstering mucosal and systemic antibody production, thereby improving disease resistance in fish.

These findings suggest that *Bacillus subtilis* may synergize with oligosaccharides by producing beneficial metabolites (e.g., SCFAs) during fermentation.

Despite obtaining promising results, the exact mechanisms of synbiotics remain controversial. The interactions between probiotics and native gut microbiota are often explained through general ecological concepts like saprophytism and competition [153].

There are three main types of interactions between probiotic effector molecules and intestinal mucosal receptors.

The first type involves the identification of probiotics, lectins, and carbohydrates on the surface of intestinal mucosa. Many probiotics express lectins as effector molecules, binding probiotics to carbohydrate conjugates on the cell surface, such as probiotic extracellular polysaccharides, capsule polysaccharides, and intestinal adhesion molecules like membrane mucin [155].

The second type involves the interaction between host cell proteins and probiotic surface proteins, such as the binding of probiotic pili to intestinal mucosal mucin [156].

The third type involves hydrophobic interactions between hydrophobic proteins on the surface of probiotics and complementary hydrophobic protein fragments on animal cells, such as the binding of probiotic surface proteins to intestinal mucosal mucins [157].

However, there is still limited systematic research on how probiotics colonize the intestine, alter microbial ecosystems, or interact synergistically with prebiotics.

Additionally, systematic research on the compatibility and synergistic effects between probiotics and prebiotics is lacking. Current selection processes for prebiotics compatible with probiotics are simplistic, limiting their ability to selectively promote probiotic proliferation [158]. There is also a need for deeper investigation into the biological activity of oligosaccharides with varying molecular weights and degrees of polymerization.

3.1.4. Postbiotics

The concept of postbiotics was first introduced in 2013. In 2021, the International Scientific Association for Probiotics and Prebiotics (ISAPP) defined postbiotics in Nature

Reviews Gastroenterology & Hepatology as "preparations of non-living microorganisms and/or their components that confer health benefits to the host" [159,160].

Postbiotics are defined as non-viable microbial formulations derived from inactivated microbial cells, bacterial cellular components, and crude metabolic byproducts generated during fermentation processes [161,162]. These compounds are primarily sourced from probiotic microorganisms spanning diverse genera and species, though not all probiotic strains are suitable for postbiotic production, necessitating a stringent selection process. Currently, only microbial strains that pass rigorous safety assessments—including bacteria (e.g., *Lactobacillus* spp., such as *L. plantarum*, *L. fermentum*, and *L. rhamnosus*; *Bifidobacterium* spp., like *B. bifidum* and *B. animalis*; and *Enterococcus*, *Bacillus*, and *Pseudomonas* strains) and fungi (primarily yeast)—are utilized. Crucially, postbiotics exclude inactivated pathogens, viruses, or isolated microbial metabolites.

Chemically, postbiotics encompass a heterogeneous mixture of bioactive components, including the following [163]:

1. Structural cell elements: e.g., peptidoglycan, teichoic acids, and cellular debris from lysed probiotics.
2. Metabolic products such as short-chain fatty acids (SCFAs: acetate, propionate, butyrate), organic acids (e.g., lactate), enzymes (proteases, lipases), antimicrobial agents (e.g., bacteriocins), vitamins (B-complex, vitamin K), and antioxidants (catalase, superoxide dismutase).
3. Other bioactive molecules, like nucleotides and their derivatives.

The selection of microbial strains for postbiotic synthesis prioritizes safety, stability, and functionality, often favoring those with a safe status. Postbiotics are distinguished from purified microbial metabolites or single-compound extracts, as their efficacy is attributed to synergistic interactions among their complex components. Current research focuses on their applications in gut microbiota modulation, immune enhancement, anti-inflammatory responses, and metabolic regulation, particularly through SCFA-mediated intestinal barrier reinforcement. However, challenges remain in standardizing production protocols and elucidating mechanistic synergies among constituent molecules.

Postbiotics offer similar health benefits to live probiotics but have several advantages:

1. Defined Chemical Structure and Stability: Postbiotics have a clear chemical composition, greater stability, and a longer shelf life [164];
2. High Safety Profile: Since postbiotics are non-living, they cannot acquire virulence genes or transmit antibiotic resistance, reducing the risk of producing harmful metabolites. This makes them safer for consumption, and they are effective at lower doses [165].
3. Lower Risk for Immunocompromised Hosts: Active probiotics require large doses to be effective, which may cause adverse reactions in immunocompromised individuals. Postbiotics, however, are safer and cause minimal impact even in higher amounts [163].
4. Ease of Storage and Transport: Postbiotics do not need to maintain viability, allowing them to remain stable across a wide range of temperatures and pH levels. This makes them easier to store and transport [166].
5. Environmental Resilience: Postbiotics retain their beneficial effects under extreme environmental conditions, such as high temperatures or exposure to digestive enzymes [167].

Postbiotics are mainly related to immune regulatory activity, which can stimulate innate and adaptive immune systems, stimulate the generation and differentiation of immune cells, release inhibitory inflammatory cytokines, and thus exert anti-inflammatory activity. Postbiotics can [168] attach to intestinal epithelial cells and directly regulate

immune function; microfold cells located in the epithelium, which are transported to immune cells; bind with dendritic cells, leading to the activation of cascade reactions, stimulating immune responses. Research has shown that adding endogenous elements to feed can promote the growth of aquatic animals by increasing their food intake, protein content, and energy, improving feed utilization efficiency and thereby achieving the goal of promoting growth; increasing the length of intestinal villi in aquatic animals, thus increasing the absorbent surface area, improving nutrient utilization efficiency and thereby enhancing growth performance [169]; and adjusting the quantity of beneficial bacteria in the intestine to maintain the balance of gut microbiota, improve nutrient absorption and feed utilization, and promote body growth [166].

Postbiotics can regulate beneficial gut microbiota, increase intestinal mucosal thickness and villus length, strengthen the body's natural defense mechanisms, and have an important impact on maintaining gastrointestinal health. For example, heat inactivated *Lactobacillus acidophilus* reduces diarrhea caused by *Escherichia coli* through space-occupying competition [169].

Postbiotics, via their bioactive components, regulate intestinal immune responses to boost mucin production. These mucins support symbiotic bacteria by providing adhesion sites and nutrients while inhibiting pathogens, restoring the gut microbiota balance, and preventing dysbiosis-related diseases [170]. A case in point is Stress–Worry-Free Concentration® (SWFC), a postbiotic derived from *Cetobacterium somerae* and Lactococcus lactis supernatants. A study on *Cyprinus carpio* fed high-fat diets (HFDs) evaluated SWFC's effects (0, 0.2, and 0.3 g/kg supplementation over 98 days) [171].

Though growth remained unaffected, SWFC improved health markers:

Skin mucosa: Enhanced C3 and antioxidant capacity (T-AOC, SOD) and reduced lipid peroxidation (MDA) ($p < 0.05$).

Liver health: The 0.2 g/kg dose significantly lowered serum ALT, AST, and hepatic triglycerides ($p < 0.05/0.01$), suppressed pro-inflammatory genes (TNF-α, IL-1β) and lipid synthesis genes (ACC, FAS, PPAR-β/γ), and upregulated anti-inflammatory TGF-β.

Gut microbiota: SWFC restored the microbial equilibrium, mitigating HFD-induced oxidative stress and inflammation [165].

In summary, postbiotics like SWFC reduce feed costs, enhance preservation, and improve animal health, offering a sustainable solution for advancing aquaculture productivity and addressing intensive farming challenges.

3.2. Antimicrobial Peptides

Antimicrobial peptides (AMPs) are small molecules with antibacterial properties. Compared to antibiotics, AMPs are less likely to lead to resistance and can be considered potential treatments for antibiotic-resistant bacteria [168]. They promote fish growth, enhance immunity, and increase disease resistance. AMPs offer a promising alternative in the livestock industry to combat antibiotic overuse. Originating from a variety of life forms, they possess antibacterial, antiviral, and antifungal properties. In aquaculture, adding AMPs to fish feed can enhance fish health and improve resistance to pathogens [172] (Figure 4).

Among the most studied AMPs are Hepcidin and Cecropin [173]. Research shows that AMPs can inhibit a variety of pathogens [174], including *Aeromonas hydrophila*, and improve fish growth and antioxidant response to better understand their effects on fish gut microbiota [175].

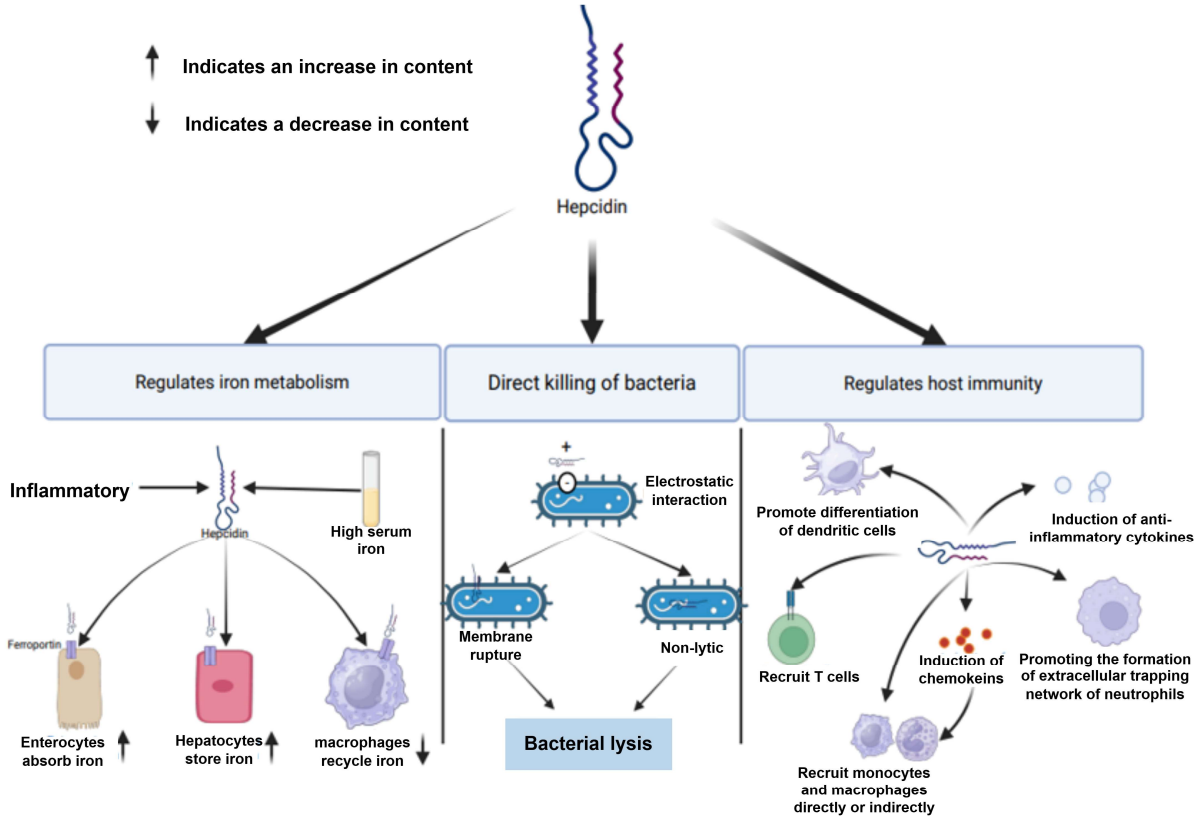


Figure 4. Antimicrobial mechanisms diagram of Hepcidin.

Hepcidin's primary functions include regulating iron metabolism during immune responses by limiting the supply of iron to bacteria, thus restricting pathogen growth; exhibiting direct antibacterial activity to kill pathogens; and modulating host immunity through interactions with the host immune system [176]. In aquaculture, Hepcidin may act as an immune modulator and an antibacterial therapeutic. Studies have shown that fish eggs expressing Hepcidin can serve as a novel feed supplement, enhancing the immunity of zebrafish against pathogenic infections [177]. Although Hepcidin shows potential as an antibiotic replacement, challenges remain in its practical application, such as its instability in terms of hemolysis and protein hydrolysis [178]. For example, in grass carp, Hepcidin inhibits bacterial growth through two mechanisms: damaging bacterial cell membranes and interacting with bacterial genomic DNA (gDNA). Other antimicrobial peptides, such as tetracycline, NK-18, and bacteriocins, also kill bacteria by binding to DNA. Research indicates that Hepcidin-25 (a mature peptide) and Hepcidin-20 (a truncated peptide) exhibit significant bactericidal activity against both Gram-positive and Gram-negative bacteria, partially through membrane disruption and binding to gDNA. The disruption of bacterial cell membranes by grass carp Hepcidin is a new discovery, as other Hepcidins do not exhibit this function. The antimicrobial peptide NK-18 kills bacteria by disrupting cell membranes and binding to DNA [177]. This dual-target strategy makes it difficult for bacteria to develop resistance to these peptides [179]. Therefore, Hepcidin may be a promising candidate for treating bacterial infections. Additionally, iron regulation promotes antibacterial defense by directly killing microorganisms and regulating iron metabolism in grass carp.

One previous study has shown that Hepcidin may also exert strong antibacterial effects in vivo through synergistic interactions with other inducible acute phase response proteins or tissue-specific antibacterial compounds, such as moxorubicin [180]. However, further research is needed to explore whether there are iron-coordinated molecules present in grass carp. As expected, the prophylactic or therapeutic administration of Hepcidin increased

the survival rate of grass carp infected with lethal doses of *Aspergillus niger*, accompanied by a reduction in bacterial numbers in the fish's foregut and spleen. The therapeutic effect of grass carp Hepcidin was found to be more effective than its preventive use [181].

In the future, it is necessary to continuously study the function of Hepcidin and optimize its expression system to obtain more easily applicable antimicrobial peptides.

4. Conclusions and Future Perspective

Current regulatory frameworks in some regions approve probiotics based solely on preliminary assessments of antibacterial activity and immunostimulatory potential. However, a 2017 U.S. FDA (Silver Spring, MD) inspection revealed >50% of probiotic manufacturers violated protocols, primarily through strain misidentification and microbial contamination, jeopardizing product safety and efficacy [182]. While antibiotic alternatives demonstrate growth-promoting potential, their performance exhibits geographic variability due to divergent farming practices, regional disease profiles, and management heterogeneity.

This review systematically analyzes microecologics (definition, mechanisms, case studies) in cyprinid aquaculture, highlighting safety concerns as the primary constraint for large-scale adoption. We emphasize science-driven development requiring the rigorous validation of novel formulations.

Sustainable aquaculture advancement necessitates integrated strategies beyond singular additives:

1. Precision nutrition.
2. Biosecurity-enhanced management.
3. Water quality optimization.
4. Preventive health protocols.

Such multidisciplinary approaches synergistically enhance host resilience, reduce disease incidence, and minimize therapeutic reliance, ultimately ensuring the eco-friendly production of premium aquatic products. Though certain additives show promise through dual antimicrobial–nutritive functions, realizing their full potential requires collaborative innovation among farmers, nutritionists, and microbiologists. Critical research priorities include

1. Cost–benefit analyses.
2. Dose–response relationships.
3. Environmental fate assessments.
4. Smallholder applicability.

Immunometabolic modulation mechanisms in practical applications that align aquaculture practices with international guidelines for antimicrobial resistance (AMR), management, and the responsible use of feed additives—including antibiotics and their alternatives—are fundamental for reducing the risk of AMR. Research should prioritize fish health with a strong focus on disease prevention. Key areas of focus should include the following:

1. Evaluating the efficacy and safety of feed additives under varying environmental conditions.
2. Assessing the environmental impact and alternative methods for antimicrobial agents.
3. Implementing the active and passive monitoring of drug withdrawal periods, wastewater treatment, residues, and AMR detection.

By focusing on these areas, aquaculture can adopt more sustainable practices, improve fish health, minimize AMR risks, and secure the long-term sustainability of aquaculture.

A major limitation of this article is its exclusive focus on antibiotic residues in freshwater cyprinid fish. The article primarily addresses strategies to improve the internal health of fish and enhance their immunity to achieve antibiotic-free aquaculture. However, it does not provide detailed information on salt-tolerant cyprinid species or discuss the interactions between beneficial bacteria and environmental factors that could improve external

water conditions. Additionally, the article does not explore how nutritional strategies could further enhance the immune systems of cyprinid fish.

These gaps will be addressed in future research.

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