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Evaluation of Probiotic Applications in Healthy Farming of Grass Carp (*Ctenopharyngodon idella*)

Xiahong Yang [1,2]
1 Yuanhua Town Yangli Family Farm of Haining City, Haining 314416, Zhejiang, China
2 Zhejiang Agronomist College, Hangzhou, 310021, Zhejiang, China
Corresponding author: 2392110213@qq.com

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Abstract Probiotics have emerged as an important strategy for promoting sustainable and healthy aquaculture by improving growth performance, enhancing immunity, and maintaining ecological balance. Grass carp (*Ctenopharyngodon idella*) is one of the most widely cultured freshwater fish species; however, intensive farming practices often lead to microbial imbalance, disease outbreaks, and reduced production efficiency. This review summarizes the application effects of probiotics in grass carp aquaculture, focusing on their regulatory mechanisms and practical benefits. Probiotics can improve feed utilization, stimulate digestive enzyme activities, maintain intestinal microbial homeostasis, and enhance nutrient absorption. Moreover, they contribute to improved antioxidant capacity, immune responses, and resistance against bacterial pathogens. The application of probiotics also plays an important role in regulating water quality by reducing harmful microorganisms and promoting environmentally friendly aquaculture systems. A case study of compound probiotic preparations demonstrates their potential in improving growth performance, intestinal microbiota composition, disease resistance, and economic benefits. Despite these advantages, challenges remain regarding strain selection, functional stability, and precise application strategies. Future research integrating omics technologies and targeted microbial regulation will further promote the development of probiotic-based green aquaculture technologies for grass carp production.

Keywords Probiotics; Grass carp (*Ctenopharyngodon idella*); Intestinal microbiota; Immune regulation; Healthy aquaculture

1 Introduction

Grass carp (*Ctenopharyngodon idella*) is one of the most important freshwater aquaculture species, with very large global production and particular importance in China as an affordable source of aquatic animal protein (Gharti et al., 2023). It is also described as an important economic fish whose germplasm resources are strategically important for the stable supply of high-quality aquatic protein and for the sustainable development of the industry. Projections indicate that grass carp production in China alone may exceed 6.4 million tons by 2030, underscoring the continuing expansion and economic weight of this farming sector. At the same time, aquaculture more broadly remains the fastest-growing animal food production sector and is under increasing pressure to provide food security while improving environmental performance (Fachri et al., 2024). This tension is especially relevant to grass carp culture, because production growth has been accompanied by rising concern over pollution, eutrophication, and resource use, with life-cycle assessment showing that feed processing and water pollution are major contributors to the environmental burden of commercial grass carp production. Farm-level studies also show that culture environment strongly shapes production outcomes: poor pond water quality reduces output, product quality, and profit, whereas improved systems and management can enhance fish quality. Because grass carp is an herbivorous species with distinctive nutritional physiology and high forage intake capacity, healthy farming must integrate suitable feeding, water-quality maintenance, welfare, and disease prevention rather than focus on yield alone (Pedrazzani et al., 2022). This broader healthy-aquaculture perspective is reinforced by welfare-based work showing that nutritional and environmental conditions are directly linked to stress resistance and gut health in cultured grass carp. Even relatively simple production studies in ponds indicate that grass carp can achieve strong growth under different plant-based feeding regimes, but survival, feed conversion, and water-quality stability depend on how culture conditions are managed. Together, these findings show that the future of the grass carp industry depends not only on maintaining high output, but on shifting from conventional expansion toward healthy aquaculture that balances productivity, fish welfare, environmental control, and product safety.

Despite its scale and value, grass carp farming faces persistent health problems that are intensified by high-density culture and environmental stress. Across aquaculture systems, disease outbreaks remain a major constraint because intensification increases host susceptibility and pathogen transmission. In grass carp specifically, bacterial diseases remain a major threat, and *Aeromonas hydrophila*-associated enteritis alone has been reported to cause substantial economic losses estimated at $164 million annually. More generally, the rapid development of aquaculture has often been accompanied by environmental degradation, disease emergence, and reduced productivity, creating strong pressure for better health management strategies. Historically, farmers have relied on antibiotics, chemotherapeutics, disinfectants, and other medicinal products to suppress these problems. However, the widespread and sometimes indiscriminate use of these agents has created serious concerns about drug residues, environmental contamination, and selection for antimicrobial resistance in aquaculture settings. Reviews focused on China further show that livestock farming and aquaculture are major areas of antibiotic misuse, with consequences including residue pollution and heightened risks of antibiotic resistance affecting animals and humans. Internationally, antibiotic governance remains uneven: many countries still permit critically important antibiotics in aquaculture, and compliance with international recommendations and certification standards is often incomplete (Luthman et al., 2024). Surveys from freshwater aquaculture likewise indicate that heavy dependence on aquaculture medicinal products can leave gaps in disease management while contributing to public-health and ecosystem risks. At the policy level, current regulatory thinking increasingly emphasizes stricter enforcement, species-appropriate supporting measures, and the development of substitutes for antibiotics rather than continued dependence on them. For grass carp farming, these regulatory and health pressures converge into a practical challenge: producers need disease-control strategies that are effective under pond conditions, compatible with food-safety expectations, and less likely to aggravate antimicrobial resistance or environmental pollution.

Within this context, probiotics have emerged as a promising green technology for healthy aquaculture because they offer a biological route to improve fish performance while reducing reliance on antibiotics and other chemical inputs. Recent reviews consistently describe probiotics as beneficial microorganisms that can enhance growth, feed utilization, immunity, disease resistance, gut microbial balance, and water quality in cultured aquatic animals. Their mechanisms are multifactorial: probiotics can modulate the intestinal microbiota, strengthen innate immune responses, produce antimicrobial substances such as bacteriocins and organic acids, and promote the breakdown of organic matter and toxic metabolites in the rearing environment. These properties make them especially relevant to healthy grass carp culture, where gut health, nutritional efficiency, stress resistance, and pond-water quality are tightly linked. Broad aquaculture syntheses therefore regard probiotics as eco-friendly alternatives to antibiotics that can support disease prevention and environmental sustainability at the same time. Interest has also shifted toward host-associated probiotic strains, because microbes derived from the host or its environment can improve digestion, inhibit pathogen colonization, and stimulate hematological and immune responses more specifically. Among candidate taxa, Bacillus spp. have drawn particular attention because they are non-pathogenic, stress tolerant, and associated with improvements in feed utilization, antioxidant defense, immune function, water quality, and disease resistance. Field evidence from freshwater farming also suggests that probiotic and fermented products can reduce disease outbreaks, improve fish growth, lower costs, and decrease dependence on conventional medicinal products. More recent syntheses place probiotics within the wider response to antimicrobial resistance, arguing that they are integral to sustainable aquaculture and to the search for non-antibiotic disease-control strategies. Although practical issues remain, including host specificity, formulation stability, delivery methods, and commercialization, the overall evidence supports probiotics as one of the most promising tools for advancing healthy grass carp farming. Accordingly, evaluating probiotic applications in grass carp is scientifically and practically important for developing culture systems that are more productive, disease-resilient, environmentally responsible, and aligned with the regulatory transition toward greener aquaculture technologies.

2 Types of Probiotics and Their Mechanisms of Action

2.1 Common probiotic species used in aquaculture and their functional characteristics

Probiotics used in aquaculture span several bacterial and yeast groups, including Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Carnobacterium, Shewanella, Bacillus, Aeromonas, Pseudomonas, and Saccharomyces, showing that fish probiotics are taxonomically broad rather than limited to a single lineage. Among these, *Bacillus* spp. are the most consistently emphasized in aquaculture because spore formation improves survival under harsh culture and feed-processing conditions, while their strains are generally described as non-pathogenic and capable of producing antimicrobial compounds (Kuebutornye et al., 2019). Bacillus is also valued as both a gut probiotic and a pond bioremediator, since it can degrade organic detritus, reduce nitrogenous waste, and in some species disrupt quorum sensing by degrading AHL signals associated with pathogen virulence (James et al., 2021). Lactic acid bacteria form the second major group, and indigenous finfish LAB such as Lactobacillus, Lactococcus, Pediococcus, Enterococcus, Weissella, and related genera are regarded as promising because they stimulate digestive function, mucosal tolerance, immune activity, and disease resistance.

Functional selection depends less on genus name alone than on strain-level traits relevant to aquaculture performance. Candidate strains are commonly screened for safety, non-hemolytic behavior, survival under acid and bile exposure, adhesion to mucosal surfaces, extracellular enzyme production, antioxidant capacity, and direct antagonism against pathogens (Coulibaly et al., 2023). In LAB, these traits have been demonstrated in host-associated isolates that survive simulated gastrointestinal conditions, form biofilms, and inhibit multiple pathogens, while also contributing enzymes such as lipases or β-galacto-sidase that can support digestion. In grass carp and other fish, host-derived strains are increasingly preferred because native probiotics tend to colonize the gut more efficiently and are less likely to disturb microbiota homeostasis than exogenous strains (Chomová et al., 2025). This shift has expanded the range of candidate probiotics beyond classical Bacillus and LAB to include autochthonous strains such as *Pseudomonas monteilii* and *Cetobacterium somerae*, both of which have recently shown probiotic potential in grass carp.

2.2 Microbiota regulation

The grass carp intestine contains a complex bacterial community involved in nutrition, host physiology, and immune-related processes, so probiotic action in this species depends first on reshaping an existing microbial ecosystem rather than simply adding one beneficial strain. Dietary probiotics can alter this ecosystem at the community level, and direct feeding trials in grass carp show that oral administration changes bacterial composition at the genus level within weeks. A recurring mechanism is selective enrichment of beneficial taxa: probiotic treatment increased *Cetobacterium* in grass carp, a genus linked to potential immune function, while also favoring other putative beneficial groups such as Streptococcus and Enterococcus in the intestine (Hao et al., 2017). At the same time, probiotics suppress undesirable members of the microbiota, as shown by reduced intestinal abundance of potential pathogens including Pseudomonas and Flavobacterium after probiotic feeding.

Microbiota regulation also affects digestive ecology, barrier function, and network stability. In grass carp fed Bacillus subtilis Ch9, probiotic supplementation increased total anaerobes as well as Lactobacillus and Bifidobacterium, supporting the view that Bacillus can promote cross-feeding and a more favorable microbial balance instead of acting alone. More recent work with host-derived *Pseudomonas monteilii* JK-1 suggests that probiotic benefits can be mediated through keystone taxa and microbiome network stability, with Cetobacterium associated with growth performance and Akkermansia associated with immune response (Qi et al., 2024). Probiotics can also support intestinal structure directly: *Cetobacterium somerae* improved digestive and absorptive capacity in juvenile grass carp, reduced intestinal permeability, and preserved tight-junction integrity, indicating that microbiota modulation and epithelial protection are mechanistically linked. This mechanism has limits under environmental stress, however, because nanoplastic exposure in grass carp weakened probiotic protection and altered microbial diversity despite measurable immune activation.

2.3 Immune and disease mechanisms

Probiotics enhance fish disease resistance chiefly through innate immune activation, especially by increasing phagocytic activity, lysozyme production, complement activity, respiratory burst, and cytokine expression. In finfish, lactic acid bacteria often increase neutrophil activity, lysozyme secretion, phagocytosis, and pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, and TNF-α, although some strains instead preferentially induce IL-10, showing that immune effects are strain and host dependent rather than uniform (Ringø et al., 2018). This host- and strain-specificity is a central feature of probiotic immunobiology in aquaculture, and immune outcomes vary with source, dose, and duration of supplementation. Beyond soluble effectors, probiotics can stimulate gut-associated immunity, including increases in Ig-positive cells and acidophilic granulocytes, thereby strengthening mucosal defense at a major portal of pathogen entry.

Experimental studies show that these mechanisms translate into higher survival after pathogen challenge. In fish fed Lactococcus lactis, probiotic supplementation increased growth and survival, upregulated anti-inflammatory cytokines, and moderated excessive pro-inflammatory cytokine responses after Aeromonas hydrophila infection. In goldfish, Exiguobacterium acetylicum elevated respiratory burst, phagocytosis, antimicrobial enzymes, immunoglobulin levels, and cytokine gene expression, increasing post-challenge survival from 33.2% in controls to 73.2% in the high-dose group. In grass carp specifically, *Pseudomonas monteilii* JK-1 reduced Aeromonas load while increasing IL-1β, IL-10, TNF-α, TGF-β, and antioxidant enzymes in gut and head kidney, linking microbial regulation with coordinated immune and oxidative-stress control. Bacillus-based products can extend beyond classical probiotics into adjuvant-like platforms, since recombinant B. subtilis spores have enhanced survival and specific IgM and IgZ responses in virus-challenged grass carp, underscoring the broader immunobiological value of Bacillus in healthy aquaculture (Soltani et al., 2019). In grass carp, probiotics therefore act through three connected mechanisms: functional taxa selection, intestinal barrier stabilization, and immune priming . These mechanisms make Bacillus, LAB, and host-derived strains especially relevant for antibiotic-reduced and health-oriented grass carp farming.

3 Effects of Probiotics on Growth Performance of Grass Carp

3.1 Effects of probiotics on feeding behavior and feed utilization efficiency

Probiotics improve feed utilization in grass carp primarily by strengthening digestive capacity rather than by simply increasing feed intake. In a 6-week trial, a duo-strain preparation of Bacillus subtilis and Lactobacillus plantarum increased protease, amylase, lipase, and trypsin activities while also lowering feed conversion ratio, indicating more efficient digestion and nutrient use (Luo et al., 2022). A broader review of aquaculture nutrition supports the same mechanism, concluding that probiotics enhance intestinal microbial balance, digestive enzyme activity, food absorption, and ultimately feed efficiency when properly applied.

Evidence from grass-carp-specific trials shows that this nutritional effect can occur with or without major changes in voluntary feed intake. Juvenile fish receiving *Cetobacterium somerae* showed significantly higher feed intake together with improved feed conversion ratio and feed efficiency ratio, suggesting that this host-associated strain promoted both appetite and utilization efficiency (Figure 1) (Chen et al., 2025). By contrast, a multi-strain probiotic improved growth and feed utilization most clearly at 0.34-1.68 g/kg, implying that the quality of nutrient conversion, not maximum consumption alone, explains much of the probiotic growth response.

3.2 Growth parameters and productivity

Across studies, probiotic supplementation consistently improves core growth indicators in grass carp, especially weight gain, specific growth rate, and feed conversion ratio. Dietary supplementation with Bacillus subtilis Ch9 for 56 days significantly increased specific growth rate and reduced feed conversion ratio relative to the unsupplemented control. Similar benefits were reported in a 2024 study of host-derived *Pseudomonas monteilii* JK-1, which significantly increased weight gain, specific growth rate, and survival rate while reducing pathogen burden, linking better productivity to better overall health status.

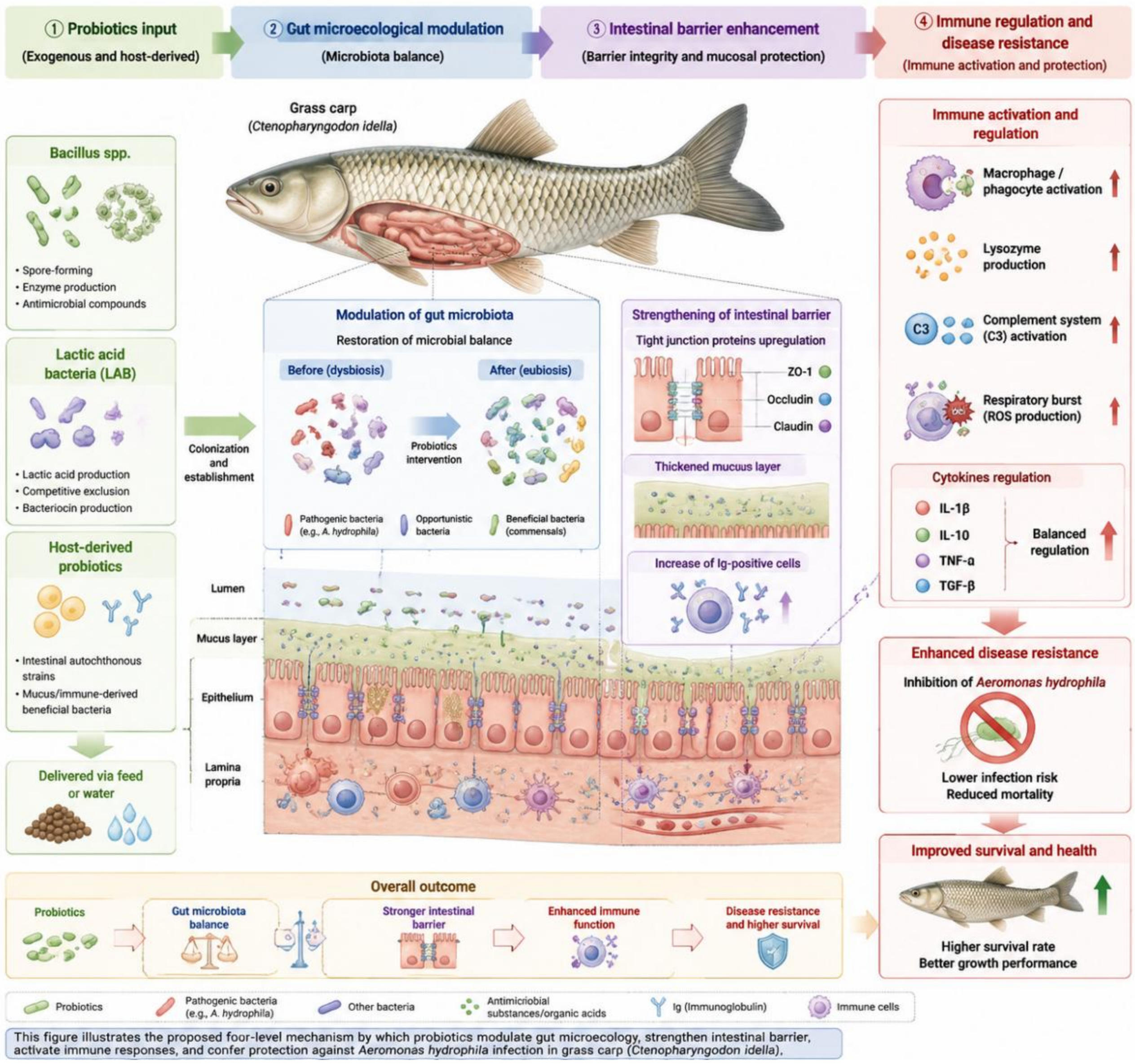

Figure 1 Mechanisms of probiotic-mediated immune enhancement and disease resistance in grass carp (*Ctenopharyngodon idella*)

The productivity benefit is not limited to one probiotic type or one production stage. In fingerling grass carp, diets containing *Bacillus coagulans*, *Rhodopseudomonas palustris*, or *Lactobacillus acidophilus* all increased final weight, daily weight gain, and relative growth rate compared with the control, although differences among the three probiotic treatments were not significant. Likewise, probiotic-fed groups in a comparative feeding study outperformed controls in weight gain and specific growth rate, and the probiotic treatment also improved protein efficiency and food conversion indices, which are directly relevant to aquaculture productivity and cost control (Abdallah et al., 2022).

3.3 Species, dose, and duration

Growth responses depend strongly on probiotic species and dosage, and the best-performing level is often intermediate rather than maximal. With *Bacillus natto*, fish in the Bn3-Bn5 groups showed better growth and lower feed conversion ratio than lower-dose groups and controls, suggesting a threshold concentration is needed before performance gains become clear. However, the response is not indefinitely linear: in grass carp fed host-derived Bacillus velezensis LH023, growth improved overall, but after week 5 the 10^8 group grew more slowly than the 10^7 group, indicating that excessive probiotic concentration can reduce the benefit (Liu et al., 2025).

Duration also shapes probiotic outcomes, because physiological gains can appear early and then plateau over longer feeding periods. In the Ch9 trial, digestive enzyme activities rose from days 14 to 56, but the increase was greatest in the shorter term and no longer continued upward by day 56, even though values remained above control fish. A similar pattern of dose optimization was quantified in other studies: broken-line analysis identified about 1.34 g/kg as the optimum level for a multi-strain probiotic, while C. somerae produced the best overall growth, intestinal development, and nutrient retention at about 1.27-1.35 × 10^9 cells/kg rather than at the highest inclusion level (Chen et al., 2025). Overall, probiotics improve grass carp growth performance mainly by enhancing digestive efficiency, nutrient retention, and feed conversion, but the magnitude of benefit depends on the probiotic strain, supplementation level, and feeding duration. For grass carp farming, the evidence supports probiotics as effective growth promoters when matched carefully to species-specific and dose-specific conditions.

4 Regulatory Effects of Probiotics on the Digestive System and Nutrient Metabolism of Grass Carp

4.1 Effects of probiotics on intestinal structure integrity and functional improvement

Probiotics improve intestinal structural integrity in grass carp by promoting mucosal development and strengthening epithelial barrier function. In juvenile grass carp, dietary *Cetobacterium somerae* increased intestinal length index, somatic index, and fold height, indicating better intestinal development and a larger absorptive surface. The same study further showed that this probiotic reduced intestinal permeability, preserved tight-junction ultrastructure, and increased tight-junction and adherens-junction biomarkers, supporting a direct role in barrier maintenance rather than only a secondary growth effect (Chen et al., 2025).

Barrier protection is also evident with other host-associated probiotics and under challenge conditions. Bacillus velezensis LH023 increased expression of ZO-2, ZO-3, and claudin-12 in the intestine of grass carp, which was interpreted as reduced mucosal permeability and improved protective function (Liu et al., 2025). In infected carp, compound probiotics alleviated villus swelling and prevented the decline of Occludin, Claudin-1, and ZO-1, showing that probiotic barrier support can persist even during pathogen-induced intestinal injury.

4.2 Digestive enzymes and absorption

Probiotics enhance digestive enzyme activity in grass carp across multiple enzyme systems, which helps explain improvements in nutrient digestion and feed use. Feeding *Bacillus subtilis* Ch9 increased protease, amylase, and lipase activities in the intestine and hepatopancreas over 14-56 days, with the best overall response reported at 3 × 10^9 CFU/kg feed (Wu et al., 2012). Similar species-dependent effects were reported in fingerlings given *Bacillus coagulans*, *Rhodopseudomonas palustris*, or *Lactobacillus acidophilus*, where all probiotics improved growth but B. coagulans produced the strongest increase in protease activity.

Improved enzyme activity is closely tied to stronger absorptive function. *C. somerae* enhanced both digestive enzymes and brush-border enzymes in juvenile grass carp, and these changes were accompanied by improved nutrient retention, indicating more effective digestion-to-absorption transfer. This pattern is consistent with broader fish evidence showing that probiotic supplementation improves intestinal microbial balance, digestive enzyme activity, and food absorption, thereby increasing feed efficiency rather than acting only as a passive microbial additive (Assan et al., 2021).

4.3 Metabolism and energy utilization

The metabolic effects of probiotics in grass carp extend beyond digestion to the regulation of lipid deposition, carbohydrate use, and microbial energy metabolism . In fish fed a high-fat diet, *Bacillus subtilis* improved growth and serum biochemical indices while reducing hepatic lipid accumulation, indicating that probiotics can redirect nutrient partitioning away from fatty liver formation (Guo et al., 2022). In parallel, intestinal microbiota studies show that the grass carp gut microbiome itself contributes substantially to nutrition metabolism, with carbohydrate metabolism increasing along the intestine and the hindgut acting as a major site of fiber fermentation that helps the host obtain nutrients and energy from plant material.

Probiotics appear to influence these metabolic processes partly by remodeling microbiota-linked pathways. In grass carp receiving *B. velezensis* LH023, KEGG analysis showed enrichment of pathways related to carbohydrate, amino acid, lipid, cofactor, and vitamin metabolism, suggesting coordinated improvement of nutrient transformation capacity. A complementary signal comes from *C. somerae*, which increased intestinal acetic acid concentration in juvenile grass carp, consistent with enhanced microbial fermentation and better local energy supply for intestinal development and absorption (Chen et al., 2025). Overall, probiotics regulate the grass carp digestive system through barrier reinforcement, enzyme activation, and metabolic reprogramming . These coordinated effects help explain why probiotic supplementation often improves nutrient utilization, intestinal resilience, and growth performance in healthy farming systems.

5 Effects of Probiotics on Immune Function and Disease Resistance of Grass Carp

5.1 Regulatory effects of probiotics on non-specific immune responses

Probiotics enhance the non-specific immune system of grass carp by stimulating humoral and cellular defense indicators that respond rapidly to infection. In a 60-day feeding trial, a multi-strain probiotic increased plasma myeloperoxidase activity and complement C3 content at higher inclusion levels, showing that immune enhancement can be dose dependent rather than identical across all supplementation levels. A separate study using autochthonous intestinal bacteria found that probiotic-fed grass carp had higher respiratory burst, phagocytic activity, and lysozyme activity than controls, together with increased complement C3, total serum protein, albumin, and globulin, indicating broad activation of innate defense pathways.

This immune stimulation is also reflected at the gene-expression level in multiple immune organs. Feeding *Pediococcus pentosaceus* SL001 significantly upregulated IgM and C3 expression in the liver, spleen, and head kidney while downregulating IL-8, suggesting stronger baseline defense with lower inflammatory burden (Gong et al., 2019). Similarly, Bacillus velezensis B8 increased serum alkaline phosphatase and SOD activity, and after 3-4 weeks it significantly upregulated IgM and TNF-α in the spleen and kidney, showing that probiotic immunoregulation can intensify over time rather than appear only immediately after feeding begins (Wu et al., 2021).

5.2 Effects of probiotics on antioxidant capacity and stress responses

Probiotics improve antioxidant capacity in grass carp by increasing key enzymatic defenses and reducing oxidative damage. Under *Aeromonas hydrophila* challenge, dietary Bacillus subtilis reduced malondialdehyde while increasing total antioxidant capacity, SOD, CAT, GSH, and the expression of antioxidant enzymes including SOD, CAT, and GPx, indicating protection against infection-induced oxidative stress. Comparable effects were observed with duo-strain supplementation of *B. subtilis* and Lactobacillus plantarum, which significantly improved SOD and CAT and lowered MDA during a 6-week feeding period, showing that antioxidant benefits are not limited to single-strain preparations (Luo et al., 2022).

These antioxidant effects appear closely linked to stress resilience and intestinal protection. In juvenile grass carp, dietary probiotics including *Bacillus cereus*, *B. subtilis*, *Paracoccus marcusii*, and *Lactobacillus plantarum* elevated serum C3/C4 or AKP, lowered intestinal MDA, and in the Bacillus groups activated the Nrf2 pathway while upregulating CAT and SOD-related genes, which supports a mechanistic role for antioxidant signaling (Xue et al., 2020). In the multi-strain trial, probiotic-fed fish also showed lower liver MDA, higher glutathione peroxidase, and a significantly stronger respiratory burst after hypoxia stress, indicating that probiotic-enhanced redox control can translate into better tolerance of environmental stressors.

5.3 Roles of probiotics in enhancing disease resistance and reducing disease incidence

A consistent outcome across studies is that probiotics increase post-challenge survival and reduce disease-related mortality in grass carp. Host-derived *Pseudomonas monteilii* JK-1 significantly increased survival rate and reduced Aeromonas pathogen load, while also upregulating IL-1β, IL-10, TNF-α, and TGF-β in the gut and head kidney, indicating that improved resistance is coupled to coordinated immune activation rather than pathogen suppression alone. Likewise, dietary supplementation with Lactobacillus buchneri L3-9 or its extracellular products significantly reduced mortality after *A. hydrophila* challenge, and the probiotic treatments also decreased Aeromonadaceae and Enterobacteriaceae abundance in the intestine, linking disease control to microbial exclusion of potential pathogens (Gao et al., 2024).

The disease-resistance effect extends across diverse probiotic taxa and delivery strategies. Autochthonous intestinal bacteria reduced cumulative mortality after A. hydrophila challenge from 80% in controls to 26.67% in the mixed-strain group, showing that consortia can outperform single strains in protection efficiency. Additional lower-ranked but relevant studies support the same pattern: Paenibacillus polymyxa S3 increased survival after *A. hydrophila* infection while upregulating C3, lysozyme, IgM, TLR-4, and MyD88. *Bacillus* methylotrophicus XA-8 also reduced *A. hydrophila* virulence by downregulating aer, act, hylA, lafA, hcp, and luxS, and supplemented fish showed 52.63% infection resistance together with lower intestinal Aeromonas abundance (Figure 2) (Khan et al., 2024). Overall, probiotics strengthen grass carp health by improving innate immunity, antioxidant defense, and pathogen resistance . The evidence across many strains supports probiotics as a practical strategy for reducing disease incidence and lowering dependence on antibiotics in healthy grass carp farming.

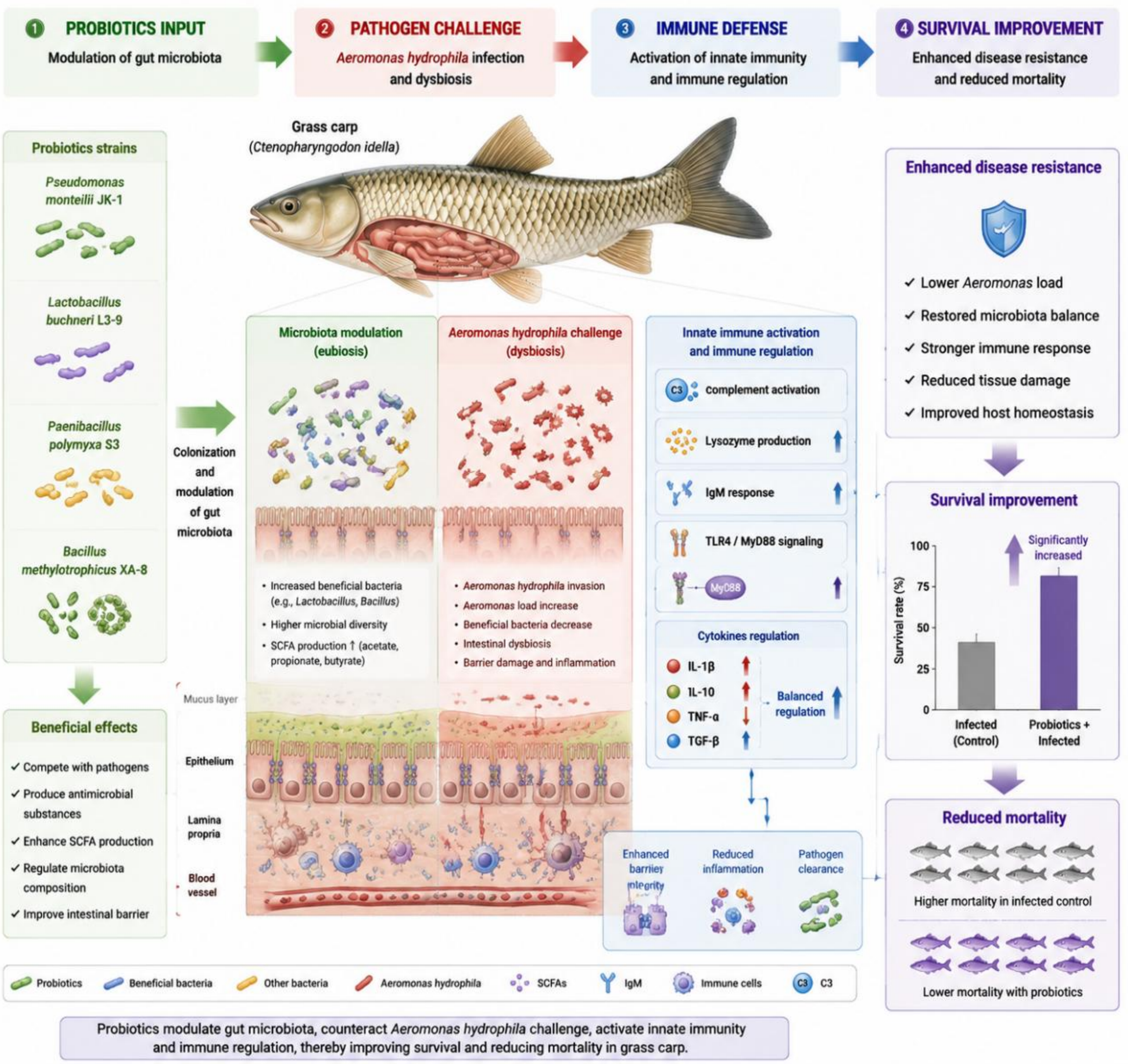

Figure 2 Mechanisms of probiotic-mediated enhancement of disease resistance in grass carp following pathogen challenge

6 Application Effects of Probiotics in Environmental Regulation of Grass Carp Aquaculture

6.1 Effects of probiotics on improving physicochemical properties of aquaculture water

Probiotics can improve the physicochemical properties of grass carp culture water, especially by reducing nitrogenous wastes that drive stress and eutrophication. In grass carp culture, complex probiotics lowered ammonia nitrogen, nitrite nitrogen, and total nitrogen throughout the experimental period, and nitrate nitrogen fell by 54.49% on day 18. A broader freshwater synthesis found that probiotics reduced ammonia by an average of 50.7% across studies, indicating that nitrogen control is one of the most reproducible environmental effects of probiotic application (Nathanailides et al., 2021).

Mechanistically, these effects appear to arise from microbial restructuring and enhanced nitrogen cycling rather than from simple dilution of pollutants. Metagenomic analysis of pond water showed that adding probiotics reduced NH4+-N, NO2−-N, and total nitrogen while increasing nitrification genes such as amoC_B and hao and denitrification genes such as nirS and nosZ (Mang et al., 2024). Mixed Bacillus systems also improved water quality by reducing NH4+-N, NO2−-N, NO3−-N, and total phosphorus by 46.3%, 76.3%, 35.6%, and 80.3%, respectively, while enriching bacterial groups associated with nitrogen and phosphorus removal.

6.2 pathogen inhibition

Probiotics suppress harmful microorganisms in aquaculture water through several direct and indirect mechanisms, including organic acid production, antimicrobial metabolites, nutrient competition, and exclusion of pathogen colonization. Reviews of disease control in aquaculture show that probiotics can inhibit pathogenic bacteria by lowering pH after organic acid production and by secreting bactericidal compounds such as short-chain fatty acids, peroxide, and bactericidal proteins. Classical probiotic theory in aquaculture also identifies competition for nutrients, energy, and adhesion sites as core mechanisms by which beneficial microbes suppress harmful species in the rearing environment (Verschuere et al., 2000).

In grass carp systems, these antimicrobial effects are supported by strain-specific evidence. Bacillus methylotrophicus WM-1, isolated from grass carp culture systems, showed strong antagonism against Aeromonas hydrophila and was considered applicable to aquaculture production because it combined pathogen inhibition with environmental safety. More broadly, quorum-quenching probiotics appear especially promising because they reduce bacterial virulence by disrupting quorum sensing rather than by killing pathogens outright, which makes them a plausible non-antibiotic strategy for controlling disease pressure in sustainable aquaculture (Lubis et al., 2024).

6.3 Ecological aquaculture applications

The main value of probiotics in ecological aquaculture is that they can couple production support with environmental remediation. Reviews focused on sustainable aquaculture consistently describe probiotics as feed additives or water supplements that improve water quality, reduce antibiotic dependence, and support environmentally friendly production. In freshwater fish farming, the environmental benefits can outweigh the environmental cost of probiotic production, especially because lower ammonia and improved feed conversion directly reduce the ecological footprint of pond systems (Nathanailides et al., 2021).

Grass-carp-relevant studies also suggest that probiotics fit well within integrated and low-pollution production models, but their use should be embedded in broader management rather than treated as a stand-alone solution. *Bacillus velezensis* LG37 degraded ammonia nitrogen and residual feed protein in grass carp culture wastewater by 55.5% and 73.6% within one week, and it also increased grass carp tolerance to ammonia toxicity. At the same time, recent cyprinid reviews emphasize that probiotic performance depends on environmental factors such as pH, temperature, and dissolved oxygen, and that sustainable aquaculture still requires integrated strategies including water-quality optimization, biosecurity, and preventive health management (Qu et al., 2025). In grass carp aquaculture, probiotics therefore appear most useful as ecological regulators that improve water quality, suppress harmful microbes, and support greener production systems. Their environmental value is strongest when strain selection, application method, and farm management are matched to the specific culture system.

7 Case Study: Evaluation of the Application Effects of Compound Probiotic Preparations in Grass Carp Aquaculture

7.1 Effects of compound probiotic supplementation on growth performance and feed efficiency of grass carp

Compound probiotic supplementation generally improves growth performance and feed utilization in grass carp. In a 6-week feeding trial, a duo-strain preparation of Bacillus subtilis and Lactobacillus plantarum significantly increased percent weight gain, specific growth rate, and survival rate, while decreasing feed conversion ratio, showing that mixed-strain delivery can enhance both production and feed efficiency. A separate grass carp study using Lactobacillus buchneri L3-9 together with its extracellular products also increased weight gain rate and reduced feed conversion ratio within only 21 days, indicating that compound microecological preparations can act rapidly under culture conditions (Gao et al., 2024).

These responses likely reflect complementary functions among strains and bioactive metabolites rather than a simple additive effect. Probiotics are understood to promote growth through improved digestion, enhanced nutrient absorption, and better feed conversion, which is especially relevant in carp farming where feed remains the major production cost. Supporting evidence from other carp models shows that mixed probiotic preparations can reduce feed conversion ratio by 5.04-6.47% and generate measurable feed savings at farm scale, suggesting that the performance gains observed in grass carp are economically meaningful when translated to commercial production (Prazdnova et al., 2025).

7.2 Intestinal microbiota and immune regulation

Compound probiotics regulate the grass carp intestinal ecosystem by enriching beneficial taxa and suppressing potential pathogens. In the duo-strain grass carp study, probiotic feeding increased the relative abundance of Bacteroidetes and Firmicutes while decreasing Proteobacteria and Cyanobacteria, indicating a shift toward a more favorable microbial structure (Luo et al., 2022). Earlier work in grass carp also showed that combined administration of Shewanella xiamenensis, Aeromonas veronii, and Bacillus subtilis increased Cetobacterium and reduced potential pathogens such as Pseudomonas and Flavobacterium, linking microbiota remodeling to lower disease risk.

Immune regulation appears to follow these microbial shifts. Dietary supplementation with L. buchneri L3-9 and its extracellular products increased Lactobacillaceae, reduced Aeromonadaceae and Enterobacteriaceae, downregulated the pro-inflammatory gene TNF-α, and upregulated anti-inflammatory markers including TGF-β1 and IL-10 (Gao et al., 2024). More broadly in juvenile grass carp, probiotic combinations or multiple candidate strains elevate complement components and antioxidant defenses while improving intestinal barrier-related signaling, suggesting that compound preparations work through coordinated effects on microbiota, mucosal integrity, and innate immunity rather than through one pathway alone.

7.3 Water quality and economic value

The broader value of compound probiotics in grass carp farming lies in coupling host benefits with environmental regulation. Probiotics can improve freshwater pond conditions by lowering ammonia, nitrite, and organic loading, and average effects across freshwater fish studies include a 50.7% reduction in ammonia and a 10.7% decrease in feed conversion ratio, both of which directly support more sustainable production. Mechanistically, water-applied probiotics can promote nitrification and denitrification by increasing functional genes such as amoC_B, hao, nirS, and nosZ, which explains why improvements in water quality often persist beyond the immediate feeding response (Mang et al., 2024).

Although direct pond-scale case studies in grass carp remain fewer than feed-based trials, related high-density carp systems show the same integrated pattern of benefit. In crucian carp, long-term use of compound probiotics under micro-water-exchange conditions kept total ammonia nitrogen and nitrite in dynamic equilibrium, improved body weight and length, and lowered mortality after bacterial challenge. Likewise, probiotic use in biofloc-based carp culture reduced NH_3-N and NO_2^-, improved growth and welfare, and supports the conclusion that compound probiotics can contribute to ecological aquaculture by reducing waste output, stabilizing culture water, and improving the economic efficiency of feed use (Ajamhasani et al., 2023). In this case-study perspective, compound probiotic preparations show clear potential to improve growth, gut health, and system sustainability in grass carp aquaculture. Their most practical value appears when feed efficiency, immune stability, and water-quality management are evaluated together rather than as isolated outcomes (Figure 3).

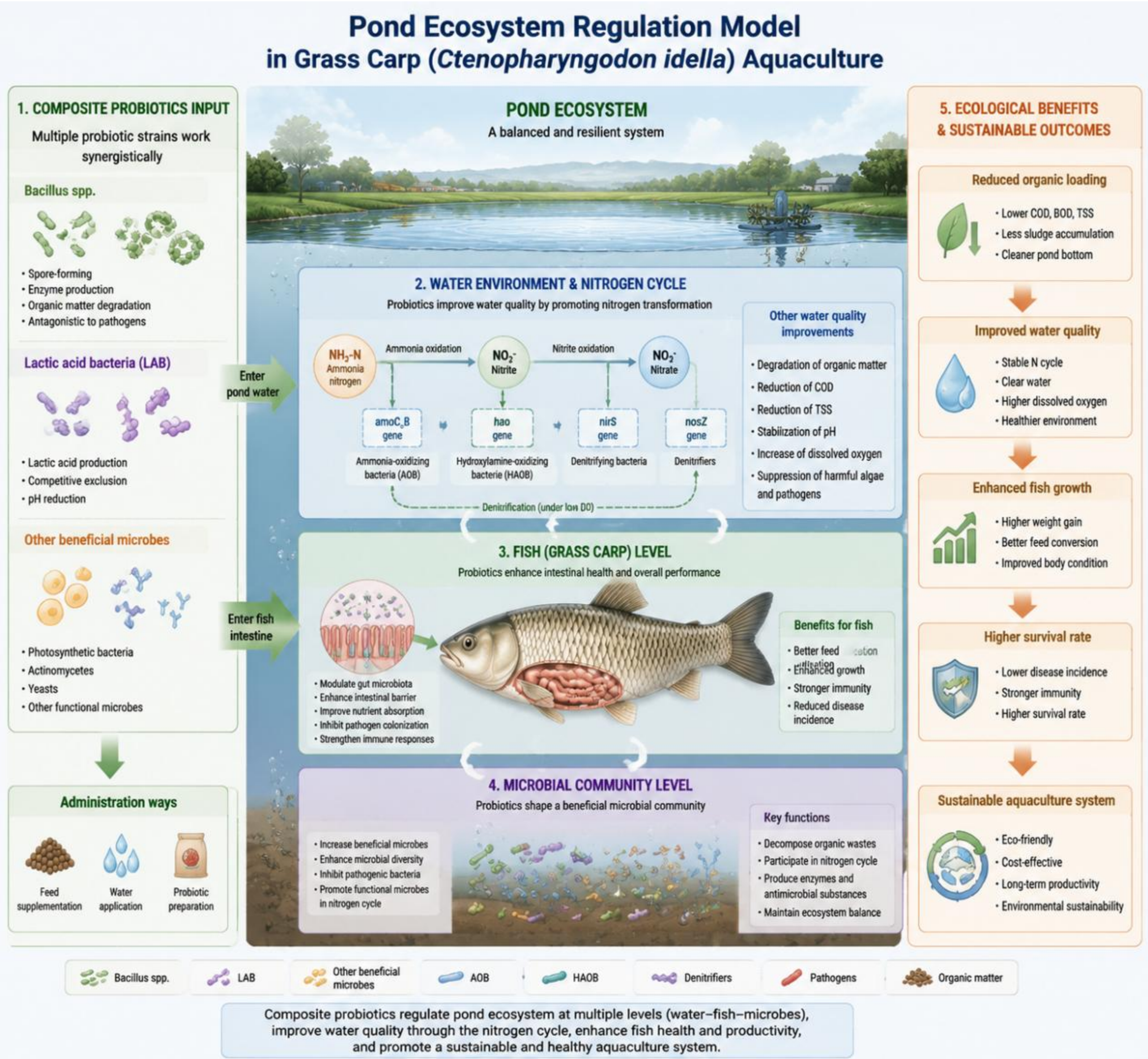

Figure 3 Ecological regulation mechanisms of compound probiotics in grass carp aquaculture system

8 Key Issues and Future Perspectives of Probiotic Applications

8.1 Probiotic strain selection, functional evaluation, and optimization of stability

A core issue in future grass carp probiotic development is that strain efficacy is species- and environment-dependent, so candidate strains cannot be selected only by taxonomic identity. Reviews of aquaculture probiotics emphasize that a strain beneficial in one host may not provide the same benefit in another, and may even become unsuitable under different rearing conditions, which makes extensive host-oriented testing essential before field use. This concern is consistent with newer overviews stressing that strain selection for aquaculture must account for safety, functional performance, and species-specific dosing, because predictable large-scale application remains a major bottleneck (Elsegeny et al., 2025).

Future screening should therefore combine biosafety evaluation with functional trait testing and formulation-oriented stability assessment. Candidate strains are now expected to show tolerance to low pH, bile or salinity stress, antagonism toward pathogens, non-hemolytic behavior, and preferably traits such as adhesion or sporulation that support colonization and storage stability. Recent work on indigenous aquaculture isolates also shows that pH tolerance, salinity resilience, gut colonization potential, and spore formation can be integrated into trait-based ranking systems, with sporulation being especially useful for feed formulation, storage, and administration stability (Rwezawula et al., 2025).

8.2 Synergistic applications with feed, water, and culture systems

The next stage of application in grass carp farming is likely to depend less on single additives and more on synergistic integration with feeds, water management, and farming systems. Recent reviews indicate that multi-strain probiotics and synbiotics often outperform single strains, and broader evidence across aquaculture shows superior efficacy of multi-strain or synbiotic strategies in 68% of trials for growth and disease resistance (Wen et al., 2026). This matters for grass carp because practical farming systems must improve several outcomes at once, including feed utilization, intestinal health, pathogen control, and water quality.

Evidence from carp and pond-system studies suggests that probiotics work best when they are matched with the production environment rather than added in isolation. In biofloc culture, combining probiotics with complex carbon sources improved ammonia, nitrite, growth, and welfare indices, showing that probiotic function can be amplified by system design and substrate availability (Ajamhasani et al., 2023). Field-oriented reviews focused on grass carp also report that water-applied probiotics such as Pseudomonas stutzeri and Bacillus strains reduced ammonia, nitrite, and total nitrogen while reshaping ambient microbial communities, supporting integration of probiotics into ecological water regulation strategies.

8.3 Precision regulation through omics

A major future direction is to move from outcome-based evaluation to mechanism-based precision regulation using genomics, metagenomics, transcriptomics, and metabolomics. Probiogenomics has been proposed as a framework for characterizing probiotic candidates through whole-genome sequencing, which can identify metabolic capacity, stress-resistance traits, and genes linked to adhesion, bacteriocin production, and immunomodulation (Fachri et al., 2024). More broadly, multi-omics integration is increasingly favored because single-omics approaches can miss regulatory links across microbial composition, host responses, and metabolite production.

For grass carp specifically, omics tools are especially promising because the intestinal microbiome already has identifiable functional structure that could guide probiotic targeting. Multi-omics work in grass carp showed that Proteobacteria and Fusobacteria/Firmicutes/Bacteroidetes form two ecological and functional groups with different capacities for carbohydrate utilization, virulence, and antibiotic resistance, and the proposed Functional Group 2/Functional Group 1 ratio may serve as a biomarker for microbiota status (Li et al., 2023). Parallel fish studies further show that combined metagenomics, metabolomics, and transcriptomics can link probiotic-induced microbial shifts to specific metabolic pathways and host phenotypes, providing a model for precision probiotic design in grass carp. Overall, the future of probiotics in healthy grass carp farming lies in selecting host-adapted stable strains, embedding them into integrated feed-water-system management, and using omics tools to predict and monitor function. These advances should make probiotic application more standardized, more mechanistically transparent, and more reliable at commercial scale.

9 Conclusions and Future Perspectives

Probiotics have emerged as one of the most promising biological tools for improving the health and production performance of grass carp aquaculture. Across existing studies, their major functions can be summarized as promoting growth, improving feed utilization, enhancing digestive enzyme activity, maintaining intestinal epithelial integrity, regulating gut microbial balance, and strengthening non-specific immune responses. These effects are closely interconnected. Improved intestinal structure and digestive function increase nutrient assimilation, while a more stable intestinal microbiota helps suppress opportunistic pathogens and supports mucosal homeostasis. At the same time, probiotics stimulate antioxidant defenses and innate immune factors, thereby increasing the resilience of grass carp under intensive farming conditions. Beyond their direct effects on the host, probiotics also play an important regulatory role at the culture-system level. By reducing ammonia, nitrite, and other harmful metabolites, and by reshaping microbial communities in water, probiotics contribute to a healthier aquaculture environment that lowers physiological stress and disease pressure. This dual action on both fish and water makes probiotics especially valuable in healthy grass carp farming, where sustainable productivity depends not only on rapid growth but also on ecological stability, reduced antibiotic reliance, and improved survival. Therefore, probiotics should be regarded not merely as feed additives, but as multifunctional biological regulators that connect nutrition, immunity, environmental management, and green production.

Despite the encouraging progress, current research on probiotic application in grass carp still has several important limitations. First, many studies remain focused on short-term feeding trials and phenotypic observations, while the long-term effects on host health, ecological safety, and farming-system stability remain insufficiently clarified. Second, the responses of grass carp to probiotics are strongly influenced by strain specificity, dosage, developmental stage, feed composition, and environmental factors such as temperature, dissolved oxygen, and water quality. As a result, probiotic effects observed under laboratory or small-scale conditions are not always fully reproducible in commercial aquaculture settings. In addition, many candidate probiotics currently used in aquaculture are not host-associated strains, which may limit their colonization ability, stability, and functional consistency in the grass carp intestinal tract. Technological and industrial constraints also restrict broader application. The screening and functional verification of superior strains remain difficult, especially when safety, antagonistic activity, digestive contribution, immune regulation, and environmental adaptability must all be evaluated together. Standardized protocols for strain selection, product formulation, dosage control, storage stability, and delivery methods are still lacking. At the same time, large-scale production of highly active and stable probiotic products remains a challenge, particularly for formulations intended for diverse aquaculture environments. Regulatory uncertainty, biosafety assessment, and the need to prevent unintended ecological impacts further complicate practical deployment. These limitations indicate that the transition from experimental success to standardized and reliable field application is still incomplete.

Future development of probiotic-based green aquaculture technologies for grass carp will likely move toward greater precision, integration, and ecological orientation. One major trend is the selection of host-adapted and functionally targeted strains, especially those derived from the grass carp intestine or culture environment, because such strains are more likely to colonize effectively and perform consistently. At the same time, probiotic development will increasingly emphasize multi-strain formulations, synbiotic combinations, and coordinated use with feed management, water regulation, and ecological farming systems such as biofloc or low-exchange aquaculture. This systems-based approach is expected to improve not only fish growth and disease resistance but also nutrient recycling, microbial stability, and overall environmental performance of production systems. Another important trend is the use of omics-based technologies to uncover the precision regulation mechanisms of probiotics. Genomics, metagenomics, transcriptomics, and metabolomics can help identify functional strains, predict biosafety, reveal host-microbe interactions, and clarify how probiotics influence immunity, metabolism, and microbial network assembly in grass carp. These tools will support the development of next-generation probiotics, paraprobiotics, postbiotics, and even engineered microbial platforms with improved stability and targeted function. In the future, probiotic technology in grass carp aquaculture is likely to evolve from empirical supplementation toward data-guided microbial management. Such a transition will provide stronger support for antibiotic-free farming, environmentally friendly production, and the construction of resilient green aquaculture systems. In conclusion, probiotics have become an important foundation for promoting healthy, efficient, and sustainable grass carp aquaculture. Their future value will depend on whether research can move beyond broad efficacy claims toward standardized, mechanism-based, and field-validated application systems.

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Conflict of Interest Disclosure
The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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