

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