

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 -galactosidase 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.