



MICROBIOME ENGINEERING: BOOSTING FISH HEALTH AND DISEASE RESISTANCE

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Introduction

Aquaculture is currently the fastest-growing food production sector worldwide. However, this expansion also brings with it a significant problem: disease outbreaks, which can result in enormous financial losses. Numerous treatments have also been employed traditionally. These include chemicals like formalin, potassium permanganate, and malachite green to combat parasite and fungal infections, as well as antibiotics like oxytetracycline and florfenicol.

Despite their short-term effectiveness, the overuse of chemicals and antibiotics has raised major issues, such as environmental harm, antimicrobial resistance (AMR), and even trade restrictions on a global scale. Researchers are now using a novel concept called microbiome engineering to solve these problems.

By influencing and controlling the microbial populations that naturally exist in and on aquatic animals, this strategy aims to enhance their health and disease resistance (Kumar et al., 2016; Das et al., 2017). Microbiome-based approaches are more sustainable than chemical therapies and are consistent with the One Health theory, which connects the health of humans, animals, and the environment.

Microbiome

The community of microorganisms residing in a particular habitat, as well as its interactions and activities, is referred to as the microbiome. Although viruses, fungi, and bacteria are all part of these microbial communities, bacteria often hold the majority of the group.

People, fish, plants, animals, the environment, and even food microbiomes are examples of the various types of microbiomes that can be distinguished based on their geographical location.

The fish microbiome creates a complex ecosystem in fish. Although bacteria make up the majority of it, it also contains viruses, fungi, and archaea. Different microbial communities are found in different areas of fish bodies, such as the gut, mouth, gills, and skin.

It's interesting to note that fish and mammals have very distinct microbial compositions. Fish microbiomes are mostly influenced by *Proteobacteria* and *Fusobacteria*, whereas humans are usually dominated by *Firmicutes* and *Bacteroidetes*.

The species, life stage, diet, feeding habits, and environment of the fish all affect the microbial communities' makeup, which is not constant.

Functions and Importance

Fish microbiome includes bacteria, fungi, viruses, and archaea that are present on the skin, gills, and in the digestive tracts of fish and shellfish (Nayak, 2010).

They are involved in various nutritional, immunological, metabolic and developmental functions (Table 1).

Table 1. Functional roles of microbiome in fish

Nutritional	Aid digestion, release enzymes, and improve nutrient absorption.
Immunological	Train the mucosal immune system, stimulate antimicrobial peptides, and block pathogen adhesion.
Metabolic	Affect lipid metabolism, feeding behavior, and stress resilience.
Developmental	Influence gut maturation, reproduction, and growth.

The disruption of this balance, known as dysbiosis, from stress, poor water quality, or overuse of antibiotics increases the likelihood of disease.

Microbiome Engineering

Microbiome engineering is the process of altering microbial populations and their metabolites to improve the functionality and health of the host. It seeks to create a balanced microbiome that benefits the animal by promoting the growth of beneficial microorganisms while suppressing dangerous pathogens, promoting the synthesis of compounds that strengthen immunity, and lowering the virulence of pathogenic microbes.

A sustainable alternative to pesticides and antibiotics, microbiome engineering offers a practical way to improve the health of fish and prawns while reducing environmental risks in aquaculture (Fig. 1).

Key Strategies in Aquaculture

Probiotics

Probiotics are live supplements of good bacteria and fungi that help with digestion, boost immunity, and improve resistance to disease. Common examples are *Lactobacillus* and *Bacillus subtilis*, which are usually added by feed or water. They defend fish and prawns by producing antimicrobial chemicals, strengthening the gut's defences, and battling dangerous infections for nutrition and attachment sites.

Based on traits like host and environmental compatibility, lack of antibiotic resistance genes, and tolerance to intestinal conditions, probiotic strains are chosen for safe and effective use. Probiotics in aquaculture assist maintain higher water quality and enhance animal health (Hai, 2015).

Prebiotics

Non-digestible compounds, including as β -glucans, fructose oligosaccharides (FOS), and mannose oligosaccharides (MOS), preferentially promote probiotics and other beneficial microorganisms. By encouraging the synthesis of short-chain fatty acids, they improve resistance to infections and promote intestinal health.

Synbiotics

Synbiotics are combinations of prebiotics (nutrients that support their growth) and probiotics (good bacteria) that are intended to improve the health of aquatic animals. Synbiotics improve growth

performance, promote immunity, and improve digestion by promoting balanced microbial communities. The chosen probiotic and prebiotic must be safe for the environment and the host, biocompatible, biodegradable, and employed at the right concentrations for optimal utilisation.

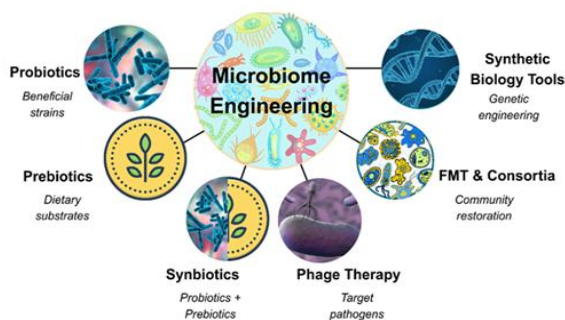


Fig. 1. Tools used in Microbiome Engineering

Microbial Consortia & Fecal Microbiota Transplantation

Innovative approaches that employ whole microbial communities rather than individual strains to restore homeostasis in the host include microbial consortia and fecal microbiota transplantation (FMT). Microbial consortia involve the introduction of carefully selected beneficial microorganisms, whereas FMT restores a stable and diversified population by transferring gut microbiota from a healthy donor to a recipient. By boosting microbial diversity and digestive enzyme activities like α -amylase and trypsin, FMT has shown remarkable benefits in aquaculture.

It also restores gut diversity more efficiently than natural recovery after antibiotic exposure (e.g., florfenicol), and it even boosts rainbow trout growth and muscle yield through gut microbiota modulation. When combined, these strategies improve resistance to infections, facilitate environmental change adaption, and promote general health by

preserving a diverse and functional microbiome.

Phage Therapy

Phage therapy uses bacteriophages, which are viruses that selectively infect bacteria, to manage hazardous infections like *Vibrio* and *Aeromonas* in aquaculture. Unlike antibiotics, phages are very host-specific, killing only the harmful bacteria and leaving the beneficial microbes unharmed. They work so fast that they can eliminate nearly half of a bacterial population in a single day.

To identify pathogens, phages employ tail-end binding, in which the viral tail attaches itself to certain bacterial surface receptors. In aquaculture, phage-enhanced feed pellets or, for optimal results, supplements and antimicrobials can be used to distribute phages. Phage-probiotic combinations have also had promising outcomes. For example, PzI-Ah152, which targets *Aeromonas hydrophila* and has been successfully utilised to treat enteritis in crucian carp.

Genomic & Synthetic Biology Tools

By enabling precise, targeted modifications of microbial genomes, genomic and synthetic biology tools like Zinc Finger Nucleases (ZFNs), TALENs, and CRISPR/Cas9 are transforming microbiome engineering.

These technologies enable the creation of customised microbial strains and engineered probiotics that can secrete molecules that boost the immune system, produce antimicrobial compounds, or improve host health in aquaculture systems. Ex-situ (lab-based) and in-situ (within the host) gene editing techniques are both utilized to precisely modify microbial functions, enhancing their efficacy and safety.

These tools not only improve microbial functions but also reduce the risk of antimicrobial resistance by decreasing dependence on antibiotics. This makes it possible to develop therapeutic strategies that are specifically suited to particular infections, hosts, and environments.

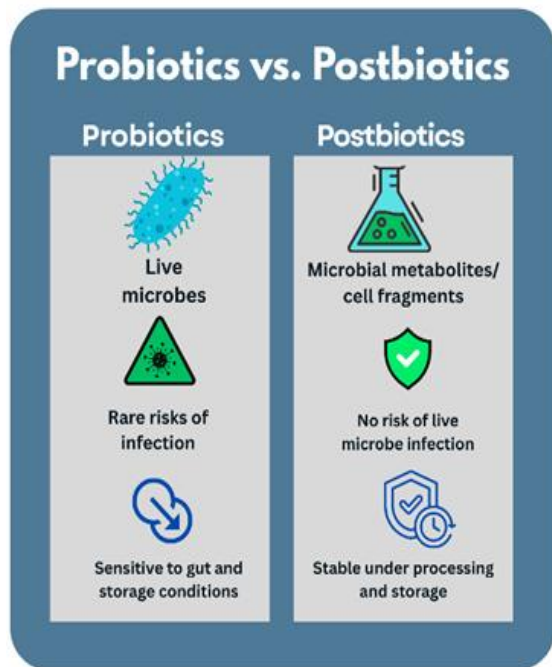


Fig.2. Difference between probiotics and postbiotics

Postbiotics

Postbiotics differ from probiotics because they contain microbial cell fragments, polysaccharides, metabolites, and peptides without live organisms (Fig.2). There is no risk of infection for immunocompromised hosts. They have a longer shelf life and are more resilient in farm conditions and they provide immunomodulatory effects, improve nutrient absorption, and have antimicrobial properties.

Factors Influencing Microbiome

The microbiome in aquaculture species is influenced by a variety of interconnected factors including the

environmental factors, host-specific factors and stressors (Table 2).

Table 2. Various factors influencing the gut microbiome

Environmental factors	Water quality, temperature, salinity, pH, season, and geography shape microbial communities.
Host-specific factors	Water quality, temperature, salinity, pH, season, and geography shape microbial communities.
Stressors	Starvation, or infections disrupt gut flora and lower immunity.

Disease Resistance Mechanisms Supported by Microbiome Engineering

Microbiome engineering improves resistance by various actions (Fig. 3)

- **Immune modulation**, stimulating both innate and adaptive responses.
- **Nutrient competition**, where beneficial microbes limit pathogen colonization.
- **Antimicrobial production**, as commensals create bacteriocins and peptides.
- **Supporting gut integrity**, which enhances digestion, barrier function, and resilience to stress.

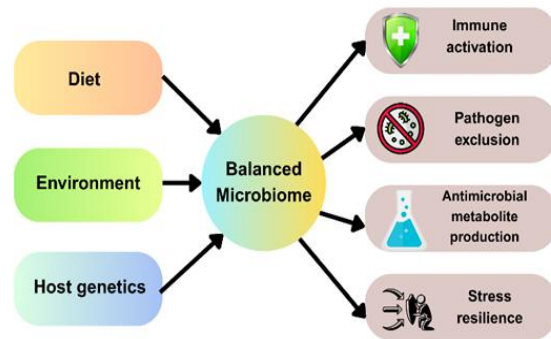


Fig. 3 Gut Microbiome–Disease Resistance Mechanism

Target pathogens include:

- Bacteria: *Vibrio* (vibriosis), *Aeromonas* (furunculosis).
- Viruses: VHSV, piscine nodavirus.
- Fungi: Opportunistic invaders that appear under stress conditions.

Challenges and Limitations

There are a number of obstacles and restrictions to using microbiome-based techniques in aquaculture. The intricacy of microbial populations is a significant worry. Because of this complexity, the outcomes of interventions are frequently unpredictable and heavily reliant on the particular situation. Significant obstacles are also presented by safety and regulatory concerns. There are ethical discussions and rigorous legal examination around genetically modified microorganisms.

Adoption at the agricultural level is difficult. For farmers to get reliable outcomes under real-world circumstances, they want formulations that are stable, inexpensive, and simple to use. Finally, there are significant ecological hazards. These include the potential for unforeseen environmental consequences, disruption of natural microbial populations, and horizontal gene transfer.

Before putting large-scale solutions into place, all of these hazards need to be carefully monitored and evaluated.

Future Directions and One Health Integration

Recent developments are altering aquaculture's approach to managing microbiomes. Comprehensive profiles of microbial populations and their roles are provided by multi-omics techniques, which integrate transcriptomics, metabolomics, and metagenomics. This enhances forecasts of the interactions between hosts, microorganisms, and surroundings. New probiotics that offer particular advantages for host health and disease resistance can now be created thanks to advancements in synthetic biology. In the meantime, helpful tools for forecasting microbiome alterations under various farming settings are provided by AI-assisted modelling. This encourages data-driven management that supports sustainability (Table 3).

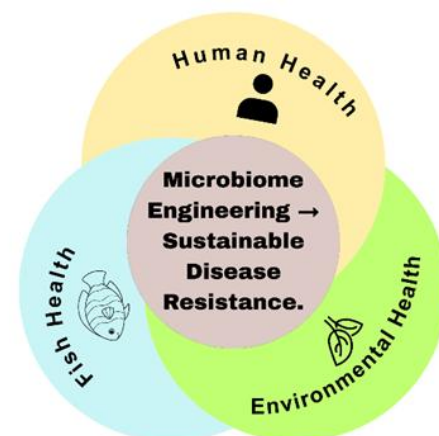


Fig. 4 One Health and Microbiome Engineering

Adopting a One Health viewpoint is also crucial (FAO, OIE & WHO, 2020). In order to safeguard human health, surrounding ecosystems, and farmed species, this strategy focuses on minimising the usage of antibiotics

(Fig. 4). It is essential to adopt international policy frameworks from agencies like as the FAO and OIE and to strengthen the oversight of zoonotic infections and antibiotic resistance. This contributes to the establishment of uniform guidelines for aquaculture's long-term resilience, biosecurity, and appropriate use of antibiotics.

Table 3. Current Practices and Emerging Innovations in Aquaculture Health Management within a One Health Framework

Aspect	Current Practice	Emerging/ Needed Innovation
Therapeutic	Antibiotic, chemicals, probiotics	Phage therapy, precision antimicrobial
Prophylactic	Vaccines (mainly fish), probiotics, SPF broodstock	RNAi, CRISPR-based resistance, microbiome modulation
Resistance Issues	AMR, residues in food chain	Stewardship, monitoring, microbiome-based alternatives

Conclusion

A viable and sustainable strategy for improving disease resistance and general health in aquaculture is microbiome engineering. Probiotics, prebiotics, synbiotics, phages, postbiotics, and genetic engineering methods like CRISPR, TALEN, and ZFN can all be used to alter the gut microbiota, increase

immunity, promote growth, and improve disease resilience in aquatic species. The lack of genomic data for many aquatic species, the cost and technical difficulty of gene-editing techniques, the difficulties of introducing genes into mixed microbial communities, and practical issues like strain identification, safety assessment, and storage are just a few of the challenges that are being addressed by ongoing advancements in multi-omics, artificial intelligence, and the One Health framework.

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