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Engineering the fish gut microbiome: could it serve as future-proof strategy for sustainable aquaculture?

Anjaly MA[1], Manomi Sarasan[1], Bhavya Kachiprath[2], Vrinda Sukumaran[3], I. S. Bright Singh[1] and Jayesh Puthumana[1*]

Abstract

As the global population rises the need for sustainable production of nutritious food intensifies. Recent estimates show that 70% more food needs to be produced to feed the projected human population of 9.1 billion by 2050. Aquaculture plays a critical role in meeting these demands through sustainable practices. The significance of the gut microbiome in maintaining the health of aquatic animals is increasingly recognized particularly within sustainable aquaculture. Modification of the gut microbiome is an emerging field that enhances the growth and health of fish and other aquatic species. Probiotics prebiotics and synbiotics are pivotal for gut microbiota modification. Additionally, biotechnological techniques such as fecal microbiota transplantation (FMT) CRISPR-Cas bacteriophage-mediated methods and synthetic biology are advancing these efforts. Looking ahead the integration of current techniques with advanced engineering approaches is expected to create optimally modified gut microbiota for aquatic species. Achieving this goal requires a comprehensive understanding of the basic composition of the intestinal microbiota and the available methods for modification. In this review, we mainly focus on the basic characteristics and significance of the gut microbiome in aquatic animal health while exploring mechanisms techniques and applications of microbiome engineering. Emerging trends challenges and limitations are also examined alongside ethical and safety considerations that are vital for progress in this field.

Keywords Fish gut, Microbiome engineering, FMT, CRISPR, Aquaculture

Introduction

The relationship between the gut microbiota and host health has gained tremendous focus in recent years. Gut microbiota comprises a complex and diverse community of microorganisms that inhabit the gastrointestinal (GI) tract of animals. It includes bacteria, fungi, viruses and other microbes. The majority of these consist of bacteria, and they can be aerobic, facultatively anaerobic, or obligately anaerobic, depending on the specific microenvironment within the GI tract [190]. It plays a significant role in various physiological functions, such as digestion, nutrient absorption, and immune system regulation. Hence, a diversified and balanced gut microbiota is always essential to the overall well-being of the host [263].

The fish gut microbiota composition is influenced by host preferences, feeding habitats, diets, environmental factors, and developmental stages. The development of the mucosal system, epithelial proliferation, and

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*Correspondence:
Jayesh Puthumana
jayesh@cusat.ac.in
[1] National Centre for Aquatic Animal Health, Cochin University of Science and Technology, Kochi, Kerala 682016, India
[2] Department of Marine Science, College of Natural Sciences, Incheon National University, Incheon 22012, Republic of Korea
[3] School of Environmental Studies, Cochin University of Science and Technology, Kochi, Kerala 682022, India

angiogenesis are all influenced by gut microbiota, which helps digestion and nutrient absorption more effectively [228]. It is important for maintaining the immune system, feeding behaviour, energy balance and disease resistance of the host (fish). By offering nutritional competition, limiting direct interaction with the gut, and providing resistance to colonization, gut microbes prevent pathogens from flourishing. Also, it is very crucial in maintaining the average health of the fish [81, 295].

The majority of research on gut microbial populations have been conducted on mammals. Studies on the fish gut microbiome have been relatively limited in comparison. However, there has been a surge in attention to understanding fish gut microbiomes in recent years due to their ecological and economic importance [93, 134, 171]. The study of gut microbiota composition and relative abundance also extends towards a wide range of animals such as birds [72], reptiles [307], amphibians, and various invertebrates [46].

Fish have a very different bacterial ecology than other animals. The human gut microbiome is dominated by bacteria and firmicutes, while the amphibian gut microbiome is represented by Firmicutes, Bacteroidetes, and Proteobacteria. In contrast, Proteobacteria is the main phylum in fish microbiota, with Fusobacteria constituting another common abundant group [37, 268, 274]. Identifying the microbial communities within the fish gut is crucial. It is equally important to understand the complex dynamics of their interactions with the host organism and also to discern the consequential impacts on the physiological processes and overall health of the host [28, 171]. Deeper insights into the functional roles of these microbiomes can provide valuable insights into their impact on digestion, immunity, metabolism and other physiological traits of the fish. This knowledge can then be leveraged to develop strategies for engineering the microbiome to enhance fish health and productivity [276].

A significant portion of our understanding of the complex interaction between the gut microbiota and fish hosts is derived from terrestrial vertebrates, with additional insights coming from studies involving gnotobiotic zebrafish (*Danio rerio*). Gnotobiotic animal models represent a highly significant in vivo experimental approach for studying the functions of the gut microbiota [55, 291]. Initially, research on fish gut microflora relied on culture-dependent techniques. However, the advent of metagenomics and next-generation sequencing (NGS) has revolutionized fish gut microbiome research in recent years [128]. These techniques enable direct analysis of microbial genomes from samples (gut or other environment), creating a deeper understanding of the relation between microorganisms and their hosts. The principal NGS platforms employed in fish gut microbiome research encompass the Illumina system, the Roche 454 system, and the Ion Torrent Personal Genome Machine (PGM). Notably, the Illumina system stands out as the most prevalent choice [55, 263].

Microbiome engineering holds a crucial role in achieving improvements in host health by leveraging the manipulation of microbial communities to achieve specific functions. Several approaches, including engineered probiotics, microbial transplantation, chemical-based approaches, and genetic engineering, play a vital role in the rapidly growing field of microbiome engineering [245]. Current practices in gut microbiota engineering mainly focus on humans, with less attention given to fish. However, applying microbiome approaches could significantly enhance fish health as well [171].

Aquaculture emerged as essential in meeting the increasing demand for high-quality animal protein [94] while alleviating pressure on wild fish stocks and marine ecosystems. Estimates indicated that 70% more food would be required to feed the projected human population of 9.1 billion by 2050, with aquaculture expected to play a key role in meeting this demand sustainably [40]. The anticipated doubling of aquaculture production by 2050 highlights its critical role in future global food security [171]. With the increasing frequency of disease outbreaks in aquaculture, farmers have turned more often to antibiotics, resulting in antimicrobial-resistant bacteria in both fish and the environment due to antibiotic residues. This practice also reduces microbiome diversity, which negatively impacts fish nutrition and health [205]. Implementing beneficial microbiome communities in the fish gut via probiotics or prebiotics could provide promising strategies for enhancing growth rates, disease resistance, and overall well-being in aquaculture settings. This comprehensive approach to fish health management offers significant potential for sustainable aquaculture practices and safeguarding the health and welfare of farmed fish populations [275]. Apart from this, engineering gut microbiome is gaining interest due to its potential to increase resistance to pathogens, enhance growth, boost metabolism, and improve immunity, thereby enhancing the overall gut physiology [178].

This review highlights the importance of gut microbiome engineering in fish health and addresses the challenges of its application in aquaculture, emphasizing the need for microbiome engineering. It also discusses various mechanisms, techniques, and applications of gut microbiome engineering while evaluating its effects on disease resistance, growth, and other physiological traits. Furthermore, this study explores emerging trends and innovations in the field, addressing the challenges and limitations for the broader adoption of gut microbiome engineering while considering ethical, regulatory, and safety considerations.

Fish gut microbiome: composition and function

Overview of the fish gut microbiome

The developing field of research concerning the gut microbiome of fish reflects a notable surge in interest. The majority of fish gut microbiomes comprise bacteria, and they play a significant role in various physiological functions, such as digestion, nutrient absorption, and immune system regulation. They have a significant impact on the overall health and physiological balance of the host, which is similar to that of humans and other animal species [263]. Hence, a long-standing, intimate, intricate relationship exists between animal hosts and their gut flora. Research conducted in humans has demonstrated the involvement of microorganisms within the gastrointestinal tract (GIT) in the gut-brain axis. The gut-brain axis is a complex bidirectional communication system that involves the interaction between the central nervous system (CNS), the enteric nervous system (ENS) of the gut, the endocrine system, and the immune system. The main component that regulates the gut-brain axis is the gut microbiome [28].

The composition of gut microbiota can fluctuate over time and can either be transient or persistent. The transient (allochthonous) microbiota refers to the temporal inhabitants of the gut, and a significant portion of these microorganisms typically enter the gastrointestinal system through diet. Even though they may not permanently colonize the gut, they can still play a role in maintaining the physiological processes of the host. In contrast, the persistent (autochthonous) microbiota establishes a more stable and long-term symbiotic association with the host's gut. They contribute to overall well-being and functioning of the gut [188, 303]. Autochthonous microbes appear to be attached to the intestinal epithelium of the gut, whereas allochthonous microbes associate with the lumen [188, 225].

The development of the gut microbiota in fish is a complex process. The first microbes that the fish eggs come into contact with are mainly determined by the surrounding water, which acts as a repository of microorganisms [268, 313]. Hence, the eggs get a chance to be colonized by these microbes. Upon hatching, the larvae come into contact with these microbes and get colonized in their GIT. This early colonization stage acts as a basic platform for the development of the gut microbiota in fish [73, 282]. Research across various fish species suggests that the diversity of the gut microbiome varies as fish progress from early larval to juvenile stages [152, 169, 257]. Although the composition of the gut microbiota appears to be significantly affected by the introduction of external food sources, a prevailing gut microbial community existed even before that [64, 83], In a study conducted on Nile tilapia, the composition of the gut microbiota seems to vary in biofloc system. However, despite these fluctuations, a stable core community of prokaryotes persists within the gut of fish. This indicates that certain microbial populations maintain a consistent presence and functionality within the gut ecosystem, even as the overall composition varies due to external factors. New microbes interact with this core community, coexisting and forming a dynamic microbiome composition within the gut. This evolving microbial community potentially contributing to host health and homeostasis [63, 295].

Roles and functions of gut microbiota in fish health and performance

The gut microbiota forms a complex ecosystem within the fish GIT, actively contributing to various aspects of host physiology, metabolism, and immune function (Fig. 1). Gut microbiota aid in the breakdown of complex dietary components, such as carbohydrates, proteins, and lipids, into simpler forms that can be absorbed by the fish. They produce enzymes that the fish host may lack, facilitating digestion. Many bacterial strains of the fish gut can secrete enzymes that aid in digestion [222]. This enhances nutrient absorption and ensures efficient utilization of nutrients from the diet. Apart from this, intestinal microbiota is also involved in epithelial proliferation and maturation which aids in the efficient absorption of nutrients from the gut [190]. Intestinal microbiota in humans is known to facilitate protein/amino acids (AAs) digestion and absorption by decomposing complex subunits, which are easy to absorb and consequently modify metabolic pathways in the host cell [12].

In the investigations using the zebrafish (*D.rerio*) model, researchers delved into the intricate interplay where the diet influences the microbial composition, inturns effects the metabolism, immunity and overall physiological processes. Semova et al. [237] demonstrated that the presence of microbiota within the zebrafish gut amplifies both the number and size of lipid droplets within the intestinal epithelium, highlighting the pivotal role of microbiota in modulating lipid absorption and energy equilibrium. Cheesman et al. [43] found that the resident microbiota and Wnt signalling jointly stimulate epithelial cell proliferation in the developing zebrafish intestine. Specifically, they revealed that gut microbiota support cell proliferation by enhancing the stability of β-catenin in intestinal epithelial cells, thus shedding light on a mechanism by which microbiota influence intestinal development.

Zhang et al. [307] explored the impact of dietary supplementation with *L. delbrueckii* on *C. carpio*, uncovering

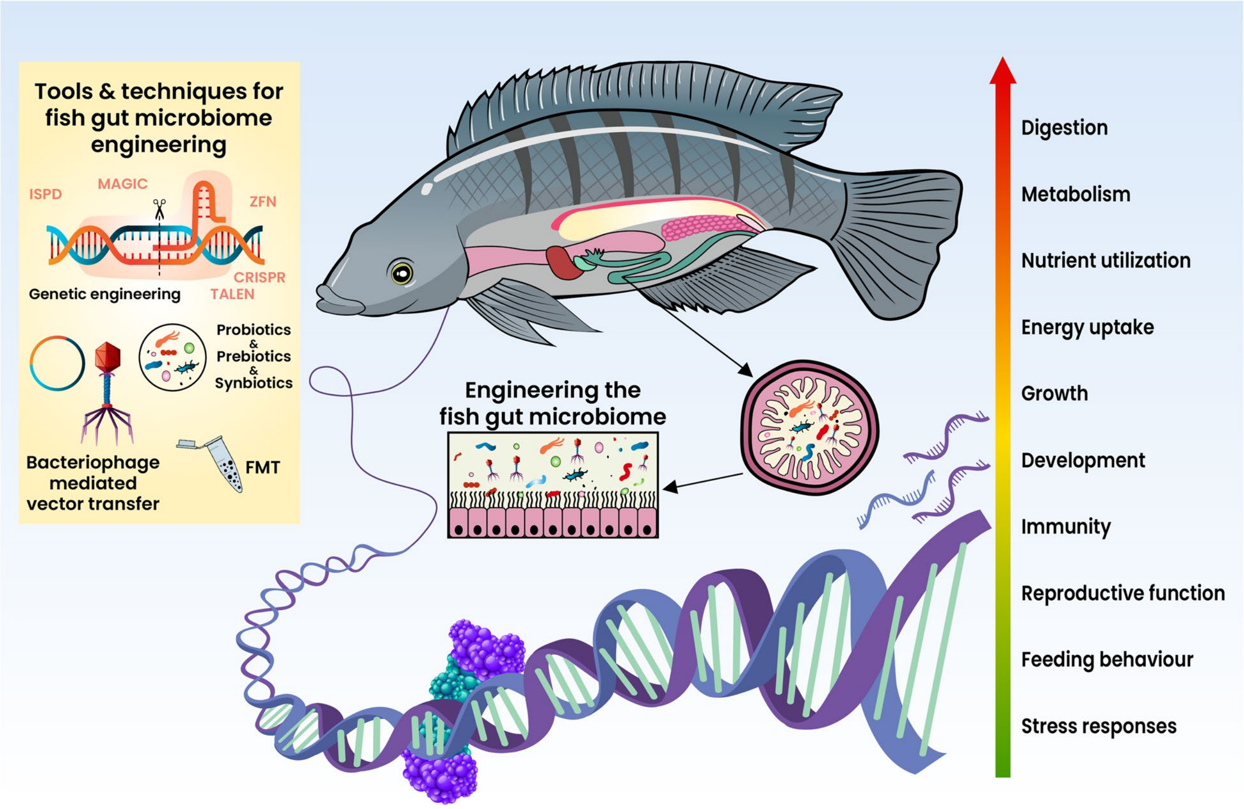

Fig. 1 Schematic representation of gut microbiome engineering methods and their impacts on fish. The figure illustrates tools and techniques for gut microbiome engineering in aquaculture species, including probiotic supplementation, prebiotic application, synbiotic approaches, microbial transplantation, and genetic engineering. Key impacts include enhanced growth, disease resistance, nutrient absorption, and gut health, emphasizing their role in sustainable aquaculture

a notable enhancement in intestinal enzyme activities alongside improved growth performance. Likewise, Xia et al. [293] found that the bile acids can also improve the gut barriers of fish through both direct and indirect effects mediated by the gut microbiota. In addition, intestinal microorganisms within fish have been shown to enhance carbohydrate digestion through the secretion of a variety of enzymes and by boosting the activities of host digestive enzymes. These processes facilitate the breakdown of carbohydrates into decomposition intermediates and fermentation products. These products can subsequently serve as substrates for secondary utilizers, influencing the intestinal environment accordingly [306]. In addition, gut microbiota exerts influence over the feeding behaviour of fish through the modulation of appetite and satiety signalling pathways. Through the production of metabolites, they impact host metabolism, facilitate energy extraction from the diet, and influence energy storage mechanisms. This dynamic interplay between gut microbiota and host metabolism significantly contributes to the maintenance of energy balance and overall metabolic well-being in fish [124, 170]. Also, a variety of factors have been implicated in appetite control, including gut microbiota, which develops complex interactions to manipulate metabolic requirements and hedonic feelings. Gut microbial metabolites and components act as appetite-related signalling molecules to regulate appetite-related hormone secretion and the immune system, or act directly on hypothalamic neurons. To date, numerous studies support the notion that gut microbiomes exert a profound influence on eating behaviour in humans and other animals [78, 106].

Gut microbiota also plays a crucial role in promoting health through the development and regulation of the mucosal innate and adaptive immune systems [114]. The mutual interactions between a host and its intestinal microbiota are pivotal in moulding immune development. The immune system of the host co-evolves with its native microorganisms, which are essential for numerous physiological functions [130]. By actively monitoring the composition and changes in intestinal microorganisms, the host's immune system regulates the balance of the gut microbiota, ensuring the health of the intestinal lining. This delicate balance is crucial for maintaining optimal GI function and overall physiological health [16, 170, 306]. The mucosal immune system of fish encompasses both innate and adaptive immunity, with all four mucosal organs harbouring mucosa-associated lymphoid tissues (MALT), with particular significance attributed to the gut-associated lymphoid tissue (GALT). Goblet cells, found throughout these mucosal organs, secrete mucus, forming a protective barrier rich in mucins (glycoproteins), proteases, antimicrobial peptides (AMPs), esterase, and lysozymes. Notably, mucosal epithelial cells are equipped with pathogen recognition receptors (PRRs) that engage with microbial entities. Furthermore, fish mucosal tissues host various immune cells including macrophages, neutrophils, natural killer cells, mast cells, eosinophils, and basophils. Immunoglobulin M (IgM) predominates in the plasma of teleost, serving as a key mediator in systemic immune responses [97]. The colonization of intestinal mucosal surfaces by indigenous microbiota exerts a favourable influence on the regulatory mechanisms of the gut immune system. Within this context, the mucosal immune system employs PRRs to distinguish between pathogenic and commensal bacterial entities. These PRRs serve as pivotal mediators in the identification of bacterial antigens, triggering intricate signalling cascades that promote immune responses within the intestinal environment [206]. The microbiota plays a crucial role in shielding the host against colonization and the proliferation of diverse pathogens through the mechanism of colonization resistance [146]. Furthermore, the microbiota exerts its protective effects via several intricate pathways, including competitive nutrient utilization, constraining direct gut interaction, and destroying the resistance mechanisms. This results in the establishment and propagation of pathogens which enables them to flourish within the host environment [81, 192, 295]. Moreover, gut bacterial species actively secrete AMPs as they compete with pathogens for niche occupancy. Consequently, a delicate equilibrium is established among the gut microbiota, pathogens, and gut mucosa. Disruption of this equilibrium not only predisposes the host to infections but also initiates the activation of the GALT. The gut microbiota serves as a pivotal factor in the progression and refinement of the GALT by playing a fundamental role in its development and maturation [28].

Dong et al. [69] investigated the effect of infectious hematopoietic necrosis virus (IHNV) infection in the digestive tract of rainbow trout (*O. mykiss*). They focused on assessing the immune response and microbial communities across various segments of the digestive tract, ranging from the buccal mucosa to the hindgut post-infection. Their observations unveiled that immune responses induced by IHNV within the mucosal linings disrupt the micro-ecological balance. This disruption facilitates the infiltration of opportunistic pathogens, leading to subsequent secondary infections. It is evident that well-established gut microbiota mitigate infections and sustain overall fish health [69]. A well-established diverse gut microbiota in fish can offer protection against infections thereby lowering the chances of infection and improving overall health. Any changes to this equilibrium of microbes inside the fish can weaken their immune response, potentially leading to disease [177, 183].

Furthermore, the intestinal microbiota exerts a significant influence on various physiological processes, extending beyond digestion and immunity. It plays a crucial role in modulating stress responses, impacting reproductive functions, and contributing to developmental processes [140]. The administration of *L. rhamnosus* probiotics to zebrafish (*D. rerio*) elicited a significant enhancement in ovarian function, notably impacting oocyte maturation and ovulation. This effect was achieved through the downregulation of genes that typically impede these reproductive processes. Additionally, *L. rhamnosus* induced substantial modifications in the zebrafish gut microbiota, leading to larval development. This is characterized by improved growth dynamics and more efficient sex differentiation within the zebrafish population [36]. The coordination between gut microorganisms and reproductive endocrine regulation is mainly based on their ability to directly modulate hormonal profiles [109]. An experiment involving the dietary incorporation of probiotics in goldfish revealed notable enhancements across multiple reproductive parameters. Specifically, supplementation of feed with probiotics led to increased gonadosomatic index (GSI), heightened fecundity, and elevated fry production among spawning females. Additionally, this intervention correlated with augmented length and weight metrics in the resultant fry population [179].

The relationship between the composition of the gut microbiome and neuroendocrine system emerges as a crucial determinant in the modulation of crowding stress and growth regulation. This symbiotic relationship influences stress response mechanisms and behavioural patterns, especially impacting anxiety-like behaviours and locomotor activities. These behavioural alterations, in turn, affects the feeding behaviours and the maintenance of energy homeostasis [28]. In zebrafish, enriching the microbiota has been shown to mitigate anxiety-related behaviours and alleviate stress responses. This effect is achieved through the downregulation of corticotropin-releasing hormone (CRH) expression and reduction in cortisol levels. Conversely, periods of feed deprivation in zebrafish have been found to adversely affect their behaviour and stress physiology, potentially increasing their susceptibility to disease outbreaks [87]. Alterations in the gut microbiota can impair fish foraging behaviour and inhibit feeding activity by elevating stress hormone levels. Changes in hormonal levels disrupt central and peripheral mechanisms involved in nutrient sensing which is crucial for metabolic appetite control. Stress-induced inhibition of these mechanisms underlies the decreased appetite observed in fish under stress conditions [53].

Factors influencing the composition of fish gut microbiome

The fish gut microbiome composition is influenced by a diverse array of factors, including host genetics, developmental stage, feeding habits, diet, stress, disease, and environmental conditions [73, 263, 295, 312]. Environmental factors viz. season, temperature, pH, water and sediment quality play a significant role in shaping microbial communities (Fig. 2). Understanding these interconnections is essential for maintaining fish health and optimizing aquaculture practices [66, 202]. Environmental parameters, including water and sediment quality, exert substantial influence on the composition and diversity of the gut microbiota of aquatic animals. Water acts as a conduit for microbial transportation, facilitating the ingestion of microbes by fish through drinking or water intake. Subsequently, a fraction of these ingested microbes can colonize the fish gut microbiota, thereby contributing to its complexity and functionality [122, 303, 315].

Geographic location

Various geographical regions exhibit divergent environmental parameters encompassing temperature, salinity levels, seasonal patterns, water chemistry, and microbial constituents. These environmental variables exert a direct influence on the diversity and abundance of microorganisms within aquatic ecosystems, subsequently impacting the composition of the gut microbiota in fish [122, 203]. Liu et al. [162, 164, 166, 167] utilized high-throughput 16S rRNA gene sequencing to investigate how genetic variation and environmental differences affect the gut microbiome of three geographic populations of the black Amur bream (*Megalobrama terminalis*) from Pearl river, Moyang river and Wanquan river. Their study suggested that prolonged geographic isolation leads to diverse environmental conditions and genetic variations, which together influence the diversity of the gut microbiome in wild *M. terminalis*. Additionally, these findings offer valuable insights for studying ecological adaptation in fish populations [162, 164, 166, 167]. In contrast, Nikouli et al. [193] studied adult *Sparus aurata* and *Dicentrarchus labrax* individuals and found no significant geographic impact on gut microbial species. They observed a strong similarity in gut microbial communities between the two species, with only minor differences noted in the luminal gut communities. When Hanson-Regan [108] compared the gut microbiomes of both native and introduced species of the freshwater fish

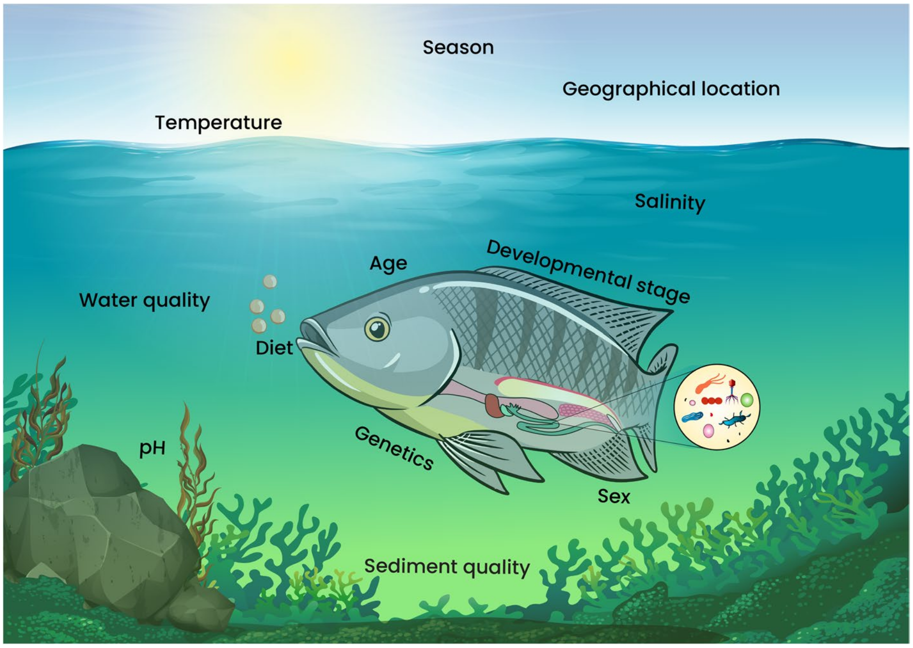

Fig. 2 Factors influencing the fish gut microbiome. The figure outlines key factors shaping the fish gut microbiome, including environmental conditions, diet composition, host genetics, health status, and microbial interactions, highlighting their combined effects on microbiome structure and function

invader *Cyprinella lutrensis,* they observed a significant change in gut microbiota between host sites. Additionally, they noted a lesser extent of variation among host species. Their findings emphasize that beyond geographical factors, the collective influence of host genetics, diet, and geography holds substantial importance in shaping the intestinal microbiota. However, in *S. salar*, a dramatic difference between environmental and gut bacterial communities was observed. Moreover, community composition was not significantly impacted by geography [168]. Gut microbial composition shift was also found in humans according to geography [238].

Season

Dulski et al. [71] performed high-throughput sequencing of 16S rRNA gene amplicons to investigate the gut microbial populations within Tench (*Tinca tinca*). They examined two distinct populations, wild Tench (lake) and Tench in semi-intensive farming (pond), during both the summer and autumn seasons. They observed a notable disparity in the abundance of *Candidatus xiphinematobacter* between lake and pond fish during the summer, but not in the autumn. Additionally, in pond Tench, the abundance of *Methylobacterium* exhibited a significant reduction during the summer compared to the autumn. Consequently, they propose that seasonal variations can exert an influence on the composition of the gut microbiome [71]. Similarly, in Nile tilapia (*O. niloticus*), conspicuous differentiations in microbial diversity were observed across diverse sampling periods and among populations inhabiting both natural habitats and captivity. These observations underline the pronounced impact exerted by environmental variables in modulating the intricate composition of the intestinal microbiota [20].

Seasonal fluctuations were noted in the gut microbiota of Atlantic salmon (*S. salar L.*). During the yearly cycle, lactic acid bacteria (LAB) consistently make up a substantial portion of the intestinal microbiota. However, bacterial species like *Vibrio* spp. were only detected at specific sampling points. The indigenous (autochthonous) microbial population appeared relatively stable across seasons, whereas the non-indigenous (allochthonous) population may change in response to different seasons. This suggests that while certain microbial groups remain consistent in the salmon gut throughout the year, others may exhibit seasonal shifts, possibly influenced by environmental factors or host physiology [120].

Temperature

Temperature is a key factor in regulating animal physiology and function. Response to temperature by each host species will be unique. Especially in the case of microbes, some may have the capacity to proliferate in elevated temperatures, while some of them get suppressed or may not have any effect. Likewise, certain gut bacteria like Firmicutes and Proteobacteria show consistent changes according to the variation in temperature [239] Hassenrück et al. [113] demonstrated that the temperature variation of 26 to 33 °C in juvenile milkfish induced changes in gut microbiota composition which in turn helped the host to acclimatise to temperature variation. Similarly, temperature variation from 8, 12, 16 and 20 °C in Chinook salmon (*O. tshawytscha*) resulted in the replacement of Vibrionaceae (*Photobacterium phosphoreum* and/or *P.pisciola*) by the Fusobacteriaceae (*C.somerae*) and Brevenemataceae (*Brevinema*) as the dominant population [256]. In addition, variation in water temperature from 8 to 18 °C in Chum Salmon (*O. keta*) showed the dominancy of Flavobacterium with an increasing level of Tenacibaculum at 8 °C and the most diverse population of other microbes with an increasing level of vibro at 18 °C. This indicates that temperature variation is very important in regulating dissolved oxygen, salinity, and pH levels in water, which is crucial for aquatic animal health [95].

In addition, most opportunistic pathogens get activated at elevated temperatures and can also cause gut dysbiosis in fish [95, 162, 164, 166, 167, 260]. In mussel larvae, (*M. coruscus*) a shift in gut microbiome composition was reported according to the temperature variation from 21 to 27 °C. As the temperature increases, the composition of several bacteria including *Delftia*, *Neptunomonas*, *Pseudoalteromonadaceae*, *Rhodococcus*, and *Stenotrophomonas*, while the composition of *Tenacibaculum* genera elevated significantly. *Tenabacibaculum* is an opportunistic pathogen that can cause the death of the larvae [320]. Elevated temperature of 28 °C was also known to impair the intestinal health of crabs [10]. Similarly, acute heat stress of 24.5 °C in Rainbow trout altered the normal gut morphology, permeability, and antioxidant capacity altogether harming metabolism (Zhou et al., 2020). Heat stress is also reported to damage the intestines, their microbiota, and overall physiology in sturgeons [162, 164, 166, 167]. However, low temperatures (14 °C) also showed a negative effect on gut microbial composition and thereby altered the normal physiological condition of largemouth bass (*Micropterus salmoides*) [172]. Cold stress is also known to affect intestinal health by reducing the intestinal microbiota and enhancing the proliferation of pathogenic Pleisomonas bacteria [121]. Moreover, many studies documented that temperature is a key factor in regulating gut microbiome health and composition [162, 164, 166, 167],).

Salinity

Salinity is regarded as another important factor which maintains the composition of intestinal microbiota. Apart from this, salinity is a significant factor that helps to maintain the osmotic pressure and, thereby, regulates respiration, metabolism, digestion, growth, and even the survival rate [98]. The effect of salinity variation among freshwater, brackish water, and marine water species may vary. Many studies have reported the change in gut microbiome in different species in different salinity ranges [133, 135, 163, 165]. Since Atlantic salmon is an anadromous fish, variation in salinity could affect its gut microbial diversity. Changes in the composition of the gut community have been reported during the transfer from seawater to freshwater. Proteobacteria were found as a dominant community in all groups, yet the presence of *Escherichia coli* or Shigella occupied a dominant population in the seawater [185]. There was a dominant community shift from Actinobacteria to Proteobacteria reported after acute other communities like phages did not show any response [98]. However, abundance of proteobacteria and lactobacillus in farmed Chinook Salmon remained unaffected by salinity shifts. Hence, some gut bacteria populations in fish can remain stable regardless of salinity [314].

Salinity stress also enhances the chances of infections among aquatic species. Low salinity stress in Pacific white shrimp made the species susceptible to pathogenic *V. parahaemolyticus* by reducing the gut microbiome richness and promoting the proliferation of pathogenic species [41]. High salinity exposure in grass carp resulted in boosting the growth of harmful bacteria with the suppression of beneficial bacterial growth. A shift in gut microbiome composition according to the salinity shift caused a deleterious effect on the overall health of the host [160, 161]. Both the low and high salinity levels induce changes in the intestinal microbiota diversity, increase the risk of susceptibility towards various diseases, and altogether disrupt the normal physiological functions of the host [56].

pH

pH level in water also has a significant role in balancing intestinal microbiota. Aquatic animals always come up with varying pH levels due to anthropogenic interventions. A change in pH level beyond the optimum level will disrupt gut-microbiota balance and also promote the enhancement of pathogenic microbes [261]. Low pH levels resulted in dysbiosis in marine sea bream, reducing the abundance of LAB in the intestine and finally promoting the growth of pathogenic strains [85]. Ocean acidification modified the intestinal community structure in *Crassostera gigas* [137] and edible oysters [57]. The relative abundance of Firmicutes declined and boosted the growth of mycoplasma in the case of *C. gigas* at low pH [137]. Elevated pH also possesses deleterious effects on gut microbiota. High alkaline conditions enhanced the growth of Bacteroides, ammonium-producing Providencia sp., and Vibrio pathogenic bacteria in common carp and disrupted the overall balance of intestinal microbiota [242]. Niu et al. investigated the changes in the gut microbiota of *C. elegans* in different acidic conditions and suggested that there exists a deep relationship between the environmental pH and microbial ecology. Extreme pH also resulted in gut dysbiosis in *C. elegans* [194]. Similarly, a pH drop caused an imbalance of intestinal microbiota in *Colossoma macropomum* (Sylvain et al., 2016). Hence, a change in pH level can cause dysbiosis of gut microbiota and can favour the growth of pathogenic microbes in aquatic animals [137].

Sediment and water quality

In aquatic systems, water and sediment are primary components that determine the composition of intestinal microbiota [124]. The microbial community present in the water and sediment represents the dominant community in the gut of the species in that habitat [124]. In the study conducted on shrimp *L. vannamei,* the bacterial community structure was different in the water, sediment, and intestine. Even though the bacterial communities were different, they shared a similar dominant bacterial profile. This indicates the deep relationship between the gut microbiota and the microbiota in sediment and water [122]. Similar findings were also observed in *P. japonicus*. The shrimp intestinal microflora closely mirrored the surrounding water microbiota and influenced the health of the host by regulating the immune activity [254]. Another study conducted by Fan et al. also demonstrates that bacterial composition is almost similar in the intestine and sediment in the *L. vannamei,* irrespective of its relative abundance [79]. Several studies documented that water and sediment are the main key factors in shaping the gut microbiome of aquatic animals [70, 117].

Host genetics, age, developmental stage, and diet

Intestinal microbiota is primarily shaped by genetics and phylogeny of the host, development, and other environmental factors [154]. Host genetics controls the responses toward the colonisation of bacteria in the gut, secretion of various enzymes, and other factors. Hence, both may have an equal role in shaping the intestinal microbial community [189]. Beyond other environmental factors, host genomics is the primary factor that shapes the gut microbiota in stickleback. Smith et al. observed that the more genetically divergent population of stickleback fish consists of the most divergent intestinal community [251]. Despite this in Channel catfish and blue catfish, host genetics played a minimal effect in regulating the gut microbial composition. This study strongly suggests that the same environmental condition and similar life history diminish the effect of host genetics in influencing gut microbial diversity [24].

In addition to the host genetics, the developmental stage is also a key factor in regulating gut microbiota. The environment of the gut will be different at different life stages and also the associated microbes. Microbial colonisation will change over time in each species. It may also be due to feeding at that specific period [313]. Gut microbiota associated with the younger and adult stages, including their relationships, were better studied than the intermittent stages [204]. In gilthead bream, the later age represented the high diversity of gut microbiota. In addition to age or developmental stage, the sex of the host also influences the microbial composition [209]. Basil et al. proved that the intestinal microbial composition mainly depends upon the fish's age more than the sex and sampling site. Age-related community shifts in the Bacteroidia and Desulfobacteria populations were also documented [19]. Similar findings can also be observed in the southern catfish. The gut microbial population underwent sequential changes according to the developmental stage in catfish [313] and also in sturgeon fish [204].

Host diet

Fish possess specific gut traits according to their diet and feeding habits. Herbivorous fish exhibit longer guts compared to the carnivorous species. The gut microbiota associated with the gut also varies according to the nutrition available through the diet [162, 164, 166, 167]. Several studies have been recorded on the modulation of gut microbiota with the application of specific feeds [94, 145, 269, 270]. Villasante et al. demonstrated the effect of a carbohydrate diet on the gut microbiome of Atlantic salmon. They found that the carbohydrate diet caused a low abundance of several bacteria except carbohydrate-dependent bacteria [281]. It was reported that the microbial shift and change in digestive enzyme activity were found in the gut of *M. amblycephala* during the feed transition. Mainly, the metabolic activity is changed during the feed transition [288]. Fasting periods also have a key role in maintaining the gut microbial balance. Proteobacteria were the representatives during the fasting period, and Firmicutes represented the feeding period in the Leopard coral grouper (*P. leopardus*). Host diet and fasting are also the main factors that shape the gut microbiome of the host [180].

Challenges in aquaculture and the need for microbiome engineering

Food security is a crucial factor required for acquiring sustainable development worldwide [80]. Aquatic food plays a significant role in global nutrition and food security [196]. However, wild fisheries are decreasing rapidly due to the overexploitation of aquatic wild stock [80]. Aquaculture offers a solution to the overexploitation of wild fisheries in a sustainable way [32, 39]. Aquaculture is regarded as a rapidly growing industry, expanding at a rate of 1.1% each year. It has been the fastest-growing food sector in the world over the past 50 years, with fish food production of more than 80 million metric tons [80].

The global aquaculture sector has thrived over the past 20 years and continues to grow by meeting critical goals of environmental, economic, and social sustainability. Aquaculture plays a significant role in meeting the need for protein sources for millions of people worldwide. Many people, especially those in coastal areas, mainly depend on aquaculture for their livelihoods [26]. Aquaculture contributed about 50% of fish to meet the needs of human beings in 2020. In addition, 62% of the global live-weight volume and 75% of the global edible weight volume will be contributed by inland freshwater aquaculture alone in 2020 [17, 27]. The rise in population has resulted in increased protein requirements, which in turn caused a surge in demand for aquaculture products [182]. About 40 million metric tons of aquatic food will have to be produced to meet the needs of people by 2030 [3, 23].

Sustainable aquaculture practices mainly focus on using low-impact farming methods, including non-fed aquaculture, recirculating aquaculture systems, or integrated agriculture–aquaculture, which helps to reduce waste and water use [175]. Although the aquaculture sector is growing, various challenges pose a serious threat to achieving sustainability [27]. Aquatic pollution, disease outbreaks, genetic degradation of aquaculture species, use of chemicals and drugs, decline in comparative profitability, lack of knowledge on market risks, and financial crises are some of the main challenges faced by aquaculture [155, 265]. Disease outbreaks are the main challenge faced by aquaculture today [243, 265]. Emerging various viral bacterial fungal diseases with changes in weather and climate make them even worse [173, 191].

Good aquaculture practices, including proper diet and maintaining optimal rearing conditions, are now being practised to mitigate disease-causing conditions. Along with this, antimicrobial products have been used to prevent microbial infections in aquaculture systems.

However, some pathogens are capable of resisting these antimicrobials. Despite this, another serious issue arising as a result of using antimicrobials is antimicrobial resistance, especially antibiotic resistance [181, 201, 290]. Vaccinations [264] have been used as an alternative to antimicrobials. Limited efficacy, ineffectiveness against juvenile fish, farmed crustaceans, and molluscs, and inefficiency in developing long-term acquired immunity become major limitations in vaccine implementation [208]. Biosecurity approaches and vaccine implementations have been used to mitigate various infectious diseases in aquaculture. Species-specific approaches and the severe contingency and efficacy of diseases still act as a barrier against these approaches [191]. The application of probiotics, prebiotics, synbiotics, and phytobiotics opened a new biological approach to replace the use of antimicrobials and vaccines [208]. Preventive measures against diseases and infections are far better than treating the diseases [5, 14]. Maintaining a healthy gut microbiome is a significant key to good health [73, 217].

Recently, there has been a significant rise in interest in gut microbiome research, as a healthy gut is considered an indication of the well-being of the host. Several studies are now going based on diversity, functions, and factors influencing the gut microbiome. Moreover, advances in high-throughput sequencing, including 16S rRNA sequencing and shotgun sequencing, have greatly contributed to deeper insights into gut microbial studies. Emerging technologies like metatranscriptomics, metabolomics, culturomics, and synthetic biology also open up a more detailed mechanistic approach to gut microbial studies than a simple phylogenetic description [86, 143]. Now, gut microbiome engineering seeks more interest due to its different approach to maintaining the health of individuals [126].

In humans, gut microorganisms have been used as biosensors to treat the dysbiosis of the microbiome against several diseases like cancer, metabolic, infectious, and auto-immune diseases. Intestinal gut microbiome engineering to produce a keystone consortium is an emerging area of research to produce healthy individuals [15]. Several application studies are ongoing in humans and other animals, rather than fish. Techniques like manipulation of the gut microbiome community, application of in vitro gut stimulators, and synthetic microbial communities are now focused mainly on humans. Similar technologies, including biotechnological approaches, can also be executed in fish, which will open a new door to gut microbiome research in fish [171]. Numerous strategies for intestinal microbiota manipulation together with probiotics in a beneficial way to maintain host health will bring solutions for various challenges in aquaculture, including disease outbreaks [93]. Emerging in situ genome engineering approaches to gut microbiome manipulation also boost the microbiome applications in fish hosts [245]. Implementations of these gut microbiome engineering techniques aim to achieve not only the maintenance of a healthy environment for fish but also the sustainable growth of the entire aquaculture sector [65].

Mechanisms and techniques of gut microbiome engineering

Gut microbiome engineering is an emerging field that aims to create a healthy host by manipulating gut microbes [304]. Microbial manipulation is done by altering gut microbiota composition or their metabolic activity to maintain host health [148, 149]. Gut microbiome engineering involves various techniques such as probiotics, prebiotics, synbiotics, phage therapy, genetic engineering, and faecal microbial transplantations [289]. Now, gut engineering studies are mainly concentrated in humans, mice, and rats [86].

Probiotics, prebiotics, and synbiotics: mechanisms and applications

Probiotics

Probiotics, prebiotics, and synbiotics possess significant applications in the aquaculture sector [60, 61]. Probiotics are living microorganisms with beneficial effects that can be administered to individuals in proper amounts. These microorganisms usually proliferate and colonize the gut of the host and help to maintain the good health of the host [116]. Probiotics are used to promote the growth of intestinal microbes and improve digestion, absorption, immune responses, and disease resistance in the host [300]. In addition, it can also remove other pathogenic microorganisms by providing a competitive environment [136]. Feed probiotics and water probiotics have been used to maintain microbial balance in the host.

Bacterial or fungal strains can be isolated and administered by mixing with feed as a probiotic. Whereas, these microbes can be simply served in the water as water probiotics. Water probiotics improve water quality by removing organic matter and other contaminants [59]. However, most of the probiotics are administered by mixing with feed. Several bacterial strains have been used in probiotics as live or dead strains [42]. Probiotic strains are selected based on several criteria. The strain should be free from antibiotic genes and could not affect host genetics. Microorganisms should be able to be administered to the host, and they should have the ability to survive the drastic conditions in the digestive tract and to proliferate in the host gut. These microbes should have antimicrobial properties and must affect promoting the health of the host [184]. In addition, these microbial communities should be native to the aquatic environment and compatible with the host. Mainly, probiotic communities must be safe for application, both for the host and the environment and free from any antibiotic-resistant gene. [59].

Several species of *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* were commonly used in probiotics. They include *L. acidophilus, L. casei, L. fermentum, L. gasseri, L. plantarum, L. reuteri, L. helveticus, L. bugaricus, L. salivarius, L. rhamnosus, L. johnsonii, L. paracasei, B.bifidum, B. breve, B. lactis, B. longum, Saccharromyces sp., S. boulardii, S. thermophiles,* and *S. cremoris* [42, 88, 272]. *B. subtilis* is regarded as one of the most potent probiotic microbes used for various applications. *B. subtilis* is used in *O. niloticus, Labeo rohita*, and *L. vannamei* for the secretion of digestive enzymes; in *M. rosenbergii* and *O. niloticus* for improving water quality. It is also used in aquaculture to improve water quality, maintain reproductive health, minimize stress, and promote antioxidant responses [96]. Streptomyces, another potent probiotic microorganism, is widely used due to its ability to secrete chemical compounds like streptomycin and oxytetracycline. Streptomyces provide disease-resistant capacity, antimicrobial activity, and enzyme modulation activity [29]. Several species of Lactobacillus were also widely used in aquaculture in therapeutic and prophylactic feed formulations [248].

Prebiotics

Prebiotics are non-digestible feed additives that can promote the growth and activity of bacteria in the gut [226]. Prebiotics are non – digestible carbohydrates that can be classified into monosaccharides, oligosaccharides, and polysaccharides based on the degree of polymerization [34]. These feed additives can promote growth, immunity, disease resistance, and stress reduction in aquatic animals. Prebiotics are mainly derived from plants and naturally occur in vegetables, fruits, beans, seaweeds, microalgae, edible mushrooms, and animal milk [285]. The beneficial effect of prebiotics is due to the by-products generated during fermentation by gut bacteria such as *Lactobacillus* and *Bifidobacterium* and gut enzymes [34, 225]. Short-chain fatty acids produced during this fermentation cause a pH drop in the gut and, in turn, affect the gut environment in a positive way that enables these prebiotics to be absorbed and utilized by the host [102].

Ideal prebiotics are selected based on several criteria. The prebiotic food additive should have the ability to overcome the enzyme degradation in the upper gastrointestinal tract. It should be utilized by the beneficial gut bacteria as an energy source and have a positive effect on the gut environment. It must be able to stimulate the immune responses in the gut environment [195]. Prebiotics help to minimize the chances of infection by promoting the growth of beneficial gut bacteria that produce inhibitory compounds, act as competent for adhesion sites, inhibit the quorum sensing in the gut and stimulate immune responses. [102]. These in-digestible carbohydrates directly interact with pattern recognition receptors (PRR) and activate the innate immune system. They can also stimulate the microbial-associated molecular patterns (MAMPs) activity [255].

Mannan-oligosaccharides (MOS), Arabinoxylan-oligosaccharides (AXOS), fructo-oligosacchrides (FOS), galacto-oligosccharides (GOS), Isomaltooligosaccharides (IMO), and trans oligosaccharides are the main oligosaccharides used as prebiotics in aquaculture. Inulin, β-glucans, stachyose, lactylol, lactosucrose, lactulose, and oligofructose constitute the other important prebiotics [8]. MOS has been used as an immunostimulant against *A. hydrophyla* infection in Nile tilapia [219]. Inulin is used to promote growth, immunization responses [301], and stress reduction [318] in Nile tilapia. In addition, XOS and GOS were known to enhance antioxidation and detoxification in Nile tilapia [297]. Fructo-oligosaccharides possess positive effects on growth performance, phagocytic activity, and resistance against *A. hydrophila* infection in *C. macropomum* (De et al., 2019). Growth performance, intestinal enzyme activity, and metabolism in *M. amblycephala* were stimulated by the use of XOS as a probiotic [1].

Synbiotics

Combinations of probiotics and prebiotics were denoted as synbiotics. Synbiotics gained much importance due to the application of live microbes into the gut along with dietary supplements [6]. Synbiotics promote the proliferation of good bacteria in the gut, which boosts intestinal enzyme activity and digestion. It also promotes the nutrient absorption ability, thereby ensuring efficient feed utilization by the host [227]. Moreover, synbiotics stimulate the intestinal epithelial cells to secrete immune functional cells and promote innate immune responses [125]. Synbiotics, as a combination of probiotics and prebiotics, offer not only growth performance and immune stimulation but also disease resistance capacity and gastrointestinal microbial balance. It also acts as a biological control agent in water and, consequently, supports the overall well-being of the animal [198].

The synbiotic composition is determined based on the prebiotic component. Prebiotics with high degrees of polymerization are highly recommended for use in synbiotics, as they can provide primary and secondary metabolites by fermentation and can be utilized by the host. In addition, more survival and action of probiotics were observed in combination with the prebiotics [30]. Synbiotics must be biocompatible and biodegradable, and they should ensure host and environmental safety [212]. Synbiotics have been applied to several aquaculture species to promote their growth and well-being [227].

Feed additives with microencapsulated Bacillus and oat β-glucan were known to improve the growth performance and feed utilization in Nile tilapia [67]. A combination of MOS with *B.* sp., *L. acidophilus,* and *E. faecium* in feed was regarded as a prophylactic alternative for fish farming [38]. Probiotic *L. casei* with *S. ferox* extract (SFE) in catfish (*C. gariepinus* var.*)* promoted growth, feed efficiency, and resistance against bacterial infections [110]. Feed supplementation of the synbiotic culture of *B. amyloliquefacien* promoted the growth rate and lyzozymal activity in Nile tilapia [92].

The concentration of prebiotics, probiotics, and synbiotics is a crucial factor to be determined before supplementation. These feed supplements beyond the optimal level have a negative effect on species and vice versa. Moreover, compatibility between the probiotics and prebiotics is also very important. These feed additives should be formulated based on the environmental conditions of the implementing species. Consequently, a long-term study is required for the formulation of probiotics, prebiotics, and synbiotics for each species [7].

Phage therapy and bacteriophage-based approaches

Bacteriophages are viruses that can infect and kill specific bacteria. They play a vital role in controlling the bacterial population and are capable of eliminating half of the bacterial population in each 24 h [214, 215]. The tail end of the virus can specifically identify and bind to the receptors on the surface of the bacteria, which makes them host-specific. The specificity can extend up to genus level, species level or to specific strains [266]. The phage life cycle comprises lytic and lysogenic cycles. Phages replicate and lyse the bacterial cell in the lytic cycle whereas in the lysogenic cycle, phage DNA gets incorporated into the host genome and transferred to the subsequent generations [218]. Phage-based microbiome engineering is a different approach that specifically reduces the targeted bacterial population by utilizing the phage life cycle as they lysis the bacterial cell [266]. Several studies on phage application to control or prevent pathogenic bacteria are well documented [141, 142, 211, 230, 249].

Phages can be isolated by using various methods including double layer agar method, concentration methods and filtration or adsorption-based separation methods from soil, water and sediment. An ideal phage must be strictly lytic, and free of antibiotic resistance and virulence gene. Moreover, they should be polyvalent, environmentally tolerant and able to co-exist with other phages. The ability to destroy the bacterial biofilm is also considered a priority in phage selection [162, 164, 166, 167]. Engineered phages through various techniques viz. Chemical mutagenesis, CRISPR/Cas and Caspe technique Cell-free transcription techniques and Bacteriophage recombineering with electroporated DNA (BRED) also can be used to improve phage specificity [127]. Broad-spectrum phages, also known as phage cocktails, are mixtures of multiple phages that can infect multiple hosts that belong to distinct species or genera [62].

Feed pellets fixed with phage are considered an effective method for delivering phages to the fish through oral administration. Application of phage therapy together with dietary supplements and anti-microbials could enhance its efficacy (Donati et al., 2022). Combination of phages with probiotics has been practised in various studies. Phages that can overcome the dry conditions in the environment are only chosen as a coating for the fish feed [214, 215]. Biopolymers like edible whey protein isolates (WPI) coating on fish feed incorporated with phages were known to improve the loading efficiency and storage stability and act as a barrier against gastrointestinal digestion. Hence, the phage activity can be limited during the storage phase and can ensure effective activation in the target site. WPI coating also helps to overcome phage dissociation in saltwater. Consequently, WPI remains a cost-effective method for treating bacterial infections in aquaculture [123]. Oral application of phages is regarded as the most convenient method of phage administration rather than the method of injection and direct application to the surrounding water [214, 215].

Phage therapy remains an excellent approach to eradicating pathogenic bacteria compared to antibiotics. Antibiotics can only avoid the infection for a particular time, but phage therapy can maintain the overall gut microbiota composition irrespective of the infection [68]. Phage therapy also provides a solution for multidrug resistance in bacteria [82]. Bacteriophage-based approaches helped to eradicate multi-antibiotic-resistant bacteria like *Aeromonas* subsp. Masoucida in aquaculture [298]. Apart from this, phage Pzl-Ah152 was known to be well effective against *A. hydrophila*, used to treat enteritis of crucian carps. Pzl-Ah152 boosted the gut microbial diversity without disrupting its balance [82]. MJG Phage are also used to treat the *A. hydrophila* infection in Rainbow trout [33] and Phage D6 in fish pellets [216]. Cocktail of Phages VB_VaC_TDDLMA (phage TDD) and VB_VaC SRILMA (phage SRI) can control the *V. alginolyticus* infection in brine shrimp culture (*A. franciscana*) [262]. Motile Aeromonas Septicemia (MAS) caused by *Aeromonas* spp. in striped cat fish (*P.hypophthalmus*) is effectively treated by the *A. hydrophila*-phage 2 and *A. hydrophila*-phage

5 [147]. Early or simultaneous administration of phage could have more effect against bacterial infections in aquaculture [141].

Microbial transplantation and other strategies

Faecal Microbial Transplantation (FMT) or simply microbial transplantation is the engraftment of a microbial community from the stool of a healthy donor to the recipient to restore or modify the normal gut microbiota [25, 132]. The FMT concept was first described in humans during the fourth century in China [310]. Microbial Transplantation also called Faecal bacteriotherapy opens a new way to treat dysbiosis in the gastrointestinal tract [49]. Apart from the faecal transplantation, the Bacterial consortium is utilized as the most convenient method of FMT in aquaculture. Bacterial transplantation is implemented through probiotics, with feed or by simply increasing their concentration in surrounding water [279]. Understanding and observing the current status of the recipient, donor selection and selection of method of implementation are the main factors taken care of during FMT. In addition, compatibility between the species and environment also should be considered [22, 279]. Though the FMT have good beneficial effects, side effects like diarrhoea and abdominal pain are reported in humans [4]. Similarly, the side effects of FMT in fish also need to be evaluated prior to the application.

FMT can restore the gut microbial diversity after antibiotic treatment or dysbiosis. Han et. al. demonstrated the restoration of gut microbiota after florfenicol-perturbation to the intestine. The microbiome diversity lost as a result of florfenicol application was successfully restored by the FMT than that of natural recovery. FMT also promotes intestinal damage repair and stabilizes intestinal metabolites [105, 107]. FMT in large yellow croaker (*L. crocea*) larvae were observed to promote intestinal health by enhancing intestinal microbial diversity. The larvae gut after FMT were shown to increase in α-amylase and trypsin, leucine-aminopeptidase activity and a well-established gut environment than normal (Zhang et al., 2023). FMT implementation in the early life stage of Rainbow trout resulted in improved muscle yield and fast growth through gut microbiota modulation [223]. Three Lactobacillus strains isolated from Patagonian fish were colonised and established in the gut of zebrafish and modified their intestinal microbiota composition [240].

Inter-species Intestinal microbial transplantation (IMT) was carried out between donor Atlantic salmon (*S.salar*) to recipient gilthead seabream (*S. aurata*), after disrupting the normal gut microbial community of seabream with antibiotics. IMT was observed to be maintained and enriches the growth of novel bacteria over time. This study suggests that IMT can be done within the species or between different species and could be used to improve the digestive mechanisms and thereby maintain the fish's health [231]. Reciprocal FMT has been carried out from the Zebrafish and Mice to the germ-free recipients showing the development of a new microbial community in the gut. A newly introduced microbial community will proliferate and shape the intestinal microbiota composition, where the host acts as a habitat or basic niche [221]. Similar reciprocal microbial transfer was also applied in Zebra fish from humans and some of the bacterial communities were known to be colonised successfully [11, 273, 278]. Lactic acid bacteria isolated from the Atlantic Salmon (*S. salar*) [9], Salmonids [280], and freshwater fish [100] can be used as probiotic candidates in aquaculture.

Application of phytogenic, immune and metabolic modulators are the other strategies used to modify the gut microbiota. Herbal seed powder supplementation consisting of fennel, fenugreek and anise seeds improved intestinal microbiota health and the overall health of European sea bass [13]. Allium-derived compounds also modulated the intestinal microbial component in Gilthead Seabream (*S. aurata*). Many studies documented the effect of phytobiont effect in shaping the gut microbiota [44, 269, 270, 294]. RNase 1 was used as a gut microbiota and metabolome modulator in Blunt snout after the *Aeromonas* infection. RNase of *M.amblycephala* with antimicrobial activity and digestive activity played a significant role in reducing inflammation of the gut, reducing pathogen invasion and improving intestinal function [90]. Similarly, other roles of RNase can also be evaluated for enhancing gut microbiota [45, 259].

Genetic engineering and biotechnological interventions

Advancement in biotechnology and genomic techniques enables researchers to modify microbial strains and improve gut microbiome diversity. CRISPR/Cas-based tools, synthetic biology techniques, and metabolic engineering are the main engineering techniques applied in gut microbial studies [321]. Synthetic biology approaches in the gut microbiome are now mainly practised in humans, where it engineers bacteria to improve their functions by modifying their natural biological components. Synthetic biology approaches mainly focus on designing the cells and enzymes thereby effectively improving cell communication [139]. Gene editing tools like CRISPR/Cas technique, Transcription-activator effector nucleases (TALEN) and Zinc finger nucleases (ZFNs) unlocked new entities of synthetic biology [139].

CRISPR-based genome editing techniques are widely used to modify the functional genes of lactic acid bacteria (LAB). CRISPR/Cas systems can induce double-strand break in the target site of nucleic acid by the action of Cas9 nuclease and repairs, which result in genome editing [160, 161]. Genome engineering is an important synthetic biology tool that can be applied to probiotics. The inducible plasmid self-destruction (IPSD) technique is one of the genome engineering or editing techniques that delivers homologous DNA into the bacteria and can result in gene knock-out or knock-in. IPSD was effectively implemented in lactobacilli and bifidobacteria to modify the probiotics [321].

Metagenomic Alteration of the Gut microbiome by In situ Conjugation (MAGIC), is a tool of synthetic biology in which genetic modification is carried out with the help of their natural DNA exchanging capability. In MAGIC, a donor strain can be modified and developed, so that they can able to transfer the genetic pyloads to other bacterial strains by normal gene transfer methods like conjugation [150, 229]. Reproducible pGT vectors also can be transferred between the bacterial strains with the help of the MAGIC tool [139]. General methods of gene transfers, including transformation, transduction, and conjugation, can be applied to insert required DNA into the bacterial cells to modify their functions to attain a modified gut microbiota. Chemical transformation and electroporation are commonly used transformation tools, whereas suicidal plasmid vectors, conjugative transposons and shuttle plasmid vectors belong to the conjugating techniques [244] [150]. Apart from this, synthetic biology utilises several tools like, molecular docking, artificial intelligence techniques, nanotechnology and post-translational modification to engineer the microbial enzyme secretions [247].

Applications

Successful applications of gut microbiome engineering in aquaculture and its impact on fish health and performance

Application of probiotics in aquaculture

Previous research shows that probiotics play a significant role in fish health (Table 1). Probiotics possess various applications in aquaculture to improve growth, immunity, and the overall health of the animal. Various probiotic strains exhibit different effects on each aquaculture species. Several species of *Bacillus*, *Streptococcus*, *Pseudomonas*, and *Enterococcus* are commonly used probiotic strains in aquaculture. A combination of several bacterial strains or individual strains can be formulated as probiotics [31], demonstrated that the application of *Lactococcus lactis* enhances the immunity in *O. niloticus* by excluding the pathogenic bacteria through competition. It also boosts the nutritional value of its food [31]. A similar effect in improving growth, disease resistance, and intestinal health was observed in *O.niloticus* with the combined application of *S. agalactiae*, *B. cereus NY5*, and *B. subtilis* [138]. A mixture of *E. xiangfangensis* (GFB-1), *P. stutzeri* (GFB-2), *B. subtilis*, *Citrobacter freundii*, and *P. aeruginosa* was known to improve growth, reproductive performance, and gut health in *B. gonionotus* [233].

B. cereus and *G. candidum* have been associated with the growth enhancement and stimulation of digestive enzyme activities in *L. rohita* [94]. Similarly, the combination of *B. amyloliquefaciens* and *B. subtilis* was reported to improve immune responses and growth in *L.rohita* and *Heteropneustes fossilis* [235, 252]. *B. amyloliquefaciens* is shown to improve the immunity growth and the lipid metabolism in *O. niloticus* [246], whereas *B.subtilis* and *B. licheniformis* together are linked to influencing reproductive performance in Red tilapia (*O. niloticus x O. mossambicus*) [74]. *Lactiplantibacillus plantarum* enhances nutrient utilisation and intestinal enzyme activities in *Larimichthys crocea* [163, 165]. All these studies highlight the different applications of probiotics in aquaculture and their potential to enhance immunity, growth, disease resistance, and feed utilisation efficacy in various aquaculture species.

Applications of prebiotics in aquaculture

Prebiotics also share similar applications with probiotics, as they enhance gut health by improving immunity, growth, disease resistance, and survival rate (Table 2). Fructooligosaccharides (FOS), galactooligosaccharides (GOS), mannanoligosaccharides (MOS), and inulin are the main prebiotics widely used in aquaculture. A combination of FOS, GOS, MOS and inulin showed a positive impact on the growth and health of *C. gariepinus* [89]. Improved stress resistance and survival rates were reported by the application of FOS, xylooligosaccharides (XOS), chitosan, β-glucan, and MOS in hybrid grouper (*Epinephelus lanceolatus* x *E. fuscoguttatus*) [319]. Similarly, in *O.niloticus,* MOS with vitamin E was found to enhance its reproductive performance [253]. Chito-oligosaccharides also showed improved growth performance, innate immunity, and intestinal health in *O. niloticus* [197], whereas it was found to improve gut health and resistance against *A. hydrophila* in hybrid tilapia (*O. niloticus x O. aureus*) [213]. Chitosan was given to *Oreochromis niloticus* to promote innate immunity, antioxidant capacity, and overall intestinal morphology [159].

Guerreiro et al., demonstrated that FOS, XOS and GOS could enhance immunity in *Diplodus sargus* [101]. A combination of Inulin, GOS with D-sorbitol is known to boost growth performance, serum complement and cytokine levels, lysozyme activity, intestinal antioxidant capacity and gut microbial diversity in *Nibea coibor* [158]. Inulin has also been reported to improve the growth, serum immunity and immune responses in *O. mykiss* [91] and promote growth and immunohematological indices in *O. niloticus* [301]. Moreover, inulin together with

Table 1 Applications of probiotics in aquaculture

Probiotic strain	Applications	Aquaculture species	Reference
Lactococcus lactis	Exclusion of pathogenic bacteria, boosting food nutritional value, enhancing the host immune response against pathogenic microorganisms	*Oreochromis niloticus*	[31]
Streptococcus agalactiae, *Bacillus cereus* NY5 and *B. subtilis*	Promotes growth and disease resistance, improves gut immune status, intestinal morphology, and intestinal microbial community composition	*Oreochromis niloticus*	[138]
Enterococcus xiangfangensis (GFB-1), *Pseudomonas stutzeri* (GFB-2), *Bacillus subtilis*, *Citrobacter freundii*, and *P. aeruginosa*	Improves growth, histological and hematological parameters, reproductive performances, enhancement of digestive enzyme activities and lactic acid bacteria composition in the gut	*Barbonymus gonionotus*	[233]
Bacillus cereus, Geotrichum candidum	Increases growth, feed utilization capacity, haematological profile and digestive enzyme activities	*Labeo rohita*	[94]
Bacillus cereus	Enhances growth performance, nutrient retention, elevated digestives enzyme activities of protease, amylase and cellulase	*Cirrhinus mrigala*	[21]
B. amyloliquefaciens BN06, B. subtilis WN07and B. megaterium CT03	Increases growth, hemato-immunological and enzymatic activity	*Labeo rohita*	[235]
B. amyloliquefaciens BN06, B. subtilis WN07and B.megaterium CT03	Enhances growth, health status, and economic viability	*Heteropneustes fossilis*	[252]
Bacillus licheniformis, *B. subtillis*, *B. polymyxa*, *B. pumilus*, *B. amyloliquefaciens*, *B. megaterium*, *B. coagulans*, *Aspergillus niger*, and *A. oryzae*, *B.licheniformis*, *Rhodococcus* spp., *Rhodobacter* spp., *Nitrosomonas*, and *Nitrobacter*	Improves growth, feed utilization, non-specific immune responses and disease resistance	*Cyprinus carpio*	[103]
Bacillus subtilis	Improves intestinal histological features, immune responses and disease resistance	*Lates calcarifer*	[232]
Bacillus amyloliquefaciens	Influences fish growth, immunity, and lipid metabolism	*Oreochromis niloticus*	[246]
Lactic Acid *Bacillus, Bacillus subtilis, Bacillus licheniformis, Aspergillus oryzae, Aspergillus niger and Saccharomyces boulardii*	Enhances growth and survival rate	*Pangasianodon hypophthalmus*	[51]
Bacillus amyloliquefaciens	Boost immunity and survival rate	*Labeo rohita*	[18]
L. plantarum	Improved the growth, survival rates, disease resistance, intestinal integrity and intestinal enzyme activities	*Larimichthys crocea*	[163, 165]
Streptococcus faecalis, Lactobacillus plantarum, Bacillus mesentericus	Enhancement of immune response, growth and antioxidant enzymatic activity	*Pagrus major*	[241]
B. subtilis	Increases immunity and survival rate	*Labeo rohita*	[250]
Bacillus coagulans	Positive regulation of intestinal immunity, barrier function and inflammatory responses	*Macrobrachium rosenbergii*	[316]
Lactiplantibacillus plantarum1 and *Lactiplantibacillus plantarum2*	Enhances nutrient utilization, digestive enzymes, and metabolism by increasing the gut surface area of juvenile fish	*Lates calcarifer*	[77]
Bacillus amyloliquefaciens	Increases feed conversion ratio, growth, survival rate	*Oncorhynchus mykiss*	[210]
Bacillus subtilis and *B. licheniformis*	Accelerates reproductive performance	Red Tilapia (*Oreochromis niloticus* x *O. mossambicus)*	[74]
Bacillus subtilis strains-BS, 6–3-1, and HAINUP40	Promotes growth and immunity	Hybrid grouper *(Epinephelus fuscpguttatus x Epinephelus lanceolatus)*	[105, 107]

Table 2 Application of prebiotics in aquaculture

Prebiotic component	Effects	Aquaculture species	Reference
Fructooligosaccharide, galactooligosaccharide, and mannooligosaccharide	Positive effect on growth and health conditions	*Clarias gariepinus*	[89]
Fructooligosaccharide, xylooligosaccharides chitosan, β-glucan and mannooligosaccharide	Improves stress resistance and survival rates	Hybrid grouper (*Epinephelus lanceolatus* × *Epinephelus fuscoguttatus*)	[319]
Mannan oligosaccharides with vit E	Enhances the reproductive performance	*Oreochromis niloticus*	[253]
Chito-oligosaccharide	Improves growth performance and innate immunity	*Oreochromis niloticus*	[197]
Chito-oligosaccharide	Improves intestinal health, changed autochthonous gut bacteria, and resistance against *A. hydrophila infection*	Hybrid tilapia (*Oreochromis niloticus* × *Oreochromis aureus*)	[213]
Chitosan	Promotes growth, intestinal morphology, innate immunity, and antioxidant capacity	*Oreochromis niloticus*	[159]
Fructooligosaccharides (scFOS), xylooligosaccharides (XOS) and galactooligosaccharides (GOS)	Enhances immunity	*Diplodus sargus*	[101]
Inulin, glactooligosacharides and D-sorbitol	Improves the growth performance, serum complement and cytokine levels, lysozyme activity and intestinal antioxidant capacity. Modified gut microbial composition	*Nibea coibor*	[158]
Inulin	Promotes growth and biochemical parameters, as well as serum immunity and mucosal immune responses	*Oncorhynchus mykkis*	[91]
Inulin	Enhances growth performance, immunohaematological indices	*Oreochromis niloticus*	[301]
Inulin and jerusalem artichoke tuber	Positive effects on growth and health	*Oreochromis niloticus*	[271]
Fructooligosaccharides	Improves growth performance, survival rate, and digestive capacity, and could contribute to the reinforcement of the intestinal barrier function	***Atractosteus tropicus***	[207]
Fructooligosaccharides	Enhances growth, digestive enzyme activities, gut morphology and intestinal barrier function	*Atractosteus tropicus*	[239]
Fucoidan	Improves the growth by promoting digestive tract maturation and maintaining an ideal intestinal microbial composition	*Larimichthys crocea*	[308, 309]
Mannan-oligosaccharide	Enhances growth and the activity of digestive enzymes	***Atractosteus tropicus***	[176]
Fructooligosaccharide	Increases the growth, digestion and immune activities	*Lateolabrax japonicus*	[283]
Fructooligosaccharide (FOS), galactooligosaccharide (GOS), mannanoligosaccharide (MOS), inulin, β-glucan, isomaltooligosaccharide (IMO) and xylooligosaccharide (XOS)	Pathogen exclusion, improve digestion and nutrient absorption and modification of gut microbiota	*Litopenaeus vannamei*	[317]
Mannan-oligosaccharide and inulin	Increase in growth	*Litopenaeus vannamei*	[277]

Jerusalem artichoke tuber enhanced the growth activities of *O. niloticus* [271]. FOS enhanced growth, survival rate, digestive capacity, intestinal barrier function, digestive enzyme activities gut morphology in *Atractosteus tropicus* [207, 239].

Fucoidan has been linked with the growth, digestive tract maturation and maintenance of an ideal intestinal microbial composition in *L. crocea* [308, 309]. The combined action of FOS, GOS, MOS, inulin, β-glucan, isomaltooligosaccharides (IMO) and XOS resulted in pathogen exclusion, improved digestion, nutrient absorption and modification of the gut microbiota in *L. vannamei* [317]. MOS application enhanced growth and digestive enzyme activity in *Atractosteus tropicus* [176]. Similarly, growth enhancement and improvement in digestion and immune activities were observed in *L. japonicus* by the action of FOS [283]. Moreover, MOS and inulin were also known to evoke growth promotion in this species [277]. All these findings emphasize the significance of probiotics in the aquaculture sector.

Synbiotics applications in aquaculture

Synbiotics, which combine probiotics and prebiotics, exhibit a significant role in aquaculture by enhancing the growth, immunity, disease resistance, and gut health of various species (Table 3). Effects of probiotics and prebiotics will vary in the same species itself based on their combinations and proportions. *Bifidobacterium sp., L. acidophilus,* and *E. faecium,* along with MOS and chitosan, have been reported to improve the immunity and resistance against the *A. hydrophila* infection in *O. niloticus* [38]. The combination of *A. oryzae* and β-glucan also promotes the immune response in the same species [60, 61]. In *A.japonica,* the administration of *B. subtilis* with MOS and *E. faecium* with FOS was found to improve its disease resistance [200]. Enhanced growth, feed utilisation, intestinal health, and non-specific immunity were the notable improvements observed in *L. vannamei* with the administration of *B. subtilis* and *S. cerevisiae* with β-glucan and MOS [299]. Whereas *L.plantarum* L20 and *S. polycystum* enhanced the growth, disease resistance, and protection of gastrointestinal structure by modulating the gut microbiota in *P. monodon* [47]. Similarly, in *M.rosenbergii*, the combination of *L. rhamnosus* and Yu-Ping-Feng polysaccharide improved lipase activity and maintained a balanced environment [156].

The inclusion of *B. subtilis* and *L. plantarum* with xylo-oligosaccharides was found to promote the immunity and overall performance of *O. niloticus* [284]. Acinetobacter KU011TH and chitosan in hybrid catfish (*C. gariepinus x C. macrocephalus*) influenced growth, immunity, and disease resistance against *A. hydrophila* [236]. A combination of galactooligosaccharides (GOS) and *P. acidilactici* was found to increase innate immune responses and skin mucus parameters in *O. mykiss* [115]. Moreover, *L. delbrueckii* subsp. *bulgaricus*, along with *A. officinalis* initiated improvements in digestive enzyme activity and overall metabolic efficiency in the same species [302]. Reproductive performance and growth of *D. rerio* were also found to be influenced by the combined action of *Lactobacillus acidophilus* and *L. delbrueckii* subsp. *bulgaricus* [305]. Overall, the combined action of prebiotics and probiotics as synbiotics brings more significant effects in aquaculture in terms of their immunity, growth, and overall development and thereby supports the sustainability of aquaculture.

Application of bacteriophage-mediated vector transfer

The applications of bacteriophage-mediated vector transfer in aquaculture also offer a promising approach to modifying gut microbial communities and enhancing the overall health of the aquaculture species (Table 4). In the same way as prebiotics, probiotics, and synbiotics, bacteriophage application also basically influences the growth, immunity, and disease resistance in aquaculture species. For instance, the application of FpV4 and FPSV-D22 targeting *F. psychrophilum* was reported to positively alter the gut microbiota of *O. mykiss*, independent of the target bacterium. This study also underlines that the intervention of phage in the gut affected their interaction with other bacteria [68]. Treatment of *A.hydrophila* with phage PZL-Ah152 positively influenced the gut microbiota in *C.carassius* [82]. Vb AsM ZHF phage targeted *S.maximus*, promoting immunity and disease resistance against *A. salmonicida subsp.* and reducing the mortality rate [298]. Introduction of FCL-2 phage against *F. columnare* increased the survival rate of *Salmo salar* by reducing the relative amounts of the pathogen in water. Similarly, phage MJG phage targeting *A. hydrophila* resulted in restoration of liver tissue damage and elimination of the clinical signs of infections in *O. mykiss* [33]. All these reports strongly denote the significance of phage-mediated vector transfer in fish gut microbiome engineering as a way forward**.**

Lessons learned and challenges encountered in real-world implementations

Gut microbiome engineering is widely applied in humans, and its implementation in aquaculture is more challenging. However, research on these gut microbiome techniques and their applications in aquaculture is focussing more now. Now, the use of probiotics, prebiotics, and synbiotics has become more common in aquaculture, where biotechnological approaches remain rare and need to be focused more [171]. Probiotics, prebiotics, and synbiotics can be applied in aquaculture to improve growth, digestion, immunity, disease resistance, nutrient absorption, and water quality [31, 74, 77, 284, 319].

Probiotic strain identification and isolation, including determination of its proportion of use and evaluation of its safety and effectiveness on required species, is the main challenge faced during the formulation of a probiotic, especially in aquatic species. Beyond the probiotic formulation, its application at the farm level in agriculture is another challenge. Knowledge of the benefits of probiotics and their administration of use among farmers is to be boosted to achieve the effective use of probiotics in aquaculture [258]. Evaluation of safety and efficacy is very important to selecting a bacterial strain as a probiotic. Temperature is a significant factor that affects the viability of the probiotic strain, especially during storage. Storage of probiotics at 4°C can maintain viability and

Table 3 Applications of Synbiotics in aquaculture

Synbiotics	Effects	Aquaculture species	Reference
Bifidobacterium sp, *Lactobacillus acidophilus* and *Enterococcus faecium* + Mannan oligosaccharides and chitosan	Boosts immunity and resistance against *A. hydrophila* infection	*Oreochromis niloticus*	[38]
Aspergillus oryzae + β-glucan	Improves immunity	*Oreochromis niloticus*	[60, 61]
Bacillus subtilis, *Saccharomyces cerevisiae* + β-glucan and mannan oligosaccharide	Improved growth, feed utilization, intestine health and non-specific immunity	*Litopenaeus vannamei*	[299]
Bacillus subtilis + mannooligosaccharides and *Enterococcus faecium* + fructooligosaccharides	Enhances disease resistance	*Anguilla japonica*	[200]
Lactobacillus plantarum L20 **+** *Sargassum polycystum*	Improves growth, disease resistance and gastrointestinal structure protection by modulating gut microbiota	*Penaeus monodon*	[47]
Lactobacillus rhamnosus + Yu-Ping-Feng polysaccharide	Improves the activity of lipase and the content of lactic acid during in vitro fermentation, maintaining balanced gut environment and gut microbiota	*Macrobrachium rosenbergii*	[156]
***Saccharomyces cerevisiae* +** Mannan Oligosaccharides, and β-Glucan	Enhances immunity including antioxidant activity and disease resistance against *P. aeruginosa* infection	***Oreochromis niloticus***	[76]
Bacillus subtilis and *Lactobacillus plantarum* + xylooligosacharides	Increases immunity growth and overall performance	*Oreochromis niloticus*	[284]
Bacillus subtilis + inulin	Greater weight gain and growth, resistance against *A. hydrophila* infection	*Pseudoplatystoma reticulatum*	[199]
Acinetobacter KU011TH + chitosan	Improves growth and immunity. stronger disease resistance against *A. hydrophila* infection	Hybrid Catfish (***Clarias gariepinus*** × ***C. macrocephalus***)	[236]
Galactooligosaccharides (GOS) + *Pediococcus acidilactici*	Increases innate immune response and skin mucus parameters	*Oncorhynchus mykiss*	[115]
L. bulgaricus FNCC–004 + alginate-probiotic mixture	Enhances growth and help to maintain good environmental conditions	*Litopenaeus vannamei*	[224]
Lactobacillus plantarum L20 + *Sargassum polycystum*	Positive effect on growth, immune response, and disease resistance	*Penaeus monodon*	[48]
Lactobacillus acidophilus + *Moringa oleifera leaf extract*	Enhanced growth and immunity	*Penaeus vannamei*	[58]
***Bacillus sp. SJ-10* +** prebiotic β-glucooligosaccharides	Boosts protection against *S. iniae* infection, improved immunity, growth rate, feed conversion ratio, and protein efficiency ratio	*Paralichthys olivaceus*	[112]
Bacillus subtilis WB60 + mannan oligosaccharide	Increase in growth performance, non-specific immune responses, disease resistance and modulates intestinal morphology	*Anguilla japonica*	[148, 149]
Lactobacillus delbrueckii subsp. *bulgaricus* + *Asparagus officinalis* L	Improved digestive enzyme activity, higher efficiency on metabolic processes and microbial function in the gut	***Oncorhynchus mykiss***	[302]
***Lactobacillus acidophilus and Lactobacillus delrueckii subsp. Bulgaricus* + button mushroom extract**	Positive influence on the growth and reproductive performances	***Danio rerio***	[305]
Lactobacillus plantarum + xanthan gum	Increases disease resistance and survival rates	*Litopenaeus vannamei*	[286]

efficiency for a long period [50]. In addition, water quality, feed utilisation, species, and other environmental factors are also affecting the viability of probiotic strains [7]. Genetic exchange between the probiotic strains changes the function of the strains, making the formulation less effective in the host. Moreover, they can also be emulated as opportunistic pathogens and can infect the host [75]. Along with this, prebiotics and synbiotics applications are also facing similar limitations in modulating gut microbiota. Formulation of prebiotics, probiotics, and synbiotics in different aged and sized fish and their application level at each species, together with the evaluation of effectiveness and safety in each administration method, are the main challenges faced in this sector [186].

Likewise, formulation and administration techniques of safe phages are the main challenge in

Table 4 Application of bacteriophage mediated vector transfer in aquaculture

Phage	Aquaculture species	Effects	Reference
FpV4 and FPSV-D22 targeting *F. psychrophilum*	*Oncorhynchus mykiss*	Phage addition positively altered the microbiota of the fish independently of the presence of their target bacterium. It affects the complex network of phage- bacteria interactions in the gut	[68]
PZL-Ah 152, infects *Aeromonas hydrophila*	*Carassius carassius*	The phage PZL-Ah152 reduced the number of colonies of the genus *Aeromonas* and also had a positive effect on the gut microbiota	[82]
FCL-2 against *F. columnare*	*Salmo salar*	Phage treatment increased survival rate and reduced the relative amounts of the pathogen in the water	[83]
MJG against *Aeromonas hydrophila*	*Oncorhynchus mykiss*	MJG treatment would restore liver tissue damages and abolish the clinical signs of infection	[33]
vB_AsM_ZHF	*Scophthalmus maximus*	Provides, immunity, reduced mortality and disease resistance against *A. salmonicida subsp.*	[298]

phage-mediated vector transfer in aquaculture. Jun et al. demonstrated that phage pVp-1 has bacteriolytic activity against three representative AHPND-V. *Parahaemolyticus* strains in *L. vannamei.* Phage therapy resulted in no such resistance against the AHPND as its proliferation is so rapid. Hence, the relationship between the period of phage division and the proliferation is an important factor to be considered in phage therapy [129]. Sometimes, single-phage-type applications will not be enough to control the infection due to the rapid growth of the pathogen. Advanced methods like phage cocktail combined with toxin binder can be applied in these situations [99]. Another main challenge faced in this technique is the safety issue due to the virulence genes associated with phages. Virulence genes associated with the bacteriophages, especially in the case of temperate phages, should be observed properly and ensure the safety of these phages before use [157]. In aquaculture, mainly phage therapy is applied to bathing, feeding, injection, and wiping. The effective optimal safety delivery method must be developed before its implementation [54].

FMT is considered a powerful tool to treat dysbiosis and to restore the gut microbiome in animals. The selection of healthy donors and receivers is the important factor considered first and can be achieved by rigorous screening experiments [171]. FMT is mainly applied in humans now, though the studies in advanced use of FMT remain in an unoccupied area [4]. Selection and screening of the desired donor strains from the donor is a very large and time-consuming method. Its application in other individuals belonging to the species or different species must be tested and screened properly to evaluate the effects [94]. Some recipient populations may not be able to accept and develop the new gut microbiome, mainly in stressful conditions. Receptance ability will be directly correlated with the health of the recipient. FMT will be successful mostly in the younger ages than in the older ones [121]. Tang et al. demonstrated the effective transplantation of young faces enhanced metabolic capacity in older ones to restore gut microbiome diversity [267].

Gut microbiome modulation through the CRISPR method is much more expensive. Inappropriate aquatic genome data is another limitation of the CRISPR technique in aquaculture [104]. It is much more difficult to incorporate genes into a group of mixed microbiotas, and it is only possible in the population of E. coli. Moreover, gene insertion can occur multiple times in multiple target sites and can also bring a negative result. Like other engineering techniques, precise standardisation of the safer CASPER editing technique is the most important challenge [244].

Environmental consequences and sustainability considerations.

As the researchers focus more on gut microbiome engineering to improve sustainability in aquaculture, environmental consequences are gaining more concern and attention. The main concern is regarding the biotechnological application in this field [151]. Intensified aquaculture production and practices began to depend on antibiotics to overcome disease outbreaks. Gradually, it led to the development of antibiotic-resistant bacteria and started transferring these resistant genes into the aquatic environment. Intrusion of these antibiotic-resistant genes into the terrestrial environment through the aquatic environment brought adverse effects not only to humans and terrestrial animals but also to aquatic animals [118]. In this circumstance, antibacterial vaccines, probiotics, and bacteriophages gained more attention

[52]. Henceforth, biotechnological interventions in the aquaculture field, especially in gut microbiome engineering, opened a new path for the improvement of aquaculture production and sustainability [131, 171].

Environmental concerns regarding genetically modified organisms also came up with the biotechnological applications. The main concerns about genetically engineered microbes were competition, habitat alteration, horizontal gene transfer, and toxicity due to their small size and rapid growth. The persistence of unwanted genes, transfer of modified genes to the wild population, and uncontrollable proliferation are the main issues that gain more attention. A solution for the gene transfer is the implementation of a toxin and antitoxin system in different parts of the genetic repository of the cell, as the plasmid that does not carry the antitoxin will kill the new host [174]. Even though the bacteriophage-mediated vector transfer technique can replace antibiotics to overcome antibiotic resistance, resistance mutation can cause environmental concerns. But it develops ten times slower than antibiotic resistance [162, 164, 166, 167]. Loss and gain of function of genes in engineered microbes were the other concerns because they can change the basic characteristics of the bacteria, which can be a challenge to the stability of the environment [119]. Altogether, the escape of the modified microbes to the environment and the disruption in native genes are the main subjects to be more concerned about and need to ensure safety [174].

Future directions and challenges

Emerging trends and innovations in gut microbiome engineering

Gut microbiome engineering has mainly been applied in humans now. Its applications in agriculture are an emerging area. Only a few studies are documented on the various microbiome engineering techniques. Now, the gut microbiome engineering techniques look forward to widening their study mainly towards therapeutic and preventive applications. In aquaculture, it's mainly targeting the enhancement of fish health, improvement of feed efficiency, immunity, disease resistance, and other sustainable practices. Deep knowledge of the composition, diversity, and factors affecting the gut microbiome is the primary data that is required prior to the application of engineering techniques. For the proper database of the gut microbiome, integrated application of multi-omics, metatranscriptomics, and metabolome single-cell genomics is required. A well-documented database provides a strong basis for gut microbiome engineering [296]. Further improvements in gnotobiotic models to study the host and microbiome interaction can help achieve this. Culturing of fish gut microbiota will promote its application through probiotics, prebiotics, and synbiotics [162, 164, 166, 167].

Biotechnological innovation is regarded as the new opening in gut microbiome engineering. The combined action of different methods together can bring a better result. Rasmussen et al. documented the combined action of vibriophage KVP40 and probiotic *P. inhibens, which* reduced the mortality of fish pathogenic vibrio thereby helping to maintain fish fry health [220]. In addition, application of probiotics with feed supplements also enhances sustainable outcomes. Ribonucleotide supplementation with probiotic strain *B. subtilis* improved the health of Asian seabass [232]. Implementation of FMT and probiotics together can bring a huge result in gut microbiome modulation. *B. subtilis* from the intestine of *Microstomus kitt* possesses a high probiotic potential [234]. *B. velezensis* isolated from the *M. salmoides* acts as a potential feed additive to promote growth disease resistance in aquaculture [292].

The combination of the CRISPR/Cas technique and phage therapy is another emerging area. As the CRISPR/Cas system works against many anti-microbial resistances of pathogens, you can use it to eliminate pathogens from the gut. Applying phage as a delivery system of the CRISPR/Cas system to improve its potential to eliminate pathogens [187]. Engineering a key consortium of single microbes that support mutually is the main emerging trend in this field [15]. CRISPR/Cas involves novel gene editing techniques like, Base editors that work on single base conversions, primer editors rely on base substitutions and precise DNA insertions and deletions; and CRISPR-associated transposases perform recombination-independent, multi-kilobase DNA insertions. These are applied in the formulation of probiotics in humans [287] and can be applied in aquaculture.

Addressing challenges and limitations for wider adoption

Safety issues are the main problems that are faced by all engineering techniques in the aquaculture field. Emergence of antibiotic-resistant pathogens together with their capability to transfer these genes to other microorganisms [272]. Genetically engineered bacteria are a huge goal, and their development is still stuck in its initial phase. Its application is much more concerned and restricted due to its potential pathogenicity and local regulations. The future challenges regarding this engineered bacterium will be its capacity to effectively proliferate and work in its target site, how to localise these bacteria only to the target site, and also its elimination after use. New advancements in technologies could address these safety challenges and can be used for therapeutic applications [287]. When it comes to aquaculture, it is very difficult to apply to water and aquatic animals, development and usage of probiotics and other techniques, their activity in aquaculture, production cost, and storage capacity include another set of challenges [66].

Ethical, regulatory, and safety considerations

Gut microbiome engineering involves various ethical, regulatory, and safety considerations. Mainly, the ethical principle includes individual good/non-maleficence that ensures the animals are not harmed intentionally and carelessly. Other principles involve collective good/beneficence that secures the proper well-being of animals; autonomy relies on individual rights and free will; and lastly, justice assures equality in availing of all benefits and costs for all [111]. The ethical view consists of different aspects, including bioethics, environmental ethics, and food ethics. Bioethics focuses on and supports the overall well-being of animals. Environmental ethics mainly supports biodiversity in terms of minimising the use of chemicals. Food ethics relies on avoiding unhealthy food that is high in fat, sugar, or any other substance that destroys the gut microbiota [144]. Ethical concern tries to avoid the potential hazards of the various techniques that cause hazards or risks towards the animal health and environment before their application. [111]. The safety controls of worldwide regulatory authority regarding genetically engineered microbes (GEM) include the minimisation or controlling of biocontainment to limit the spread and persistence of GEM in the environment. Genetic instability is ensured for the stability of GEM regardless of the loss or gain of function. In addition, uncontrolled growth of GEM and competition with stable and long-term eubiotic communities are also evaluated before the application of GEM [151].

Conclusion

Summary of key findings and implications

Gut microbiome studies in aquaculture are still in their infant stage, where more studies have been documented on its composition and diversity. More studies are required to develop microbiome applications for the sustainable growth of aquaculture species. In this review, we summarise the details and knowledge about the gut microbiome composition, and structure, its importance in aquaculture, and the various engineering techniques for modulating the intestinal microbiota. We also focus on its applications in aquaculture and other ethical and safety concerns regarding these techniques. As we concentrate on the gut microbiome in fishes, Fusobacteria, proteobacteria, Firmicutes, and Bacteroidetes constitute the abundant group. They have a significant influence on host physiology, digestion, feed utilisation, metabolism, immune function, and stress responses. Several abiotic and biotic factors, including host genetics, developmental stage, feeding habits, diet, stress, disease, and environmental conditions consisting of season, temperature, pH, water, and sediment quality, play a significant role in shaping intestinal microbial communities. Hence, factors such as seasonal shifts and other environmental factors that influence the non-indigenous species of the gut microbiome highlight the need for dynamic, environmentally adaptive strategies-based approaches in microbiome engineering. As disease outbreaks become more frequent in aquaculture, farmers increasingly mainly depend on antibiotics and vaccines, leading to antimicrobial-resistant bacteria in fish and the environment becoming a serious issue. Maintaining the proper health of individuals and good aquaculture practices helps to avoid many of these disease conditions. Well-balanced gut microbiota composition and diversity promote the better health of an individual. At this point, modulation of these intestinal microbiotas emerges as a new platform for achieving the sustainable growth of aquaculture.

Gut microbiome engineering is a new approach towards the modulation of intestinal microbial diversity that involves various techniques such as probiotics, prebiotics, synbiotics, phage therapy, genetic engineering, and faecal microbial transplantations. Among these, probiotics, prebiotics, and synbiotics constitute those practices much more explored in the aquaculture sector. Techniques are well utilised to promote the growth of intestinal microbes and improve digestion, absorption, immune responses, and disease resistance in the host. Bacteriophage-mediated vector transfer is another biotechnological approach also utilised in aquaculture. It mainly helped to eradicate antibiotic-resistant bacteria. FMT studies are mainly documented in humans, and only a few studies have been reported in aquaculture. Likewise, CRISPR-CasPER-mediated engineering techniques are also trapped in their initial state; more concentration is required for their advanced application in aquaculture.

The main challenge regarding these techniques is their application in farm conditions. Development of these techniques for each aquatic species in consideration of the safety issues is also time-consuming and more difficult. Combining these different techniques towards the development of key consortiums for each species can be looked forward to in the future. Much more advanced studies and application of each technique in gut microbial studies enhance the sustainability of aquaculture.

Prospects and recommendations for further research and application

To achieve further advancements in gut microbiome studies, improvements from the basic level of experiments are required. Applying advanced techniques with multi-omics approaches provides greater insights into the role, function, and composition of gut microbiota and will help to optimise the intestinal community towards its future applications [202]. Gnotobiotic fish models, including zebrafish and stickleback, can be utilised for the same [311]. Various engineering techniques can be applied to achieve a healthy microbiota or to treat dysbiosis in animals. Yet, deep knowledge of the relationship between the host disease condition and the intestinal fauna is required for its proper implementation [84]. A combination of various engineering techniques and its target implementation, gut microbiome modulation, is the main area kept before performing in the future [187]. Beyond the usual engineering techniques, metabolic engineering strategies can also be included for better outputs from gut microbiome modulation [153]. CRISPR-related tools can expand engineering techniques in a much more precise and efficient way [35]. Next-generation probiotics are another emerging area in aquaculture. The application of synthetic biology with genetic modification of probiotics will boost the personalised enhanced future in next-generation probiotic therapies [2]. Natural bacteria-based drugs in treating different diseases are also an upcoming therapeutic application in humans and can also be applied in aquaculture in the future. More advancement in gut microbiome engineering techniques together with artificial intelligence (AI) and synthetic biology can bring great development in sustainable aquaculture [139, 202]. The development of comprehensive guidelines and well-structured training programs incorporating advanced strategies and addressing safety concerns is essential for the successful real-world application of microbiome engineering. With its potential to enhance aquaculture sustainability through improved productivity, disease resistance, and environmental balance, microbiome engineering emerges as a robust, future-proof strategy for advancing sustainable aquaculture practices.

Authors' contributions

AMA: Investigation and writing the original draft. MS, BK, VS: Investigation and editing the manuscript. ISB: Supervision and editing the manuscript. JP: Conceptualization, Supervision and editing the manuscript.

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References

1. Abasubong KP, Liu WB, Adjoumani YJJ, Xia SL, Xu C, Li XF. Xylooligosaccharides benefit the growth, digestive functions and TOR signaling in *Megalobrama amblycephala* fed diets with fish meal replaced by rice protein concentrate. Aquaculture. 2019;500:417–28.
2. Abouelela ME, Helmy YA. Next-generation probiotics as novel therapeutics for improving human health: current trends and future perspectives. Microorganisms. 2024;12(3):430.
3. Aich N, Nama S, Biswal A, Paul T. A review on recirculating aquaculture systems: Challenges and opportunities for sustainable aquaculture. Innovative Farming. 2020;5(1):17–24.
4. Almeida C, Oliveira R, Baylina P, Fernandes R, Teixeira FG, Barata P. Current trends and challenges of fecal microbiota transplantation—an easy method that works for all? Biomedicines. 2022;10(11): 2742.
5. Aly SM, Fathi M. Advancing aquaculture biosecurity: a scientometric analysis and future outlook for disease prevention and environmental sustainability. Aquac Int. 2024:32(7):1–27.
6. Amenyogbe E, Chen G, Wang Z, Huang J, Huang B, Li H. The exploitation of probiotics, prebiotics and synbiotics in aquaculture: present study, limitations and future directions.: a review. Aquacult Int. 2020;28:1017–41.
7. Amenyogbe E, Droepenu EK, Ayisi CL, Boamah GA, Duker RQ, Abarike ED, Huang JS. Impact of probiotics, prebiotics, and synbiotics on digestive enzymes, oxidative stress, and antioxidant defense in fish farming: current insights and future perspectives. Front Mar Sci. 2024;11: 1368436.
8. Amillano-Cisneros JM, Fuentes-Valencia MA, Leyva-Morales JB, Davizón YA, Marquéz-Pacheco H, Valencia-Castañeda G, et al. Prebiotics in global and Mexican fish aquaculture: a review. Animals. 2023;13(23):3607.
9. Amin M, Adams M, Bolch CJ, Burke CM. In vitro screening of lactic acid bacteria isolated from gastrointestinal tract of Atlantic Salmon (*Salmo salar*) as probiont candidates. Aquacult Int. 2017;25:485–98.
10. Amouri RE, Tu Z, Abo-Raya MH, Wang X, Shi Y, Hu M, Wang Y. Nano-TiO 2 and elevated temperature impair intestinal health in crabs via a mussel-based food chain. Environ Sci: Nano. 2024;11(12):4698–716.
11. Arias-Jayo N, Alonso-Saez L, Ramirez-Garcia A, Pardo MA. Zebrafish axenic larvae colonization with human intestinal microbiota. Zebrafish. 2018;15(2):96–106.
12. Armstrong LE, Casa DJ, Belval LN. Metabolism, bioenergetics and thermal physiology: Influences of the human intestinal microbiota. Nutr Res Rev. 2019;32(2):205–17.
13. Ashry AM, Habiba MM, Abdel-wahab A, Younis EM, Davies SJ, Elnakeeb MA, et al. Dietary effect of powdered herbal seeds on zootechnical performance, hemato-biochemical indices, immunological status, and intestinal microbiota of European sea bass (*Dicentrarchus labrax*). Aquac Rep. 2024;36:102074.
14. Assefa A, Abunna F. Maintenance of fish health in aquaculture: review of epidemiological approaches for prevention and control of infectious disease of fish. Vet Med Int. 2018;2018(1):5432497.
15. Bai X, Huang Z, Duraj-Thatte AM, Ebert MP, Zhang F, Burgermeister E, et al. Engineering the gut microbiome. Nat Rev Bioeng. 2023;1(9):665–79.

16. Banerjee G, Ray AK. Bacterial symbiosis in the fish gut and its role in health and metabolism. Symbiosis. 2017;72:1–11.
17. Bartley DM. World Aquaculture 2020–A brief overview. 2022. FAO Fisheries and Aquaculture Circular No. 1233. NFIAP/C1233
18. Barui K, Choudhury TG, Kamilya D, Devi AA, Monsang SJ, Rathore G, et al. Paraprobiotic supplementation to fish feed: effects on the immune support system and control of *Aeromonas hydrophila* infection in *Labeo rohita*. Aquac Int. 2024;32(4):4225–48.
19. Basili M, Sabatini L, Pellini G, Massaccesi N, Manini E, Donato F, et al. Age and environment are the main drivers shaping the wild common sole (*Solea solea*) microbiota. BMC Ecol Evol. 2024;24(1):118.
20. Bereded NK, Abebe GB, Fanta SW, Curto M, Waidbacher H, Meimberg H, Domig KJ. The impact of sampling season and catching site (wild and aquaculture) on gut microbiota composition and diversity of Nile tilapia (*Oreochromis niloticus*). Biology. 2021;10(3): 180.
21. Bhatnagar A, Lamba R. Antimicrobial ability and growth promoting effects of feed supplemented with probiotic bacterium isolated from gut microflora of *Cirrhinus mrigala*. J Integr Agric. 2015;14(3):583–92.
22. Bibbò S, Settanni CR, Porcari S, Bocchino E, Ianiro G, Cammarota G, Gasbarrini A. Fecal microbiota transplantation: screening and selection to choose the optimal donor. J Clin Med. 2020;9(6):1757.
23. Bjørndal T, Dey M, Tusvik A. Economic analysis of the contributions of aquaculture to future food security. Aquaculture. 2024;578: 740071.
24. Bledsoe JW, Waldbieser GC, Swanson KS, Peterson BC, Small BC. Comparison of channel catfish and blue catfish gut microbiota assemblages shows minimal effects of host genetics on microbial structure and inferred function. Front Microbiol. 2018;9: 1073.
25. Borody TJ, Khoruts A. Fecal microbiota transplantation and emerging applications. Nat Rev Gastroenterol Hepatol. 2012;9(2):88–96.
26. Boyd CE, D'Abramo LR, Glencross BD, Huyben DC, Juarez LM, Lockwood GS, et al. Achieving sustainable aquaculture: Historical and current perspectives and future needs and challenges. J World Aquac Soc. 2020;51(3):578–633.
27. Boyd CE, McNevin AA, Davis RP. The contribution of fisheries and aquaculture to the global protein supply. Food security. 2022;14(3):805–27.
28. Butt RL, Volkoff H. Gut microbiota and energy homeostasis in fish. Front Endocrinol. 2019;10:9.
29. Butt UD, Khan S, Liu X, Sharma A, Zhang X, Wu B. Present status, limitations, and prospects of using Streptomyces bacteria as a potential probiotic agent in aquaculture. Probiotics and Antimicrobial Proteins. 2024;16(2):426–42.
30. Butt UD, Lin N, Akhter N, Siddiqui T, Li S, Wu B. Overview of the latest developments in the role of probiotics, prebiotics and synbiotics in shrimp aquaculture. Fish Shellfish Immunol. 2021;114:263–81.
31. Cano-Lozano JA, Diaz LMV, Bolivar JFM, Hume ME, Pardo RYR. Probiotics in tilapia (*Oreochromis niloticus*) culture: Potential probiotic *Lactococcus lactis* culture conditions. J Biosci Bioeng. 2022;133(3):187–94.
32. Cánovas-Molina A, García-Frapolli E. Socio-ecological impacts of industrial aquaculture and ways forward to sustainability. Mar Freshw Res. 2021;72(8):1101–9.
33. Cao Y, Li S, Han S, Wang D, Zhao J, Xu L, et al. Characterization and application of a novel Aeromonas bacteriophage as treatment for pathogenic *Aeromonas hydrophila* infection in rainbow trout. Aquaculture. 2020;523:735193.
34. Carbone D, Faggio C. Importance of prebiotics in aquaculture as immunostimulants. Effects on immune system of *Sparus aurata* and *Dicentrarchus labrax*. Fish Shellfish Immunol. 2016;54:172–8.
35. Careaga MBL. Engineering the Microbiome: CRISPR LEADS THE WAY. Scientist. 2024;38(1):30–8.
36. Carnevali O, Avella MA, Gioacchini G. Effects of probiotic administration on zebrafish development and reproduction. Gen Comp Endocrinol. 2013;188:297–302.
37. Castaner O, Goday A, Park YM, Lee SH, Magkos F, Shiow SA, Schröder H. The gut microbiome profile in obesity: a systematic review. Int J Endocrinol. 2018;2018(1):4095789.
38. Cavalcante RB, Telli GS, Tachibana L, de Carla Dias D, Oshiro E, Natori MM, et al. Probiotics, prebiotics and synbiotics for Nile tilapia: growth performance and protection against *Aeromonas hydrophila* infection. Aquac Rep. 2020;17:100343.
39. Cavalli LS, da Rocha AF, de Brito BG, de Brito KCT, Rotta MA. Major sustainable development goals applied to aquaculture. Pesq Agrop Gaúcha. 2021;27(1):110–26.
40. Chandrababu A, Puthumana J. CRISPR-edited, cell-based future-proof meat and seafood to enhance global food security and nutrition. Cytotechnology. 2024;76:619–52.
41. Chang YT, Huang WT, Wu PL, Kumar R, Wang HC, Lu HP. Low salinity stress increases the risk of *Vibrio parahaemolyticus* infection and gut microbiota dysbiosis in Pacific white shrimp. BMC Microbiol. 2024;24(1):275.
42. Chauhan A, Singh R. Probiotics in aquaculture: a promising emerging alternative approach. Symbiosis. 2019;77(2):99–113.
43. Cheesman SE, Neal JT, Mittge E, Seredick BM, Guillemin K. Epithelial cell proliferation in the developing zebrafish intestine is regulated by the Wnt pathway and microbial signaling via Myd88. Proc Natl Acad Sci. 2011;108(supplement_1):4570–7.
44. Chen CC, Lin CY, Lu HY, Liou CH, Ho YN, Huang CW, et al. Transcriptomics and gut microbiome analysis of the edible herb Bidens pilosa as a functional feed additive to promote growth and metabolism in tilapia (*Oreochromis* spp.). BMC Genom. 2024;25(1):785.
45. Chen J, Huang X, Geng R, Zhu D, Wang W, Liu H. Ribonuclease1 contributes to the antibacterial response and immune defense in blunt snout bream (*Megalobrama amblycephala*). Int J Biol Macromol. 2021;172:309–20.
46. Chen Z, Chen JQ, Liu Y, Zhang J, Chen XH, Qu YF. Comparative study on gut microbiota in three Anura frogs from a mountain stream. Ecol Evol. 2022;12(4): e8854.
47. Chin YK, Azzam-Sayuti M, Mohamad A, Haifa-Haryani WO, Ahmad MI, Nazarudin MF, et al. Elucidation of synbiotic diet comprising of *Lactobacillus plantarum* L20 and *Sargassum polycystum* on gastrointestinal microbiota, tissue structures and AHPND associated dysbiosis susceptibility in black tiger shrimp (*Penaeus monodon*). Aquaculture. 2025;594:741339.
48. Chin YK, Haifa-Haryani WO, Nazarudin MF, Ahmad MI, Azzam-Sayuti M, Mohamad A, Ina-Salwany MY. The synergistic *Lactobacillus plantarum* L20 and *Sargassum polycystum*-added diet for improvement of Black tiger shrimp, *Penaeus monodon*'s growth, immune responses, bacterial profiles, and resistance against *Vibrio parahaemolyticus* associated Acute hepatopancreatic necrosis disease (AHPND) infection. Aquaculture Reports. 2024;34:101903.
49. Choi HH, Cho YS. Fecal microbiota transplantation: current applications, effectiveness, and future perspectives. Clinical endoscopy. 2016;49(3):257–65.
50. Chomová N, Pavloková S, Sondorová M, Mudroňová D, Fečkaninová A, Popelka P, et al. Development and evaluation of a fish feed mixture containing the probiotic *Lactiplantibacillus plantarum* prepared using an innovative pellet coating method. Front Vet Sci. 2023;10:1196884.
51. Chowdhury MA, Roy NC. Probiotic supplementation for enhanced growth of striped catfish (*Pangasianodon hypophthalmus*) in cages. Aquac Rep. 2020;18: 100504.
52. Chuah LO, Effarizah ME, Goni AM, Rusul G. Antibiotic application and emergence of multiple antibiotic resistance (MAR) in global catfish aquaculture. Curr Environ Health Rep. 2016;3:118–27.
53. Conde-Sieira M, Chivite M, Míguez JM, Soengas JL. Stress effects on the mechanisms regulating appetite in teleost fish. Front Endocrinol. 2018;9: 416277.
54. Cui H, Cong C, Wang L, Li X, Li J, Yang H, et al. Protective effectiveness of feeding phage cocktails in controlling *Vibrio harveyi* infection of turbot *Scophthalmus maximus*. Aquaculture. 2021;535:736390.
55. Cui X, Zhang Q, Zhang Q, Zhang Y, Chen H, Liu G, Zhu L. Research progress of the gut microbiome in hybrid fish. Microorganisms. 2022;10(5): 891.
56. Dai W, Zhang Z, Dong Y, He L, Xue Q, Lin Z. Acute salinity stress disrupts gut microbiota homeostasis and reduces network connectivity and cooperation in razor clam sinonovacula constricta. Mar Biotechnol. 2023;25(6):1147–57.
57. Dang X, Huang Q, He YQ, Gaitán-Espitia JD, Zhang T, Thiyagarajan V. Ocean acidification drives gut microbiome changes linked to species-specific immune defence. Aquat Toxicol. 2023;256: 106413.
58. Das SP, Abidin Z, Huang HT, Lin YR, Huang CY, Wu YS, et al. Deciphering the influence of dietary synbiotics in white shrimp gut and its

effects in regulating immune signalling pathways. Front Mar Sci. 2024;10:1342708.

59. Das S, Mondal K, Haque S. A review on application of probiotic, prebiotic and synbiotic for sustainable development of aquaculture. Growth. 2017;14:15.
60. Dawood MA, Abo-Al-Ela HG, Hasan MT. Modulation of transcriptomic profile in aquatic animals: Probiotics, prebiotics and synbiotics scenarios. Fish Shellfish Immunol. 2020;97:268–82.
61. Dawood MA, Eweedah NM, Moustafa EM, Shahin MG. Synbiotic effects of *Aspergillus oryzae* and β-glucan on growth and oxidative and immune responses of nile Tilapia, *Oreochromis niloticus*. Prob Antimicrobial Proteins. 2020;12:172–83.
62. de Lima Paz A, da Silva JM, da Silva KMM, Val AL. Protective effects of the fructooligosaccharide on the growth performance, hematology, immunology indicators and survival of tambaqui (*Colossoma macropomum*, *Characiformes: Serrasalmidae*) infected by *Aeromonas hydrophila*. Aquac Rep. 2019;15: 100222.
63. Deng Y, Kokou F, Eding EH, Verdegem MC. Impact of early-life rearing history on gut microbiome succession and performance of Nile tilapia. Animal Microbiome. 2021;3:1–17.
64. Derome N, Filteau M. A continuously changing selective context on microbial communities associated with fish, from egg to fork. Evol Appl. 2020;13(6):1298–319.
65. Diwan AD, Harke SN, Gopalkrishna AM, Panche AN. Aquaculture industry prospective from gut microbiome of fish and shellfish: an overview. J Anim Physiol Anim Nutr. 2022;106(2):441–69.
66. Diwan A, Harke SN, Panche A. Impact of climate change on the gut microbiome of fish and shellfish. In: Microbiome of finfish and shellfish. Singapore: Springer Nature Singapore. 2023:255-294.
67. do Carmo Alves AP, Orlando TM, de Oliveira IM, Libeck LT, Silva KKS, Rodrigues RAF, et al. Synbiotic microcapsules of *Bacillus subtilis* and oat β-glucan on the growth, microbiota, and immunity of Nile tilapia. Aquaculture Int. 2024;32(4):3869–88.
68. Donati VL, Madsen L, Middelboe M, Strube ML, Dalsgaard I. The gut microbiota of healthy and *Flavobacterium psychrophilum*-infected rainbow trout fry is shaped by antibiotics and phage therapies. Front Microbiol. 2022;13: 771296.
69. Dong S, Ding LG, Cao JF, Liu X, Xu HY, Meng KF, et al. Viral-infected change of the digestive tract microbiota associated with mucosal immunity in teleost fish. Front Immunol. 2019;10:2878.
70. Duan Y, Xiong D, Li Y, Ding X, Dong H, Wang W, Zhang J. Changes in the microbial communities of the rearing water, sediment and gastrointestinal tract of *Lateolabrax maculatus* at two growth stages. Aquac Rep. 2021;20: 100742.
71. Dulski T, Kozłowski K, Ciesielski S. Habitat and seasonality shape the structure of tench (*Tinca tinca* L.) gut microbiome. Sci Rep. 2020;10(1):4460.
72. Edwards J, Hoffbeck C, West AG, Pas A, Taylor MW. 16S rRNA gene-based microbiota profiles from diverse avian faeces are largely independent of DNA preservation and extraction method. Front Microbiol. 2023;14:1239167.
73. Egerton S, Culloty S, Whooley J, Stanton C, Ross RP. The gut microbiota of marine fish. Front Microbiol. 2018;9:873.
74. Eissa ES, El-Sayed AF, Hendam BM, Ghanem SF, Abd Elnabi HE, Abd El-Aziz YM, et al. The regulatory effects of water probiotic supplementation on the blood physiology, reproductive performance, and its related genes in Red Tilapia (*Oreochromis niloticus* X *O. mossambicus*). BMC Vet Res. 2024;20(1):351.
75. Elgendy MY, Ali SE, Dayem AA, Khalil RH, Moustafa MM, Abdelsalam M. Alternative therapies recently applied in controlling farmed fish diseases: mechanisms, challenges, and prospects. Aquac Int. 2024;32:1–62.
76. El-Nobi G, Hassanin M, Khalil AA, Mohammed AY, Amer SA, Montaser MM, El-Sharnouby ME. Synbiotic effects of *Saccharomyces cerevisiae*, Mannan oligosaccharides, and β-glucan on innate immunity, antioxidant status, and disease resistance of Nile tilapia, *Oreochromis niloticus*. Antibiotics. 2021;10(5):567.
77. Emam SM, Mohammadian B, Mohammadian T, Tabande MR. Autochthonous probiotic bacteria improve intestinal pathology and histomorphology, expression of immune and growth-related genes and resistance against *Vibrio alginolyticus* in Asian seabass (*Lates calcarifer*). Vet Res Commun. 2024:48(5):1–19.
78. Falcinelli S, Rodiles A, Hatef A, Picchietti S, Cossignani L, Merrifield DL, et al. Influence of probiotics administration on gut microbiota core: a review on the effects on appetite control, glucose, and lipid metabolism. J Clin Gastroenterol. 2018;52:S50–6.
79. Fan L, Wang Z, Chen M, Qu Y, Li J, Zhou A, et al. Microbiota comparison of Pacific white shrimp intestine and sediment at freshwater and marine cultured environment. Sci Total Environ. 2019;657:1194–204.
80. Fao, F. A. O. S. T. A. T. Food and agriculture organization of the United Nations. Rome, URL: http://faostat.fao.org. 2018:403-403.
81. Fassarella M, Blaak EE, Penders J, Nauta A, Smidt H, Zoetendal EG. Gut microbiome stability and resilience: elucidating the response to perturbations in order to modulate gut health. Gut. 2021;70(3):595–605.
82. Feng C, Jia K, Chi T, Chen S, Yu H, Zhang L, et al. Lytic bacteriophage PZL-Ah152 as biocontrol measures against lethal *Aeromonas hydrophila* without distorting gut microbiota. Front Microbiol. 2022;13:898961.
83. Fiedler AW, Drågen MK, Lorentsen ED, Vadstein O, Bakke I. The stability and composition of the gut and skin microbiota of Atlantic salmon throughout the yolk sac stage. Front Microbiol. 2023;14:1177972.
84. Filardo S, Di Pietro M, Sessa R. Current progresses and challenges for microbiome research in human health: a perspective. Front Cell Infect Microbiol. 2024;14: 1377012.
85. Fonseca F, Cerqueira R, Fuentes J. Impact of ocean acidification on the intestinal microbiota of the marine sea bream (*Sparus aurata* L.). Front Physiol. 2019;10: 1446.
86. Foo JL, Ling H, Lee YS, Chang MW. Microbiome engineering: Current applications and its future. Biotechnol J. 2017;12(3): 1600099.
87. Forsatkar MN, Nematollahi MA, Rafiee G, Farahmand H, Lawrence C. Effects of the prebiotic mannan-oligosaccharide on the stress response of feed deprived zebrafish (*Danio rerio*). Physiol Behav. 2017;180:70–7.
88. Gao Y, Qiang L, Wu N, Tan R, Sun Y, Li Z, et al. Study on the potential probiotics isolated from marine aquaculture system and evaluation for aquaculture application. Aquac Res. 2024;2024(1):9555271.
89. Genç E, Genç MA, Kaya D, Secer FS, Qaranjiki A, Güroy D. Effect of prebiotics on the growth performance, haematological, biochemical, and histological parameters of African catfish (*Clarias gariepinus*) in recirculating aquaculture system. Turkish J Vet Anim Sci. 2020;44(6):1222–31.
90. Geng R, Liu H, Wang W. Differential expression of six Rnase2 and three Rnase3 paralogs identified in blunt snout bream in response to *Aeromonas hydrophila* infection. Genes. 2018;9(2): 95.
91. Ghafarifarsani H, Rashidian G, Bagheri T, Hoseinifar SH, Van Doan H. Study on growth enhancement and the protective effects of dietary prebiotic inulin on immunity responses of rainbow trout () fry infected with. Ann An Sci. 2021;21(2):543–59.
92. Ghaly FM, Hussein SH, Awad SM, El-Makhzangy AA. Growth promoter, immune response, and histopathological change of prebiotic, probiotic and synbiotic bacteria on Nile tilapia. Saudi J Biolog Sci. 2023;30(2): 103539.
93. Ghanbari M, Kneifel W, Domig KJ. A new view of the fish gut microbiome: advances from next-generation sequencing. Aquaculture. 2015;448:464–75.
94. Ghori I, Tubassam M, Ahmad T, Zuberi A, Imran M. Gut microbiome modulation mediated by probiotics: Positive impact on growth and health status of Labeo rohita. Front Physiol. 2022;13: 949559.
95. Ghosh SK, Wong MKS, Hyodo S, Goto S, Hamasaki K. Temperature modulation alters the gut and skin microbial profiles of chum salmon (*Oncorhynchus keta*). Front Mar Sci. 2022;9:1027621.
96. Ghosh T. Recent advances in the probiotic application of the *Bacillus* as a potential candidate in the sustainable development of aquaculture. Aquaculture. 2025;594:741432.
97. Gomez D, Sunyer JO, Salinas I. The mucosal immune system of fish: the evolution of tolerating commensals while fighting pathogens. Fish Shellfish Immunol. 2013;35(6):1729–39.
98. Gong J, Xu F, Li Y, He Y, Liang Z, Chen X, et al. Metagenomic analysis of intestinal microbial function and key genes responsive to acute high-salinity stress in Nile tilapia (*Oreochromis niloticus*). Gene. 2024;913:148371.
99. González-Gómez JP, Soto-Rodriguez SA, Gomez-Gil B, Serrano-Hernández JM, Lozano-Olvera R, López-Cuevas O, et al. Effect of phage therapy on survival, histopathology, and water microbiota of *Penaeus vannamei*

challenged with *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease (AHPND). Aquaculture. 2023;576:739851.

100. Govindaraj K, Samayanpaulraj V, Narayanadoss V, Uthandakalaipandian R. Isolation of lactic acid bacteria from intestine of freshwater fishes and elucidation of probiotic potential for aquaculture application. Prob Antimicrob Proteins. 2021;13(6):1598–610.
101. Guerreiro I, Couto A, Machado M, Castro C, Pousao-Ferreira P, Oliva-Teles A, Enes P. Prebiotics effect on immune and hepatic oxidative status and gut morphology of white sea bream (*Diplodus sargus*). Fish Shellfish Immunol. 2016;50:168–74.
102. Guerreiro I, Oliva-Teles A, Enes P. Prebiotics as functional ingredients: focus on Mediterranean fish aquaculture. Rev Aquac. 2018;10(4):800–32.
103. Gupta A, Gupta P, Dhawan A. Dietary supplementation of probiotics affects growth, immune response and disease resistance of *Cyprinus carpio* fry. Fish Shellfish Immunol. 2014;41(2):113–9.
104. Gutási A, Hammer SE, El-Matbouli M, Saleh M. recent applications of gene editing in fish species and aquatic medicine. Animals. 2023;13(7):1250.
105. Han C, Song S, Cui C, Cai Y, Zhou Y, Wang J, et al. Strain-specific benefits of bacillus probiotics in hybrid grouper: growth enhancement, metabolic health, immune modulation, and vibrio harveyi resistance. Animals. 2024;14(7):1062.
106. Han H, Yi B, Zhong R, Wang M, Zhang S, Ma J, et al. From gut microbiota to host appetite: gut microbiota-derived metabolites as key regulators. Microbiome. 2021;9:1–16.
107. Han Z, Sun J, Jiang B, Chen K, Ge L, Sun Z, Wang A. Fecal microbiota transplantation accelerates restoration of florfenicol-disturbed intestinal microbiota in a fish model. Commun Biol. 2024;7(1):1006.
108. Hanson-Regan W. Geographical, ecological, and genetic drivers of gut microbial diversity in native and invasive minnows of the genus *Cyprinella*. Actinopterygii: Leuciscidae; 2023. Thesis, Faculty of the University of Tennessee at Chattanooga in Partial
109. Haque R, Das II, Sawant PB, Chadha NK, Sahoo L, Kumar R, Sundaray JK. Tenets in microbial endocrinology: a new vista in teleost reproduction. Front Physiol. 2022;13: 871045.
110. Hardi EH, Nugroho RA, Rostika R, Mardliyaha CM, Sukarti K, Rahayu W, et al. Synbiotic application to enhance growth, immune system, and disease resistance toward bacterial infection in catfish (*Clarias gariepinus*). Aquaculture. 2022;549:737794.
111. Hardwick A, Cummings C, Graves J Jr, Kuzma J. Can societal and ethical implications of precision microbiome engineering be applied to the built environment? A systematic review of the literature. Environ Syst Decis. 2024;44:1–24.
112. Hasan MT, Jang WJ, Kim H, Lee BJ, Kim KW, Hur SW, Lim SG, et al. Synergistic effects of dietary *Bacillus* sp. SJ-10 plus β-glucooligosaccharides as a synbiotic on growth performance, innate immunity and streptococcosis resistance in olive flounder (*Paralichthys olivaceus*). Fish Shellfish Immunol. 2018;82:544–53.
113. Hassenrück C, Reinwald H, Kunzmann A, Tiedemann I, Gärdes A. Effects of thermal stress on the gut microbiome of juvenile milkfish (*Chanos chanos*). Microorganisms. 2020;9(1):5.
114. Hoque F, Das A, Sundaray JK. Gut microbiome and fish health: an overview in finfish aquaculture prospective. In: Diwan, A., Harke, S.N., Panche, A. (eds) Microbiome of Finfish and Shellfish. Springer, Singapore. Microbiome of finfish and shellfish. 2023:47–74.
115. Hoseinifar SH, Mirvaghefi A, Amoozegar MA, Sharifian M, Esteban MÁ. Modulation of innate immune response, mucosal parameters and disease resistance in rainbow trout (*Oncorhynchus mykiss*) upon synbiotic feeding. Fish Shellfish Immunol. 2015;45(1):27–32.
116. Hoseinifar SH, Yousefi S, Van Doan H, Ashouri G, Gioacchini G, Maradonna F, Carnevali O. Oxidative stress and antioxidant defense in fish: the implications of probiotic, prebiotic, and synbiotics. Rev Fisher Sci Aquac. 2020;29(2):198–217.
117. Hossain A, Al Zahid M, Sanyal SK, Haque MIM, Habibullah-Al-Mamun M, Mandal SC, Watanabe K. Heterogeneous bacterial communities in gills and intestine of Nile tilapia (*Oreochromis niloticus*) and in water and sediments of aquaculture ponds in Bangladesh. Aquac Fisher. 2024.
118. Hossain A, Habibullah-Al-Mamun M, Nagano I, Masunaga S, Kitazawa, et al. Antibiotics, antibiotic-resistant bacteria, and resistance genes in aquaculture: risks, current concern, and future thinking. Environ Sci Pollut Res. 2022;29:1–22.
119. Hottes AK, Freddolino PL, Khare A, Donnell ZN, Liu JC, Tavazoie S. Bacterial adaptation through loss of function. PLoS Genet. 2013;9(7): e1003617.
120. Hovda MB, Fontanillas R, McGurk C, Obach A, Rosnes JT. Seasonal variations in the intestinal microbiota of farmed Atlantic salmon (*Salmo salar* L.). Aquac Res. 2012;43(1):154–9.
121. Hu C, Sun B, Liu M, Yu J, Zhou X, Chen L. Fecal transplantation from young zebrafish donors efficiently ameliorates the lipid metabolism disorder of aged recipients exposed to perfluorobutanesulfonate. Sci Total Environ. 2022;823: 153758.
122. Huang F, Pan L, Song M, Tian C, Gao S. Microbiota assemblages of water, sediment, and intestine and their associations with environmental factors and shrimp physiological health. Appl Microbiol Biotechnol. 2018;102:8585–98.
123. Huang K, Nitin N. Edible bacteriophage based antimicrobial coating on fish feed for enhanced treatment of bacterial infections in aquaculture industry. Aquaculture. 2019;502:18–25.
124. Huang Z, Hou D, Zhou R, Zeng S, Xing C, Wei D, et al. Environmental water and sediment microbial communities shape intestine microbiota for host health: the central dogma in an anthropogenic aquaculture ecosystem. Front Microbiol. 2021;12:772149.
125. Huynh TG, Shiu YL, Nguyen TP, Truong QP, Chen JC, Liu CH. Current applications, selection, and possible mechanisms of actions of synbiotics in improving the growth and health status in aquaculture: a review. Fish Shellfish Immunol. 2017;64:367–82.
126. Inda ME, Broset E, Lu TK, de la Fuente-Nunez C. Emerging frontiers in microbiome engineering. Trends Immunol. 2019;40(10):952–73.
127. Jia PP, Yang YF, Junaid M, Jia HJ, Li WG, Pei DS. Bacteriophage-based techniques for elucidating the function of zebrafish gut microbiota. Appl Microbiol Biotechnol. 2023;107(7):2039–59.
128. Johny TK, Puthusseri RM, Bhat SG. A primer on metagenomics and next-generation sequencing in fish gut microbiome research. Aquac Res. 2021;52(10):4574–600.
129. Jun JW, Han JE, Giri SS, Tang KF, Zhou X, Aranguren LF, et al. Phage application for the protection from acute hepatopancreatic necrosis disease (AHPND) in *Penaeus vannamei*. Indian J Microbiol. 2018;58:114–7.
130. Kaetzel CS. Coevolution of mucosal immunoglobulins and the polymeric immunoglobulin receptor: evidence that the commensal microbiota provided the driving force. ISRN Immunology. 2014;2014:1–20.
131. Khan MIR, Choudhury TG. Biotechnological approaches in fish health management. In: Biotechnological advances in aquaculture health management. Singapore: Springer Nature Singapore. 2022. pp. 1-24
132. Khoruts A, Sadowsky MJ. Understanding the mechanisms of faecal microbiota transplantation. Nat Rev Gastroenterol Hepatol. 2016;13(9):508–16.
133. Kim JA, Park YS, Kim JH, Choi CY. Hyposalinity elicits physiological responses and alters intestinal microbiota in Korean rockfish *Sebastes schlegelii*. Fish Physiol Biochem. 2024: 50: 2315-2326
134. Kim PS, Shin NR, Lee JB, Kim MS, Whon TW, Hyun DW, et al. Host habitat is the major determinant of the gut microbiome of fish. Microbiome. 2021;9(1):166.
135. Kivistik C, Knobloch J, Käiro K, Tammert H, Kisand V, Hildebrandt JP, Herlemann DP. Impact of salinity on the gastrointestinal bacterial community of *Theodoxus fluviatilis*. Front Microbiol. 2020;11:683.
136. Knipe H, Temperton B, Lange A, Bass D, Tyler CR. Probiotics and competitive exclusion of pathogens in shrimp aquaculture. Rev Aquac. 2021;13(1):324–52.
137. Kong N, Han S, Fu Q, Yu Z, Wang L, Song L. Impact of ocean acidification on the intestinal microflora of the Pacific oyster *Crassostrea gigas*. Aquaculture. 2022;546: 737365.
138. Kong Y, Gao C, Du X, Zhao J, Li M, Shan X, Wang G. Effects of single or conjoint administration of lactic acid bacteria as potential probiotics on growth, immune response and disease resistance of snakehead fish (*Channa argus*). Fish Shellfish Immunol. 2020;102:412–21.
139. Kumar P, Sinha R, Shukla P. Artificial intelligence and synthetic biology approaches for human gut microbiome. Crit Rev Food Sci Nutr. 2022;62(8):2103–21.
140. Kumari K, Nair SM. Gut microbes and its physiological role in fish: adaptive strategies for climatic variability. In: Sinha, A., Kumar, S., Kumari, K. (eds) Outlook of Climate Change and Fish Nutrition. Springer, Singapore. 2023. pp. 99-119.

141. Kumari R, Yadav R, Kumar D, Chaube R, Nath G. Evaluation of bacteriophage therapy of *Aeromonas hydrophila* infection in a freshwater fish. Pangasius Buchanani Front Aquac. 2023;2:1201466.
142. Kunttu HM, Runtuvuori-Salmela A, Middelboe M, Clark J, Sundberg LR. Comparison of delivery methods in phage therapy against *Flavobacterium columnare* infections in rainbow trout. Antibiotics. 2021;10(8):914.
143. Kwa WT, Sundarajoo S, Toh KY, Lee J. Application of emerging technologies for gut microbiome research. Singapore Med J. 2023;64(1):45–52.
144. Lange L, Berg G, Cernava T, Champomier-Vergès MC, Charles T, Cocolin L, et al. Microbiome ethics, guiding principles for microbiome research, use and knowledge management. Environ Microbiome. 2022;17(1):50.
145. Larios-Soriano E, Zavala RC, López LM, Gómez-Gil B, Ramírez DT, Sanchez S, et al. Soy protein concentrate effects on gut microbiota structure and digestive physiology of *Totoaba macdonaldi*. J Appl Microbiol. 2022;132(2):1384–96.
146. Lawley TD, Walker AW. Intestinal colonization resistance. Immunology. 2013;138(1):1–11.
147. Le TS, Nguyen TH, Vo HP, Doan VC, Nguyen HL, Tran MT, et al. Protective effects of bacteriophages against *Aeromonas hydrophila* causing motile Aeromonas septicemia (MAS) in striped catfish. Antibiotics. 2018;7(1):16.
148. Lee HL, Shen H, Hwang IY, Ling H, Yew WS, Lee YS, Chang MW. Targeted approaches for in situ gut microbiome manipulation. Genes. 2018;9(7): 351.
149. Lee S, Katya K, Hamidoghli A, Hong J, Kim DJ, Bai SC. Synergistic effects of dietary supplementation of *Bacillus subtilis* WB60 and mannanoligosaccharide (MOS) on growth performance, immunity and disease resistance in Japanese eel, *Anguilla japonica*. Fish Shellfish Immunol. 2018;83:283–91.
150. Leggieri PA, Liu Y, Hayes M, Connors B, Seppälä S, O'Malley MA, Venturelli OS. Integrating systems and synthetic biology to understand and engineer microbiomes. Annu Rev Biomed Eng. 2021;23(1):169–201.
151. Lerner A, Benzvi C, Vojdani A. The potential harmful effects of genetically engineered microorganisms (gems) on the intestinal microbiome and public health. Microorganisms. 2024;12(2): 238.
152. Li X, Zhou L, Yu Y, Ni J, Xu W, Yan Q. Composition of gut microbiota in the gibel carp (*Carassius auratus gibelio*) varies with host development. Microb Ecol. 2017;74:239–49.
153. Li P, Roos S, Luo H, Ji B, Nielsen J. Metabolic engineering in human gut microbiome: recent developments and future perspectives. Metabolic Engineering. 2023;79:1-3
154. Li W, Liu J, Tan H, Yang C, Ren LI, Liu Q, et al. Genetic effects on the gut microbiota assemblages of hybrid fish from parents with different feeding habits. Front Microbiol. 2018;9:2972.
155. Li X, Li J, Wang Y, Fu L, Fu Y, Li B, Jiao B. Aquaculture industry in China: current state, challenges, and outlook. Rev Fish Sci. 2011;19(3):187–200.
156. Li Z, Liu M, Zhou Q, Sun C, Zheng X, Chen J, et al. Synbiotic effects of *Lactobacillus rhamnosus* fermented with different prebiotics on the digestive enzyme activities, SCFAs and intestinal flora of *Macrobrachium rosenbergii* in vitro. Aquaculture Rep. 2024;38:102303.
157. Li Z, Ren H, Li Q, Murtaza B, Li X, Zhang J, Xu Y. Exploring the effects of phage cocktails in preventing Vibrio infections in juvenile sea cucumber (*Apostichopus japonicus*) farming. Aquaculture. 2020;515: 734599.
158. Li Z, Tran NT, Ji P, Sun Z, Wen X, Li S. Effects of prebiotic mixtures on growth performance, intestinal microbiota and immune response in juvenile chu's croaker, *Nibea coibor*. Fish Shellfish Immunol. 2019;89:564–73.
159. Linh NV, Lubis AR, Dinh-Hung N, Wannavijit S, Montha N, Fontana CM, et al. Effects of shrimp shell-derived chitosan on growth, immunity, intestinal morphology, and gene expression of nile tilapia (*Oreochromis niloticus*) reared in a biofloc system. Marine Drugs. 2024;22(4):150.
160. Liu D, Zhang Z, Song Y, Yang J, Lu Y, Lai W, et al. Effects of salinity on growth, physiology, biochemistry and gut microbiota of juvenile grass carp (*Ctenopharyngodon idella*). Aquat Toxicol. 2023;258:106482.
161. Liu L, Helal SE, Peng N. CRISPR-Cas-based engineering of probiotics. BioDesign Res. 2023;5:0017.
162. Liu R, Han G, Li Z, Cun S, Hao B, Zhang J, Liu X. Bacteriophage therapy in aquaculture: Current status and future challenges. Folia Microbiol. 2022;67(4):573–90.
163. Liu R, Wang S, Huang D, Huang Y, He T, Chen X. The probiotic roles of *Lactiplantibacillus plantarum* E2 as a dietary supplement in growth promotion and disease resistance of juvenile large yellow croaker (*Larimichthys crocea*). Aquaculture. 2024;578: 740082.
164. Liu Y, Feng J, Pan H, Zhang X, Zhang Y. Genetically engineered bacterium: Principles, practices, and prospects. Front Microbiol. 2022;13: 997587.
165. Liu Y, Gao J, Nie Z, Wang J, Sun Y, Xu G. Integration of metagenome and metabolome analysis reveals the correlation of gut microbiota, oxidative stress, and inflammation in *Coilia nasus* under air exposure stress and salinity mitigation. Comp Biochem Physiol D. 2024;49: 101175.
166. Liu Y, Li X, Li Y, Li J, Zhu S. Gut microbiomes of cyprinid fish exhibit host-species symbiosis along gut trait and diet. Front Microbiol. 2022;13: 936601.
167. Liu Y, Li Y, Li J, Zhou Q, Li X. Gut microbiome analyses of wild migratory freshwater fish (*Megalobrama terminalis*) through geographic isolation. Front Microbiol. 2022;13: 858454.
168. Llewellyn MS, McGinnity P, Dionne M, Letourneau J, Thonier F, Carvalho GR, et al. The biogeography of the atlantic salmon (*Salmo salar*) gut microbiome. ISME J. 2016;10(5):1280–4.
169. Lokesh J, Kiron V, Sipkema D, Fernandes JMO, Moum T. Succession of embryonic and the intestinal bacterial communities of Atlantic salmon (*Salmo salar*) reveals stage-specific microbial signatures. Microbiologyopen. 2019;8:1–16.
170. López Nadal A, Ikeda-Ohtsubo W, Sipkema D, Peggs D, McGurk C, Forlenza M, et al. Feed, microbiota, and gut immunity: using the zebrafish model to understand fish health. Front Immunol. 2020;11:512428.
171. Luna GM, Quero GM, Kokou F, Kormas K. Time to integrate biotechnological approaches into fish gut microbiome research. Curr Opin Biotechnol. 2022;73:121–7.
172. Ma S, Lv Y, Hou L, Jia Z, Lin S, Wang S, ... Hou J. Effect of acute temperature stress on energy metabolism, immune performance and gut microbiome of largemouth bass (*Micropterus salmoides*). Aquac Fish. 2023;10(2):260–70.
173. Magnano San Lio R, Favara G, Maugeri A, Barchitta M, Agodi A. How antimicrobial resistance is linked to climate change: an overview of two intertwined global challenges. Int J Environ Res Public Health. 2023;20(3):1681.
174. Mahdizade Ari M, Dadgar L, Elahi Z, Ghanavati R, Taheri B. Genetically engineered microorganisms and their impact on human health. Int J Clin Pract. 2024;2024(1):6638269.
175. Mair GC, Halwart M, Derun Y, Costa-Pierce BA. A decadal outlook for global aquaculture. J World Aquac Soc. 2023;54(2):196-205.
176. Maytorena-Verdugo CI, Peña-Marín ES, Alvarez-Villagómez CS, Pérez-Jiménez GM, Sepúlveda-Quiroz CA, Alvarez-González CA. Inclusion of mannan-oligosaccharides in diets for tropical gar *Atractosteus tropicus* larvae: Effects on growth, digestive enzymes, and expression of intestinal barrier genes. Fishes. 2022;7(3):127.
177. Medina-Félix D, Garibay-Valdez E, Vargas-Albores F, Martínez-Porchas M. Fish disease and intestinal microbiota: A close and indivisible relationship. Rev Aquac. 2023;15(2):820–39.
178. Meharoof M, Siju R, Reshma KJ. Chapter-3 manipulation of gut microbiota: an aquaculture perspective. 2020;8:37-53.
179. Mehdinejad N, Imanpour MR, Jafari V. Combined or individual effects of dietary probiotic, *Pediococcus acidilactici* and nucleotide on reproductive performance in goldfish (*Carassius auratus*). Probiotics and antimicrobial proteins. 2019;11:233–8.
180. Mekuchi M, Asakura T, Sakata K, Yamaguchi T, Teruya K, Kikuchi J. Intestinal microbiota composition is altered according to nutritional biorhythms in the leopard coral grouper (*Plectropomus leopardus*). PLoS ONE. 2018;13(6): e0197256.
181. Mishra SS, Rakesh D, Dhiman M, Choudhary P, Debbarma J, Sahoo SN, Mishra CK. Present status of fish disease management in freshwater aquaculture in India: state-of-the-art-review. Journal of Aquaculture & Fisheries. 2017;1(003):14.
182. Mitra S, Khan MA, Nielsen R, Kumar G, Rahman MT. Review of environmental challenges in the Bangladesh aquaculture industry. Environ Sci Pollut Res. 2024;31(6):8330–40.
183. Montalban-Arques A, De Schryver P, Bossier P, Gorkiewicz G, Mulero V, Gatlin DM III, Galindo-Villegas J. Selective manipulation of the gut microbiota improves immune status in vertebrates. Front Immunol. 2015;6:512.

184. Monzón-Atienza L, Bravo J, Serradell A, Montero D, Gómez-Mercader A, Acosta F. Current status of probiotics in European sea bass aquaculture as one important Mediterranean and Atlantic commercial species: A review. Animals. 2023;13(14): 2369.
185. Morales-Rivera MF, Valenzuela-Miranda D, Nuñez-Acuña G, Benevento BP, Gallardo-Escárate C, Valenzuela-Muñoz V. Atlantic Salmon (*Salmo salar*) transfer to seawater by gradual salinity changes exhibited an increase in the intestinal microbial abundance and richness. Microorganisms. 2022;11(1):76.
186. Munni MJ, Akther KR, Ahmed S, Hossain MA, Roy NC. Effects of probiotics, prebiotics, and synbiotics as an alternative to antibiotics on growth and blood profile of Nile tilapia (*Oreochromis niloticus*). Aquac Res. 2023;2023(1):2798279.
187. Nath A, Bhattacharjee R, Nandi A, Sinha A, Kar S, Manoharan N, et al. Phage delivered CRISPR-Cas system to combat multidrug-resistant pathogens in gut microbiome. Biomed Pharmacother. 2022;151:113122.
188. Nava GM, Stappenbeck TS. Diversity of the autochthonous colonic microbiota. Gut microbes. 2011;2(2):99–104.
189. Naya-Català F, Piazzon MC, Torrecillas S, Toxqui-Rodríguez S, Calduch-Giner JÀ, Fontanillas R, et al. Genetics and nutrition drive the gut microbiota succession and host-transcriptome interactions through the gilthead sea bream (*Sparus aurata*) production cycle. Biology. 2022;11(12):1744.
190. Nayak SK. Role of gastrointestinal microbiota in fish. Aquac Res. 2010;41(11):1553–73.
191. Nguyen NH. Genetics and genomics of infectious diseases in key aquaculture species. Biology. 2024;13(1): 29.
192. Nie L, Zhou QJ, Qiao Y, Chen J. Interplay between the gut microbiota and immune responses of ayu (*Plecoglossus altivelis*) during Vibrio anguillarum infection. Fish Shellfish Immunol. 2017;68:479–87.
193. Nikouli E, Meziti A, Antonopoulou E, Mente E, Kormas KA. Gut bacterial communities in geographically distant populations of farmed sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). Microorganisms. 2018;6(3):92.
194. Niu X, Dang Z, Hong M, Shi H, Ding L. Effects of Freshwater Acidification on the Gut Microbial Community of *Trachemys scripta elegans*. Animals. 2024;14(13):1898.
195. Noman M, Kazmi SSUH, Saqib HSA, Fiaz U, Pastorino P, Barcelò D, et al. Harnessing probiotics and prebiotics as eco-friendly solution for cleaner shrimp aquaculture production: a state of the art scientific consensus. Sci Total Environ. 2024;915:169921.
196. Norman R, Crumlish M, Stetkiewicz S. The importance of fisheries and aquaculture production for nutrition and food security. Revue scientifique et technique (International Office of Epizootics). 2019;38(2):395–407.
197. Nurmalasari, Liu CH, Maftuch IM, Hu SY. Dietary supplementation with prebiotic chitooligosaccharides enhances the growth performance, innate immunity and disease resistance of nile tilapia (*Oreochromis niloticus*). Fishes. 2022;7(6):313.
198. Okey IB, Gabriel UU, Deekae SN. The use of synbiotics (Prebiotic and probiotic) in aquaculture development. Sumerianz Journal of Biotechnology. 2018;1(2):51–60.
199. Oliveira FC, Soares MP, Oliveira BPN, Pilarski F, de Campos CM. Dietary administration of *Bacillus subtilis*, inulin and its synbiotic combination improves growth and mitigates stress in experimentally infected *Pseudoplatystoma reticulatum*. Aquac Res. 2022;53(12):4256–65.
200. Olowe OS, Hamidoghli A, Choi W, Bae J, Lee Y, Folorunso EA, et al. The effects of two dietary synbiotics on growth performance, hematological parameters, and nonspecific immune responses in Japanese Eel. J Aquat Anim Health. 2024;36(2):136–50.
201. Opiyo M, Mziri V, Musa S, Kyule D, Hinzano S, Wainaina M, et al. Fish disease management and biosecurity systems. In: Munguti et al., (Eds). State of Aquaculture in Kenya 2020: Towards Nutrition -Sensitive Fish Food Systems; Chapter 7: Pages 97 126
202. Ou W, Yu G, Zhang Y, Mai K. Recent progress in the understanding of the gut microbiota of marine fishes. Marine life science & technology. 2021;3:434–48.
203. Pan B, Han X, Yu K, Sun H, Mu R, Lian CA. Geographical distance, host evolutionary history and diet drive gut microbiome diversity of fish across the Yellow River. Mol Ecol. 2023;32(5):1183–96.
204. Parata L, Nielsen S, Xing X, Thomas T, Egan S, Vergés A. Age, gut location and diet impact the gut microbiome of a tropical herbivorous surgeonfish. FEMS Microbiology Ecology. 2020;96(1):fiz179.
205. Pepi M, Focardi S. Antibiotic-resistant bacteria in aquaculture and climate change: A challenge for health in the Mediterranean area. Int J Environ Res Public Health. 2021;18(11): 5723.
206. Pérez T, Balcázar JL, Ruiz-Zarzuela I, Halaihel N, Vendrell D, de Blas I, Múzquiz JL. Host–microbiota interactions within the fish intestinal ecosystem. Mucosal Immunol. 2010;3(4):355–60.
207. Pérez-Jiménez GM, Peña-Marín ES, Maytorena-Verdugo CI, Sepúlveda-Quiroz CA, Jiménez-Martínez LD, De la Rosa-García S, et al. Incorporation of fructooligosaccharides in diets influence growth performance, digestive enzyme activity, and expression of intestinal barrier function genes in tropical gar (*Atractosteus tropicus*) larvae. Fishes. 2022;7(3):137.
208. Pérez-Sánchez T, Mora-Sánchez B, Balcázar JL. Biological approaches for disease control in aquaculture: advantages, limitations and challenges. Trends Microbiol. 2018;26(11):896–903.
209. Piazzon MC, Naya-Català F, Simó-Mirabet P, Picard-Sánchez A, Roig FJ, Calduch-Giner JA, et al. Sex, age, and bacteria: how the intestinal microbiota is modulated in a protandrous hermaphrodite fish. Front Microbiol. 2019;10:2512.
210. Ponomareva EN, Sorokina MN, Grigoriev VA, Mazanko M, Chistyakov VA, Rudoy DV. Probiotic *Bacillus amyloliquefaciens* B-1895 Improved Growth of Juvenile Trout. Food science of animal resources. 2024;44(4):805.
211. Preenanka R, Safeena MP, Krishnan R. Evaluation of therapeutic potential of Streptococcus phage-1A against Streptococcus agalactiae infection in Nile tilapia (*Oreochromis niloticus*). Aquacult Int. 2023;31(3):1439–56.
212. Puri P, Sharma JG, Singh R. Biotherapeutic microbial supplementation for ameliorating fish health: developing trends in probiotics, prebiotics, and synbiotics use in finfish aquaculture. Anim Health Res Rev. 2022;23(2):113–35.
213. Qin C, Zhang Y, Liu W, Xu L, Yang Y, Zhou Z. Effects of chito-oligosaccharides supplementation on growth performance, intestinal cytokine expression, autochthonous gut bacteria and disease resistance in hybrid tilapia *Oreochromis niloticus*♀× *Oreochromis aureus*♂. Fish Shellfish Immunol. 2014;40(1):267–74.
214. Rai S, Kaur B, Singh P, Singh A, Benjakul S, Vijay Kumar Reddy S, et al. Perspectives on phage therapy for health management in aquaculture. Aquacult Int. 2024;32(2):1349–93.
215. Rai S, Tyagi A, BT NK. Oral feed-based administration of phage cocktail protects rohu fish (*Labeo rohita*) against *Aeromonas hydrophila* infection. Arch Microbiol. 2024;206(5):219.
216. Rai S, Tyagi A, Kumar BN. Isolation and characterization of *Aeromonas hydrophila* lytic phage, and evaluation of a phage cocktail against *A. hydrophila* contamination in fish fillet. Food Control. 2023;145:109460.
217. Rajeev R, Adithya KK, Kiran GS, Selvin J. Healthy microbiome: a key to successful and sustainable shrimp aquaculture. Rev Aquac. 2021;13(1):238–58.
218. Ramos-Vivas J, Superio J, Galindo-Villegas J, Acosta F. Phage therapy as a focused management strategy in aquaculture. Int J Mol Sci. 2021;22(19):10436.
219. Ranzani-Paiva MJT, de Carla Dias D, Cavalcante RB, Telli G, Oshiro E, Ishikawa CM, et al. Prebiotic, probiotic and marine algae supplementation in juvenile tilapia diet. Boletim do Instituto de Pesca. 2024;50:1-16.
220. Rasmussen BB, Kalatzis PG, Middelboe M, Gram L. Combining probiotic Phaeobacter inhibens DSM17395 and broad-host-range vibriophage KVP40 against fish pathogenic vibrios. Aquaculture. 2019;513: 734415.
221. Rawls JF, Mahowald MA, Ley RE, Gordon JI. Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. Cell. 2006;127(2):423–33.
222. Ray AK, Ghosh K, Ringø EJAN. Enzyme-producing bacteria isolated from fish gut: a review. Aquac Nutr. 2012;18(5):465–92.
223. Raymo G, Ali A, Ahmed RO, Salem M. Early-life fecal transplantation from high muscle yield rainbow trout to low muscle yield recipients accelerates somatic growth through respiratory and mitochondrial efficiency modulation. Microorganisms. 2024;12(2): 261.
224. Ridlo A, Firdaus MLM, Sumarwan J. Growth of *Litopenaeus vannamei* using synbiotics supplementation diet in outdoor low-salinity ponds concerning water quality parameters and phytoplankton communities. Journal of Marine Biotechnology and Immunology. 2024;2(2):32–6.

225. Ringø EZ, Zhou Z, Vecino JG, Wadsworth S, Romero J, Krogdahl Å, et al. Effect of dietary components on the gut microbiota of aquatic animals A never-ending story? Aquaculture Nutr. 2016;22(2):219–82.
226. Ringø E, Olsen RE, Gifstad TØ, Dalmo RA, Amlund H, Hemre GI, Bakke AM. Prebiotics in aquaculture: a review. Aquac Nutr. 2010;16(2):117–36.
227. Rohani MF, Islam SM, Hossain MK, Ferdous Z, Siddik MA, Nuruzzaman M, et al. Probiotics, prebiotics and synbiotics improved the functionality of aquafeed: Upgrading growth, reproduction, immunity and disease resistance in fish. Fish Shellfish Immunol. 2022;120:569–89.
228. Romero J, Ringø E, Merrifield DL. The gut microbiota of fish. in: Aquaculture nutrition: Gut health, probiotics and prebiotics. John Wiley & Sons, Ltd. Published 2014 by John Wiley & Sons, Ltd.2014:75-100.
229. Ronda C, Chen SP, Cabral V, Yaung SJ, Wang HH. Metagenomic engineering of the mammalian gut microbiome in situ. Nat Methods. 2019;16(2):167–70.
230. Rørbo N, Rønneseth A, Kalatzis PG, Rasmussen BB, Engell-Sørensen K, Kleppen HP, et al. Exploring the effect of phage therapy in preventing *Vibrio anguillarum* infections in cod and turbot larvae. Antibiotics. 2018;7(2):42.
231. Ruiz A, Gisbert E, Andree KB. Impact of the diet in the gut microbiota after an inter-species microbial transplantation in fish. Sci Rep. 2024;14(1):4007.
232. Saengrung J, Bunnoy A, Du X, Huang L, An R, Liang X, Srisapoome P. Effects of ribonucleotide supplementation in modulating the growth of probiotic *Bacillus subtilis* and the synergistic benefits for improving the health performance of Asian seabass (*Lates calcarifer*). Fish Shellfish Immunol. 2023;140: 108983.
233. Salam MA, Islam MA, Paul SI, Rahman MM, Rahman ML, Islam F, et al. Gut probiotic bacteria of *Barbonymus gonionotus* improve growth, hematological parameters and reproductive performances of the host. Sci Rep. 2021;11(1):10692.
234. Saravanakumar S, Prabakaran NN, Ashokkumar R, Jamuna S. Unlocking the gut's treasure: lipase-producing *Bacillus subtilis* probiotic from the intestine of *Microstomus kitt* (lemon sole). Appl Biochem Biotechnol. 2024;196(7):4273–86.
235. Saravanan K, Sivaramakrishnan T, Praveenraj J, Kiruba-Sankar R, Haridas H, Kumar S, Varghese B. Effects of single and multi-strain probiotics on the growth, hemato-immunological, enzymatic activity, gut morphology and disease resistance in Rohu. *Labeo rohita* Aquaculture. 2021;540:736749.
236. Say P, Nimikul S, Bunnoy A, Na-Nakorn U, Srisapoome P. Long-term application of a synbiotic chitosan and acinetobacter KU011TH mixture on the growth performance, health status, and disease resistance of hybrid catfish (*Clarias gariepinus*× *C. macrocephalus*) during Winter. Microorganisms, 11(7), 1807. and mitigates stress in experimentally infected *Pseudoplatystoma reticulatum*. Aquac Res. 2023;53(12):4256–65.
237. Semova I, Carten JD, Stombaugh J, Mackey LC, Knight R, Farber SA, Rawls JF. Microbiota regulate intestinal absorption and metabolism of fatty acids in the zebrafish. Cell Host Microbe. 2012;12(3):277–88.
238. Senghor B, Sokhna C, Ruimy R, Lagier JC. Gut microbiota diversity according to dietary habits and geographical provenance. Human Microbiome Journal. 2018;7:1–9.
239. Sepúlveda-Quiroz CA, Peña-Marín ES, Pérez-Morales A, Martínez-García R, Alvarez-Villagomez CS, Maytorena-Verdugo CI, et al. Fructooligosaccharide supplementation in diets for tropical gar (*Atractosteus tropicus*) juvenile: Effects on morphophysiology and intestinal barrier function. Aquac Res. 2021;52(1):37–50.
240. Sequeiros C, Garcés ME, Fernández M, Marcos M, Castaños C, Moris M, Olivera NL. Zebrafish intestinal colonization by three lactic acid bacteria isolated from Patagonian fish provides evidence for their possible application as candidate probiotic in aquaculture. Aquacult Int. 2022;30(3):1389–405.
241. Shadrack RS, Manabu I, Koshio S, Yokoyama S, Zhang Y, Mzengereza K. et al. Effects of single and mixture probiotic supplements on growth, digestive activity, antioxidative status, immune and growth-related genes, and stress response of Juvenile Red Sea Bream (*Pagrus major*). Aquac Nutr. 2022;2022(1):8968494.
242. Shang X, Geng L, Wei H, Che X, Xing L, Xing M, et al. Selenium-enriched *Lactobacillus plantarum* alleviate of high alkalinity-induced microbiota-gut-blood systems affect by improving the gut microbiota. Aquaculture. 2024;593:741294.
243. Shen Y, Ma K, Yue GH. Status, challenges and trends of aquaculture in Singapore. Aquaculture. 2021;533: 736210.
244. Sheridan PO, Scott KP. Establishing genetic manipulation for novel strains of human gut bacteria. Microbiome Res Rep. 2023;2(1):1.
245. Sheth RU, Cabral V, Chen SP, Wang HH. Manipulating bacterial communities by in situ microbiome engineering. Trends Genet. 2016;32(4):189–200.
246. Shija VM, Zakaria GE, Amoah K, Yi L, Huang J, Masanja F, et al. Dietary effects of probiotic bacteria, *Bacillus amyloliquefaciens* AV5 on growth, serum and mucus immune response, metabolomics, and lipid metabolism in nile tilapia (*Oreochromis niloticus*). Aquac Nutr. 2024;2024(1):4253969.
247. Shukla P. Synthetic biology perspectives of microbial enzymes and their innovative applications. Indian journal of microbiology. 2019;59(4):401–9.
248. Sidorova NA. Results of the study on the antimicrobial activity of probiotic strains of *lactobacilli* and their potential application in feed formulations for aquaculture species. In *BIO Web of Conferences*. EDP Sciences. 2024; 82:03007.
249. Silva YJ, Costa L, Pereira C, Mateus C, Cunha Â, Calado R, et al. Phage therapy as an approach to prevent *Vibrio anguillarum* infections in fish larvae production. PloS One. 2014;9(12):e114197.
250. Singh A, PavanKalyan M, Choudhury TG, Kamilya D, Khan MIR, Chouhan N. Supplementation of autochthonous potential probiotic *Bacillus subtilis* COFCAU_BSP3 to Labeo rohita feed: effect on immune-biochemical responses and resistance against *Aeromonas hydrophila*. Aquacult Int. 2024;32(4):3785–800.
251. Smith CC, Snowberg LK, Gregory Caporaso J, Knight R, Bolnick DI. Dietary input of microbes and host genetic variation shape among-population differences in stickleback gut microbiota. ISME J. 2015;9(11):2515–26.
252. Sohel AM, Shahjahan M, Hossain MK, Sumi KR, Hossain MS, Abdul Kari Z, Tahiluddin AB, et al. Effects of multispecies probiotics on growth, hematology, and gut health of stinging catfish (*Heteropneustes fossilis*) in biofloc system. Water. 2023;15(14):2519.
253. Sommuek T, Kasamawut K, Ngamsnae P, Sreeputhorn K, Saowakoon S. Impacts of feed additives, prebiotics and vitamin E on growth and reproductive performance of nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758). J Fish Environ. 2023;47(3):1-10.
254. Song M, Pan L, Zhang M, Huang F, Gao S, Tian C. Changes of water, sediment, and intestinal bacterial communities in *Penaeus japonicus* cultivation and their impacts on shrimp physiological health. Aquacult Int. 2020;28:1847–65.
255. Song SK, Beck BR, Kim D, Park J, Kim J, Kim HD, Ringø E. Prebiotics as immunostimulants in aquaculture: a review. Fish Shellfish Immunol. 2014;40(1):40–8.
256. Steiner K, Laroche O, Walker SP, Symonds JE. Effects of water temperature on the gut microbiome and physiology of Chinook salmon (*Oncorhynchus tshawytscha*) reared in a freshwater recirculating system. Aquaculture. 2022;560:738529.
257. Stephens WZ, Burns AR, Stagaman K, Wong S, Rawls JF, Guillemin K, et al. The composition of the zebrafish intestinal microbial community varies across development. ISME J. 2016;10:644–54.
258. Subedi B, Shrestha A. A review: Application of probiotics in aquaculture. Int J For Anim Fish Res. 2020;4(5)1-55.
259. Sun J, Chen M, Hu Z, Xu N, Wang W, Ping Z, et al. Ribonuclease 4 functions as an intestinal antimicrobial protein to maintain gut microbiota and metabolite homeostasis. Nat Commun. 2024;15(1):5778.
260. Suzzi AL, Stat M, Gaston TF, Siboni N, Williams NL, Seymour JR, Huggett MJ. Elevated estuary water temperature drives fish gut dysbiosis and increased loads of pathogenic vibrionaceae. Environ Res. 2023;219:115144.
261. Sylvain FÉ, Cheaib B, Llewellyn M, Gabriel Correia T, Barros Fagundes D, Luis Val A, Derome N. pH drop impacts differentially skin and gut microbiota of the Amazonian fish tambaqui (*Colossoma macropomum*). Sci Rep. 2016;6(1):32032.
262. Tadeu AD, Duarte J, Trindade D, Costa P, Venâncio C, Lopes I, et al. Bacteriophages to control *Vibrio alginolyticus* in live feeds prior to their administration in larviculture. J Appl Microbiol. 2024;135(5):lxae115.

263. Talwar C, Nagar S, Lal R, Negi RK. Fish gut microbiome: current approaches and future perspectives. Indian journal of microbiology. 2018;58:397–414.
264. Tammas I, Bitchava K, Gelasakis AI. Transforming aquaculture through vaccination: a review on recent developments and milestones. Vaccines. 2024;12(7): 732.
265. Tan SY, Sethupathi S, Leong KH, Ahmad T. Challenges and opportunities in sustaining aquaculture industry in Malaysia. Aquacult Int. 2024;32(1):489–519.
266. Tanaka T, Sugiyama R, Sato Y, Kawaguchi M, Honda K, Iwaki H, Okano K. Precise microbiome engineering using natural and synthetic bacteriophages targeting an artificial bacterial consortium. Front Microbiol. 2024;15:1403903.
267. Tang L, Li J, Sun B, Bai Y, Zhou X, Chen L. Transcriptomic interaction between young fecal transplantation and perfluorobutanesulfonate in aged zebrafish gonads. Toxics. 2022;10(11):631.
268. Tao L, Chai J, Liu H, Huang W, Zou Y, Wu M, et al. Characterization and dynamics of the gut microbiota in rice fishes at different developmental stages in rice-fish coculture systems. Microorganisms. 2022;10(12):2373.
269. Tawfik MM, Betancor MB, McMillan S, Norambuena F, Tocher DR, Douglas A, Martin SA. Modulation of metabolic and immunoregulatory pathways in the gut transcriptome of Atlantic salmon (*Salmo salar* L.) after early nutritional programming during first feeding with plant-based diet. Front Immunol. 2024;15:1412821.
270. Tawfik MM, Lorgen-Ritchie M, Król E, McMillan S, Norambuena F, Bolnick DI, et al. Modulation of gut microbiota composition and predicted metabolic capacity after nutritional programming with a plant-rich diet in Atlantic salmon (*Salmo salar*): insights across developmental stages. Anim Microbiome. 2024;6(1):38.
271. Tiengtam N, Khempaka S, Paengkoum P, Boonanuntanasarn S. Effects of inulin and Jerusalem artichoke (*Helianthus tuberosus*) as prebiotic ingredients in the diet of juvenile Nile tilapia (*Oreochromis niloticus*). Anim Feed Sci Technol. 2015;207:120–9.
272. Todorov SD, Lima JMS, Bucheli JEV, Popov IV, Tiwari SK, Chikindas ML. Probiotics for aquaculture: hope, truth, and reality. Probiot Antimicrobial Proteins. 2024;27:1–14.
273. Toh MC, Goodyear M, Daigneault M, Allen-Vercoe E, Van Raay TJ. Colonizing the embryonic zebrafish gut with anaerobic bacteria derived from the human gastrointestinal tract. Zebrafish. 2013;10(2):194–8.
274. Tong Q, Dong WJ, Xu MD, Hu ZF, Guo P, Han XY, Cui LY. Characteristics and a comparison of the gut microbiota in two frog species at the beginning and end of hibernation. Front Microbiol. 2023;14: 1057398.
275. Torres-Maravilla E, Parra M, Maisey K, Vargas RA, Cabezas-Cruz A, Gonzalez A, et al. Importance of probiotics in fish aquaculture: towards the identification and design of novel probiotics. Microorganisms. 2024;12(3):626.
276. Uma A, Subash P, Abraham TJ. Importance of gut microbiota in fish–a review. Indian J Anim Health. 2020;59:181–94.
277. Utami DAS, Wahyu W, Insani L, Yudana IGPGR, Harijono T. Growth performances and intestinal bacterial populations of pacific white shrimp (*Penaeus vannamei*) fed with different dietary prebiotics-supplemented feed. Jurnal Riset Akuakultur. 2024;19(1):1–13.
278. Valenzuela MJ, Caruffo M, Herrera Y, Medina DA, Coronado M, Feijóo CG, et al. Evaluating the capacity of human gut microorganisms to colonize the zebrafish larvae (*Danio rerio*). Front Microbiol. 2018;9:1032.
279. Vargas-Albores F, Martínez-Córdova LR, Hernández-Mendoza A, Cicala F, Lago-Lestón A, Martínez-Porchas M. Therapeutic modulation of fish gut microbiota, a feasible strategy for aquaculture? Aquaculture. 2021;544: 737050.
280. Vargas-González A, Barajas M, Pérez-Sánchez T. Isolation of lactic acid bacteria (LAB) from salmonids for potential use as probiotics: in vitro assays and toxicity assessment of *Salmo trutta* embryonated eggs. Animals. 2024;14(2):200.
281. Villasante A, Ramírez C, Catalán N, Opazo R, Dantagnan P, Romero J. Effect of dietary carbohydrate-to-protein ratio on gut microbiota in Atlantic salmon (*Salmo salar*). Animals. 2019;9(3): 89.
282. Wang AR, Ran C, Ringø E, Zhou ZG. Progress in fish gastrointestinal microbiota research. Rev Aquac. 2018;10(3):626–40.
283. Wang CY, Li ZB. Growth performance, digestive enzyme activity and immune response of Japanese sea bass, *Lateolabrax japonicus* fed with fructooligosaccharide. Aquac Nutr. 2020;26(2):296–305.
284. Wangkahart E, Nontasan S, Phudkliang J, Pholchamat S, Sunthamala P, Taesuk N, et al. New insights into the effect of xylooligosaccharide derived from agricultural waste, single or combined dietary supplementation with mixed probiotics on growth, flesh quality, health condition and disease resistance in Nile tilapia (*Oreochromis niloticus*). Carbohydr Polymer Technol Appl. 2024;7:100471.
285. Wee W, Hamid NKA, Mat K, Khalif RIAR, Rusli ND, Rahman MM, et al. The effects of mixed prebiotics in aquaculture: a review. Aquac Fisher. 2024;9(1):28–34.
286. Wei G, Tay MYF, Kamaja VK, Chan-Park MB. Synbiotic encapsulation against *Vibrio parahaemolyticus* infection in whiteleg shrimps. Aquaculture. 2024;590: 741051.
287. Wei J, Li Y. CRISPR-based gene editing technology and its application in microbial engineering. Engineering Microbiology. 2023;3(4): 100101.
288. Wei J, Guo X, Liu H, Chen Y, Wang W. The variation profile of intestinal microbiota in blunt snout bream (*Megalobrama amblycephala*) during feeding habit transition. BMC Microbiol. 2018;18:1–14.
289. Wolter M, Grant ET, Boudaud M, Steimle A, Pereira GV, Martens EC, Desai MS. Leveraging diet to engineer the gut microbiome. Nat Rev Gastroenterol Hepatol. 2021;18(12):885–902.
290. Wright A, Li X, Yang X, Soto E, Gross J. Disease prevention and mitigation in US finfish aquaculture: A review of current approaches and new strategies. Rev Aquac. 2023;15(4):1638–53.
291. Xia H, Chen H, Cheng X, Yin M, Yao X, Ma J, et al. Zebrafish: an efficient vertebrate model for understanding role of gut microbiota. Mol Med. 2022;28(1):161.
292. Xia L, Cheng G, Wang P, Wang X, Dong Z, Mu Q, et al. Screening and identification of probiotics from the intestinal tract of largemouth bass (*Micropterus salmoides*) for use as a feed additive and bacterial infection control. Aquaculture. 2024;584:740661.
293. Xia R, Zhang Q, Xia D, Hao Q, Ding Q, Ran C, et al. The direct and gut microbiota-mediated effects of dietary bile acids on the improvement of gut barriers in largemouth bass (*Micropterus salmoides*). Anim Nutr. 2023;14:32–42.
294. Xie M, Xie Y, Li Y, Zhou W, Zhang Z, Yang Y, et al. The effects of fish meal replacement with ultra-micro ground mixed plant proteins (uPP) in practical diet on growth, gut and liver health of common carp (*Cyprinus carpio*). Aquaculture Rep. 2021;19:100558.
295. Xiong JB, Nie L, Chen J. Current understanding on the roles of gut microbiota in fish disease and immunity. Zool Res. 2019;40(2):70.
296. Xu J, Ma B, Su X, Huang S, Xu X, Zhou X, et al. Emerging trends for microbiome analysis: from single-cell functional imaging to microbiome big data. Engineering. 2017;3(1):66–70.
297. Xu W, Lutz CG, Taylor CM, Ortega MC. Improvement of fish growth and metabolism by oligosaccharide prebiotic supplement. Aquac Nutr. 2022;2022(1):5715649.
298. Xu Z, Jin P, Zhou X, Zhang Y, Wang Q, Liu X, et al. Isolation of a virulent *Aeromonas salmonicida* subsp. masoucida bacteriophage and its application in phage therapy in turbot (*Scophthalmus maximus*). Appl Environ Microbiol. 2021;87(21):e01468-21.
299. Yao W, Li X, Zhang C, Wang J, Cai Y, Leng X. Effects of dietary synbiotics supplementation methods on growth, intestinal health, non-specific immunity and disease resistance of Pacific white shrimp, *Litopenaeus vannamei*. Fish Shellfish Immunol. 2021;112:46–55.
300. Yilmaz S, Yilmaz E, Dawood MA, Ringø E, Ahmadifar E, Abdel-Latif HM. Probiotics, prebiotics, and synbiotics used to control vibriosis in fish: A review. Aquaculture. 2022;547: 737514.
301. Yones AMAS, Mohamed Eissa IAM, Ghobashy MA, Marzok SS. Effects of dietary inulin as prebiotic on growth performance, immuno-haematological indices and ectoparasitic infection of fingerlings Nile tilapia, *Oreochromis niloticus*. Egypt J Histol. 2020;43(1):88–103.
302. Yousefi M, Farsani MN, Afzali-Kordmahalleh A, Ghafarifarsani H. Effects of dietary synbiotic supplementation on growth performance, digestive enzyme activities, and physiological resistance against high stocking density in rainbow trout (*Oncorhynchus mykiss*). Aquacult Int. 2024;32(3):3295–315.
303. Yukgehnaish K, Kumar P, Sivachandran P, Marimuthu K, Arshad A, Paray BA, Arockiaraj J. Gut microbiota metagenomics in aquaculture: factors

influencing gut microbiome and its physiological role in fish. Rev Aquac. 2020;12(3):1903–27.

304. Yuze H, Bo H. Microbiome Engineering: Role in Treating Human Diseases. Fusion of Multidisciplinary Research, An International Journal. 2020;1(01):14–24.
305. Zakariaee H, Sudagar M, Hosseini SS, Paknejad H, Baruah K. In vitro selection of synbiotics and in vivo investigation of growth indices, reproduction performance, survival, and ovarian Cyp19α gene expression in Zebrafish *Danio rerio*. Front Microbiol. 2021;12: 758758.
306. Zhang BY, Cai GH, Yang HL, Nie QJ, Liu ZY, Sun YZ. New insights on intestinal microorganisms and carbohydrate metabolism in fish. Aquacult Int. 2024;32(2):2151–70.
307. Zhang B, et al. Comparative analysis and characterization of the gut microbiota of four farmed snakes from southern China. PeerJ. 2019;7: e6658.
308. Zhang C, Wang J, Huang M, Liu M, Zhang J. Evaluation of growth performance and lipid metabolism in zebrafish fed fructooligosaccharide using RNA sequencing. Aquac Nutr. 2019;25(6):1194–206.
309. Zhang C, Zhang J, Fan W, Huang M, Liu M. Effects of dietary Lactobacillus delbrueckii on growth performance, body composition, digestive and absorptive capacity, and gene expression of common carp (*Cyprinus carpio Huanghe var*). Aquac Nutr. 2019;25(1):166–75.
310. Zhang F, Luo W, Shi Y, Fan Z, Ji G. Should we standardize the 1,700-year-old fecal microbiota transplantation? Am J Gastroenterol. 2012;107(11):1755.
311. Zhang M, Shan C, Tan F, Limbu SM, Chen L, Du ZY. Gnotobiotic models: powerful tools for deeply understanding intestinal microbiota-host interactions in aquaculture. Aquaculture. 2020;517: 734800.
312. Zhang Y, Wen B, Meng LJ, Gao JZ, Chen ZZ. Dynamic changes of gut microbiota of discus fish (*Symphysodon haraldi*) at different feeding stages. Aquaculture. 2021;531: 735912.
313. Zhang Z, Li D, Refaey MM, Xu W, Tang R, Li L. Host age affects the development of southern catfish gut bacterial community divergent from that in the food and rearing water. Front Microbiol. 2018;9: 495.
314. Zhao R, Symonds JE, Walker SP, Steiner K, Carter CG, Bowman JP, Nowak BF. Salinity and fish age affect the gut microbiota of farmed Chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture. 2020;528:735539 Dai, W., Zhang, Z., Dong, Y., He, L., Xue, Q., & Lin, Z. (2023). Acute Salinity Stress Disrupts Gut Microbiota Homeostasis and Reduces Network Connectivity and Cooperation in Razor Clam Sinonovacula constricta. Marine Biotechnology, 25(6), 1147-1157.
315. Zhao Y, Duan C, Zhang X, Chen H, Ren H, Yin Y, Ye L. Insights into the gut microbiota of freshwater shrimp and its associations with the surrounding microbiota and environmental factors. 2018;:46-956.
316. Zheng X, Liu B, Wang N, Yang J, Zhou Q, Sun C, Zhao Y. Low fish meal diet supplemented with probiotics ameliorates intestinal barrier and immunological function of *Macrobrachium rosenbergii* via the targeted modulation of gut microbes and derived secondary metabolites. Front Immunol. 2022;13: 1074399.
317. Zhou L, Han F, Lu K, Qiao Y, Li E. Comparative study on prebiotic effects of different types of prebiotics in an in vitro fermentation by gut microbiota of shrimp (*Litopenaeus vannamei*). Aquaculture. 2023;574: 739687.
318. Zhou L, Zhang J, Yan M, Tang S, Wang X, Qin JG, et al. Inulin alleviates hypersaline-stress induced oxidative stress and dysbiosis of gut microbiota in Nile tilapia (*Oreochromis niloticus*). Aquaculture. 2020;529:735681.
319. Zhu L, Wang S, Cai Y, Shi H, Zhou Y, Zhang D, et al. Effects of five prebiotics on growth, antioxidant capacity, non-specific immunity, stress resistance, and disease resistance of juvenile hybrid grouper (*Epinephelus fuscoguttatus*♀× *Epinephelus lanceolatus*♂). Animals. 2023;13(4):754.
320. Zhu YT, Liang X, Liu TT, Power DM, Li YF, Yang JL. The mussel larvae microbiome changes in response to a temperature rise. Front Mar Sci. 2024;11:1367608.
321. Zuo F, Marcotte H. Advancing mechanistic understanding and bioengineering of probiotic *lactobacilli* and *bifidobacteria* by genome editing. Curr Opin Biotechnol. 2021;70:75–82.

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