



Review

# Probiotics in Fish Nutrition—Long-Standing Household Remedy or Native Nutraceuticals?

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**Abstract:** Over the last decades, aquaculture production increased rapidly. The future development of the industry highly relies on the sustainable utilization of natural resources. The need for improving disease resistance, growth performance, food conversion, and product safety for human consumption has stimulated the application of probiotics in aquaculture. Probiotics increase growth and feed conversion, improve health status, raise disease resistance, decrease stress susceptibility, and improve general vigor. Currently, most probiotics still originate from terrestrial sources rather than fish. However, host-associated (autochthonous) probiotics are likely more persistent in the gastrointestinal tract of fish and may, therefore, exhibit longer-lasting effects on the host. Probiotic candidates are commonly screened in in vitro assays, but the transfer to in vivo assessment is often problematic. In conclusion, modulation of the host-associated microbiome by the use of complex probiotics is promising, but a solid understanding of the interactions involved is only in its infancy and requires further research. Probiotics could be used to explore novel ingredients such as chitin-rich insect meal, which cannot be digested by the fish host alone. Most importantly, probiotics offer the opportunity to improve stress and disease resistance, which is among the most pressing problems in aquaculture.

**Keywords:** probiotics; aquaculture; microbiome; living gut; species-specific bacteria; mode-of-action



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## 1. Introduction

In view of stagnating fishery landings reported over the past 50 years, only the rapidly developing aquaculture industry can meet the increasing per capita demand for fish worldwide. Over the past decades, global aquaculture production has nearly doubled every ten years, which reflects the fastest growth in the food-producing sector [1]. Undoubtedly, the sustainable utilization of scarce natural resources is a challenge for the future development of the industry. Among the obstacles for future expansion, fish nutrition and the management of fish diseases and health are among the most critical. Sustainable development of the industry requires advanced disease and health management because the aquatic environment renders fish particularly susceptible to ubiquitous pathogens [2]. However, the administration of drugs such as antibiotics is associated with human health concerns, and prophylactic alternatives are highly desirable.

Feeding costs represent 40–70% of expenditure in intensive fish farming [3], mainly attributed to the protein-rich ingredients. In the past, fishmeal was the main protein source in fish nutrition, but, nowadays, it has become a scarce, costly ingredient. As a consequence, but also with regard to the vulnerable status of several industrial species, such as the Peruvian anchoveta, alternative plant ingredients are used in the diets [4–6]. Unfortunately, plant-based ingredients can have several negative effects on fish nutrition

that involve the antinutritional effects of secondary plant metabolites, suboptimal amino acid composition, as well as mineral imbalances, which, in turn, may impact health and immune status [7–9]. Such restraints can be remedied, at least partly, by improving the digestion of these feedstuffs by making use of probiotic supplements and adjusting the gut microbiota.

In 1907, Metchnikoff was the first to point out the positive role of bacteria in milk and yogurt products. He assumed that these beneficial bacteria replace harmful microbes and are, therefore, responsible for the prolonged life of Balkan farmers who consumed high quantities of these products. In 1953, Kollath introduced the term probiotics, originating from the Latin word *pro* and the Greek word *bios* “for life” [10]. Traditionally, probiotics have thus been regarded as bioactive food additives, especially living bacteria, which have a positive influence on digestion and, moreover, the microbiome of the gastrointestinal tract (GIT) in general [11]. Verschuere et al. [12] expanded this definition, stating that probiotics are live microbial additives that have a beneficial effect on the host by (1) modifying the host-associated microbial community, (2) ensuring improved use of the feed or enhancing its nutritional value, (3) enhancing the host response towards disease, and/or (4) improving the quality of its ambient environment. Merrifield et al. proposed a slightly modified definition for probiotics in aquaculture [13]. They defined that “a probiotic organism can be regarded as a live or dead component of a microbial cell, which is administered via the feed or to the rearing water, benefiting the host by improving disease resistance, health status, growth performance, feed utilization, stress response, or general vigor. This is achieved, at least in part, by improving the host’s microbial balance or the microbial balance of the ambient environment”.

Intensification of aquaculture production exacerbates health threats of infectious diseases, including those arising from immunosuppression by plant ingredients. Over the last two decades, disease management has addressed new vaccines, immunostimulants, and disinfection strategies; in particular, probiotics have a huge potential in today’s disease management strategies. Administered probiotic strains can counteract the colonization of pathogens by competitive exclusion. This may involve either competition for binding sites, synthesis of antibacterial compounds, immune stimulation, or competition for nutrients [14]. Detailed reviews on the probiotic species and the respective fish host species have been provided elsewhere [14–19]

In this review, we summarize the current knowledge and recent findings in probiotic research. In particular, we address the concept of the core microbiome of the digestive system, discuss the utilization of host-associated, native (autochthonous) bacteria, and present modes of action that focus on the main site of host–microbe interaction, the gastrointestinal tract (GIT).

## 2. The “Living Gut” and Its Core Microbiome

A proverbial galaxy of living microorganisms colonizes every aquatic habitat and every aquatic organism. Constant confrontation and interaction with the microbes of the environment surrounding an individual are an unavoidable fate throughout its life, starting from an immature egg to the adult animal. The taxonomic composition of the ambient microbiota is, therefore, highly variable, depending on climatic and seasonal conditions, including environmental factors such as salinity or temperature [20–22]. Aquatic organisms, such as fish, experience this variability and face continuous microbial invasions. However, to maintain its basic intestinal functionality, the host also actively selects for symbiotic and commensal microorganisms. Therefore, the gut microbiota is influenced by both external factors and the selective pressure exerted by the host [23–26]. Compared to the surrounding environment, the microbial communities associated with an organism are regarded as relatively stable, particularly those in the GIT [27,28], which assures metabolic, nutritional, and immunologic functionality. Nevertheless, to a certain degree, variable chemical and physiological factors (e.g., pH, salinity, temperature) influence its composition

dynamically [24,29–31]. Depending on the duration of their presence, either temporarily or permanently, microbes are referred to as transient or persistent.

Potential microbial colonization in teleosts starts at the exposed host surfaces and their apertures. In fish, these are primarily the skin, gills, and GIT, with mouth, pharynx, and intestinal compartments. These surfaces are covered with protective mucous, which, in association with a specialized resident microflora, usually prevents the penetration of deleterious microbes [32]. Often, the persistent microbes strongly associated with the intestinal membrane are symbiotic bacteria [33]. Indeed, persistent microbes in their entirety must provide the host with immunogenic and metabolic integrity and functionality. However, due to food intake and the large surface area, the GIT is the main entry site for exogenous microorganisms, symbiotic, commensal, and pathogenic.

The microbial interactions within the GIT drastically affect the development and performance of the host animal. It has been shown that the GIT crucially affects nutritional conversion, gut physiology, and immune and stress response [34–36]. The pioneering work of Rawls et al. [37,38] identified a core microbiome in zebrafish for the first time. Accordingly, striking similarities in the microbiome of wild and farmed fish exist, which implies a role for host selection of microbiota [39]. The concept of a core microbiome also suggests that the coevolution of the microbiota and the fish host is stronger than the possible influence of environmental bacteria. Several studies on gut bacterial community censuses agree that the fish GIT harbors a bacterial load of approximately  $10^8$  bacterial cells per gram [40–44], representing ~500 species that consist mainly of aerobic or facultative anaerobe microbes. Indeed, the oxygen content in the fish gut is higher than in the human gut, which, in part, explains the low abundance of anaerobic bacteria [45]. In many fish species, Bacteroidetes, Firmicutes, and Proteobacteria comprise the dominant proportion of the gut microbiota [46–48]. Among fish, herbivores harbor the highest diversity of the microbiome [49] because they require bacteria such as *Clostridium*, *Leptotrichia*, or *Citrobacter* to support the digestion of plant-derived cellulose [50]. Due to its compartmentalization and microstructuring, the GIT provides multiple habitats with varying pH and O<sub>2</sub> concentrations and, hence, realizes multiple ecological niches. This explains the huge species diversity observed. Recent studies imply that the higher the microbial diversity in the gut, the healthier the gut [51,52].

Although there is a broad consensus on the concept of a core microbiome, gut composition shifts dynamically. For example, the diversity of the gut microbiome in zebrafish and Southern catfish increases with host age [36,53], while the microbial diversity in *Oncorhynchus mykiss* decreases with a reduction in nutrients [54]. Surrounding habitats, such as water and sediment, are the reservoirs for gut microbiota acquisition and enrichment [55,56]. Although the taxonomic composition of the microbiota in terms of species composition differs substantially, the functional composition measured from bacterial RNA data is well conserved [49].

### 3. Species-Specific, Native Probiotics

Most of the probiotics used in aquaculture do not originate from the aquatic host organism itself but from terrestrial sources or different environments. Some evidence suggests that host-associated, native (autochthonous) probiotics reveal a higher performance than those isolated from other sources (allochthonous). One possible explanation is that autochthonous probiotics are better adapted to their natural habitat, the gut, than allochthonous probiotics. Hence, autochthonous probiotics are expected to be readily able to colonize the host's GIT and perform to a better extent [57]. Furthermore, microorganisms seem to obtain the highest physiological activities in their natural habitat [58]. In addition, the survival rate of probiotics is likely higher when they are applied in their natural environment [57]. This suggests that large proportions of the gut microbiota have coevolved with the host and consequently reveal some degree of species-specificity. Indeed, interactions between multicellular organisms and microorganisms may promote beneficial mutations to the bacteria [46].

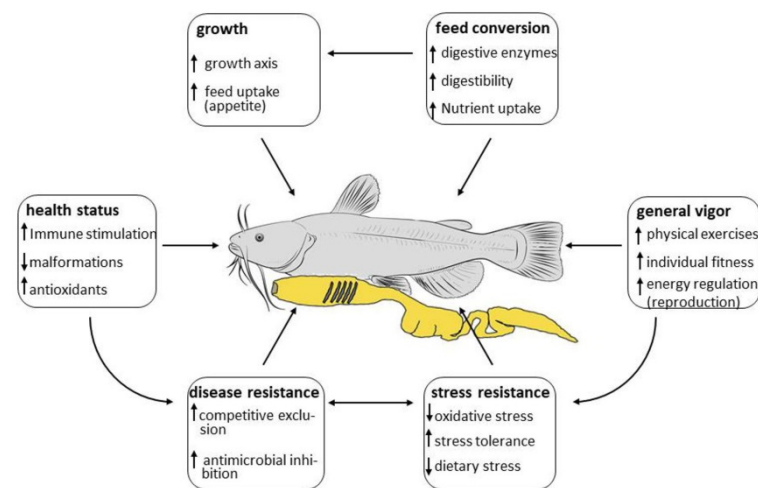
In the past, most studies regarding the specificity of probiotics have focused on lactic acid bacteria (LAB); host specificity of the adhesion and colonization of microorganisms remains controversial. Fuller was the first author to state that epithelial attachment is host-specific [11], but later studies reported that LAB from one host may indeed adhere to the epithelium of another species [59–63]. In sturgeon, the host specificity of LAB has been reported repeatedly [64,65]. This led to the controversial hypothesis that specificity is most apparent in ancient taxa such as sturgeon, whereas modern teleosts do not exhibit such stringent specificity [19]. Nevertheless, due to their beneficial effects on livestock and humans, several allochthonous LAB strains of terrestrial origin have been used successfully in fish [19]. Such an administration is particularly effective during early development [66]. Nevertheless, disruption of gut integrity occurs for some LAB strains [65]. It seems, therefore, advisable to check probiotics for pathomorphological modification of the gut during their evaluation. Several studies suggest that LAB strains are suitable probiotic candidates due to their ability to withstand acidity and bile salts and adhere to the gut, their lactic acid production (which partially inhibits the growth of pathogenic bacteria), and their strengthening of the mucosal barrier. Still, one should keep in mind that other studies could not confirm the beneficial effects (particularly when LAB from terrestrial sources were used), which, in part, may be attributed to the relative aerobic conditions in the fish gut (compared to terrestrial animals) [45]. Here, LAB may be outcompeted by other bacteria.

Mucus is a gel-like layer that functions as a transit tissue and pathogen barrier [67,68]. It consists mainly of water, glycoproteins, lipids, and salts. Among the glycoproteins, mucins are important for the successful adherence of microbes. Not surprisingly, *Lactobacillus* species were reported to adhere to mucin as well as intestinal cells with specific surface-associated proteins, including mucus-binding proteins (MUBs), surface layer proteins (SLPs), surface-layer-associated proteins (SLAPs), and moonlighting proteins [69–71]. As a screening criterion, hydrophobicity is a desirable trait of probiotics that should generally be used in the analysis [72,73].

The isolation of probiotic candidates from fish relies on culture-dependent techniques using selective and nonselective growth media. In practice, one should use several media and differing culture conditions (e.g., nutrient and pH gradients) in order to increase the diversity of isolates [74]. In fish, due to the dominance of aerobic and facultative anaerobic bacteria in the gut (see above), but also because anaerobic bacteria are difficult to handle, aerobic and facultative anaerobic bacteria are the prime targets of screening. Once a probiotic candidate is established, the cultivation protocol can easily be adapted to biotechnological production in a bioreactor. Most investigations on host-associated bacteria have used farmed fish because the isolation of bacteria from wild fish must be performed in the field and sterile work near the capture site is often difficult. Previous findings indicate that the use of multistrain probiotics improves functionality and efficacy. Provided that mutual inhibition can be excluded, the combination of multiple beneficial isolates is recommended [75–77].

#### 4. Modes of Action

Beneficial effects for finfish farmers exerted by probiotic applications encompass nutritional, metabolic, and health effects. This particularly includes increased growth performance and appetite, enhanced food conversion (e.g., by an enzymatic contribution to digestion), improved feed value (macro- and micronutrients made available by the probiotic), inhibition of pathogenic microorganisms (adherence and colonization), stimulation of the immune system, increased stress resistance, and improved general vigor (Figure 1). Furthermore, higher reproductive output, reduced malformations, and higher flesh quality have been reported.



**Figure 1.** Mode of action of probiotics, including increased growth performance and appetite, enhanced feed conversion (e.g., by secretion of microbial digestive enzymes) or improved feed value (macro- and micronutrients), increased stress tolerance, improved disease resistance due to the inhibition of pathogenic microorganisms (adherence and colonization), improved health status by stimulating the fish’s immune system, and enhancement of general vigor.

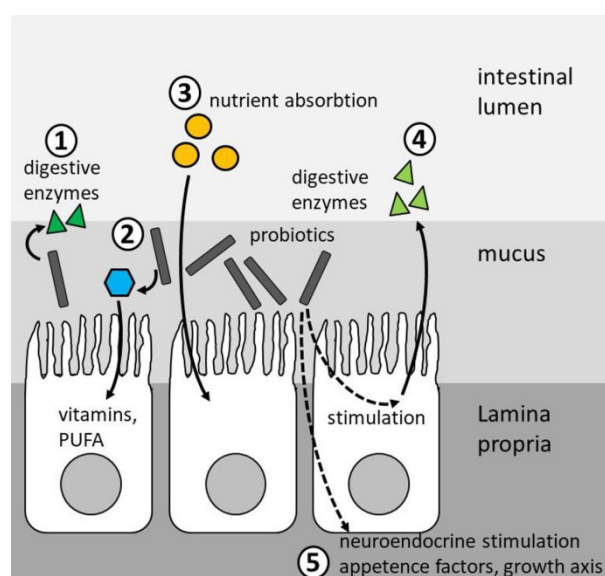
#### 4.1. Growth

Numerous studies have found that probiotic administration often results in improved growth performance [78–82]. In this regard, probiotics can act either directly by increasing the appetite and growth regulation or indirectly via improved digestibility (see feed conversion). In tilapia, weight can increase by up to 115.3% [78], but growth performance might have been linked to the better feed conversion reported. Nevertheless, probiotics actually stimulate the growth axis, increasing the transcription of insulin-like growth factor 1 (IGF-1) and the growth hormone receptor [81]. Furthermore, metabolism-related key enzymes such as glucokinase, hexokinase, glucose-6-phosphatase, and pyruvate kinase were upregulated in the host. In addition, an extract of probiotic *Rhodobacter sphaeroides* induced growth-associated genes and stimulated muscle growth, in particular [83]. The use of an additive containing *Bacillus subtilis*, *Bifidobacterium bifidum*, *Enterococcus faecium*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus lactis*, *Lactobacillus plantarum*, and *Pediococcus acidilactici* reduced the expression of myostatin, thereby enhancing the growth of white muscle [80]. Still, in a study on *Lactobacillus acidophilus*, the food-intake-stimulating hormone ghrelin was downregulated [84]. In contrast, correlated to increased food intake, Giorgia et al. [85] reported an upregulation of orexigenic genes (neuropeptide Y, agouti-related protein, ghrelin) and a decrease in anorexigenic leptin. In this context, we recommend that potential probiotics for use in practice be routinely tested for palatability [86,87]. If one detects any adverse effects, it is advisable to enhance the taste and, thus, the appetite with stimulating additives. In conclusion, probiotics modulate the regulation of growth and appetite, either directly or indirectly (Figure 1). Additionally, the host-associated microbiota seems to play an important role in the proper development and differentiation of gut components. Here, an optimal microbiota is suggested to positively influence the proliferation of epithelia cells, including the formation of mucosal layers [38,88]. For example, Merrifield et al. [89,90] observed that feed-supplemented *Pediococcus acidilactici* significantly enlarged the absorptive surface of the gut via an increased microvilli length in the proximal gut of rainbow trout, whereas *Bacillus subtilis*, *Bacillus licheniformis*, and *Enterococcus faecium* did not exhibit such an effect.

#### 4.2. Feed Conversion

There are several studies on probiotics that report increased feed conversion, but the actual mechanism is rarely revealed [91,92]. Significantly, germ-free zebrafish were arrested in their differentiation and were subsequently unable to absorb proteins [88].

Only establishing a complex microbiota restored nutrient uptake, suggesting that the gut microbiota contributes substantially to the nutrient uptake and assimilation of the host. Probiotics also convert less degradable compounds into forms that can be easily digested by the host (Figure 2). Here, various microbial enzymes, such as lipases, phytases, amylases, cellulases, trypsin, and other proteases, can be involved [93–97]. In addition, microbes can stimulate the activity and secretion of host enzymes directly [98–100]. In modern aquafeeds, supplemented with high amounts of plant ingredients, specific probiotics may increase the digestion of feed components such as nonstarch hydrocarbons, cellulose, or chitin, which are indigestible for the fish host. In addition, probiotics such as LAB may be sources of vitamins [101–103]. However, it remains controversial if the host actually absorbs these vitamins [104]. Furthermore, bacteria may represent a source of PUFA, but concentrations vary substantially between bacteria species [74]. The *Vibrio* species are especially rich in EPA and DHA [105–109]. Interestingly, high contents of DHA are observed particularly in deep-sea fish and seem to be an evolutionary adaptation towards high pressure and low temperature. Although screening techniques have improved, screening for PUFA-producing bacteria is not commonly addressed [74,110,111]. These examples show that probiotics can increase the nutritional value of the feed by increasing digestibility or providing microbial metabolites such as cofactors, vitamins, or essential fatty acids.



**Figure 2.** Nutrition- and growth-related effects (modes of action) of probiotics in the gastrointestinal tract (GIT), including direct effects such as (1) secretion of digestive enzymes; (2) absorption of (micro)nutrients such as cofactors, vitamins, and polyunsaturated fatty acids; (3) indirect effects, including elevated nutrient uptake/absorption; (4) stimulation of enzyme secretion, as well as (5) neuroendocrine stimulation of appetite and growth. For further details, refer to the text.

#### 4.3. Stress Resistance

Stress resistance and immune stimulation may translate into improved disease resistance, observed upon pathogen challenge. Therefore, in practice, it is often difficult to differentiate between these modes of action. Here, we will focus on the probiotic effects that modulate the hypothalamus–pituitary–interrenal axis (HPI axis) and regulate the oxidative stress system. In fact, the use of probiotics in counteracting stress is desirable since stress in the rearing facility often translates into an increased risk of disease transmission. In Nile tilapia, administration of *Saccharomyces cerevisiae* and *Bacillus* spp. revealed lower plasma cortisol concentrations upon transportation stress, suggesting better stress resistance after probiotic treatment [112]. Similarly, after an evoked stress, sea bream larvae pretreated with *Lactobacillus plantarum* and *Lactobacillus fructivorans* exhibited lower plasma cortisol levels [113], suggesting higher stress tolerance upon probiotic treatment. Since cortisol has an

immunosuppressive effect on antibody production, there is a clear linkage with the health status (Figure 1). In a stress challenge trial, yeast-fed fish exhibited high tolerance to acute heat stress as well as hypoxia exposure [114], but neither cortisol nor components of the oxidative stress defense were determined. Still, typical signs of stress such as the darkening of the skin or behavioral responses such as disoriented swimming, accelerated ventilation, or flaring of the gills indicate an increased stress tolerance after probiotic treatment, but no quantitative data exist. In gnotobiotic European sea bass (*Dicentrarchus labrax*) larvae, *Vibrio lentus* in the diet lowered the glucocorticoid baseline level [34]. Similarly, feeding of LAB *Lactobacillus delbrueckii delbrueckii* resulted in lower cortisol levels [115]. Before and after a hypoxia challenge, decreased glucose and cortisol levels were also reported in Nile tilapia treated with *Aspergillus oryzae* [116]. Controversially, antioxidant enzymes, as well as heat shock protein HSP70, increased upon treatment. In contrast, in zebrafish treated with *Bacillus amyloliquefaciens* R8, mRNA of antioxidant enzymes and HSP70 decreased [117]. Lower oxidative stress levels were also observed in zebrafish after probiotic treatment [118], suggesting increased hepatic stress tolerance.

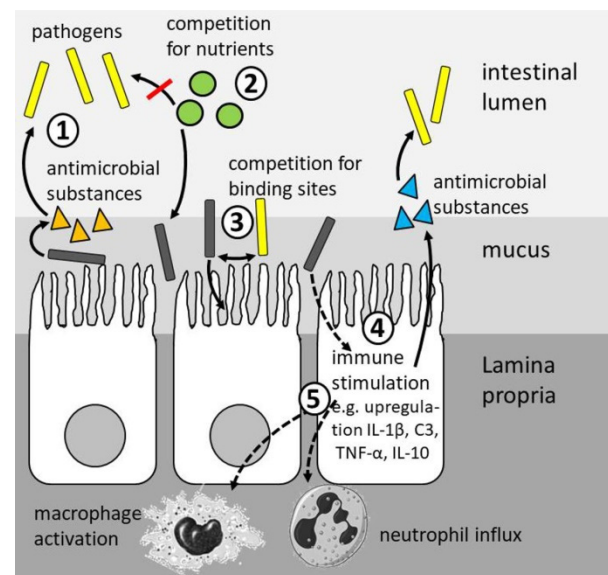
#### 4.4. Health Status

We distinguish between an immunostimulation of the host's immune system and direct interactions of the probiotic with a respective pathogen after challenge. Below, we refer to the latter as disease resistance. Still, there is a close interlinkage (Figures 1 and 3). For example, probiotic-treated fish revealed an increased expression of innate immune-related genes (IL-1 $\beta$ , IL-6, IL-21, TNF- $\alpha$ , TLR-1, -3, and -4) and, after exposure to pathogens *Aeromonas hydrophila* and *Streptococcus agalactiae*, showed a higher survival rate than control fish, which confirms that the immune stimulation leads to an enhanced immune response towards pathogens [117]. On the other hand, immunostimulation, including lysozyme, serum peroxidase, alternative peroxidase, phagocytosis, and respiratory burst activities, may not result in increased survival upon pathogen challenge [119]. Still, it is widely accepted that the stimulation of innate immunity in terms of either gene or protein synthesis, enzyme activity, or cellular response may benefit the organism during pathogen exposure. Immune stimulation frequently involves immune parameters such as a higher number of leukocytes [120,121], increased phagocytic activity [122–124], respiratory bursts [122,125], immunoglobulin M (IgM) [126,127],  $\beta$ -defensins [128], proinflammatory cytokines (interleukin IL-8 and IL-1  $\beta$ ) [124,129], and the modulation of immunity-related genes [129,130]. Probiotics can stimulate elements of the nonspecific immune system, such as mononuclear phagocytes (monocytes, macrophages), neutrophils, and natural killer (NK) cells [131]. It seems that different probiotics have different effects on the expression of immune-related genes of a respective fish species [79]. Still, differences could also be attributed to differences in experimental conditions and the strains of fish used.

Among the components of the innate immune response, the mucosal epithelium is one of the oldest and most common. Accordingly, mucin glycoproteins form a physical barrier that covers the epithelium and prevents the adhesion of pathogens. Mucin-producing cells influence the distribution of the microbes by secretion of antimicrobial peptides (AMPs). In striped catfish *Pangasianodon hypophthalmus*, the administration of a *Bacillus* mixture increased AMP levels [132]. Germ-free fish can exhibit both an undeveloped immune system and a downregulated immune response due to undeveloped intestinal vasculature and gut-associated lymphoid tissue (GALT) [133]. However, the immune status is largely restored once the microbiota is re-established [37,88].

Additionally, some microorganisms produce volatile, short-chain fatty acids (SCFAs), mainly acetic, propionic, and butyric acids, which stimulate the immune system of the fish [134,135]. Gut cell proliferation and differentiation, apoptosis, mucin production, and lipid metabolism are largely mediated by SCFAs. Moreover, SCFAs are potent immunostimulators and improve lymphocyte function. Indeed, SCFAs play a key role in the modulation of the immune system in higher vertebrates, including T-cell differentiation, stimulation of heat shock proteins, and evocation of immune-related effects upon binding

to G-coupled protein receptors, which are expressed in neutrophils, macrophages, and monocytes [136,137].



**Figure 3.** Health- and disease-resistance-related effects (modes of action) of probiotics in the gastrointestinal tract (GIT), including direct effects such as (1) secretion of antimicrobial substances, (2) competition for nutrients, and (3) binding sites, as well as (4) indirect effects such as stimulation of immune parameters and (5) activation of macrophages and the influx of neutrophils. For further details, refer to the text.

#### 4.5. Disease Resistance

In recent decades, efficient vaccines against common viral, bacterial, or fungal diseases infecting commercial livestock have been developed. However, especially in the early life stages, fish vaccination is not possible due to an incomplete immune system [63]. Larvae and fry are most susceptible to pathogens at these stages, and the resulting mortality is highly relevant [138,139]. Here, probiotics may be particularly useful.

Most surveys on probiotic applications in aquaculture consider the ability to inhibit or exclude pathogenic bacteria. The majority of the studies addressing disease resistance use in vitro assays to identify antagonistic effects as one of the most important selection criteria for candidate probiotics. However, it is essential to confirm such effects in an in vivo challenge experiment. Improved resistance towards pathogens, expressed as higher survival rates, occurs in several aquaculture species, including tilapia [124,140], carp [141,142], rainbow trout [143], cod [144], and several other species.

Prophylactic treatment of the host can lead to competitive exclusion of pathogen bacteria. Here, the probiotics compete with the pathogens for nutrients, oxygen, and binding sites to mucosa or epithelial surfaces (Figure 3). Thereby, bacteria are able to prevent potential infections. Competition for binding sites inhibits pathogen adherence and colonization, involving factors such as hydrophobicity, electrostatic interactions, lipoteichoic acids, and passive and steric forces [145]. Indeed, Brunt and Austin [146] demonstrated that the inhibition of pathogenic *Lactobacillus garvieae* and *Streptococcus iniae* by *Bacillus* species was not a result of antibiosis via the production of antimicrobial compounds. Instead, under iron-limited conditions, siderophore-producing probiotics such as *Bacillus* spp. outcompete pathogens by depriving them of available iron ions [147]. Furthermore, several probiotic organisms produce secondary metabolites that may inhibit pathogen growth, e.g., natural antibiotics, hydrogen peroxide, organic acids, bacteriocins or proteases, antibiotics, and lytic enzymes, which can eradicate harmful bacteria directly [14]. For example, *Bacillus velezensis* produced four bacteriocin gene clusters and, additionally, lytic enzymes such as



$\beta$ -1,3-glucanase [148]. In the genome of *Bacillus subtilis*, 4–5% of the genes are devoted to the production of antibiotics [149].

Undoubtedly, the innate immune system plays a major role in shaping the microbiota and, therefore, keeps the occurrence of pathogenic infections under control [150]. However, adult wild-type zebrafish display a decreased abundance of *Vibrio* spp. compared to zebrafish lacking adaptive immunity (*rag1*<sup>-/-</sup>), indicating that the innate immune system alone cannot fully regulate all members of the microbiota in the intestine [150]. It has also been suggested that the adaptive immune system, e.g., via T-cells, is involved in the regulation of the microbial composition.

Since farmers cannot predict the onset of a disease, it is of utmost importance that the probiotic is either administered as prophylactic during a susceptible life stage (e.g., larval stage) or is persistent for a long time. Here, a persistent contribution to the microbiome is highly desirable. In this context, the effects of a probiotic administered after the onset of a disease should be investigated. We would recommend probiotic applications that exert persistent proliferation in the GIT since prolonged administration could have adverse effects (dysbiosis) on the host [97]. In addition, long-term administration of probiotics could become obsolete.

#### 4.6. General Vigor (Fitness)

Undoubtedly, immune stimulation and disease resistance are closely interlinked with the physical performance of an individual. Still, few studies exist that indicate better physical fitness due to probiotic administration [151,152]. In mice and humans, probiotics can enhance exercise performance and reduce fatigue after exercise [153,154]. For example, a swimming exercise endurance test revealed improved muscle strength and endurance, including increased hepatic and muscular glycogen and decreased lactate, after four weeks of probiotic supplementation [153]. At the same time, dramatic changes in the microbial community occurred. Indeed, several studies have reported increased microbial diversity in athletes compared to non-athletes [155,156]. The mechanisms that influence physical performance and potentially support improved general vigor upon probiotic supplementation are still unknown. Additionally, probiotics may increase maximum oxygen uptake, aerobic capacity, and training load [151]. Probiotic supplementation can significantly increase SCFA production to generate more ATP required for exercise, but with regard to the limited number of studies, it is unknown if other pathways might be involved and further studies are needed. Other effects observed after probiotic applications include a reduction of malformations [157,158] and accelerated maturation [159–161].

Interestingly, *Lactobacillus rhamnosus* IMC 501<sup>®</sup> accelerates the reproductive performance of female zebrafish (*Danio rerio*), as indicated by the gonadosomatic index and gene expression of reproductive parameters such as aromatase, vitellogenin, and estradiol receptors [162,163]. This may be driven by increased energy availability, supporting the energy demand of developing gonads. Indeed, treatment with *L. rhamnosus* modulates lipid metabolism, decreasing cholesterol and triglyceride content and rising fatty acid levels [164].

## 5. In Vitro Screening for Candidate Probiotics

To reduce the actual costs, screening commonly involves in vitro assays. In general, these assays should ensure that the probiotic candidates meet the following criteria: (1) harmlessness towards the host, (2) absence of virulent and antibiotic resistance genes, (3) reach target habitat intact (e.g., survival of stomach passage at low pH), (4) acceptance by the host (adherence and host-specificity), and (5) in vivo as opposed to in vitro findings (e.g., pathogen antagonism, dietary enzymes). In humans, regulatory authorities have tried to standardize the in vitro assays by publishing detailed protocols and directives, but tests are performed in a rather arbitrary manner. Here, a standardization of tests will improve future screening efforts.

For easy handling, culturing is carried out in aerobic conditions because target candidates are aerobic and facultative anaerobic bacteria. In addition, 16S-rRNA sequencing is used for taxonomic classification. Moreover, genes involved in the response to low pH, e.g., F1F0-ATPase or heat shock proteins such as DnaK, GroES, and GroEL, are determined. However, to assess pH and bile tolerance in vitro, culturing approaches are easy to implement and are mostly cost-effective. Here, simple approaches with modified PBS have been carried out, but strategies including artificial gastric and pancreatic juices should be preferred [165–167]. An utmost important aspect of candidate screening is the safety of the respective candidate. Here, monitoring the minimum inhibitory concentrations (MICs) for the most relevant antibiotics usually relies on EFSA (European Food Safety Authority) protocols [168]. Additionally, blood agar plates are used to determine hemolytic activity [169]. The screening of pathogenic traits may also assess the ability of a candidate to bind to host cells, such as platelets [170]. The problem with the in vitro safety assays is that virulence may simply not develop under the specific conditions of the assay and, thus, remain undetected. Indeed, interaction with the host in vivo may only trigger virulence. Adherence to the mucus or epithelium is essential to persistently colonize the GIT of the host. Some probiotic strains are equipped with protein surface appendages such as pili or fimbriae, which are identified microscopically or by screening the genome for the respective encoding sequences. The adhesive capacity correlates with the hydrophobicity of the bacterial cell surface, which is determined according to the partition into hydrocarbons [171,172]. However, easy-to-perform surface hydrophobicity is often regarded as rather outdated [173]. Recently, high-throughput adhesion screening methods have utilized immobilized commercially available mucin [174].

As outlined, several bacteria contain high contents of essential fatty acids, cofactors, or vitamins [175,176]. An analysis is usually carried out under standard culture conditions using chromatographic methods, but conditions mimicking the proximal intestine during culturing are preferential. Similarly, candidate screening may target extracellular enzyme activity, allowing high throughput analysis. Most studies involve the monitoring of antagonistic activity towards selected pathogens. This is commonly assessed using simple inhibition assays performed on solid media, e.g., paper-disk diffusion assay or well diffusion assay [74]. In vitro pathogen adhesion assays are used to assess competitive inhibition. Again, conditions should mimic those in the intestine. Nevertheless, in vitro production of antimicrobial compounds may not result in similar observations in vivo since adverse effects may not be triggered. Vice versa, in vitro assays may not detect adverse effects. In addition, the desired antimicrobial effects may not be restricted to pathogens and, consequently, affect the entire microbiota, resulting in adverse effects in vivo.

## 6. Administration

Currently, in most studies, single-species probiotics are evaluated, but, as outlined above, multistrain probiotics are recommended in many application areas. For spore-forming species such as *Bacillus*, spores should be used as the delivery form because they exhibit improved resistance towards hostile environments (reduced moisture of dry feeds, low pH in the stomach). Probiotics, for which the intestinal tract is the intended site of action, are primarily administered orally via the diet. Here, the enrichment of probiotic cells on or in feeding pellets is the most common practice. In larvae, probiotics are typically added to the rearing water or incorporated into the live feed, for example, *Artemia*, as biological vectors [144]. For feed pellets, an easy-to-perform stepwise top coating technique has been described [74].

Although the pH of the GIT is not as low in fish as in higher vertebrates [103], pH sensitivity is an issue in probiotic administration. There are interspecific variations in pH along the different compartments of the GIT. In general, herbivores tend to have higher pH than omnivorous and carnivores. The channel catfish, as a typical representative of carnivores, reveals a pH between 2–4 in the stomach and 7–9 in the duodenum, a stable value of 8.6 in the upper intestine, and a near-neutral pH in the lower intestine [177].

In larvae, particularly those with prominent metamorphosis, the pH is generally higher. Hoehne-Reitan [178] reported, for example, an alkaline pH throughout the GIT until Day 24 after hatching. Still, after weaning, the pH in the stomach dropped to a minimum of 3.5. Furthermore, several teleosts are agastric (approximately 20% of the species) and do not have acidic digestion. Experimentally, artificial gastric juices stimulate the passage of the stomach [165,166,179,180]. As a rule of thumb, the lower the actual pH, the more sophisticated the strategies of administration required, most of which are adopted from application to higher vertebrates. These include delivery systems such as microencapsulation in cellulose sulfate or calcium alginate [180], legume protein encapsulation [181], hydrogels [182], and coated mucoadhesive films [183] or bioencapsulation in live biological vectors [184]. The probiotic should be retained within the delivery system, and the system should be stable until it is exposed to a specific set of environmental conditions (most importantly pH, bile salts, and enzyme activities). Consequently, the biomaterials used should be stable under acidic conditions in the stomach, and decomposition should only occur after subjecting them to the pH of the small intestine or to pancreatic enzymes.

## 7. Conclusions

Undoubtedly, in the context of rapidly increasing global aquaculture production, alternative ecofriendly methods for the prevention and therapy of diseases as well as the improvement of growth performance are pressing issues. Here, probiotics offer the tempting opportunity to modulate the GIT microbiome persistently, exerting beneficial effects such as increased growth, feed conversion, health, disease, and stress resistance. Interestingly, probiotics may be capable of degrading compounds that the fish host cannot digest alone. Carbohydrate digestion and detoxification of antinutrients are the focus of aquaculture research. Probiotic-derived chitinase, for example, may allow the use of novel feedstuff such as insect meal or krill. Similarly, the probiotic digestion of secondary plant metabolites with antinutritional effects may improve the rate of fishmeal replacement with a respective plant feed ingredient. Multispecies probiotics exhibit better probiotic effects than single-species applications. The modulation of the gastrointestinal microbiome through dietary administration of probiotics represents a potential strategy to improve microbial metabolite production, stimulate immune signaling, and increase defense mechanisms against pathogens. However, modulation of the microbiome may induce adverse effects and may even bear the risk of paving the road for pathogens. Therefore, we need to deepen our knowledge of microbiome regulation. Additionally, species-specific studies are required before a given probiotic is applied in a novel species. Moreover, the antiviral activity of several *Lactobacillus* strains towards murine norovirus (MNV) has been reported, and it will be interesting to see if microbes can also alter fish-specific viral infectivity [185].

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## References

1. FAO. *The State of World Fisheries and Aquaculture 2020. Sustainability in Action*; FAO: Rome, Italy, 2020; pp. 2–36.
2. Turnbull, J.F. Stress and resistance to infectious diseases in fish. In *Infectious Disease in Aquaculture*; Woodhead Publishing: Amsterdam, The Netherlands, 2012; pp. 111–125. [[CrossRef](#)]

3. Kroeckel, S.; Harjes, A.G.E.; Roth, I.; Katz, H.; Wuertz, S.; Susenbeth, A.; Schulz, C. When a turbot catches a fly: Evaluation of a pre-pupae meal of the Black Soldier Fly (*Hermetia illucens*) as fish meal substitute—Growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). *Aquaculture* **2012**, *364*, 345–352. [[CrossRef](#)]
4. Nasr, M.; Reda, R.; Ismail, T.; Moustafa, A. Growth, hemato-biochemical parameters, body composition, and myostatin gene expression of *Clarias gariepinus* fed by replacing fishmeal with plant protein. *Animals* **2021**, *11*, 889. [[CrossRef](#)] [[PubMed](#)]
5. Zhao, W.; Liu, Z.-L.; Niu, J. Growth performance, intestinal histomorphology, body composition, hematological and antioxidant parameters of *Oncorhynchus mykiss* were not detrimentally affected by replacement of fish meal with concentrated dephenolization cottonseed protein. *Aquac. Rep.* **2021**, *19*, 100557. [[CrossRef](#)]
6. Tusche, K.; Nagel, F.; Arning, S.; Wuertz, S.; Susenbeth, A.; Schulz, C. Effect of different dietary levels of potato protein concentrate supplemented with feed attractants on growth performance of rainbow trout (*Oncorhynchus mykiss*). *Anim. Feed. Sci. Technol.* **2013**, *183*, 202–209. [[CrossRef](#)]
7. Azeredo, R.; Machado, M.; Kreuz, E.; Wuertz, S.; Oliva-Teles, A.; Enes, P.; Costas, B. The European seabass (*Dicentrarchus labrax*) innate immunity and gut health are modulated by dietary plant-protein inclusion and prebiotic supplementation. *Fish Shellfish. Immunol.* **2017**, *60*, 78–87. [[CrossRef](#)]
8. Estruch, G.; Collado, M.C.; Monge-Ortiz, R.; Tomás-Vidal, A.; Jover-Cerdá, M.; Peñaranda, D.S.; Martínez, G.P.; Martínez-Llorens, S. Long-term feeding with high plant protein based diets in gilthead seabream (*Sparus aurata*, L.) leads to changes in the inflammatory and immune related gene expression at intestinal level. *BMC Veter Res.* **2018**, *14*, 302. [[CrossRef](#)]
9. Piazzon, M.C.; Caldach-Giner, J.A.; Fouz, B.; Estensoro, I.; Simó-Mirabet, P.; Puyalto, M.; Karalazos, V.; Palenzuela, O.; Sitjà-Bobadilla, A.; Pérez-Sánchez, J. Under control: How a dietary additive can restore the gut microbiome and proteomic profile, and improve disease resilience in a marine teleostean fish fed vegetable diets. *Microbiome* **2017**, *5*, 1–23. [[CrossRef](#)]
10. Kollath, W. The increase of the diseases of civilization and their prevention. *Münch Med. Wochenschr.* **1953**, *95*, 2.
11. Fuller, R. Probiotics in man and animals. *J. Appl. Bacteriol.* **1989**, *66*, 365–378.
12. Verschuere, L.; Rombaut, G.; Sorgeloos, P.; Verstraete, W. Probiotic Bacteria as Biological Control Agents in Aquaculture. *Microbiol. Mol. Biol. Rev.* **2000**, *64*, 655–671. [[CrossRef](#)] [[PubMed](#)]
13. Merrifield, D.L.; Dimitroglou, A.; Foey, A.; Davies, S.J.; Baker, R.T.; Bøgdal, J.; Castex, M.; Ringø, E. The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture* **2010**, *302*, 1–18. [[CrossRef](#)]
14. Hoseinifar, S.H.; Sun, Y.-Z.; Wang, A.; Zhou, Z. Probiotics as Means of Diseases Control in Aquaculture, a Review of Current Knowledge and Future Perspectives. *Front. Microbiol.* **2018**, *9*, 2429. [[CrossRef](#)] [[PubMed](#)]
15. Van Hai, N. The use of probiotics in aquaculture. *J. Appl. Microbiol.* **2015**, *119*, 917–935. [[CrossRef](#)]
16. Ringø, E.; Van Doan, H.; Lee, S.H.; Soltani, M.; Hoseinifar, S.H.; Harikrishnan, R.; Song, S.K. Probiotics, lactic acid bacteria and bacilli: Interesting supplementation for aquaculture. *J. Appl. Microbiol.* **2020**, *129*, 116–136. [[CrossRef](#)] [[PubMed](#)]
17. Ringø, E.; Hoseinifar, S.H.; Ghosh, K.; Van Doan, H.; Beck, B.R.; Song, S.K. Lactic Acid Bacteria in Finfish—An Update. *Front. Microbiol.* **2018**, *9*, 1818. [[CrossRef](#)]
18. Soltani, M.; Ghosh, K.; Hoseinifar, S.H.; Kumar, V.; Lymbery, A.J.; Roy, S.; Ringø, E. Genusbacillus, promising probiotics in aquaculture: Aquatic animal origin, bio-active components, bioremediation and efficacy in fish and shellfish. *Rev. Fish. Sci. Aquac.* **2019**, *27*, 331–379. [[CrossRef](#)]
19. Li, X.; Ringø, E.; Hoseinifar, S.H.; Lauzon, H.L.; Birkbeck, H.; Yang, D. The adherence and colonization of microorganisms in fish gastrointestinal tract. *Rev. Aquac.* **2019**, *11*, 603–618. [[CrossRef](#)]
20. Zhou, S.; Sun, Y.; Yu, M.; Shi, Z.; Zhang, H.; Peng, R.; Li, Z.; Cui, J.; Luo, X. Linking Shifts in Bacterial Community Composition and Function with Changes in the Dissolved Organic Matter Pool in Ice-Covered Baiyangdian Lake, Northern China. *Microorganisms* **2020**, *8*, 883. [[CrossRef](#)] [[PubMed](#)]
21. Wang, X.; Wang, P.; Wang, C.; Chen, J.; Miao, L.; Yuan, Q.; Liu, S.; Feng, T. Do bacterioplankton respond equally to different river regulations? A quantitative study in the single-dammed Yarlung Tsangpo River and the cascade-dammed Lancang River. *Environ. Res.* **2020**, *191*, 110194. [[CrossRef](#)]
22. Schulhof, M.A.; Allen, A.E.; Allen, E.E.; Mladenov, N.; McCrow, J.P.; Jones, N.T.; Blanton, J.; Cavalheri, H.B.; Kaul, D.; Symons, C.C.; et al. Sierra Nevada mountain lake microbial communities are structured by temperature, resources and geographic location. *Mol. Ecol.* **2020**, *29*, 2080–2093. [[CrossRef](#)]
23. Breen, P.; Winters, A.D.; Nag, D.; Ahmad, M.M.; Theis, K.R.; Withey, J.H. Internal Versus External Pressures: Effect of Housing Systems on the Zebrafish Microbiome. *Zebrafish* **2019**, *16*, 388–400. [[CrossRef](#)]
24. Riiser, E.S.; Haverkamp, T.H.A.; Varadharajan, S.; Borgan, Ø.; Jakobsen, K.S.; Jentoft, S.; Star, B. Metagenomic Shotgun Analyses Reveal Complex Patterns of Intra- and Interspecific Variation in the Intestinal Microbiomes of Codfishes. *Appl. Environ. Microbiol.* **2020**, *86*. [[CrossRef](#)] [[PubMed](#)]
25. Xiao, F.; Zhu, W.; Yu, Y.; He, Z.; Wu, B.; Wang, C.; Shu, L.; Li, X.; Yin, H.; Wang, J.; et al. Host development overwhelms environmental dispersal in governing the ecological succession of zebrafish gut microbiota. *NPJ Biofilms Microbiomes* **2021**, *7*, 1–12. [[CrossRef](#)] [[PubMed](#)]
26. Zha, Y.; Lindström, E.S.; Eiler, A.; Svanbäck, R. Different Roles of Environmental Selection, Dispersal, and Drift in the Assembly of Intestinal Microbial Communities of Freshwater Fish With and Without a Stomach. *Front. Ecol. Evol.* **2020**, *8*, 8. [[CrossRef](#)]
27. Fogarty, C.; Burgess, C.M.; Cotter, P.D.; Cabrera-Rubio, R.; Whyte, P.; Smyth, C.; Bolton, D.J. Diversity and composition of the gut microbiota of Atlantic salmon (*Salmo salar*) farmed in Irish waters. *J. Appl. Microbiol.* **2019**, *127*, 648–657. [[CrossRef](#)] [[PubMed](#)]

28. Webster, T.M.U.; Consuegra, S.; Hitchings, M.; De Leaniz, C.G. Interpopulation Variation in the Atlantic Salmon Microbiome Reflects Environmental and Genetic Diversity. *Appl. Environ. Microbiol.* **2018**, *84*, 84. [[CrossRef](#)]
29. Dulski, T.; Kujawa, R.; Godzieba, M.; Ciesielski, S. Effect of Salinity on the Gut Microbiome of Pike Fry (*Esox lucius*). *Appl. Sci.* **2020**, *10*, 2506. [[CrossRef](#)]
30. 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*, 5. [[CrossRef](#)]
31. Zhang, Z.; Li, D.; Refaey, M.M.; Xu, W. High Spatial and Temporal Variations of Microbial Community along the Southern Catfish Gastrointestinal Tract: Insights into Dynamic Food Digestion. *Front. Microbiol.* **2017**, *8*, 1531. [[CrossRef](#)]
32. Kuebutornye, F.K.A.; Wang, Z.W.; Lu, Y.S.; Abarike, E.D.; Sakyi, M.E.; Li, Y.; Xie, C.X.; Hlordzi, V. Effects of three host-associated *Bacillus* species on mucosal immunity and gut health of Nile tilapia, *Oreochromis niloticus* and its resistance against *Aeromonas hydrophila* infection. *Fish Shellfish. Immunol.* **2020**, *97*, 83–95. [[CrossRef](#)]
33. Zhang, C.; Derrien, M.; Levenez, F.; Brazeilles, R.; Ballal, S.A.; Kim, J.; Degivry, M.-C.; Quéré, G.; Garault, P.; Vlieg, J.E.T.V.H.; et al. Ecological robustness of the gut microbiota in response to ingestion of transient food-borne microbes. *ISME J.* **2016**, *10*, 2235–2245. [[CrossRef](#)]
34. Aerts, J.; Schaeck, M.; De Swaef, E.; Ampe, B.; Decostere, A. *Vibrio lentus* as a probiotic candidate lowers glucocorticoid levels in gnotobiotic sea bass larvae. *Aquaculture* **2018**, *492*, 40–45. [[CrossRef](#)]
35. Pérez-Ramos, A.; Mohedano, M.L.; Pardo, M.Á.; López, P.  $\beta$ -Glucan-Producing *Pediococcus parvulus* 2.6: Test of Probiotic and Immunomodulatory Properties in Zebrafish Models. *Front. Microbiol.* **2018**, *9*, 1684. [[CrossRef](#)]
36. Zhang, Z.; Li, D.; Refaey, M.M.; 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. [[CrossRef](#)] [[PubMed](#)]
37. Rawls, J.F.; Samuel, B.S.; Gordon, J.I. Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 4596–4601. [[CrossRef](#)]
38. Rawls, J.F.; Mahowald, M.A.; Ley, R.E.; Gordon, J.I. Reciprocal Gut Microbiota Transplants from Zebrafish and Mice to Germ-free Recipients Reveal Host Habitat Selection. *Cell* **2006**, *127*, 423–433. [[CrossRef](#)] [[PubMed](#)]
39. Roeselers, G.; Mittge, E.K.; Stephens, W.Z.; Parichy, D.M.; Cavanaugh, C.M.; Guillemin, K.; Rawls, J.F. Evidence for a core gut microbiota in the zebrafish. *ISME J.* **2011**, *5*, 1595–1608. [[CrossRef](#)]
40. Etyemez, M.; Balcázar, J.L. Bacterial community structure in the intestinal ecosystem of rainbow trout (*Oncorhynchus mykiss*) as revealed by pyrosequencing-based analysis of 16S rRNA genes. *Res. Veter. Sci.* **2015**, *100*, 8–11. [[CrossRef](#)]
41. Larsen, A.M.; Mohammed, H.H.; Arias, C.R. Comparison of DNA extraction protocols for the analysis of gut microbiota in fishes. *FEMS Microbiol. Lett.* **2014**, *362*, 1–8. [[CrossRef](#)] [[PubMed](#)]
42. Li, T.; Long, M.; Gatesoupe, F.-J.; Zhang, Q.; Li, A.; Gong, X. Comparative Analysis of the Intestinal Bacterial Communities in Different Species of Carp by Pyrosequencing. *Microb. Ecol.* **2015**, *69*, 25–36. [[CrossRef](#)] [[PubMed](#)]
43. Liu, L.; Gong, Y.-X.; Zhu, B.; Liu, G.-L.; Wang, G.-X.; Ling, F. Effect of a new recombinant *Aeromonas hydrophila* vaccine on the grass carp intestinal microbiota and correlations with immunological responses. *Fish Shellfish. Immunol.* **2015**, *45*, 175–183. [[CrossRef](#)]
44. Narrowe, A.B.; Albuthi-Lantz, M.; Smith, E.P.; Bower, K.J.; Roane, T.M.; Vajda, A.M.; Miller, C.S. Perturbation and restoration of the fathead minnow gut microbiome after low-level triclosan exposure. *Microbiome* **2015**, *3*, 1–18. [[CrossRef](#)] [[PubMed](#)]
45. Llewellyn, M.S.; Boutin, S.; Hoseinifar, S.H.; Derome, N. Teleost microbiomes: The state of the art in their characterization, manipulation and importance in aquaculture and fisheries. *Front. Microbiol.* **2014**, *5*, 207. [[CrossRef](#)]
46. Huang, Q.; Sham, R.C.; Deng, Y.; Mao, Y.; Wang, C.; Zhang, T.; Leung, K.M.Y. Diversity of gut microbiomes in marine fishes is shaped by host-related factors. *Mol. Ecol.* **2020**, *29*, 5019–5034. [[CrossRef](#)]
47. Dehler, C.E.; Secombes, C.J.; Martin, S.A. Environmental and physiological factors shape the gut microbiota of Atlantic salmon parr (*Salmo salar* L.). *Aquaculture* **2017**, *467*, 149–157. [[CrossRef](#)]
48. Dulski, T.; Kozłowski, K.; Ciesielski, S. Habitat and seasonality shape the structure of tench (*Tinca tinca* L.) gut microbiome. *Sci. Rep.* **2020**, *10*, 1–11. [[CrossRef](#)]
49. Wang, A.R.; Ran, C.; Ringø, E.; Zhou, Z.G. Progress in fish gastrointestinal microbiota research. *Rev. Aquac.* **2018**, *10*, 626–640. [[CrossRef](#)]
50. Liu, H.; Guo, X.; Gooneratne, S.R.; Lai, R.; Zeng, C.; Zhan, F.; Wang, W. The gut microbiome and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels. *Sci. Rep.* **2016**, *6*, 24340. [[CrossRef](#)] [[PubMed](#)]
51. Bajinka, O.; Tan, Y.; Abdelhalim, K.A.; Özdemir, G.; Qiu, X. Extrinsic factors influencing gut microbes, the immediate consequences and restoring eubiosis. *AMB Express* **2020**, *10*, 1–11. [[CrossRef](#)] [[PubMed](#)]
52. Vasemägi, A.; Visse, M.; Kisand, V. Effect of Environmental Factors and an Emerging Parasitic Disease on Gut Microbiome of Wild Salmonid Fish. *mSphere* **2017**, *2*, e00418-17. [[CrossRef](#)]
53. Stephens, W.Z.; Burns, A.R.; Stagaman, K.; Wong, S.; Rawls, J.F.; Guillemin, K.; Bohannan, B.J.M. The composition of the zebrafish intestinal microbial community varies across development. *ISME J.* **2016**, *10*, 644–654. [[CrossRef](#)]
54. Michl, S.C.; Ratten, J.-M.; Beyer, M.; Hasler, M.; Laroche, J.; Schulz, C. The malleable gut microbiome of juvenile rainbow trout (*Oncorhynchus mykiss*): Diet-dependent shifts of bacterial community structures. *PLoS ONE* **2017**, *12*, e0177735. [[CrossRef](#)] [[PubMed](#)]

55. Wu, S.; Wang, G.; Angert, E.R.; Wang, W.; Li, W.; Zou, H. Composition, Diversity, and Origin of the Bacterial Community in Grass Carp Intestine. *PLoS ONE* **2012**, *7*, e30440. [[CrossRef](#)]
56. Xing, M.; Hou, Z.; Yuan, J.; Liu, Y.; Qu, Y.; Liu, B. Taxonomic and functional metagenomic profiling of gastrointestinal tract microbiome of the farmed adult turbot (*Scophthalmus maximus*). *FEMS Microbiol. Ecol.* **2013**, *86*, 432–443. [[CrossRef](#)]
57. Murall, C.L.A.; Touzel, M.P.; Allen-Vercos, E.; Alizon, S.; Froissart, R.; McCann, K. Chapter five—Invasions of host-associated microbiome networks. In *Advances in Ecological Research*; Bohan, D.A., Dumbrell, A.J., Massol, F., Eds.; Academic Press: Cambridge, MA, USA, 2017; Volume 57, p. 80.
58. Ibrahim, M.D. Evolution of probiotics in aquatic world: Potential effects, the current status in Egypt and recent prospectives. *J. Adv. Res.* **2015**, *6*, 765–791. [[CrossRef](#)] [[PubMed](#)]
59. Nikoskelainen, S.; Ouwehand, A.; Salminen, S.; Bylund, G. Protection of rainbow trout (*Oncorhynchus mykiss*) from furunculosis by *Lactobacillus rhamnosus*. *Aquaculture* **2001**, *198*, 229–236. [[CrossRef](#)]
60. Nikoskelainen, S.; Salminen, S.; Bylund, G.; Ouwehand, A.C. Characterization of the Properties of Human- and Dairy-Derived Probiotics for Prevention of Infectious Diseases in Fish. *Appl. Environ. Microbiol.* **2001**, *67*, 2430–2435. [[CrossRef](#)]
61. Rinkinen, M.; Mättö, J.; Salminen, S.; Westermarck, E.; Ouwehand, A.C. In vitro adhesion of lactic acid bacteria to canine small intestinal mucus. *J. Anim. Physiol. Anim. Nutr.* **2000**, *84*, 43–47. [[CrossRef](#)]
62. Rinkinen, M.; Westermarck, E.; Salminen, S.; Ouwehand, A.C. Absence of host specificity for in vitro adhesion of probiotic lactic acid bacteria to intestinal mucus. *Veter Microbiol.* **2003**, *97*, 55–61. [[CrossRef](#)]
63. Gildberg, A.; Mikkelsen, H. Effects of supplementing the feed to Atlantic cod (*Gadus morhua*) fry with lactic acid bacteria and immuno-stimulating peptides during a challenge trial with *Vibrio anguillarum*. *Aquaculture* **1998**, *167*, 103–113. [[CrossRef](#)]
64. Askarian, F.; Kousha, A.; Salma, W.; Ringø, E. The effect of lactic acid bacteria administration on growth, digestive enzyme activity and gut microbiota in Persian sturgeon (*Acipenser persicus*) and beluga (*Huso huso*) fry. *Aquac. Nutr.* **2011**, *17*, 488–497. [[CrossRef](#)]
65. Salma, W.; Zhou, Z.; Wang, W.; Askarian, F.; Kousha, A.; Ebrahimi, M.T.; Myklebust, R.; Ringø, E. Histological and bacteriological changes in intestine of beluga (*Huso huso*) following ex vivo exposure to bacterial strains. *Aquaculture* **2011**, *314*, 24–33. [[CrossRef](#)]
66. Ringø, E.; Birkbeck, T.; Munro, P.; Vadstein, O.; Hjelmeland, K. The effect of early exposure to *Vibrio pelagius* on the aerobic bacterial flora of turbot, *Scophthalmus maximus* (L.) larvae. *J. Appl. Bacteriol.* **1996**, *81*, 207–211. [[CrossRef](#)]
67. Bansil, R.; Turner, B.S. Mucin structure, aggregation, physiological functions and biomedical applications. *Curr. Opin. Colloid Interface Sci.* **2006**, *11*, 164–170. [[CrossRef](#)]
68. Van Tassel, M.L.; Miller, M.J. *Lactobacillus* adhesion to mucus. *Nutrients* **2011**, *3*, 613–636. [[CrossRef](#)]
69. Buck, B.L.; Altermann, E.; Svingerud, T.; Klaenhammer, T.R. Functional Analysis of Putative Adhesion Factors in *Lactobacillus acidophilus* NCFM. *Appl. Environ. Microbiol.* **2005**, *71*, 8344–8351. [[CrossRef](#)]
70. Etzold, S.; Juge, N. Structural insights into bacterial recognition of intestinal mucins. *Curr. Opin. Struct. Biol.* **2014**, *28*, 23–31. [[CrossRef](#)]
71. Johnson, B.; Selle, K.; O’Flaherty, S.; Goh, Y.J.; Klaenhammer, T. Identification of extracellular surface-layer associated proteins in *Lactobacillus acidophilus* NCFM. *Microbiol.-Sgm* **2013**, *159*, 2269–2282. [[CrossRef](#)]
72. Bautista-Gallego, J.; Arroyo-López, F.; Rantsiou, K.; Jiménez-Díaz, R.; Garrido-Fernández, A.; Cocolin, L. Screening of lactic acid bacteria isolated from fermented table olives with probiotic potential. *Food Res. Int.* **2013**, *50*, 135–142. [[CrossRef](#)]
73. Son, S.-H.; Jeon, H.-L.; Yang, S.-J.; Lee, N.-K.; Paik, H.-D. In vitro characterization of *Lactobacillus brevis* KU15006, an isolate from kimchi, reveals anti-adhesion activity against foodborne pathogens and antidiabetic properties. *Microb. Pathog.* **2017**, *112*, 135–141. [[CrossRef](#)]
74. Wanka, K.M.; Damerou, T.; Costas, B.; Krueger, A.; Schulz, C.; Wuertz, S. Isolation and characterization of native probiotics for fish farming. *BMC Microbiol.* **2018**, *18*, 119. [[CrossRef](#)]
75. Chowdhury, A.; Roy, N.C. Probiotic supplementation for enhanced growth of striped catfish (*Pangasianodon hypophthalmus*) in cages. *Aquac. Rep.* **2020**, *18*, 100504. [[CrossRef](#)]
76. Wanka, K.M.; Schulz, C.; Kloas, W.; Wuertz, S. Administration of host-derived probiotics does not affect utilization of soybean meal enriched diets in juvenile turbot (*Scophthalmus maximus*). *J. Appl. Ichthyol.* **2019**, *35*, 1004–1015. [[CrossRef](#)]
77. Chen, X.; Xie, J.; Liu, Z.; Yin, P.; Chen, M.; Liu, Y.; Tian, L.; Niu, J. Modulation of growth performance, non-specific immunity, intestinal morphology, the response to hypoxia stress and resistance to *Aeromonas hydrophila* of grass carp (*Ctenopharyngodon idella*) by dietary supplementation of a multi-strain probiotic. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2020**, *231*, 108724. [[CrossRef](#)] [[PubMed](#)]
78. El-Haroun, E.R.; Goda, A.M.A.-S.; Chowdhury, M.A.K. Effect of dietary probiotic Biogen(R) supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). *Aquac. Res.* **2006**, *37*, 1473–1480. [[CrossRef](#)]
79. Kuebutornye, F.K.A.; Abarike, E.D.; Sakyi, M.E.; Lu, Y.; Wang, Z. Modulation of nutrient utilization, growth, and immunity of Nile tilapia, *Oreochromis niloticus*: The role of probiotics. *Aquac. Int.* **2020**, *28*, 277–291. [[CrossRef](#)]
80. Silva, V.V.; Salomão, R.A.S.; Mareco, E.A.; Pai, M.D.; Santos, V.B. Probiotic additive affects muscle growth of Nile tilapia (*Oreochromis niloticus*). *Aquac. Res.* **2021**, *52*, 2061–2069. [[CrossRef](#)]

81. Yi, C.-C.; Liu, C.-H.; Chuang, K.-P.; Chang, Y.-T.; Hu, S.-Y. A potential probiotic *Chromobacterium aquaticum* with bacteriocin-like activity enhances the expression of indicator genes associated with nutrient metabolism, growth performance and innate immunity against pathogen infections in zebrafish (*Danio rerio*). *Fish Shellfish. Immunol.* **2019**, *93*, 124–134. [[CrossRef](#)]
82. Guidoli, M.G.; Mendoza, J.A.; Falcón, S.L.; Boehringer, S.I.; Sánchez, S.; Macías, M.E.F.N. Autochthonous probiotic mixture improves biometrical parameters of larvae of *Piaractus mesopotamicus* (Caracidae, Characiforme, Teleostei). *Ciência Rural* **2018**, *48*. [[CrossRef](#)]
83. Chiu, K.-H.; Liu, W.-S. Dietary administration of the extract of *Rhodobacter sphaeroides* WL-APD911 enhances the growth performance and innate immune responses of seawater red tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*). *Aquaculture* **2014**, *418–419*, 32–38. [[CrossRef](#)]
84. Hosseini, M.; Miandare, H.K.; Hoseinifar, S.H.; Yarahmadi, P. Dietary *Lactobacillus acidophilus* modulated skin mucus protein profile, immune and appetite genes expression in gold fish (*Carassius auratus gibelio*). *Fish Shellfish. Immunol.* **2016**, *59*, 149–154. [[CrossRef](#)]
85. Giorgia, G.; Elia, C.; Andrea, P.; Cinzia, C.; Stefania, S.; Ana, R.; Daniel, M.L.; Ike, O.; Oliana, C. Effects of Lactogen 13, a New Probiotic Preparation, on Gut Microbiota and Endocrine Signals Controlling Growth and Appetite of *Oreochromis niloticus* Juveniles. *Microb. Ecol.* **2018**, *76*, 1063–1074. [[CrossRef](#)]
86. Irianto, A.; Austin, B. Probiotics in aquaculture. *J. Fish Dis.* **2002**, *25*, 633–642. [[CrossRef](#)]
87. Irianto, A.; Austin, B. Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Dis.* **2002**, *25*, 333–342. [[CrossRef](#)]
88. Bates, J.M.; Mittge, E.; Kuhlman, J.; Baden, K.N.; Cheesman, S.E.; Guillemin, K. Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. *Dev. Biol.* **2006**, *297*, 374–386. [[CrossRef](#)] [[PubMed](#)]
89. Merrifield, D.; Bradley, G.; Harper, G.; Baker, R.; Munn, C.; Davies, S. Assessment of the effects of vegetative and lyophilized *Pediococcus acidilactici* on growth, feed utilization, intestinal colonization and health parameters of rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquac. Nutr.* **2009**, *17*, 73–79. [[CrossRef](#)]
90. Merrifield, D.L.; Harper, G.M.; Dimitroglou, A.; Ringã, E.; Davies, S.J. Possible influence of probiotic adhesion to intestinal mucosa on the activity and morphology of rainbow trout (*Oncorhynchus mykiss*) enterocytes. *Aquac. Res.* **2009**, *41*, 1268–1272. [[CrossRef](#)]
91. Tan, H.Y.; Chen, S.-W.; Hu, S.-Y. Improvements in the growth performance, immunity, disease resistance, and gut microbiota by the probiotic *Rummeliibacillus stabekisii* in Nile tilapia (*Oreochromis niloticus*). *Fish Shellfish. Immunol.* **2019**, *92*, 265–275. [[CrossRef](#)]
92. Wang, Y.; Wang, Q.; Xing, K.; Jiang, P.; Wang, J. Dietary cinnamaldehyde and *Bacillus subtilis* improve growth performance, digestive enzyme activity, and antioxidant capability and shape intestinal microbiota in tongue sole, *Cynoglossus semilaevis*. *Aquaculture* **2021**, *531*, 735798. [[CrossRef](#)]
93. Santos, K.O.; Costa-Filho, J.; Spagnol, K.L.; Nornberg, B.F.; Lopes, F.M.; Tesser, M.B.; Marins, L.F. The inclusion of a transgenic probiotic expressing recombinant phytase in a diet with a high content of vegetable matter markedly improves growth performance and the expression of growth-related genes and other selected genes in zebrafish. *Aquaculture* **2020**, *519*, 734878. [[CrossRef](#)]
94. Zhang, M.; Dong, B.; Lai, X.; Chen, Z.; Hou, L.; Shu, R.; Huang, Y.; Shu, H. Effects of *Clostridium butyricum* on growth, digestive enzyme activity, antioxidant capacity and gut microbiota in farmed tilapia (*Oreochromis niloticus*). *Aquac. Res.* **2021**, *52*, 1573–1584. [[CrossRef](#)]
95. Wang, Y.; Al Farraj, D.A.; Vijayaraghavan, P.; Hatamleh, A.A.; Biji, G.D.; Rady, A.M. Host associated mixed probiotic bacteria induced digestive enzymes in the gut of tiger shrimp *Penaeus monodon*. *Saudi J. Biol. Sci.* **2020**, *27*, 2479–2484. [[CrossRef](#)]
96. Tarkhani, R.; Imani, A.; Hoseinifar, S.H.; Ashayerizadeh, O.; Moghanlou, K.S.; Manaffar, R.; Van Doan, H.; Reverter, M. Comparative study of host-associated and commercial probiotic effects on serum and mucosal immune parameters, intestinal microbiota, digestive enzymes activity and growth performance of roach (*Rutilus rutilus caspicus*) fingerlings. *Fish Shellfish. Immunol.* **2020**, *98*, 661–669. [[CrossRef](#)]
97. Niu, K.-M.; Kothari, D.; Lee, W.-D.; Zhang, Z.; Lee, B.-J.; Kim, K.-W.; Wu, X.; Han, H.-S.; Khosravi, S.; Lee, S.-M.; et al. Probiotic Potential of the Farmed Olive Flounder, *Paralichthys olivaceus*, Autochthonous Gut Microbiota. *Probiotics Antimicro* **2021**. [[CrossRef](#)] [[PubMed](#)]
98. Hmani, H.; Daoud, L.; Jlidi, M.; Jalleli, K.; Ben Ali, M.; Brahim, A.H.; Bargui, M.; Dammak, A.; Ben Ali, M. A *Bacillus subtilis* strain as probiotic in poultry: Selection based on in vitro functional properties and enzymatic potentialities. *J. Ind. Microbiol. Biotechnol.* **2017**, *44*, 1157–1166. [[CrossRef](#)]
99. Kong, Y.; Li, M.; Chu, G.; Liu, H.; Shan, X.; Wang, G.; Han, G. The positive effects of single or conjoint administration of lactic acid bacteria on *Channa argus*: Digestive enzyme activity, antioxidant capacity, intestinal microbiota and morphology. *Aquaculture* **2021**, *531*, 735852. [[CrossRef](#)]
100. Tarkhani, R.; Imani, A.; Hoseinifar, S.H.; Moghanlou, K.S.; Manaffar, R. The effects of host-associated *Enterococcus faecium* CGMCC1.2136 on serum immune parameters, digestive enzymes activity and growth performance of the Caspian roach (*Rutilus rutilus caspicus*) fingerlings. *Aquaculture* **2020**, *519*, 734741. [[CrossRef](#)]
101. Nguyen, T.L.; Chun, W.-K.; Kim, A.; Kim, N.; Roh, H.J.; Lee, Y.; Yi, M.; Kim, S.; Park, C.-I.; Kim, D.-H. Dietary Probiotic Effect of *Lactococcus lactis* WFLU12 on Low-Molecular-Weight Metabolites and Growth of Olive Flounder (*Paralichthys olivaceus*). *Front. Microbiol.* **2018**, *9*, 2059. [[CrossRef](#)]

102. Ray, A.; Ghosh, K.; Ringø, E. Enzyme-producing bacteria isolated from fish gut: A review. *Aquac. Nutr.* **2012**, *18*, 465–492. [[CrossRef](#)]
103. Ray, A.K.R.E. Fishes—Digestive organs. In *Aquaculture Nutrition—Gut Health, Probiotics, and Prebiotics*; Merrifield, D., Ringø, E., Eds.; John Wiley & Sons Ltd.: West Sussex, UK, 2014.
104. Eck, P.; Friel, J. Should probiotics be considered as vitamin supplements? *Vitam. Miner.* **2013**, *2*, e124. [[CrossRef](#)]
105. Ringø, E.; Jelstensen, J.P.; Olsen, R.E. Production of eicosapentaenoic acid by freshwater *Vibrio*. *Lipids* **1992**, *27*, 564–566. [[CrossRef](#)]
106. Ringø, E.; Sinclair, P.D.; Birkbeck, H.; Barbour, A. Production of Eicosapentaenoic Acid (20:5 n-3) by *Vibrio pelagius* Isolated from Turbot (*Scophthalmus maximus* (L.)) Larvae. *Appl. Environ. Microbiol.* **1992**, *58*, 3777–3778. [[CrossRef](#)]
107. Yano, Y.; Nakayama, A.; Saito, H.; Ishihara, K. Production of docosahexaenoic acid by marine bacteria isolated from deep sea fish. *Lipids* **1994**, *29*, 527–528. [[CrossRef](#)]
108. Yano, Y.; Nakayama, A.; Yoshida, K. Distribution of polyunsaturated Fatty acids in bacteria present in intestines of deep-sea fish and shallow-sea poikilothermic animals. *Appl. Environ. Microbiol.* **1997**, *63*, 2572–2577. [[CrossRef](#)]
109. Estupiñán, M.; Hernández, I.; Saitua, E.; Bilbao, M.E.; Mendibil, I.; Ferrer, J.; Alonso-Sáez, L. Novel *Vibrio* spp. Strains Producing Omega-3 Fatty Acids Isolated from Coastal Seawater. *Mar. Drugs* **2020**, *18*, 99. [[CrossRef](#)] [[PubMed](#)]
110. Nichols, D.S.; Davies, N.W. Improved detection of polyunsaturated fatty acids as phenacyl esters using liquid chromatography-ion trap mass spectrometry. *J. Microbiol. Methods* **2002**, *50*, 103–113. [[CrossRef](#)]
111. Nichols, D.S.; McMeekin, T.A. Biomarker techniques to screen for bacteria that produce polyunsaturated fatty acids. *J. Microbiol. Methods* **2002**, *48*, 161–170. [[CrossRef](#)]
112. Sutthi, N.; Van Doan, H. *Saccharomyces cerevisiae* and *Bacillus* spp. effectively enhance health tolerance of Nile tilapia under transportation stress. *Aquaculture* **2020**, *528*, 735527. [[CrossRef](#)]
113. Rollo, A.; Sulpizio, R.; Nardi, M.; Silvi, S.; Orpianesi, C.; Caggiano, M.; Cresci, A.; Carnevali, O. Live microbial feed supplement in aquaculture for improvement of stress tolerance. *Fish Physiol. Biochem.* **2006**, *32*, 167–177. [[CrossRef](#)]
114. Abass, D.A.; Obirikorang, K.A.; Campion, B.B.; Edziyie, R.E.; Skov, P.V. Dietary supplementation of yeast (*Saccharomyces cerevisiae*) improves growth, stress tolerance, and disease resistance in juvenile Nile tilapia (*Oreochromis niloticus*). *Aquac. Int.* **2018**, *26*, 843–855. [[CrossRef](#)]
115. Carnevali, O.; de Vivo, L.; Sulpizio, R.; Gioacchini, G.; Olivotto, I.; Silvi, S.; Cresci, A. Growth improvement by probiotic in European sea bass juveniles (*Dicentrarchus labrax*, L.), with particular attention to IGF-1, myostatin and cortisol gene expression. *Aquaculture* **2006**, *258*, 430–438. [[CrossRef](#)]
116. Dawood, M.A.; Eweedah, N.M.; Moustafa, E.M.; Farahat, E.M. Probiotic effects of *Aspergillus oryzae* on the oxidative status, heat shock protein, and immune related gene expression of Nile tilapia (*Oreochromis niloticus*) under hypoxia challenge. *Aquaculture* **2020**, *520*, 734669. [[CrossRef](#)]
117. Lin, Y.-S.; Saputra, F.; Chen, Y.-C.; Hu, S.-Y. Dietary administration of *Bacillus amyloliquefaciens* R8 reduces hepatic oxidative stress and enhances nutrient metabolism and immunity against *Aeromonas hydrophila* and *Streptococcus agalactiae* in zebrafish (*Danio rerio*). *Fish Shellfish. Immunol.* **2019**, *86*, 410–419. [[CrossRef](#)]
118. Gioacchini, G.; Giorgini, E.; Olivotto, I.; Maradonna, F.; Merrifield, D.L.; Carnevali, O. The Influence of Probiotics on Zebrafish *Danio rerio* Innate Immunity and Hepatic Stress. *Zebrafish* **2014**, *11*, 98–106. [[CrossRef](#)]
119. Van Doan, H.; Hoseinifar, S.H.; Khanongnuch, C.; Kanpiengjai, A.; Unban, K.; Van Kim, V.; Srichaiyo, S. Host-associated probiotics boosted mucosal and serum immunity, disease resistance and growth performance of Nile tilapia (*Oreochromis niloticus*). *Aquaculture* **2018**, *491*, 94–100. [[CrossRef](#)]
120. Veiga, P.T.D.N.; Owatari, M.S.; Nunes, A.L.; Rodrigues, R.A.; Kasai, R.Y.D.; Fernandes, C.E.; De Campos, C.M. Short communication: *Bacillus subtilis* C-3102 improves biomass gain, innate defense, and intestinal absorption surface of native Brazilian hybrid Surubim (*Pseudoplatystoma corruscans* x *P. reticulatum*). *Aquac. Int.* **2020**, *28*, 1183–1193. [[CrossRef](#)]
121. Nunes, A.L.; Owatari, M.S.; Rodrigues, R.A.; Fantini, L.E.; Kasai, R.Y.D.; Martins, M.L.; Mourinho, J.L.P.; De Campos, C.M. Effects of *Bacillus subtilis* C-3102-supplemented diet on growth, non-specific immunity, intestinal morphometry and resistance of hybrid juvenile *Pseudoplatystoma* sp. challenged with *Aeromonas hydrophila*. *Aquac. Int.* **2020**, *28*, 2345–2361. [[CrossRef](#)]
122. Meidong, R.; Nakao, M.; Sakai, K.; Tongpim, S. *Lactobacillus paraplantarum* L34b-2 derived from fermented food improves the growth, disease resistance and innate immunity in *Pangasius bocourti*. *Aquaculture* **2021**, *531*, 735878. [[CrossRef](#)]
123. Zhou, S.; Song, D.; Zhou, X.; Mao, X.; Zhou, X.; Wang, S.; Wei, J.; Huang, Y.; Wang, W.; Xiao, S.-M.; et al. Characterization of *Bacillus subtilis* from gastrointestinal tract of hybrid Hulong grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*) and its effects as probiotic additives. *Fish Shellfish. Immunol.* **2019**, *84*, 1115–1124. [[CrossRef](#)] [[PubMed](#)]
124. Zhang, D.-X.; Kang, Y.-H.; Zhan, S.; Zhao, Z.-L.; Jin, S.-N.; Chen, C.; Zhang, L.; Shen, J.-Y.; Wang, C.-F.; Wang, G.-Q.; et al. Effect of *Bacillus velezensis* on *Aeromonas veronii*-Induced Intestinal Mucosal Barrier Function Damage and Inflammation in Crucian Carp (*Carassius auratus*). *Front. Microbiol.* **2019**, *10*, 2663. [[CrossRef](#)] [[PubMed](#)]
125. Tachibana, L.; Telli, G.S.; Dias, D.D.C.; Gonçalves, G.S.; Guimarães, M.C.; Ishikawa, C.M.; Cavalcante, R.B.; Natori, M.M.; Alarcon, M.F.F.; Tapia-Paniagua, S.; et al. *Bacillus subtilis* and *Bacillus licheniformis* in diets for Nile tilapia (*Oreochromis niloticus*): Effects on growth performance, gut microbiota modulation and innate immunology. *Aquac. Res.* **2021**, *52*, 1630–1642. [[CrossRef](#)]
126. Arani, M.M.; Salati, A.P.; Keyvanshokoo, S.; Safari, O. The effect of *Pediococcus acidilactici* on mucosal immune responses, growth, and reproductive performance in zebrafish (*Danio rerio*). *Fish Physiol. Biochem.* **2021**, *47*, 153–162. [[CrossRef](#)] [[PubMed](#)]



127. Zhang, H.-P.; Dong, W.-L.; Chen, L.; Wang, Y.-M.; Muhammad, I.; Ju, A.-Q.; Shan, X.-F.; Ma, H.-X.; Kong, L.-C. Effects of dietary *Lactobacillus plantarum* C20015 on growth, immunity, and disease resistance in koi carp. *Aquac. Int.* **2020**, *28*, 1797–1809. [[CrossRef](#)]
128. Abarike, E.D.; Jian, J.; Tang, J.; Cai, J.; Yu, H.; Lihua, C.; Jun, L. Influence of traditional Chinese medicine and *Bacillus* species (TCMBS) on growth, immune response and disease resistance in Nile tilapia, *Oreochromis niloticus*. *Fish Shellfish. Immunol.* **2019**, *91*, 417. [[CrossRef](#)]
129. Midhun, S.J.; Neethu, S.; Arun, D.; Vysakh, A.; Divya, L.; Radhakrishnan, E.; Jyothis, M. Dietary supplementation of *Bacillus licheniformis* HGA8B improves growth parameters, enzymatic profile and gene expression of *Oreochromis niloticus*. *Aquaculture* **2019**, *505*, 289–296. [[CrossRef](#)]
130. Abarike, E.D.; Cai, J.; Lu, Y.; Yu, H.; Chen, L.; Jian, J.; Tang, J.; Jun, L.; Kuebutornye, F.K. Corrigendum to “Effects of a commercial probiotic BS containing *Bacillus subtilis* and *Bacillus licheniformis* on growth, immune response and disease resistance in Nile tilapia, *Oreochromis niloticus*” [Fish Shellfish Immunol. 82 (2018) 229–238]. *Fish Shellfish. Immunol.* **2019**, *84*, 1180. [[CrossRef](#)] [[PubMed](#)]
131. Aly, S.M.; Mohamed, M.F.; John, G. Effect of probiotics on the survival, growth and challenge infection in *Tilapia nilotica* (*Oreochromis niloticus*). *Aquac. Res.* **2008**, *39*, 647–656. [[CrossRef](#)]
132. Thy, H.T.T.; Tri, N.N.; Quy, O.M.; Fotedar, R.; Kannika, K.; Unajak, S.; Areechon, N. Effects of the dietary supplementation of mixed probiotic spores of *Bacillus amyloliquefaciens* 54A, and *Bacillus pumilus* 47B on growth, innate immunity and stress responses of striped catfish (*Pangasianodon hypophthalmus*). *Fish Shellfish. Immunol.* **2017**, *60*, 391–399. [[CrossRef](#)]
133. Ha, C.W.Y.; Lam, Y.Y.; Holmes, A.J. Mechanistic links between gut microbial community dynamics, microbial functions and metabolic health. *World J. Gastroenterol.* **2014**, *20*, 16498–16517. [[CrossRef](#)]
134. Liu, W.; Yang, Y.; Zhang, J.; Gatlin, D.M.; Ringø, E.; Zhou, Z. Effects of dietary microencapsulated sodium butyrate on growth, intestinal mucosal morphology, immune response and adhesive bacteria in juvenile common carp (*Cyprinus carpio*) pre-fed with or without oxidised oil. *Br. J. Nutr.* **2014**, *112*, 15–29. [[CrossRef](#)]
135. Montalban-Arques, A.; De Schryver, P.; Bossier, P.; Gorkiewicz, G.; Mulero, V.; Gatlin, D.M.I.; Galindo-Villegas, J. Selective Manipulation of the Gut Microbiota Improves Immune Status in Vertebrates. *Front. Immunol.* **2015**, *6*, 512. [[CrossRef](#)]
136. Louis, P.; Hold, G.L.; Flint, H.J. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat. Rev. Genet.* **2014**, *12*, 661–672. [[CrossRef](#)]
137. Shapiro, H.; Thaïss, C.A.; Levy, M.; Elinav, E. The cross talk between microbiota and the immune system: Metabolites take center stage. *Curr. Opin. Immunol.* **2014**, *30*, 54–62. [[CrossRef](#)]
138. Gomez-Gil, B.; Roque, A.; Turnbull, J.F. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. *Aquaculture* **2000**, *191*, 259–270. [[CrossRef](#)]
139. Picchiatti, S.; Fausto, A.M.; Randelli, E.; Carnevali, O.; Taddei, A.R.; Buonocore, F.; Scapigliati, G.; Abelli, L. Early treatment with *Lactobacillus delbrueckii* strain induces an increase in intestinal T-cells and granulocytes and modulates immune-related genes of larval *Dicentrarchus labrax* (L.). *Fish Shellfish. Immunol.* **2009**, *26*, 368–376. [[CrossRef](#)]
140. Chu, T.-W.; Chen, C.-N.; Pan, C.-Y. Antimicrobial status of tilapia (*Oreochromis niloticus*) fed *Enterococcus avium* originally isolated from goldfish intestine. *Aquac. Rep.* **2020**, *17*, 100397. [[CrossRef](#)]
141. Feng, J.; Chang, X.; Zhang, Y.; Yan, X.; Zhang, J.; Nie, G. Effects of *Lactococcus lactis* from *Cyprinus carpio* L. as probiotics on growth performance, innate immune response and disease resistance against *Aeromonas hydrophila*. *Fish Shellfish. Immunol.* **2019**, *93*, 73–81. [[CrossRef](#)] [[PubMed](#)]
142. Chauhan, A.; Singh, R. Isolation and evaluation of putative probiotic strains from different teleost to prevent *Pseudomonas aeruginosa* infection in *Cyprinus carpio*. *Aquac. Res.* **2019**, *50*, 3616–3627. [[CrossRef](#)]
143. Ghosh, B.; Cain, K.D.; Nowak, B.F.; Bridle, A.R. Microencapsulation of a putative probiotic *Enterobacter* species, C6-6, to protect rainbow trout, *Oncorhynchus mykiss* (Walbaum), against bacterial coldwater disease. *J. Fish Dis.* **2016**, *39*, 1–11. [[CrossRef](#)] [[PubMed](#)]
144. Puvanendran, V.; Rud, I.; Msw, B.; Arnesen, J.; Axelsson, L. Probiotic *Carnobacterium divergens* increase growth parameters and disease resistance in farmed Atlantic cod (*Gadus morhua*) larvae without influencing the microbiota. *Aquaculture* **2021**, *532*, 736072. [[CrossRef](#)]
145. Salyers, A.A.; Whitt, D.D.; Whitt, D.D. *Bacterial Pathogenesis: A Molecular Approach*; American Society for Microbiology (ASM): Washington, DC, USA, 2011.
146. Brunt, J.; Austin, B. Use of a probiotic to control lactococcosis and streptococcosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Dis.* **2005**, *28*, 693–701. [[CrossRef](#)]
147. Kesarcodi-Watson, A.; Kaspar, H.; Lategan, M.J.; Gibson, L. Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. *Aquaculture* **2008**, *274*, 1–14. [[CrossRef](#)]
148. Kuebutornye, F.K.A.; Abarike, E.D.; Lu, Y.; Hlordzi, V.; Sakyi, M.E.; Afriyie, G.; Wang, Z.; Li, Y.; Xie, C.X. Mechanisms and the role of probiotic *Bacillus* in mitigating fish pathogens in aquaculture. *Fish Physiol. Biochem.* **2020**, *46*, 819–841. [[CrossRef](#)]
149. Stein, T. *Bacillus subtilis* antibiotics: Structures, syntheses and specific functions. *Mol. Microbiol.* **2005**, *56*, 845–857. [[CrossRef](#)]
150. Nadal, A.L.; Ikeda-Ohtsubo, W.; Sipkema, D.; Peggs, D.; McGurk, C.; Forlenza, M.; Wiegertjes, G.F.; Brugman, S. Feed, Microbiota, and Gut Immunity: Using the Zebrafish Model to Understand Fish Health. *Front. Immunol.* **2020**, *11*, 11. [[CrossRef](#)]

151. Lee, M.-C.; Hsu, Y.-J.; Ho, H.-H.; Hsieh, S.-H.; Kuo, Y.-W.; Sung, H.-C.; Huang, C.-C. Lactobacillus salivarius Subspecies salicinius SA-03 is a New Probiotic Capable of Enhancing Exercise Performance and Decreasing Fatigue. *Microorganisms* **2020**, *8*, 545. [[CrossRef](#)] [[PubMed](#)]
152. Wosinska, L.; Cotter, P.D.; O'Sullivan, O.; Guinane, C. The Potential Impact of Probiotics on the Gut Microbiome of Athletes. *Nutrients* **2019**, *11*, 2270. [[CrossRef](#)]
153. Chen, Y.M.; Wei, L.; Chiu, Y.S.; Hsu, Y.J.; Tsai, T.Y.; Wang, M.F.; Huang, C.C. Lactobacillus plantarum TWK10 supplementation improves exercise performance and increases muscle mass in mice. *Nutrients* **2016**, *8*, 205. [[CrossRef](#)]
154. Lee, M.-C.; Hsu, Y.-J.; Chuang, H.-L.; Hsieh, P.-S.; Ho, H.-H.; Chen, W.-L.; Chiu, Y.-S. In Vivo Ergogenic Properties of the Bifidobacterium longum OLP-01 Isolated from a Weightlifting Gold Medalist. *Nutrients* **2019**, *11*, 2003. [[CrossRef](#)] [[PubMed](#)]
155. Barton, W.; Penney, N.C.; Cronin, O.; Garcia-Perez, I.; Molloy, M.G.; Holmes, E.; Shanahan, F.; Cotter, P.D.; O'Sullivan, O. The microbiome of professional athletes differs from that of more sedentary subjects in composition and particularly at the functional metabolic level. *Gut Microbiota* **2018**, *67*, 625–633. [[CrossRef](#)]
156. Petersen, L.M.; Bautista, E.J.; Nguyen, H.; Hanson, B.M.; Chen, L.; Lek, S.H.; Sodergren, E.; Weinstock, G.M. Community characteristics of the gut microbiomes of competitive cyclists. *Microbiome* **2017**, *5*, 1–13. [[CrossRef](#)]
157. Tovar-Ramírez, D.; Mazurais, D.; Gatesoupe, J.; Quazuguel, P.; Cahu, C.; Zambonino-Infante, J. Dietary probiotic live yeast modulates antioxidant enzyme activities and gene expression of sea bass (*Dicentrarchus labrax*) larvae. *Aquaculture* **2010**, *300*, 142–147. [[CrossRef](#)]
158. Lamari, F.; Castex, M.; Larcher, T.; Ledevin, M.; Mazurais, D.; Bakhrouf, A.; Gatesoupe, F.-J. Comparison of the effects of the dietary addition of two lactic acid bacteria on the development and conformation of sea bass larvae, *Dicentrarchus labrax*, and the influence on associated microbiota. *Aquaculture* **2013**, *376–379*, 137–145. [[CrossRef](#)]
159. Carnevali, O.; Maradonna, F.; Gioacchini, G. Integrated control of fish metabolism, wellbeing and reproduction: The role of probiotic. *Aquaculture* **2017**, *472*, 144–155. [[CrossRef](#)]
160. Gioacchini, G.; Ciani, E.; Pessina, A.; Cecchini, C.; Silvi, S.; Rodiles, A.; Merrifield, D.L.; Olivotto, I.; Carnevali, O. Correction to: Effects of Lactogen 13, a New Probiotic Preparation, on Gut Microbiota and Endocrine Signals Controlling Growth and Appetite of *Oreochromis niloticus* Juveniles. *Microb. Ecol.* **2018**, *76*, 1075. [[CrossRef](#)]
161. Mehrim, A.I.; Khalil, F.F.; Hassan, M.E. Hydroyeast Aquaculture<sup>®</sup> as a reproductive enhancer agent for the adult Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758). *Fish Physiol. Biochem.* **2014**, *41*, 371–381. [[CrossRef](#)] [[PubMed](#)]
162. Gioacchini, G.; Giorgini, E.; Merrifield, D.L.; Hardiman, G.; Borini, A.; Vaccari, L.; Carnevali, O. Probiotics Can Induce Follicle Maturational Competence: The *Danio rerio* Case. *Biol. Reprod.* **2012**, *86*, 65. [[CrossRef](#)] [[PubMed](#)]
163. Gioacchini, G.; Maradonna, F.; Lombardo, F.; Bizzaro, D.; Olivotto, I.; Carnevali, O. Increase of fecundity by probiotic administration in zebrafish (*Danio rerio*). *Reproduction* **2010**, *140*, 953–959. [[CrossRef](#)] [[PubMed](#)]
164. Falcinelli, S.; Picchiatti, S.; Rodiles, A.; Cossignani, L.; Merrifield, D.L.; Taddei, A.R.; Maradonna, F.; Olivotto, I.; Gioacchini, G.; Carnevali, O. Lactobacillus rhamnosus lowers zebrafish lipid content by changing gut microbiota and host transcription of genes involved in lipid metabolism. *Sci. Rep.* **2015**, *5*, 1–11. [[CrossRef](#)] [[PubMed](#)]
165. Geraylou, Z.; Vanhove, M.P.M.; Souffreau, C.; Rurangwa, E.; Buyse, J.; Ollevier, F. In vitro selection and characterization of putative probiotics isolated from the gut of *Acipenser baerii* (Brandt, 1869). *Aquac. Res.* **2012**, *45*, 341–352. [[CrossRef](#)]
166. Kavitha, M.; Raja, M.; Perumal, P. Evaluation of probiotic potential of *Bacillus* spp. isolated from the digestive tract of freshwater fish *Labeo calbasu* (Hamilton, 1822). *Aquac. Rep.* **2018**, *11*, 59–69. [[CrossRef](#)]
167. Linh, N.T.H.; Sakai, K.; Taoka, Y. Screening of lactic acid bacteria isolated from fermented food as potential probiotics for aquacultured carp and amberjack. *Fish. Sci.* **2018**, *84*, 101–111. [[CrossRef](#)]
168. EFSA. 2008 Annual report on pesticide residues according to Article 32 of Regulation (EC) No 396/2005. *EFSA J.* **2010**, *8*, 1646. [[CrossRef](#)]
169. Pisano, M.B.; Viale, S.; Conti, S.; Fadda, M.E.; Deplano, M.; Melis, M.P.; Deiana, M.; Cosentino, S. Preliminary Evaluation of Probiotic Properties of Lactobacillus Strains Isolated from Sardinian Dairy Products. *BioMed Res. Int.* **2014**, *2014*, 1–9. [[CrossRef](#)]
170. Harty, D.; Oakey, H.; Patrikakis, M.; Hume, E.; Knox, K. Pathogenic potential of lactobacilli. *Int. J. Food Microbiol.* **1994**, *24*, 179–189. [[CrossRef](#)]
171. Jena, P.K.; Trivedi, D.; Thakore, K.; Chaudhary, H.; Giri, S.S.; Seshadri, S. Isolation and characterization of probiotic properties of Lactobacilli isolated from rat fecal microbiota. *Microbiol. Immunol.* **2013**, *57*, 407–416. [[CrossRef](#)] [[PubMed](#)]
172. Kaushik, J.K.; Kumar, A.; Duary, R.K.; Mohanty, A.K.; Grover, S.; Batish, V.K. Functional and Probiotic Attributes of an Indigenous Isolate of *Lactobacillus plantarum*. *PLoS ONE* **2009**, *4*, e8099. [[CrossRef](#)]
173. Papadimitriou, K.; Zoumpopoulou, G.; Folign, B.; Alexandraki, V.; Kazou, M.; Pot, B.; Tsakalidou, E. Discovering probiotic microorganisms: In vitro, in vivo, genetic and omics approaches. *Front. Microbiol.* **2015**, *6*, 58. [[CrossRef](#)]
174. Laparra, J.M.; Sanz, Y. Comparison of in vitro models to study bacterial adhesion to the intestinal epithelium. *Letts. Appl. Microbiol.* **2009**, *49*, 695–701. [[CrossRef](#)] [[PubMed](#)]
175. Mosso, A.; Jimenez, M.; Vignolo, G.; Leblanc, J.; Samman, N. Increasing the folate content of tuber based foods using potentially probiotic lactic acid bacteria. *Food Res. Int.* **2018**, *109*, 168–174. [[CrossRef](#)] [[PubMed](#)]
176. Solopova, A.; Bottacini, F.; Degli Esposti, E.V.; Amaretti, A.; Raimondi, S.; Rossi, M.; Van Sinderen, D. Riboflavin Biosynthesis and Overproduction by a Derivative of the Human Gut Commensal *Bifidobacterium longum subsp. infantis* ATCC. *Front. Microbiol.* **2020**, *11*, 11. [[CrossRef](#)] [[PubMed](#)]

177. Page, J.W.; Andrews, J.W.; Murai, T.; Murray, M.W. Hydrogen ion concentration in the gastrointestinal tract of channel catfish. *J. Fish Biol.* **1976**, *8*, 225–228. [[CrossRef](#)]
178. Hoehne-Reitan, K.; Kjorsvik, E.; Reitan, K.I. Development of the pH in the intestinal tract of larval turbot. *Mar. Biol.* **2001**, *139*, 1159–1164.
179. Kaktcham, P.M.; Temgoua, J.-B.; Zambou, F.N.; Diaz-Ruiz, G.; Wachter, C.; Pérez-Chabela, M.D.L. In Vitro Evaluation of the Probiotic and Safety Properties of Bacteriocinogenic and Non-Bacteriocinogenic Lactic Acid Bacteria from the Intestines of Nile Tilapia and Common Carp for Their Use as Probiotics in Aquaculture. *Probiotics Antimicrob. Proteins* **2018**, *10*, 98–109. [[CrossRef](#)]
180. Gunzburg, W.H.; Aung, M.M.; Toa, P.; Ng, S.; Read, E.; Tan, W.J.; Brandtner, E.M.; Dangerfield, J.; Salmons, B. Efficient protection of microorganisms for delivery to the intestinal tract by cellulose sulphate encapsulation. *Microb. Cell Factories* **2020**, *19*, 1–14. [[CrossRef](#)]
181. Gharibzahedi, S.M.T.; Smith, B. Legume proteins are smart carriers to encapsulate hydrophilic and hydrophobic bioactive compounds and probiotic bacteria: A review. *Compr. Rev. Food Sci. Food Saf.* **2021**, *20*, 1250–1279. [[CrossRef](#)] [[PubMed](#)]
182. Kwiecień, I.; Kwiecień, M. Application of Polysaccharide-Based Hydrogels as Probiotic Delivery Systems. *Gels* **2018**, *4*, 47. [[CrossRef](#)] [[PubMed](#)]
183. Tan, E.W.; Tan, K.Y.; Phang, L.V.; Kumar, P.V.; In, L.L.A. Enhanced gastrointestinal survivability of recombinant *Lactococcus lactis* using a double coated mucoadhesive film approach. *PLoS ONE* **2019**, *14*, e0219912. [[CrossRef](#)]
184. Rosas-Ledesma, P.; León-Rubio, J.M.; Alarcón, F.J.; Moriñigo, M.A.; Balebona, M.C. Calcium alginate capsules for oral administration of fish probiotic bacteria: Assessment of optimal conditions for encapsulation. *Aquac. Res.* **2011**, *43*, 106–116. [[CrossRef](#)]
185. Lee, H.; Ko, G. Antiviral effect of vitamin A on norovirus infection via modulation of the gut microbiome. *Sci. Rep.* **2016**, *6*, 25835. [[CrossRef](#)] [[PubMed](#)]