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Research Article

Probiotic bacteria as a healthy alternative for fish and biological control agents in aquaculture

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Abstract
Aquaculture is a rapidly expanding sector that contributes significantly to the global food supply, with Asia accounting for 90% of production and India being the second largest aquaculture fish producer. Probiotics are used in aquaculture techniques because of the need for aquatic organism development, higher disease resistance, and feed efficiency. Several potential probiotics have been shown to produce antimicrobial substances that inhibit the growth of harmful microbes and preserve intestinal microecological equilibrium by allowing them to stick to stomach surfaces, thus impairing pathogenic growth. Aquaculture has benefited various industries, pharmaceuticals, and global food security in recent decades. Nevertheless, the stress conditions that aquatic creatures experience in aquaculture cause fish to become less resilient to illness and weaken their immune systems. Therefore, impacts local people's socioeconomic conditions and economic progress in many nations. Several approaches, including conventional practices, artificial chemicals, and antibiotics, have been used to control disease in the aquaculture sector. Hence, alternative techniques are far more important to keep the microbial ecosystem in aquaculture systems healthy. This review presents the current understanding of probiotic use in aquaculture as a healthy alternative for fish and biological control agents in aquaculture and to find out its future direction for research.

Keywords: Aquaculture, Fish disease, Growth, Probiotic, Lactic acid bacteria

INTRODUCTION

Microorganisms linked to advantageous outcomes for the host are referred to as probiotics. Aquaculture has decreased the use of antibiotics due to environmental problems created by these agents such as antibiotic-resistant bacteria strains, which adversely affect other ecosystems (Okeke *et al*., 2022). The addition of antibiotics to the culture species eliminates pathogenic microorganisms and helpful microorganisms that are beneficial to the same organism. Consequently, accumulating these chemicals in organisms is not safe for human beings, who are the final consumers. Nowadays, there is a growing trend for people to consume 100% natural foods to live a healthier and longer life. Similarly, environmental care has been driven by countless regulations over time in a variety of environments. Probiotics have been studied for fish disease treatment for several decades, mostly for commercial purposes (Iorizzo *et al*., 2022). Microorganisms that benefit the body are replacing the need for growth promoters and antimicrobial drugs. The production of antibacterial compounds by some candidate probiotics inhibits the growth of harmful microorganisms that are harmful to the intestinal microbiome. As a result, they adhere to stomach surfaces, preventing pathogenic growth. To prevent pathogenic growth, fish pathogenic bacteria compete for nutrients and attachment sites in the stomach. When tested for direct antibacterial activity against known pathogens, several probiotics used in aquaculture have shown direct antibacterial activity (Xu *et al*., 2014; Lin *et al*., 2020).

Aquaculture is a significant contributor to the worldwide food supply. Asia currently provides approximately 90% of the worldwide aquaculture production, making it one of the sectors with the most rapid expansion. Compared to other sources of animal proteins, fish is more readily available and less expensive in tropical nations (Pegu *et al*., 2023). The escalating global demand for fish presents both a challenge and an opportunity (Naylor *et al*., 2021). Several experiments show that beneficial bacteria can enhance aquaculture production in addition to disease-causing bacteria (Allameh *et al*., 2017). Regarding the significant group of microorganisms used as animal probiotics, lactic acid bacteria (LAB) are the most prevalent. LABs are used in the fish production industry to enhance resistance to pathogens, survivability, protein efficiency ratio, feed conversion efficiency, and digestion. Furthermore, they prevent intestinal disorders and neutralize antinutritional factors present in feeds. They also improve the immune system of fish. When used in aquaculture, LABs can improve bacterial growth and monitoring by improving microbial efficiency (Allameh *et al*., 2017). Probiotic-fortified meals also assist in promoting fish growth, in addition to increasing the metabolic rate of the fish body.

Today, it is known that probiotics possess antimicrobial properties by affecting the intestinal microbiota, secreting antibacterial substances (bacteriocins and organic acids), competing for essential nutrients that enable pathogen survival, and producing antitoxin effects. They inhibit pathogen adhesion to the intestine, compete with pathogens for essential nutrients, and produce antitoxin effects. Furthermore, probiotics can modulate the immune system, regulate allergic responses, and reduce the proliferation of cancer in mammals. Thus, when provided at specific concentrations and viability, probiotics positively affect host health (Razafindralambo *et al*., 2023). Probiotics are commonly described as "friendly bacteria," "friendly," or "healthy". Studies have been focused on the characteristics of the microorganisms in the intestinal microbiota for many years. However, the term “probiotic” has primarily been applied to gram-positive lactic acid bacteria (Hoseinifar *et al*., 2018), particularly those belonging to *Bifidobacterium*, *Lactobacillus* and *Streptococcus* genera.

As opposed to terrestrial animals, aquatic species' gastrointestinal microbiota is particularly dependent on the external environment due to water flow through their digestive tracts. Therefore, there is a great deal of transient bacterial growth in the intestines due to constant intake of water and food, together with microorganisms that live there. Several bacteria have been identified in the gastrointestinal tracts (GIT) of aquatic animals, including *Salmonella, Listeria*, and *Escherichia coli*, which may be pathogenic, but probiotic bacteria and various other microorganisms have also been identified. A number of these bacteria are gram-positive, such as *Bacillus, Carnobacterium, Enterococcus*, and *several Lactobacillus* species; some of these bacteria are also gram-negative, facultatively anaerobic, such as *Vibrio* and *Pseudomonas,* as well as certain fungi, yeasts, and algae belonging to the genera *Debaryomyces, Saccharomyces*, and *Tetraselmis* (El-Saadony *et al*., 2021). There is an increasing interest in aquaculture probiotics at present, (Loh *et al*., 2017) proposed extending the definition of these probiotics to include “living microbial additives that benefit the health of hydrobionts and therefore increase productivity.” Probiotics are often defined as beneficial microorganisms that may survive in the digestive tract due to their resistance to bile salts and acids. In aquaculture, probiotics are relatively new, but interest in them has grown because they reduce disease. The present review presents the effects of probiotic bacteria on immune system responses, rearing parameters, and inhibitory effects on pathogenic bacteria in fish.

AQUATIC PROBIOTICS- SELECTION AND ADMINISTRATION OF THE RIGHT PROBIOTICS

It is very important to select the right strains of probiotics because inappropriate strains may lead to undesirable effects on the host if the strain is not right. The primary objective of selecting probiotics is to ensure they are safe and capable of producing desirable benefits. Furthermore, they must maintain their ability during production, manufacturing, distribution, and storage before reaching consumers (Shewale *et al*., 2014). The characteristics of a good and successful probiotic are outlined below, and they should all have a few things in common.

What makes a good probiotic?

(Michael, 2014; Torres-Maravilla *et al*., 2024)

It should be a strain capable of positively affecting the host animal, such as increased growth or disease resistance.

It should be non-pathogenic and non-toxic.

A large number of viable cells should be present, preferably in a large quantity.

It should be capable of surviving and metabolizing in the gut environment, such as being resistant to low pH and organic acids.

It should be stable and viable for longer when stored and used in the field.
As recommended by the United Nations, the following specifications should be considered in selecting and approving a probiotic product (Aly, 2010):
The viability of the probiotics should be demonstrated as efficacious and very stable when they pass through the gastrointestinal tract (GIT).
When the probiotic is present in the host's gut, it should colonize.
Competition between probiotic cultures and pathogenic bacteria for attachment to intestinal surfaces
According to in vitro tests, the probiotic should inhibit pathogenic bacteria.
It is important that probiotics are resistant to other sanitary agents or disinfectants.
The genus and species names should be indicated according to international nomenclature on the label of probiotic products.
Labels should indicate doses and expiration dates.
There should be data indicating that the product is not likely to infect an immunocompromised animal.

MATERIALS AND METHODS

METHODS OF ADMINISTRATION OF PROBIOTICS IN AQUACULTURE

Various methods of administering probiotics in aquaculture exist, such as feeding, injection, or direct immersion (Hai *et al*., 2015), individually or in combination, as presented in the Fig.1.

Water additives, feed additives, and injection

A probiotic mixture is most administered by incorporating it into the feed (92.8%), followed by direct incorporation into the water (4.8%), and by incorporating it into live food (1.6%) (Melo *et al*., 2020). Bacterial or fungal strains can be mixed with feeding pellets, encapsulated into live feedstock, or administered orally in feed for rearing animals (Das *et al*., 2017). A variety of probiotics, including bacterial strains, yeasts, and substances extracted from them, are generally provided as feed additives in aquaculture. In a recent study, *Lactobacillus plantarum* CR1T5 was applied as a feed additive to improve black-eared catfish growth performance and immunity (Silarudee *et al*., 2019). Rainbow trout fry's growth performance and nutrient utilization were enhanced by feed additives such as *Bifidobacterium strains* (Sahandi *et al*., 2018). Gobi *et al*. (2016) and Gupta *et al*. (2016) suggest that probiotics can be added to water as an additive. At 106 CFU/ml, *Vibrio lentus* leads to significant changes in gene expression in sea bass related to immune response, cell proliferation and death, cell adhesion, ROS metabolism, and iron transport (Schaeck *et al*., 2017). Furthermore, applying the product through injection in addition to the methods mentioned above is also possible. Probiotic injection with *Enterobacter sp.* through intramuscular and intraperitoneal injection of strain C6-6 (LaPatra *et al*., 2014) enhances the immune system of rainbow trout.

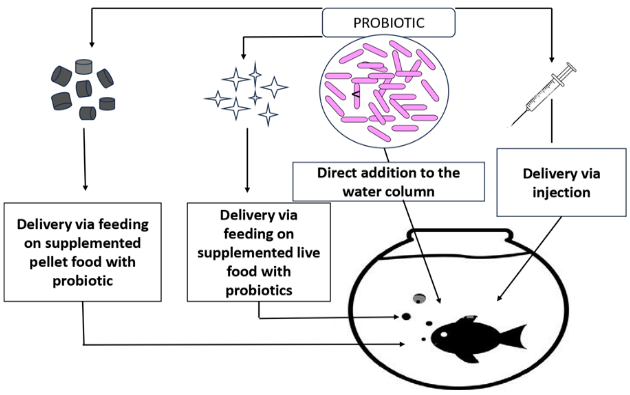

Fig. 1. *Different routes for administration of probiotic bacteria*

Single and combination

Probiotics can be applied singly or in combination in a variety of forms, such as multi-strain probiotics, probiotics that contain plant extract, and probiotics that contain yeast extract. It has been found that the combination of probiotics is more effective than using single probiotics, according to most studies on probiotics. Multi-strain probiotics are highly sensitive to pathogens and active against various aquaculture animals (Pannu *et al*., 2014). The multi-strain probiotics positively affects the growth and survival of rohu at the hatchling and fry stages, but not at the later stages of larval development (Jha *et al*., 2014). Compared to the application of *Bacillus megaterium* PTB 1.4 alone or *Pediococcus pentosaceus* E2211 alone as a probiotic to catfish *Clarias sp*., the combination of *B. megaterium* PTB 1.4 and *Pediococcus pentosaceus* E2211 in the feed showed better results than either of the probiotics alone (Hamka *et al*., 2020). *Lactobacillus plantarum* N11 and *B. velezensis* H3.1 were both combined with *Nile tilapia* (*Oerochromis niloticus*) to promote a higher survival rate (583.33%) when compared to the single application *of L. plantarum* (54.17%) and *B. velezensis* (41.67%) (Doan *et al*., 2018). Combined application of *B. coagulans* and *Mentha piperita* in *Catla catla* improved growth performance, nutrient retention, and immunity (Bhatnagar and Saluja, 2019).

Dosage of probiotics

Depending on the species, the appropriate probiotic dosage should be administered. Probiotic levels must be determined according to the probiont species, the fish species, their physiological status, the rearing conditions, and the specific application (Hai, 2015). The growth performance of rainbow trout was improved when probiotic *Bifidobacterium* strains were fed at a level of 107 CFU/g to the trout, but not when 100 CFU/

g was fed to the trout (Sahandi *et al*., 2018). As a result, this suggests that increasing the number of probiotics in feed does not always equate to increased growth. When supplementing functional nutrients into aquafeeds, it is important to consider the dose while adding these nutrients. The weight gain of pabda catfish, *Ompok pabda*, was reduced when more than 0.2% of dietary commercial probiotics was provided to the fish (Chowdhury *et al*., 2020). A study revealed that a concentration greater than a certain level might result in disturbances in the overall body physiology of the fish as well as disturbances in the metabolic process of carbohydrates and fats.

EFFECTS OF PROBIOTIC BACTERIA ON THE PERFORMANCE OF FISH

Effects of probiotics on fish growth

The effects of probiotics on both human health and the environment have been investigated in several studies (Nayak *et al*., 2010; El-Kady *et al*., 2022). Aquatic animals are often treated with probiotics such as *Enterobacter, Pseudomonas, Bifidobacteria, Carnobacterium, Shewanella, Lactobacillus, Lactococcus, Leuconostoc, Bacillus, Aeromonas, Vibrio, Clostridium, and Saccharomyces* (Nayak *et al.,* 2010, Hai *et al*., 2015; Hasan *et al*., 2020).

It investigated how microbial probiotics affect the growth, nutrient digestibility, enzymes, and intestinal microbiota of Rohu fingerlings (*Labeo rohita*) in their diet (Mohapatra *et al*., 2012). Fish fed a combination of three probiotics (*Bacillus subtilis, Lactococcus lactis,* and *Saccharomyces cerevisiae*) had a higher growth rate, protein efficiency ratio, nutrient digestibility, and feed conversion ratio. Several probiotic-containing diets have been shown to increase the growth rates of tilapia (*Oreochromis niloticus* L.) compared to control diets (Anee *et al*., 2021). Fish performance was improved by adding probiotics to diets containing yeast. In the intestines of wild rainbow trout, yeast was isolated similarly. When introduced into the digestive tract of cultured rainbow trout, this probiotic significantly accelerated their growth. Compared to yeast and control treatments, no significant growth increase was observed with bacterial mixtures in conjunction with optimum protein levels. The results may be explained by yeast's adaptability to aquatic environments more than bacteria. In addition to probiotic-fortified diets, the best growth response was obtained when the bacterium was used instead of yeast. In a study conducted by Zhang *et al*. (2021), gram-positive probiotic bacteria were found to enhance the survival, growth, and speed of marine fish larvae. Two kinds of probiotics on the growth of juvenile dentex (*Dentex dentex*) but found no significant improvement over the control group (Ibrahem *et al*., 2015).

Feed conversion ratio (FCR)

Adding probiotics to feed can improve feed utilization, even under stressful conditions (Anee *et al*., 2021). Nile tilapia (Oreochromis niloticus L.) showed the greatest FCR values in a probiotic-containing diet. The feed conversion ratio improved when Spirulina was added to N. tilapia's diet as a probiotic (Reque *et al*., 2022). Furthermore, *N. tilapia* treated with commercial probiotics showed significantly higher feed conversion efficiency than controls. Maniat *et al*. (2022) found that shrimp-fed probiotics had a lower feed conversion ratio than controls. In a similar study, Mohapatra *et al*. (2012) found that probiotic diets reduced FCRs in Rohu fingerlings (*Labeo rohita*).

Protein efficiency ratio (PER)

Food fortified with probiotics may improve the protein efficiency ratio (PER) or apparent nitrogen utilization (ANU) of animals (Anee *et al*., 2021). Consequently, protein consumption is optimized, which is the most expensive nutrient in feed. Among other benefits, probiotics improve feed nutritional efficiency under stress conditions (Anee *et al*., 2021). In Nile tilapia (*Oreochromis niloticus* L.), probiotics significantly improved protein efficiency ratios (Reque *et al*., 2022).

Digestibility

The use of probiotics in diets has been found to increase nutrient digestibility (Mohapatra *et al*., 2012). In addition to improving protein digestibility, probiotics may also increase performance (Anee *et al*., 2021). The positive effects of probiotics on feed nutrient digestion in fish diets can be attributed to the activation of digestive enzymes in bacteria by probiotics (Zhao *et al*., 2023). Askarian et al. (2012) state that chitin affects Atlantic salmon's adherent aerobic intestinal microbiota (*Salmo salar L.*). Chitin was also tested for protease, amylase, cellulose, phytase, and chitinase activity. LABs have been reported to produce digestive enzymes such as amylase, lipase, and protease. An increase in nutrient digestibility was observed in Rohu fish (*Labeo rohita*) when different types of probiotics were added to their diets (Mohapatra *et al*., 2012). In terrestrial animals (such as poultry), using probiotics in a diet has been shown to increase digestibility (Wang *et al*., 2017). Feeding live yeast to sea bass (*Dicentrachus labrax*) increased their digestive enzyme activity (Tovar-Ramírez e*t al*., 2010; Perdichizzi *et al*., 2023). In a study conducted by Wang and Xu, probiotics from *Bacillus sp.* were shown to significantly increase digestive enzyme activities in common carp by affecting protease, amylase, and lipase activity (Luo *et al*., 2020). probiotic treatment significantly affected amylase and trypsin activity in shrimp after probiotic treatment. Probiotics enhance digestion by increasing the population of beneficial microorganisms and the activity of microbial enzymes, thereby improving feed digestibility and absorption. In addition, they demonstrated that digestion enzyme activity is enhanced by high growth performance.

Body composition

Probiotics were administered to increase protein and lipid content in Nile tilapia (*Oreochromis niloticus* L.) diets. However, the administration of probiotics had no significant effect on the fish's moisture and ash content. When commercial probiotics were used, no statistically significant differences were observed in carcass moisture, ash, and protein content among the treatments (Noshair *et al*., 2023). The researchers observed differences in the amount of lipids and gross energy in the carcasses, with the highest value measured in fish fed a control diet. The probiotic diets did not significantly alter the body composition of *Nile tilapia* (Anee *et al*., 2021). *Bacillus subtilis* also increased fat content in carcasses but not moisture, ash, or protein. *B. subtilis* also improved fat content, not moisture, ash, or protein. Probiotics did not significantly affect fish body composition or tissue synthesis.

Immune system response

Nutritional factors and feed additives have been studied since the 1980s about fish's immune system. Several of these additives have been investigated by researchers for their ability to protect fish against stressors or diseases (Kiron *et al*., 2012). Probiotics have a wide range of benefits, but one of their most important applications is their ability to modulate immune systems (Nayak *et al*., 2010). Alternative methods have gained popularity in recent years to control disease problems. Antibiotic bacteria, such as LAB, are used to control pathogen populations by enhancing immunity or excluding them competitively. To achieve this goal, aquaculture can enrich larval food, include probiotics in the diet, or add them to the water. Fish's immune systems can be stimulated by probiotics both specifically and non-specifically. Fish can benefit from mono-species or multi-species probiotics by enhancing phagocytic and lysozyme enzyme activity and cytokine production. Moreover, probiotic bacteria increase immunoglobulin cells and acidophilic granulocytes in the fish gut, stimulating the immune system. It is possible to influence the immunomodulatory activity of probiotics by varying the source, the type, the dose, and the duration of supplementation (Nayak *et al*., 2010).

Studies have shown that fish and shellfish contain several immunostimulants that are beneficial to the body. In aquaculture, autochthonous microbiota may augment immune responses to pathogens living in the water. Microbial cell walls contain muramyl dipeptide, glucans, and lipopolysaccharides. Accordingly, probiotic LAB is considered an immunostimulant in aquatic animals. LAB, as a probiotic stimulates the immune system and improves the quality of the water and nutrition, resulting in a higher rate of larval survival and increased aquaculture production (Yu *et al*., 2022). *Bacillus* S11 protects shrimp from disease by activating both cellular and humoral immune defenses inside the shrimp's gut and presumably providing competitive exclusion. Dead bacterial cells and yeast-glucan have also been shown to stimulate immune responses in shrimp (*Penaeus monodon*). Yu *et al*. (2022) found that different strains of *Lactobacillus rhamnosus* (JCM 1136) induce immune responses in rainbow trout (*Oncorhynchus mykiss*). Fish-feeding supplements such as probiotics and immunostimulants can prevent infectious diseases. Dietary administration of probiotics can enhance the body's natural immune function. The bacteria may adhere transiently to the gastrointestinal tract and colonize it, increasing antibody production (Dysin *et al*., 2023). Probiotics have been shown to improve disease resistance and animal growth in animal rearing. Consequently, probiotics affect the immune system responses and bacterial population of aquatic organisms and the environment.

Further, some studies suggest that the fish gut microbiota prevents fish from becoming ill from food poisoning by inhibiting the growth of pathogenic bacteria in the digestive system. These results deserve attention because the digestive tract is a key entry point for pathogenic bacteria (Ringo *et al*., 2006). A list of different probiotic bacteria with their source/medium/fish tested as well as their role in promoting growth is presented in Table 1.

USE OF PROBIOTICS IN AQUACULTURE

Using probiotics on aquatic organisms has been promoted by the need for sustainable aquaculture. Initial interest was focused on growth promoters and animal health improvements; however, new areas such as reproduction and stress tolerance, require more research. A variety of applications of probiotics in aquaculture are illustrated in Fig. 2 and Table 2.

Growth promoter

Aquaculture uses probiotics to stimulate the growth of cultivated species, but it is unclear whether these products increase appetites or improve digestion. When administered over time, microorganisms colonising gastrointestinal tracts can multiply more rapidly than those expelled. Fish cultured with probiotics exhibit various benefits when these bacteria adhere to their intestinal mucosa (El-Saadony *et al*., 2021). Several studies have explored the effect of probiotics on phytoplankton (microalgae), a primary source of aquatic food chains, because it is capable of producing nutrient-producing photosynthetic machinery that higher organisms cannot synthesize on their own, such as polyunsaturated fatty acids and vitamins. One of the best microalgae for aquaculture is *Chaetoceros spp*. a primary source of live food. Their nutritional needs, however, have limited production. In the presence of *Chaetoceros muelleri* microalgae, *Vibrio alginolyticus* C7b probiotic achieves a high density. Since rotifers are small, most cultured aquatic species rely on them for their first live food, since they are more accessible to larvae. Rotifers *Brachionus plicatilis* have grown faster when they have been given brine shrimp nauplii, a lactic acid bacterium commonly fed to rotifers. By adding *Lactococcus casei sp.*, *Pediococcus acidilactici,* and *Lactobacillus lactis sp.* lactis best results were achieved (Contreras Tapia *et al*., 2020). Probiotics have been used as growth promoters for edible fish. A probiotic *Streptococcus* strain was fed to *Nile tilapia* (*Oreochromis niloticus*) for 9 weeks, resulting in increased crude protein and crude lipid content and a weight increase from 0.154 g to 6.164 g. Commercial probiotic products were found to increase weight 115.3% with a 2% concentration (Sîrbu *et al*., 2022). In addition to swordtails (*Xiphophorus maculatus, Xiphophorus helleri*), and guppys (*P. sphenops, Poecilia reticulate*), the feed was supplemented with *Streptomyces* and *Bacillus subtilis,* and significant survival and growth were found after 90 and 50 days, respectively.

Use of probiotics to prevent various diseases

In the aquaculture industry, antibiotics have been used for a long time to prevent diseases from affecting the crop. In addition, this led to the development of bacteria's resistance mechanisms, and an imbalance in aquatic species' gastrointestinal microbiota, which affected their health (Nakano *et al*., 2007; Sarker et al., 2023). Currently, the European Union regulates the use of antibiotics in organisms for human consumption. Consumers today prefer natural products, free from additives like antibiotics; disease prevention is more common than disease treatment. The use of probiotics in aquaculture is, therefore a viable alternative for inhibiting pathogens and controlling diseases. The probiotic microorganisms release chemicals that are bactericidal or bacteriostatic against pathogenic bacteria in the intestine of the host, thus constituting a barrier to opportunistic pathogen proliferation. Several factors cause the antibacterial effect, including the production of antibiotics, bacteriocins, siderophores, enzymes (lysozymes, proteases), and/or hydrogen peroxide, as well as the generation of organic acids that alter the intestinal pH. The viable probiotics administered to tilapia *Oreochromis niloticus*, improved non-specific immune response, as measured by parameters such as lysozyme activity, neutrophil migration, and bactericidal activity, which improved fish resistance to infection by *Edwardsiella tarda* (Taoka *et al*., 2006; Sîrbu *et al.,* 2022).

An isolated strain of Carnobacterium sp. from salmon intestine was administered to rainbow trout and Atlantic salmon, demonstrating *in vitro* antagonism against *Aeromonas hydrophila*, *A. salmonicida*, *Flavobac terium psychrophilum, Photobacterium damselae*, and *Vibrio species*. The control of furunculosis in rainbow trout was demonstrated to be effective with dead probiotic cultures containing *Vibrio fluvialis* A3-47S, *A. hydrophila* A3-51 and Carnobacterium BA211. It appears that in this particular case, the number of leukocytes was greater than with live bacteria, suggesting that humoral factors were less important than cellular immunity in these preparations of inactivated bacteria (Knobloch *et al*., 2022). In the case of shrimp, studies have focused on the evaluation of probiotics such as *Bacillus cereus, Paenibacillus polymyxa*, and *Pseudomonas sp.* PS-102 biocontrol agents against pathogens of various *Vibrio species*. It has been found that probiotic strains isolated from the gastrointestinal tract of clownfish (*Amphiprion percula*) are effective in inhibiting several pathogens, including *A. hydrophila* and *V. alginolyticus*. The bacteria isolated from clownfish can colonize the intestinal mucus and, therefore can be used as a prophylactic and/or therapeutic agent (Knobloch *et al*., 2022). Because probiotics generate a density in vivo that permits the production of antimicrobial metabolites. Also, it has been shown that concentrations of probiotics containing 106 to 108 cells per cell per unit of dietary fiber promote the growth of healthy microbiota in ornamental fish from the genera *Poecilia* and *Xiphophorus*, decreasing the number of heterotrophic microbes. The use of *V. alginolyticus* strains as probiotics to increase survival and growth of white shrimp (*Litopenaeus vannamei*), as well as probiotics in Ecuadorian shrimp hatcheries, increased production by 35%, whereas antimicrobials decreased production by 94% (Pereira *et al*., 2022).

Improvement in nutrient digestion

Probiotics can improve the digestive processes of aquatic animals. Apart from extracellular enzymes, probiotic strains also produce vitamin, fatty acid, and amino acid growth factors (El-Saadony *et al*., 2021). Nutrient absorption is increased when probiotics are added to feed (Sîrbu *et al*., 2022). European bass larvae (*Dicentrarchus labrax*) have been treated with probiotics, including several cases in which they are used as food. *A strain of bacteria known as Debaryomyces hansenii HF1, which produces spermine and spermidine, grows in the gastrointestinal tracts of mammals.* The enzymes secreted by this yeast also aid the digestion of sea bass larvae. 0.5 g of *Bacillus cereus* strain Ekg+1 supplemented in the diet increased the growth of juvenile common dentex *Dentex dentex L.* (Meng *et al*., 2023). *B licheniformis*, *B. subtilis,* and *Enterococcus faecium* were also found to produce similar results for

Table 1. Showing different probiotic bacteria with their source/medium/fish tested as well as their role in promoting growth

Probiotic	Source organism/Medium/ Fish tested	Growth promoting role	Reference
Lactobacillus acidophilus	Added in rearing water	Enhancing water quality in the culture pond of *Clarias gariepinus*	Dohail *et al*. (2009)
B. subtilis, B. licheniformis, and *Enterococcus faecium*	Supplemented with a traditional meal of Rainbow trout	Enhance fish growth by increasing feed consumption efficiency	Merrifield *et al*. (2010)
L. sakei	Intestines of *Paralichthys olivaceus*	Macrophage phagocytic activity " Peroxidase activity	Harikrishnan *et al.* (2010)
Bacillus NL 110, *Vibrio* Sp. NE 17	Added in rearing water	Enhancing water quality in the culture pond of *Macrobrachium rosenbergii*	Mujeeb *et al.* (2010)
Bacillus cereus	Added to the meal of Juvenile Dentex dentex L	Increases fish growth due to a greater food consumption capacity	Merrifield *et al*. (2010)
Methylococcus capsulatus	Added to the diet of Atlantic salmon (Salmo salar)	Enhance growth by supplying abundant protein to Salmo salar	Romarheim *et al*. (2011)
Bacillus spp.	Intestines of Penaeus monodon and shrimp pond sediment	Increase Growth and survival Beneficial microbial probiotics	Nimrat *et al*. (2011)
Carnobacterium Sp.	Supplemented to salmon bowel	Promote growth by showing in vitro antagonism against known fish pathogens in rainbow trout and Atlantic salmon	Martinez *et al.* (2012)
Nitrosomonas Nitrobacter	Applied in culture pond.	Promote growth by Oxidizing ammonia and nitrite in the cultured pond	Kaiser *et al*. (2013)
Enterococcus faecalis	*Oncorhynchus mykiss*	Favoring growth, stimulation of the immune system, and protection of diseases	Rodriguez-Estrada *et al.* (2013)
Bacillus Sp. (HFH4, CPF3) *Bacillus licheniformis* (CPH6, CPF4)	The gastrointestinal tract of *Channa punctatus* and *Heteropneustes fossils*	Proteolytic, Cellulolytic, and amylolytic enzyme activity	Banerjee *et al.* (2013)
Lactobacillus mesenteroides SMM69 Weissella cibaria P71	*Scophthalmus maximus L.*	Antimicrobial activity against the turbot pathogens *T. maritimum* and *V. splendidus*	Muñoz-Atienza *et al.* (2014)
Bacillus subtilis Bacillus licheniformis Bacillus sp. *Pediococcus* sp.	*Oreochromis* sp.	Resistance to S. agalactiae	Ng WK *et al.* (2014)
Lactobacillus mesenteroides SMM69 Weissella cibaria P71	*Cophthalmus maximus L.*	Antimicrobial activity against the turbot pathogens *T. maritimum* and *V. splendidus*	Muñoz-Atienza *et al.* (2014)
Lactobacillus Plantarum and *Pseudomonas* fluorescent	Added in pond water	Increase survival rate by 96.22% in *Clarias gariepinus*	Omenwa *et al*. (2015)
Bacillus sp. *Pediococcus* sp.	*Solea senegalensis*	improvement protection against pathogen outbreaks	Batista *et al*. (2015)
Lactobacillus plantarum (LP20)	*Seriola dumerili*	Improves immune response and stress	Dawood *et al*. (2015)
Lactobacillus rhamnosus	Pagrus major	Growth-promoting agent and Increases growth	Dawood *et al*. (2016)
Bacillus megaterium PTB 1.4	Catfish	Increased the activity of digestive enzymes and the growth of catfish	Afrilasari *et al*. (2016)
Bacillus megaterium, Bacillus polymyxa Lactobacillus delbrueckii	*Oreochromis* sp.	Increased the performance of zootechnical parameters	Gutiérrez *et al.* (2016)
Lactobacillus acidophilus Bacillus subtilis Lactobacillus bulgaricus Saccharomyces cerevisiae	*C. gariepinus*	Increases larval survival	Dennis *et al.* (2016)

Table 1. Cont.

Pseudoalteromonas sp.	Fish	Inhibitory activity against fish pathogens	Sayes *et al*., 2016
Enterococcus casseliflavus	*Oncorhynchus mykiss*.	Capability of improving growth performance and enhancing disease resistance by immunomodulation	Safari *et al.* (2016)
Pseudoalteromonas sp. *Cepa MLms* gA3	Fish	Inhibitory activity against the pathogen *V. anguillarum*	Wesseling *et al*. (2016)
Nitrosomonas Sp. *Nitrobacters* Sp.	The gut of Catla (*Catla catla*), Rohu (*Labeo rohita*), and Grass carp (*Ctenopharyngodon idella*)	Block the pathogen entry in the culture pond and raise the concentration of dissolved oxygen.	Sunitha and Krishna, (2016)
Escherichia coli, Proteus vulgaris, Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa and *Staphylococcus aureus*	*Scomberomorus guttatus*	-	Karthiga *et al.* (2016)
Bacillus licheniformis (TSB27) *Lactobacillus thuringiensis Bacillus Plantarum* *Bacillus subtilis* (B46).	*Sparus aurata* L.	Enhances the immune system	Bahi *et al.* (2017)
Bacillus subtilis WB60	*Anguilla japonica*	Increased in weight, efficiency in food and protein	Lee *et al*. (2017)
Bacillus pumilus H2	Fish	Anti-Vibrio activity	Gao *et al*. (2017)
Pseudoalteromonas sp	*Seriola lalandi*	Increased larval surviva	Leyton *et al*. (2017; Mata *et al.* (2017)
Lac. pentosus BD6, *Lac. fermentum* LW2, *Bacillus subtilis* E20, *Saccharomyces cerevisiae* P13	*Asian seabass*	Improved either the growth performance or disease resistance of Asian seabass against *A. hydrophila*	Lin *et al.* (2017)
Bacillus subtilis Lactobacillus rhamnosus	*Labeo rohita*	Increased the value of biochemical components	Munirasu *et al*. (2017)
Lactobacillus plantarum	*Oreochromis niloticus*	Decreases mortality and improves growth	Meidong *et al.* (2017)
Bacillus sp. MVF1	*Labeo rohita*	Decreased susceptibility to disease	Nandi *et al.* (2017)
Lactobacillus casei	*Tor tambra*	Growth performance and feed efficiency increased	Muchlisin *et al*. (2017)
Kocuria SM1 *Rhodococcus* SM2	*Oncorhynchus mykiss*	Produces extracellular enzymes that may have a role in the host digestive processes	Sharifuzzaman *et al.* (2017)
Bacillus subtilis *Bacillus licheniformis*	Juvenile rainbow trout	Resistance against *A. salmonicida*	Park *et al*. (2017)
Lactobacillus plantarum	Tilapia (*Oreochromis* sp.)	Enhanced the growth performance and modulated some hematological parameters.	Yamashita *et al.* (2017)
Vibrio lentus	*Dicentrarchus labrax*	Protective effect against Vibriosis caused by *V. harveyi* in sea bass larvae	Schaeck *et al*. (2017)
Psychrobacter spp	digestive system of Turbot, wild (*Scophthalmus maximus*)	antagonistic activity towards pathogen *Tenacibaculum maritimum*	Wanka *et al.* (2018)
Clostridium butyricum	Kuruma shrimp (*Marsupenaeus japonicas*)	Increased the content of intestinal short-chain fatty acid along with propionic acid and butyric acid and increased the levels of body crude protein.	Duan *et al.* (2018)
Pseudomonas sp. (GP21) and *Psychrobacter* sp. (GP12)	The gastrointestinal tract of Atlantic Cod	Elicit the immune response, particularly in mucosal surfaces such as the skin and intestine.	Butt, *et al.* (2021)
Lactobacillus rhamnosus	Administrated to *Oreochromis niloticus*	Stimulate the phagocytic activity in Tilapia	Cano-Lozano *et al.* (2022)

Table 1. Cont.

Bacillus Sp.	*Common carp, Cyprinus carpio*	Increase Survival rate Protease activity Lipase activity	Sumon *et al.* (2022)
Bacillus spp	Added in the surrounding water	Increase growth performance in cultured pond by phosphate solubilization and phosphate production	Armandeh *et al.* (2022)
Bacillus cereus, Paenibacillus polymyxa, and Pseudomonas Sp. PS-102	-	Act as the antagonist against various pathogens in shrimp	Sanches-Fernandes *et al.* (2022)
Lactobacillus acidophilus (Fla) and *Saccharomyces boulardii* (FSB)	Supplemented with the diet of Acipenser baerii	Enhance growth, immunity, and survival rate	Mocanu *et al.* (2022)
B. coagulans SC8168	Added in rearing water	Enhancing water quality in the culture pond of *Penaeuss vannamei*	Monier *et al.* (2023)
Bacillus subtilis	Gut of *Cirrhinus mrigala*	Increase the length and weight of guppies (Poecilia reticulata, P. sphenops), and swordtail (Xiphophorus helleri, X. maculatus), and increase the proteases and amylases activity in ornamental fish	Hoseinifar *et al.* (2023)
Debaryomyces hansenii HF1	Supplemented to Sea bass larvae	Promote growth by secreting amylase and trypsin, enzymes that help in digestion	Perdichizzi *et al.* (2023)
Psychrobacter Sp. (GP11), *Shewanella* Sp. (GS11), *Photobacterium* Sp. (GP31), and *Vibrio* Sp. (GV11),	Atlantic cod's intestine	Differential expression of an immune gene associated with bacterial defense and inflammation in the head kidney leukocytes	Coulibaly *et al.* (2023)
Bacillus sp.	Added in rearing water	Increasing growth through improving the water quality in the Penaeus monodon culture pond	Torres-Maravilla *et al.* (2024)

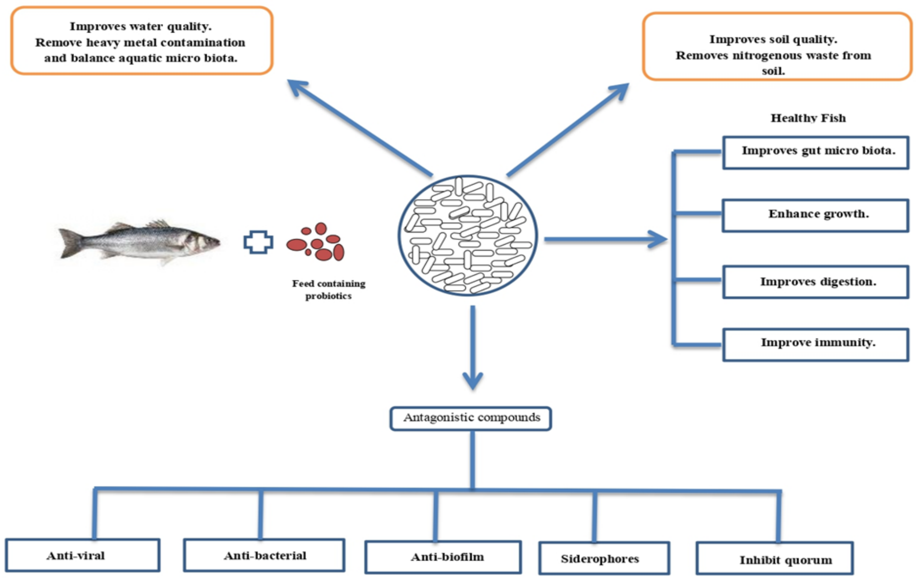

Fig. 2. *Use of probiotics in aquaculture*

rainbow trout when given with a diet that included *Bacillus subtilis*, *B. licheniformis,* and *Enterococcus faecium* for 10 weeks (Merrifield *et al*., 2010). There have been some studies where European sea bass larvae have been fed a probiotic yeast diet (*Saccharomyces cerevisiae* strain X2180), evaluating growth and antioxidant enzyme activity (glutathione peroxidase, catalase, and superoxide dismutase), indicating that probiotic supplementation may alter enzyme activity and gene expression patterns (Tovar-Ramírez *et al*., 2010). Various strains of *Bacillus* have been used as probiotics to increase the apparent digestibility of crude protein, dry matter, and phosphorus in white shrimp *Litopenaeus vannamei* Boone and Fen *Neropenaeus indicus*. The organisms' size increased when the diet was supplemented with 50 grams of probiotics per kilogram of food (Lin *et al*., 2020). To maintain a constant effect on digestive enzyme production, the probiotic should be managed throughout the shrimp's development (Ziaei-Nejad *et al*., 2006). *Bacillus subtilis*, isolated from the intestine of *Cirrhinus mrigala*, has been incorporated into the diet of guppies (*Poecilia reticulata, P. sphenops),* and swordtails (*Xiphophorus helleri, X. maculatus*) to assess their intestinal health. A significant increase has been observed in the length and weight of ornamental fish, as well as an increase in the specific activity of proteases and amylases in their digestive tracts. A wide range of exoenzymes are secreted by *Bacillus* to complement the activity of the fish and to enhance its ability to digest nutrients enzymatically.Vadstein *et al*. (2018) have found that bacteria isolated from the digestive tracts of aquatic animals produce chitinases, proteases, cellulases, lipases, and trypsins.

Improving the quality of the water

Probiotic bacteria directly absorb or decompose organic matter, improving water quality. Probiotics maintain a good water quality environment by decomposing fish or prawn excreta, food materials, plankton remains, and other organic matter by releasing CO2, nitrates, and phosphates. In culture water, Nimrat *et al.* (2012) found that mixed Bacillus probiotics significantly improved white shrimp's nitrite, pH, and ammonia levels. Kewcharoen and Srisapoome (2019) found that total ammonia was reduced when the probiotic *Bacillus subtilis* was added to shrimp culture water at 103-105 CFU/ml. At 106 CFU/ml, *Bacillus cereus* and *Pediococcus acidilactici* probiotics significantly decreased nitrate, ammonia, and biological oxygen demand in pond water (Khademzade *et al*., 2020). Several studies have shown that probiotic strains improve water quality, especially those of the gram-positive genus Bacillus. Converting organic matter into CO2 makes this bacterial group more efficient than gram-negative bacteria. A high level of probiotics in production ponds can minimize the accumulation of dissolved and particulate organic carbon. Therefore, phytoplankton production can be balanced . El-Saadony *et al*., 2021). This hypothesis cannot be confirmed by cultivating shrimp or channel catfish with *Pseudomonas, Bacillus, Nitrobacter, Cellulomonas, Enterobacter, or Rhodopseudomonas.* Nitrification is the only aspect of improving water quality that has received substantial attention (Pereira *et al*., 2022).

Stress tolerance

Probiotics significantly improve stress tolerance in aquaculture species. Fish-fed *Lactobacillus plantarum* had a lower cortisol concentration than those fed control diets, which could be because cortisol is a stress hormone. Probiotics generally help fish to tolerate ammonia stress (Razafindralambo *et al*., 2023). *Nile tilapia* defense against hypoxia stress is significantly improved by *Aspergillus oryzae* probiotics (Dawood *et al*., 2019). Chowdhury *et al*. (2020) found that Pabda catfish supplemented with dietary commercial probiotics at 0.2 % were significantly more tolerant to saline water stress than those supplemented with higher and lower doses. As a result, probiotics-supplemented groups had lower plasma glucose levels, a stress indicator.

Reproduction effects on aquatic species

The reproduction capacity of breeding aquaculture species depends on adequate lipids, proteins, fatty acids, vitamins C and E, and carotenoids. In addition, the interaction between these components affects reproduction in several ways, including fertility, fertilization, and larval development. Cultured fish species can be fed larger diets known as broodstock diets. Supplementing fresh fish with products from commercial diets improves the nutrition of fish hatcheries' broodstock. Fresh organisms used to feed broodstock fish include squid, cuttlefish, mussels, krill, and small crustaceans. Using unprocessed fish products often results in poor nutrition for broodstock fish, increasing the risk of parasites, bacteria, and viruses infecting the offspring and parents. Probiotics were added to food or water to prevent infections and study their effects on reproduction (Vidhya *et al.,* 2019; Leong *et al.,* 2023). An important study on the effects of probiotic supplements on fish reproductive performance was conducted by (Hasan *et al.,* 2020), which included a strain of *B. subtilis* isolated from *Cirrhinus mrigala's* intestine, incorporated at different concentrations into four ornamental fish species: *Poecilia reticulata, P. sphenops, Xiphophorus helleri,* and *X. maculates*. Mixing *B. subtilis* at 106–108 cells per gram of food increased the gonadosomatic index, fecundity, viability, and larval production in all four species. Probiotics synthesize complex B vitamins, particularly thiamine (vitamin B1) and vitamin B12, which reduce the number of dead or deformed fry. Abasali *et al*. (2010) conducted a similar study using a commercial probiotic containing *Lactobacillus acidophilus, L. casei, Enterococcus faecium,* and *Bifidobacterium thermophilum.* Each female was evaluated for fry production and relative fecundity. Comparing the probiotic-treated group with the control group, 105 and 150 fry, on average, were found in the probiotic-treated group. In the second study, 28 females were fecundated under control conditions, and 41 were fecundated under probiotic conditions.

Enhancement of the immune response

In aquaculture species, probiotics can enhance a variety of immunological parameters. Through stimulation of the body's non-specific and cellular immunity, probiotics can inhibit pathogen infection (Hamka *et al*., 2020). By increasing the proportion of phagocytic cells in the head kidney and activating complement receptor expression, the administration of viable lactic acid bacteria, *Lactococcus lactis, Leuconostoc mesenteroides*, and *Lactobacillus sakei* improved both cellular and hormonal immune functions in rainbow trout. According to Doan *et al*., (2018), host-associated probiotics *Lactobacillus plantarum* and *Bacillus velezensis* improve skin mucus lysozyme and peroxidase activity, serum lysozyme and peroxidase activity, alternative complement, phagocytosis, and respiratory burst activity in *Nile tilapia*. The administration of *Bacillus pumilus*, a host gut-derived probiotic, to juvenile golden pompano, *Trachinotus ovatus* increased their lysozyme activity and total protein levels (Liu *et al*., 2019).

Improve feed utilization

The use of probiotics alters enzymes and improves feed utilization, according to much research. *Lactobacillus pentosus* supplementation improved feed utilization in white shrimp, *Litopenaeus vannamei* (Zheng and Wang, 2016). *Nile tilapia* were shown to exhibit superior amylase, lipase, and protease activity after consuming heat-killed *L. plantarum* for 12 weeks (Dawood *et al*., 2019). *Astacus leptodactylus* fed with *L. plantarum* at concentrations of 107, 108, and 109 CFU/gm had elevated amylase, alkaline phosphatase, protease, and lipase activities (Valipour *et al*., 2019).

Conclusion

Probiotics have been seen to positively impact the host's gut defense system, an important factor for preventing disease and treating conditions linked to inflammation within the digestive system. Probiotics have significantly improved aquatic animals' development performance and feed consumption by activating their digestive enzymes. Aquaculture species can benefit from probiotics in terms of their immunological characteristics. Boosting the host's immune system and promoting both cellular and non-specific immunity in the body, probiotics help prevent the spread of disease. As a result of their ability to absorb or break down harmful substances or organic matter, probiotic bacteria help clean water. As a result of their ability to break down organic debris like leftover food items, plankton remnants, and fish or prawn excreta, probiotics contribute to maintaining a healthy water quality environment for cultured animals by assisting the nutrition cycle. As a substitute for pathogen inhibition and disease management in aquaculture species, probiotics are an effective alternative. Probiotic microorganisms can act as a barrier against opportunistic infections by releasing chemicals that kill or inhibit pathogenic bacteria in the host's intestine. There is a lot of pressure on crop species regarding aquaculture, which requires a high output level within a short period. The purpose of administering probiotics to food and water is to prevent illness and investigate their effects on reproduction. The probiotic supplements have a profound impact on fish reproduction.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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