Probiotics in Aquaculture

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Background

Aquaculture is a major food-producing activity that helps to cater to the needs of an ever-increasing populace. Nevertheless, infectious diseases have become a serious issue in aquaculture, resulting in significant financial losses for the industry. Treatment with costly chemotherapy medications has a detrimental effect on the aquatic ecosystem. As a result, there is an increasing need for finding alternatives that are safe, non-antibiotic-based, and environmentally. Probiotics are a possible alternative to antibiotics for controlling infectious agents and treating disorders. Growth promotion, better metabolism, improved immunological response, and water quality maintenance are all advantages of probiotics. Probiotics help aquatic animals to fight diseases and promote well-being since they have antibacterial, antifungal, and antiviral capabilities. Probiotics are a unique concept in aquaculture, and their effectiveness in an aquatic setting is still to be well investigated. This article presents current information about using probiotics, including selection criteria, kinds of probiotics utilized in fish farming, the mechanisms underlying, and probiotics administration methods.

Definition of probiotic

Probiotic is originally a Greek term, where 'Pro' means benefit and 'bios' means life. In 1907, a Russian analyst named Elie Metchnikoff noted that Bulgarian laborers lived long lives because they ate fermented dairy products. Lilly and Stillwell (1965) used the term "probiotic" to describe unknown growth-promoting chemicals produced by a ciliated protozoan. In 1974, Parker defined probiotics as organisms and substances that add to intestinal balance.

In 1992, Fuller redefined the definition as a live microbial feed supplement that beneficially affects the host by improving the intestinal microbiological balance. According to a joint working group of the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (FAO/ WHO,2001). By releasing compounds such as bacteriocins and other inorganic compounds, probiotics defend the host body from harmful microorganisms. Aquaculture probiotics are live, dead or 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 vigour, which is achieved via improving the hosts microbial balance or the microbial balance of the ambient environment (Merrifield et al. 2010a).

Probiotics have gained increasing prominence as an alternate to antibacterial drugs in the aquaculture sector for increasing productivity and preventing disease. Whenever probiotics are fed to the fish, they have a positive effect on the fish host. Dietary intake of probiotics aids in the modification of the intestinal tract's microbes balance, as well as the immune modulation and also offers several nutritional advantages (Kesarcodi-Watson et al., 2008). Probiotics have a wide range of applications in aquaculture, in addition to their health and growth-promoting characteristics. Because of the complex link between an aquatic organism and its surroundings, the notion of probiotic use in fish culture has been developed to encompass water quality enhancement by directly introducing probiotics in ponds. For these reasons probiotics are defined as “water additives” (Moriarty, 1998). It is assumed that microorganisms that improve water quality also improve the health of aquatic animals, and various commercial products labeled as "probiotics" have attempted to capitalize on this theory. The research and potential application of probiotics in aquaculture have continued to grow during the last couple of decades. Representatives of roughly 20 bacterial genera have recently been recognized as prospective probiotic candidates, with *Bacillus* spp. and *Lactobacillus* spp. (LAB) representing the bulk of promising species (Knipe et al., 2020).

Characteristics of an ideal probiotic

The vital role of probiotics is to establish or maintain a healthy intestinal microbial flora in the fish (Thirumurugan and Vignesh, 2015). The following are the characteristics of an ideal probiotic.

- They should offer a beneficial effect on growth, maturation, and immunity against pathogens. Probiotics should have no negative consequences for the host.
- Antibiotic resistance should never be a feature of probiotics; instead, they must be able to maintain inherited features.

Probiotics should have the following characteristics in order to be used as an effective feed probiotic.

- ✓ Withstand acidic conditions
- ✓ Resistant to gastric secretions
- ✓ Attach to the epithelium of the digestive tract
- ✓ Antagonism towards pathogenic microorganisms
- ✓ Immune system stimulation
- ✓ Increase in gut movement
- ✓ Able to survive in mucus
- ✓ Probiotics should have fermentative activity, resistance to drying, and viability in food during transport and storage.

Organisms obtained from various sources are submitted to a series of tests in order to determine their suitability as ideal probiotic. The screening procedure includes Gram’s reaction, in-vitro assessment of antagonistic characters, tolerance to acids and bile and susceptibility to antimicrobial drugs. If all of these criteria are met, they are considered a promising probiotic for use in fish culture.

Sources of bacterial probiotics

Bacteria can be found in humans, animals, soil, sediment, aquatic environment and different numbers of bacteria (10^2 -10^{11} CFU/g) were observed in different environments (Liu et al., 2010). Bacteria from atmosphere, soil and anthropogenic activities can enter the aquatic system and alter the microbial load in the water, which further leads to the colonization of different bacteria in the gastrointestinal tract of aquatic organisms. The microbial load in the GIT of aquatic animals is normally 10^2 -10^9 CFU/g (Kim et al., 2007). Probiotic candidates’ potential has been evaluated in a variety of settings, including semi-intensive culture systems, intensive fish farms, and natural water bodies (Chantharasophon et al., 2011), where microbes obtained from outside of the hosts are referred to as "allochthonous or exogenous," and the ones recovered from the host are referred to as "autochthonous or indigenous” (Ringo et al., 2016).

Selection of probiotics

The selection process of probiotics can be represented as follows.

Source organism

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Isolation of probiotic strain

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In-vitro examination of probiotic strain

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In-vivo assessment

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Challenge study, Performance study

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Approval from the government agency

↓

Commercialization of probiotics

Types of probiotics

Probiotics are grouped into two categories based on their mechanism of action. They are gut probiotics and water probiotics. Gut probiotics are normally administered through feed which helps to improve the gut Microflora. Water probiotics are administered in the aquatic environment which intakes all nutrients from the water and the harmful bacteria are eliminated from the system due to lack of nutrients.

Types	Description
Non-viable probiotics	Probiotics with dead microorganisms
Freeze-dried probiotics	These probiotics will rapidly die upon leaving refrigeration
Fermented probiotics	These are probiotics that are produced through fermentation
Viable probiotics	These are live microorganisms, have a protocol to be counting, and are very stable and efficacious

Probiotics in aquaculture

Fish raised in an aquaculture facility are highly influenced by the microorganisms in the surrounding water (Verschuere et al., 2010). Eukaryotes and commensal bacteria thrive in the aquaculture habitat, while opportunistic pathogens grow under favorable environmental conditions (Moraity 1998). Opportunistic pathogens such as *Vibrio* spp. invade the host through the gut and invade fish through the gills and skin (Weber et al., 2010). The Firmicutes phylum contains some of the most investigated probiotic candidates, such as LAB (lactic acid-producing bacteria) and *Bacillus* spp (Amoa et al., 2019, Azad et al., 2019, Balcazar et al., 2008, Venkat et al., 2004). Lactic acid bacteria can survive acidic pH and bile salts, allowing them to live in the gastrointestinal tract despite not being acclimated to the aquatic environment

(Merrifield et al., 2010b). These bacteria can colonize the intestinal mucus, whereupon they aid in the digestion and absorption of food, boosting the fish's growth and development.

Mode of administration of probiotics

Probiotics in aquaculture could be given in a variety of ways, including feed, injections, and direct exposure to water. Probiotics can be used alone or in combinations (Hai et al., 2015).

Feed additives, water additives and injection

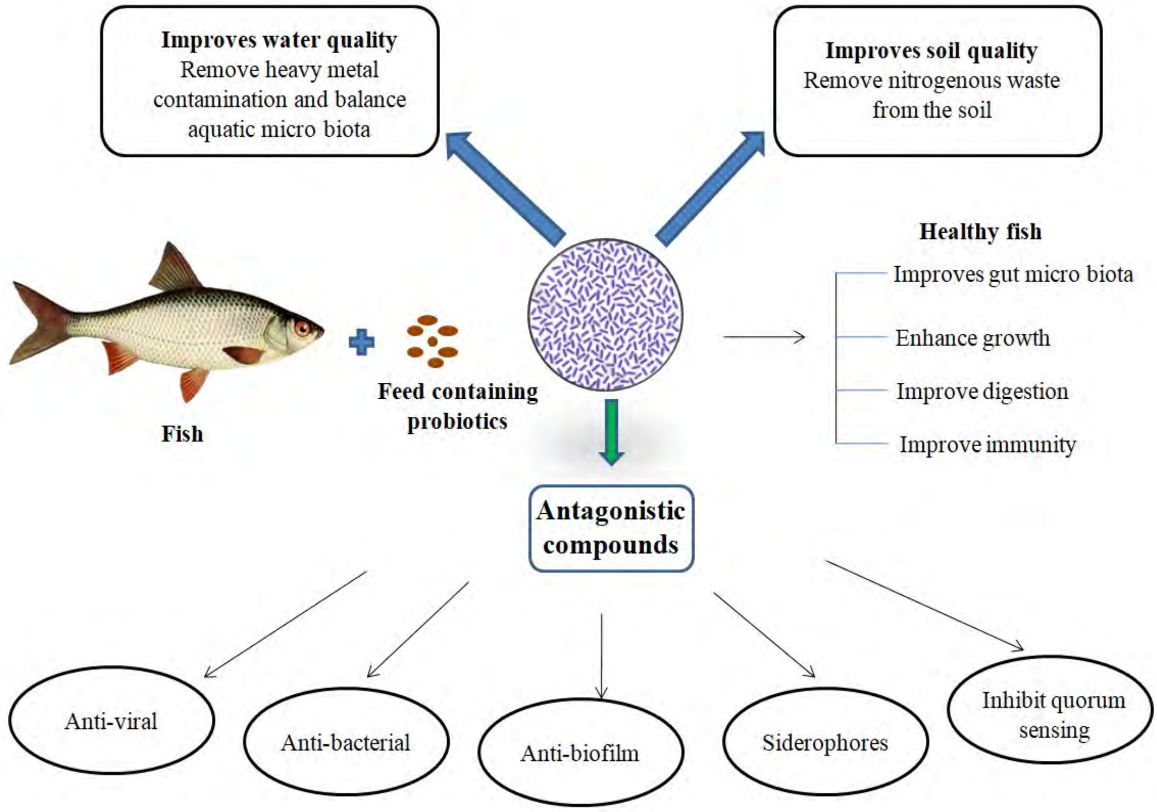
Incorporation of probiotic combinations into the feed is by far the most typical way of probiotic administration. Melo et al., 2021 reported that 92.8 % of probiotics are given as feed, followed by direct addition to water (4.8%) and in live food (1.8%) in fish culture systems. In aquaculture; probiotics such as bacterial strains, yeast, and extracted compounds are commonly used as food supplements. Dietary supplementation of probiotic strains of *Lactobacillus plantarum* has resulted in better growth and increased immunity in *Pangasius larnaudii* (Silarudee et al., 2019). Sahandi et al., 2019 reported that *Bifodobactrium* strains given as feed additive have improved the growth and nutrient utilization in rainbow trout fry. There are several reports suggesting that probiotics can also be administered through the water as an additive (Gopi et al., 2016, Gupta et al., 2016). In the sea bream, probiotic *Vibrio lentus* administered through water at a concentration of 10^6 CFU/ ml significantly altered gene expression, including immune response, cell proliferation adhesion, Reactive Oxygen Species, and iron transfer (Schaek et al., 2017). In addition to the above methods, probiotics can also be given as injection. Injection of *Enterobacter* spp. through intramuscular route enhanced the immunity in rainbow trout (Laptra et al., 2014).

Single and combinations of probiotics

Probiotics come in a variety of forms, including multi-strain probiotics, probiotics with bioactive compounds, and probiotics with fermented products. The majority of research on probiotics in aquaculture has concentrated on single probiotics, while probiotic combinations are more effective**.** Multi-strain probiotics have the benefit of being more sensitive to pathogenic organisms and active against a variety of hosts (Pannu et al., 2014). Multi-strain probiotic has a positive effect on the growth and survival of *Labeo rohita* fingerlings (Jha et al., 2014).

Beneficial effects and mode of action of probiotics in aquaculture: Figure 1

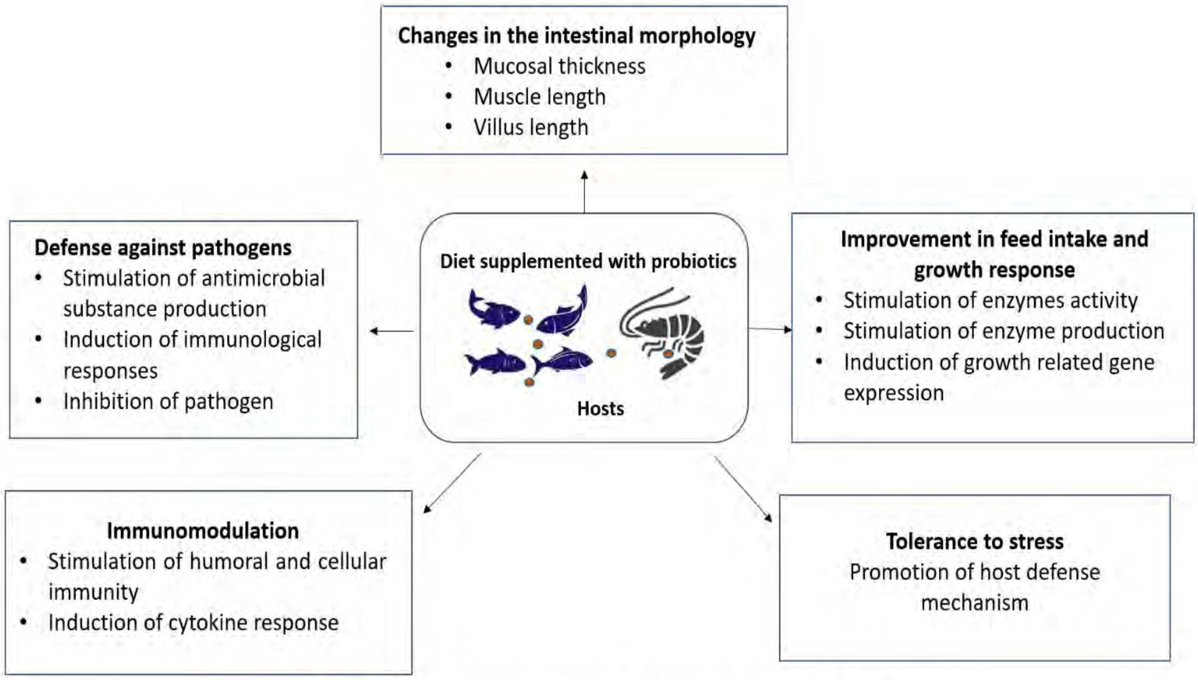
The threat of disease development inside the aquaculture sector stimulates probiotic research and analysis to build more sustainable aquaculture. With the increasing public awareness of the use of antibiotics, it's not surprising that the probiotics for aquaculture are growing at a quick pace. Probiotics have now been recommended by the Food and Agricultural Organization (FAO) for improving aquatic environmental quality by reducing mortality (Subasinghe, 2005). *Bacillus*, *Lactobacillus,* and *Bifidobacterium* are by far the most often used probiotic bacteria. Different species of *Lactobacillus*, *Bifidobacterium* and *Streptococcus* are used as probiotics in aquaculture which include *L acidophilus, L. casei, L fermentum, L. plantarum, L. salivarius, B. bifidum, B.lactum, B. breve, S. boulardii, S. thermophiles* and *S. cremonis* (Reda et al.,2018).



Application of probiotics in Aquaculture: Figure 1

In the Indian aquaculture industry, intensive and semi-intensive farming practices have emerged as one of the most practical and viable choices for meeting the nutritional needs of a rapidly growing population. Furthermore, the use of new techniques, such as the administration of probiotics, has increased total production and quality (Bandyopadhyay et al., 2015). *Bacillus* spp., *Lactobacillus* spp., *Bifidobacterium* spp., *Enterococcus* spp., *Streptomyces* spp., *Carnobacterium* spp., and yeast are the most often employed probiotic bacteria in aquaculture today (Van Doan et al., 2020).

According to studies, Gram +ve bacteria (*Bacillus* species) are used as probiotics to improve water quality. Gram-positive bacteria, particularly *Bacillus* species, were shown to be highly efficient at converting organic materials to CO_2, slime, or microbial biomass. Gram-positive appears to be superior to Gram-negative in investigations. Producers can also manage the development of gaseous and particulate organic carbon during the growth period by ensuring a high standard of probiotics inside the production pond, according to the researchers (Mohapatra et al.,2013). Nitrifying probiotic bacteria are advantageous because they can substantially increase the microbial content in the water and improve the water quality by removing ammonia and nitrate toxicity (Zorriehzahra et al. 2016). Temperatures, acidity, dissolved oxygen, ammonia, and hydrogen sulphide in rearing water were also determined to be of higher quality after the administration of probiotics. Probiotics provide a favorable and healthy environment in aquatic systems for prawn and shrimp larval rearing (Banerjee et al. 2010).



Overview of beneficial effects of probiotics on fish and shellfish

Table 1. Probiotic species used in finfish aquaculture, source and beneficial effects to the host species

Probiotic species	Host	Beneficial effects	Reference
Bacillus amyloliquefaciens COFCAU-P1	*Labeo rohita*	Disease resistance against *A.hydrophila*	Khan et al., 2022
Bacillus amyloliquefaciens *Bacillus subtilis* *Bacillus megaterium*	*Labeo rohita*	Increased survival against *A. hydrophila* infection	Saravanan et al.,2021
Saccharomyces cerevisiae	*Labeo rohita*	Growth performance, hematological parameters, improved feed utilization	Jahan et al.,2021
Lactobacillus fermentum	*Cirrhinus mrigala*	Better growth, hematological parameters, improved feed utilization	Krishnaveni et al., 2021
Bacillus methyllotrophicus *Bacillus licheniformes*	*Labeo rohita*	Increased survival against *A. hydrophila* infection	Mukherjee et al., 2019
Bacillus amyloliquefaciens	*Labeo rohita*	Increased antibody concentration, stress reduction	Nandi et al.,2018
Bacillus subtilis *Lactobacillus rhamnosus*	*Labeo rohita*	Enhanced feed digestibility	Munirasu et al., 2017
Bacillus subtilis *Terribacillus saccharophillus*	*Labeo rohita*	Increased growth and immunity	Kalarani et al.,2016

Saccharomyces cerevisiae	*Labeo rohita*	Increased growth and immunity	Bandopadhyay et al., 2015
Bacillus subtilis FPTB13	*Catla catla*	Immunomodulation and disease resistance	Sangama et al., 2015
Bacillus subtilis *Pseudomonas aeruginosa* *Lactobacillus plantarum*	*Labeo rohita*	Highest survival rate against *A. hydrophila* infection	Giri et al., 2014
Bacillus subtilis *Lactobacillus lactis* *Saccharomyces cerevisiae*	*Labeo rohita*	Increased survival against *A. hydrophila* infection	Mohapatra et al., 2014
Bacillus cereus	*Penaeus monodon*	Growth promoter	Navinchandran et al., 2014
Lactobacillus plantarum VSG3	*Labeo rohita*	Improved growth, immunity and disease resistance	Giri et al.,2013
Bacillus amyloliquefaciens	*Catla catla*	Improved growth, immunity and disease resistance	Das et al., 2013
Lactobacillus rhamnosus	*Oncorhynchu s mykiss*	Improved Blood parameters	Panigrahi et al., 2010
Bacillus NL 110 *Vibrio NE 17*	*Macrobrachi um rosenbergii*	Increased growth and immunity	Mujeeb et al.,2010

Conclusion

Even though there are substantial research on the efficacy and actions of probiotic strains, many aspects remain unanswered. Additional and future research could focus on gut bacteria transcriptome and proteome profiling, host/microbe interactions, interactions among gut microorganisms, gut immune status, antioxidant status, antagonistic activity, and knowledge on the side effects of probiotics. Aquaculture is indeed one of the world's fastest-growing industries, accounting for more than 50% of world seafood production. Aquaculture offers a vital supply of nutritious food for human consumption; however, diseases in the fish farming have a negative impact on the nation's socioeconomic status and economic development. Because antimicrobial agents used in therapeutic strategies have side effects including residual toxic effects, emerging antimicrobial resistance, immune system suppression, and reduced customer desire for drug-treated fishery products available in the market, non-antibiotic-based, eco-friendly alternatives are in high demand for aquatic animal health management.

Probiotics are an excellent alternative sustainable option of beneficial microorganisms with strong antimicrobial activity, and immunostimulatory abilities to boost health and wellbeing to enhance growth and yield, strengthen the immune function, and mitigate the adverse effects of reactive oxygen species. In order to recommend potent therapeutic, bacteria-based approaches to enhance the health, production, and economic growth of the aquaculture sector, an interactive approach among academics, researchers, growers, and fish sector owners is needed to concentrate and start exploring the specific elements of bacteria host interactions bestowing the potential significant improvements in various immune function triggered by different bacterial species. The synthesis of probiotics ought to be feasible on a broad scale with low operating costs. They ought not to be regarded as just a 'magic elixir,' but instead as a source of nourishment.

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