

Application of some Essential Bacterial Probiotics in Aquaculture: A Review

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Abstract

Aquaculture plays an essential role in maintaining food security and sustainable lives for the world's ever-growing population. Due to the expansion, intensification and variety of aquaculture methods, aquatic animals confront significant challenges, including as disease outbreaks, decreasing output, and risks to the sustainability of the sector. Poor soil conditions in aquaculture cause higher mortality, a higher prevalence of diseases and lower yields of aquatic organisms. Overfeeding, dead animals and feces from cultured organisms like fish and shrimp are the main causes of soil deterioration in aquaculture ponds. The two main compounds that cause issues are hydrogen sulfide (H₂S) and ammonia (NH₃). In order to prevent diseases and promote growth, antimicrobial medications, insecticides and disinfectants have been used in aquaculture. This has resulted in the evolution of bacterial strains that are resistant to these chemicals. Probiotic usage has been suggested as a potential remedy for various problems. Probiotics are a live beneficial bacterium that are ingested through food or water which helps to maintain a healthy balance of microorganisms inside the body. The growth of dangerous pathogens is inhibited by the bacteriocins, siderophores, lysozymes, proteases, and hydrogen peroxides produced by probiotic bacteria. This review contains various ways in which probiotics can be used in aquaculture systems. Thereby, probiotics assist the host animals by improving development and nutritional digestibility, boosting disease resistance, and improving the quality of the culture water.

Keywords: Probiotics, Aquaculture, Application, growth promoter, Improved water quality

INTRODUCTION

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Aqua feeds and aquaculture health management are extremely important to a sustainable aquaculture economy. Aquaculture produced over half of the world's fish in 2019, and it is still the industry with the strongest growth rate [1]. The Food and Agricultural Organization (FAO) of the United Nations [2] reports that the proportion of aquaculture's contribution to global fish production climbed from 25.7% in 2000 to 46% in 2018. The average yearly increase in fish consumption from 1961 to 2017 was 3.1%, exceeding the 1.6% global population growth rate as well as increases in the consuming of all other foods high in animal protein (meat, dairy, milk, etc.). Fish consumption contributed 7% of all proteins consumed and 17% of the global population's animal protein intake in 2017 [2]. In the short time of the antibiotic era—roughly 60 years—tons of antibiotics have been dispersed across the biosphere globally. Twelve thousand tons of the eighteen thousand antibiotics

generated annually in the United States for medicinal and agricultural which contribute toward the non-therapeutic treatment of cattle for growth promotion [3]. Global aquaculture production has generally increased faster than global population expansion, and it is regarded as one of the most important food production industries. Fish health is now a major concern for aqua culturists due to the rising commercialization and intensity of aquaculture practices [4]. Degradation of the rearing environment, particularly water quality is a major problem because it is crucial to aquatic creature's health [5,6]. Poor feed conversion ratios and high mortality rates can lead to a decline in fish output [7]. In the past few decades, antibiotics have been utilized as a conventional approach to manage fish illnesses and to enhance development and feed conversion efficiency. Nonetheless, there is ample evidence of the growth and proliferation of bacteria resistant to antimicrobial agents [8,9]. However, antibiotics also cause antibiotic residue to build up in fish products, making them unsafe for human consumption. Antibiotics hinder or kill the beneficial micro biota in the gastrointestinal (GI) ecosystem. Excess ammonia in pond water and sediment, which is brought about by an overabundance of feed, feces, and dead algae deposited on the bottom of the pond, is another significant issue our Indian shrimp culture farmer must deal with. As a result, shrimps are exposed to harmful gasses such NH_3 , NO_2 , and H_2S which further contributes to eutrophication in the culture system and causes stress in the animals. This leads to the development of microbiological illnesses and high mortality rates [10]. Aside from the impacts of drug residues, antimicrobial-resistant microorganisms in aquaculture pose a public health issue. For instance, conjugation between the human-originating bacteria *Escherichia coli* and the bacteria causing fish diseases *Aeromonas salmonicida* has resulted in the transfer of plasmid-borne resistance genes, some of which are harmful to humans [11]. There are two methods by which antibiotic resistance is developed: chromosomal mutation or plasmid acquisition. While resistance plasmids can spread quickly to other bacteria, chromosomal changes cannot be passed laterally, leading to a large proportion of pathogenic bacteria that quickly acquire mediated plasmid-resistance [12]. Utilizing biotechnological approaches, the concerns of antibiotic resistance, disease, and seafood demand have been managed. Probiotic utilization is one of the more successful biotechnological applications in aquaculture. Probiotics are safe, live microorganisms that improve water quality, enhance nutrition and immune response in host species, and aid in the competitive exclusion of pathogenic bacteria by producing compounds that inhibit them [13–16].

PROBIOTICS

The word probiotic is Greek term in which "pro" and "bios," in the name "probiotic" signifies "for life" [17]. Parker was the first to coin the term "probiotic" (1974). Probiotics are defined as "organisms and substances which contribute to intestinal microbial balance" in his original definition [18]. According to a recent proposal by [19], probiotics in the context of aquaculture can be defined as "live or dead, or even a component of the microorganisms that act under different modes of action in conferring beneficial effects to the host or to its environment." The term "live microbial food product which benefits its host (human or animals) by enhancing the microbial balance in the body" was added by Fuller [20], who also stated that it would work in a variety of extreme salinity and temperature fluctuations.

Probiotics are defined by the World Health Organization [21] as living organisms that, when given in appropriate amounts, boost the host's health. The notion of probiotics in aquaculture was suggested to include microbial "water additives" by [22]. According to Anjana and Tiwari [23], probiotics can be administered orally, intravenously, or immediately submerged in water. They can be used separately or in combinations [24]. Probiotics have been the subject of an increasing number of studies, and it is now possible to review the status of the science around them, from their empirical application to their scientific methodology [25, 26].

SELECTION OF PROBIOTICS

The safety and production of desired effects should be the main criteria for choosing probiotics. Prior to reaching the consumer, they must also maintain their ability during production, manufacture,

distribution, and storage [27]. Probiotics must be carefully chosen because the incorrect strains may negatively impact their hosts [28][29]. The following sequence is involved in the process of choosing probiotics [30]. Sequence of probiotics selection depicts in Figure 1.

- It is necessary to choose a microorganism source.
- By applying selective culture, the microbe that will be used for the task is isolated and identified.
- A new culture with just the colonies of interest is made for the purpose to perform an in-vitro analysis (pathogen inhibition, pathogenicity targeting species, host resistance conditions, etc.).
- Research on a small and large scale exploring in-vivo supplementation is carried out to determine whether the host species really benefits from it, provided that there are no restrictions on the application of the target species. The probiotic with the most favorable results may eventually be produced and marketed.

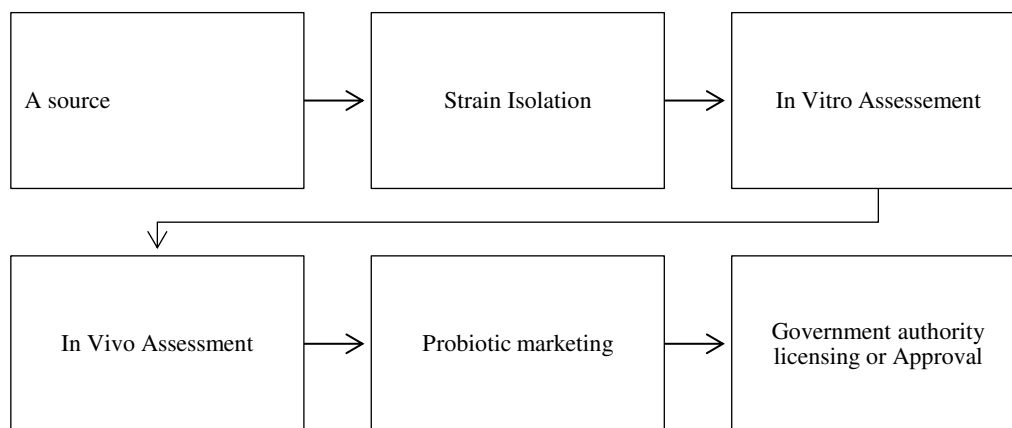


Figure 1. Sequence of probiotics selection
Source: adopted from modified [31]

ROLE OF PROBIOTICS IN AQUACULTURE

Aquaculture uses probiotics as part of a practice known as bio prophylaxis, which is the use of living microscopic organisms to prevent illnesses in aquatic animals. The production of bacteriocins by the beneficial microorganism prevents pathogenic organisms from infecting the host or causing diseases is how bio prophylaxis is accomplished [32] (Figure 2).

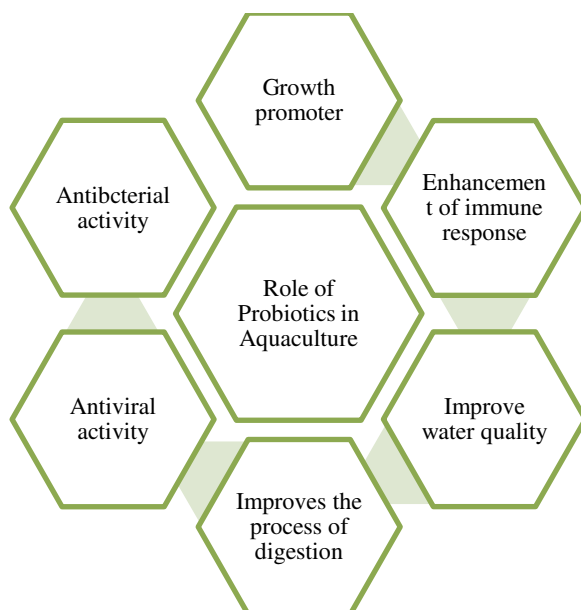


Figure 2. Schematic representation of the role of probiotics in aquaculture.

Growth promoter: The direct impact of probiotics on fish development performance, whether through increased nutrition intake or by supplying nutrients, is one of the most anticipated effects of utilizing bacterial probiotics [33]. Unlike the risk factors linked to commonly used commercially available growth enhancers, [34, 35], the growth-promoting action of probiotics on the fish is not connected with detrimental health effects [36, 37]. As probiotics are continually added to fish cultures, they stick to the intestinal mucosa of the fish and develop and exercise their multiple benefits. The author [31] observed that probiotic microorganisms have a more substantial multiplication rate compared to the rate of expulsion, which allows them to colonize the intestinal tract when administered over an extended period of time. Additionally, probiotics are thought to be an eco-friendly form of treatment. Probiotics can be added to food in the form of living microbes in the gastrointestinal system, establish a balanced native micro floral population [38]. The probiotic *Streptococcus* strain added to the diet of Nile tilapia (*Oreochromis niloticus*) resulted in a considerable increase in the fish's crude protein and crude fat content and in just nine weeks of culture, the fish's weight jumped from 0.154 g to 6.164 g [39]. In an effort to employ probiotics as a tilapia (*Oreochromis niloticus*) growth promoter, Yassir *et al* [40] found that *Micrococcus luteus* produced the greatest growth output when used as a probiotic and the best feed conversion ratio when the same organism was used. The application of *Bacillus sp.* as a probiotic has been shown to increase fish survival by allowing the bacteria to colonize the fish's digestive system and culture water [41–43]. Probiotics exhibit varying degrees of performance. For instance, *Saccharomyces cerevisiae* as well as *Bacillus subtilis* showed higher final weights (255.31 g) and specific rate of growth (SGR) (0.77%) at varied concentrations [44]. The effectiveness of probiotic application varied depending on a number of characteristics, including species composition, treatment level, frequency of administration, and environmental circumstances [45, 43], making it challenging to directly evaluate different probiotic research.

Enhancement of immune response: Probiotics have the ability to improve aquaculture species' numerous immunological characteristics. By boosting the host immune system and stimulating both cellular and non-specific immunity in the body, probiotics can prevent the spread of pathogens [46]. According to Taoka *et al* [47], Tilapia (*Oreochromis niloticus*) treated with viable probiotics exhibited enhanced non-specific immune response as measured by lysozyme activity, neutrophil migration, and bactericidal activity. Lysozyme, a protein present in many vertebrates, including fish, serves as the primary defense against the invasion of Gram-positive and some Gram-negative bacteria, it does this by cleaving the β -1, 4 glycosidic bond between N-acetyl glucosamine and N-acetylmuramic acid in the peptidoglycan layer of bacterial cell walls [48,49]. The lysozyme levels can rise resulting from feeding with diets supplemented with probiotics. It has been shown that giving *Clostridium butyricum* bacteria to rainbow trout orally increased their ability to fend off vibriosis by boosting leucocyte phagocytic activity [50]. Studies have also shown that providing high doses of probiotics through diet for an extended period of time periods influences the respiratory burst activity of *O. niloticus* [51], *R. canadum* [52], and *L. rohita* [53,54]. Probiotic bacteria also compete with one another for resources in aquatic conditions and locations for attachment in the gut. They change the infections' enzymatic activity. They have demonstrated immune-stimulatory properties [55]. Probiotics have a major positive impact on the development of juvenile aquaculture species' immune. It is noted that because the immune systems of fish larvae, shrimp, and other invertebrates are not as developed as those of adults, their immunity to infection is mostly derived from nonspecific immunological responses [56].

Improved water quality: According to Zhou *et al* [57], probiotics have a lot of potential and it's crucial to give fish a healthy habitat. Water quality was monitored in a number of experiments when probiotic strains, particularly those belonging to the gram-positive species *Bacillus*, were added. Most likely because this type of bacterium converts organic stuff into CO₂ more effectively than gram-negative bacteria. It has been proposed that fish farmers can reduce the build-up of soluble and insoluble carbon from organic matter during the season of growth by keeping high probiotic levels in their production ponds. Furthermore, this may maintain a balance in phytoplankton production [58]. Ammonia compounds, which are extremely harmful to fish, quickly accumulate when fish excrete nitrogen waste

in the form of NH_3 or NH_2^+ [59]. In their investigation, Nimrat *et al* [60] examined the water quality in treatment and control ponds and they demonstrated that how the introduction of blended probiotics (*Bacillus sp.*) impacts the development of advantageous bacteria. Since the levels of ammonia, pH, and nitrite in the water were notably lower, they serve as enhancers to improve the quality of the water. Through the nitrification process, ammonia-oxidizing bacteria convert ammonia to nitrite NO_2^- while nitrite-oxidizing bacteria convert nitrite to nitrate NO_3^- . By doing this, hazardous ammonia buildup in fish rearing facilities is avoided. In an aquaculture plant, aerobic denitrifiers are thought to be ideal choices for reducing nitrate to nitrogen gas (N_2) [61]. Probiotics derived from plant sources, such as yucca extract, potassium ricinoleate, tannic acid, and citrus seed extract, as well as beneficial bacterial species from the genera *Nitrobacter*, *Pseudomonas*, *Enterobacter*, *Cellulomonas*, and *Rhodopseudomonas*, have also been reported to have been used in culture systems noted to have a significant improvement in water quality [62,63]. Other factors including such as pH, temperature, dissolved oxygen, ammonia, and hydrogen sulfide in culturing water were shown to be of better quality after the application of probiotics. Thus, probiotics keep the aquatic system's shrimp and prawn larval culture in a good and healthy environment [64,65].

Improve the process of Digestion: It is said that through the processes of both aerobic and anaerobic decomposition, microbial metabolism contributes significantly to biogeochemical processes and cycles in the aquatic environment. Probiotics are crucial for the recycling of organic materials. They also aid in the regeneration of nutrients and the dissolution of insoluble inorganic salt. [66]. According to El-Haroun *et al* [67] and Mohapatra *et al* [68], the majority of probiotics colonizes the host and influence the digestive processes by increasing the number and generation of microbial enzymes. This improves the gastrointestinal microbial balance and as a result the digestibility, absorption, and utilization of feed. Exogenous enzymes are defined as those that are released by the probiotic bacterium. According to Mohapatra *et al* [69], these exogenous enzymes can aid in the induction of endogenous enzymes that can withstand a wider pH range than those indigenous enzymes, thereby delaying the digesting phase [70]. Proteases are an example of an enzyme that secretes and breaks peptide bonds to release free amino acids that the host can absorb [71]. Because *Bacillus* secretes a variety of exoenzymes, including lipases, carbohydrases, and proteases, which enhance enzymatic digestion and complement the actions of fish, it can help with digestion [72]. Thus, adding probiotics to the meal, improves the efficiency of nutrient absorption [73].

LIMITATIONS OF USING PROBIOTICS

Probiotics in aquaculture are limited by the inability of strains to be produced commercially and subsequently demonstrated on a large scale, the challenge of demonstrating efficacy at the farm level, and the incapability of companies to undertake comprehensive research on how to make products specifically for aquaculture [74]. Probiotics can be developed in either static or low water exchange environments (re-circulatory system); they can be used as proactive preventive measures in advance, preventing the disease rather than treating it. They work best when applied as soon as the water medium is sterilized before becoming contaminated with other microbes [75]. The risk that pathogenic bacteria used as probiotics will mutate and contaminate aquaculture products; probiotic overuse; and finally, the high production costs that will be a burden for small-scale aquaculture farms are all mentioned in an article published in International Magazine for Animal Feed and Additives Industry (2023). These factors could lead to the introduction of non-native microbes into the aquatic environment, which could alter the ecology and have an impact on native species [76].

CONCLUSIONS

Probiotics have potential applications in aquaculture, but furthermore some studies are yet essential as to how we can use some selected probiotics in aquaculture which does not have any negative effect on the health of an organism. It is a viable substitute for chemotherapy and antibiotics, which are harmful and have unfavorable long-term effects. As is well known, 60–80% of operating costs in intensive aquaculture operations are related to feeding and thereby probiotic usage can reduce feeding

costs by improving feed consumption and, consequently, which in turn improve the growth performance. Probiotics assist the host animals by improving development and nutritional digestibility, boosting disease resistance, and improving the quality of the culture water. Selecting the appropriate strain from the favorable traits is the most important stage in boosting the effectiveness of probiotics. Nonetheless, further investigation should be done in estimate of the right amounts and doses added to meals, in a group of bacteria, and in a variety of physical and chemical elements that influence the probiotics need to be researched. Probiotics have potential applications in aquaculture, but further study is necessary. They may have a beneficial or detrimental effect on the surrounding environment as well as the aquaculture animals. Although there are metal removal methods using physio-chemical approaches, they are costly and have a negative environmental impact. Understanding mechanisms is necessary to ensure its long-term commercial use, despite of a slightly less rigorous approach. This is because it helps determine any potential environmental side effects, such as whether adding probiotics will permanently alter the community of microbial species and how this will affect the turnover in inorganic and organic molecules in the specific environment. Numerous studies have demonstrated that adding the organisms more frequently and not relying solely on a single probiotic culture treatment is necessary. However, the strength of the systems, such as the necessary concentration of probiotics, the frequency of additions, the impact of temperature changes, etc. has not been measured therefore some aspects in detail are yet to be studied for further development.

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