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Exploring the Impact of Probiotics on Aquaculture: A Comprehensive Overview

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ABSTRACT

Aquaculture is the fastest growing Agricultural sector which provide food security to the growing population of the world. Disease outbreak is the main challenge to the aqua farmers. Probiotic is alternative way to prevent the diseases and also increase the production of aquaculture. The purpose of probiotic bacteria in aquaculture is to prevent infectious illnesses as outlined in this review article.; improvement of water quality; improve the digestion, nutrients absorption and finally improve the overall growth of the cultured species and also increase the stress tolerance power of the aquaculture species etc. The review article includes general knowledge such as definition of probiotic, history behind the probiotics, probiotic strains, mechanism of action, the selection criteria of probiotic for particular species and their dose to get desired benefits and their application methods. Data was gathered and organized chronologically from a variety of secondary sources, including journals, research papers, articles, reports, and electronic media. This review's primary goal is to raise knowledge of the benefits of probiotics in aquaculture as a substitute for other chemicals and antibiotics.

KEYWORDS

Aquaculture, Probiotics, Beneficial microorganisms, Disease control, immunostimulants, Growth promoters, Antibacterial activity, Fish health.

Introduction

Aquaculture stands out as a rapidly growing sector, holding great potential to meet the demand for animal protein and contribute to food security for the growing global population. High populational country such as India have great demand of these Aquaculture product but due to limited resources, the production can't meet the demand. The farmers are turn into intensification of aquaculture with high stocking density to get more yield. As aquaculture methods have become more intensive, stressful conditions have emerged for the ecosystem and the cultured animals [Dawood, M. A. O, 2016]. As a result, disease outbreak being progressively documented, now a days which is one of the most challenging threads for the aquaculture industry. In recent decades, there has been an increased use of chemical compounds and antibiotics in managing fish diseases and enhancing growth and feed efficiency (Suguna, 2020). However, this has raised several issues, such as the development of bacterial resistance, the presence of antibiotic residues in animal tissues, and disruptions to the gut microbiota of aquatic species (Martínez Cruz, 2012). Consequently, the adoption of probiotics emerges as a practical alternative for pathogen inhibition and disease control in aquaculture species.

Definition of probiotics and its history

The term "probiotic" originates from the Greek words "pro" and "bios," meaning 'pro-life' [Gismondo, M. R., Drago, L., & Lombardi, A; 1999]. Coined by Parker in 1974, the term was initially defined as "organisms and substances that give intestinal microbial balance" [Suguna, 2020]. Fuller (1989) [4] later refined the definition as a "live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance" [1]. Probiotics are commonly referred to as "friendly," "beneficial," "good," or "helpful" bacteria due to their role in maintaining gut health [1]. More recently, probiotics have been defined as "live microorganisms" that, when administered in sufficient amounts, provide health benefits to the host (FAO / WHO, 2001). [1]. In single word 'probiotic is the live microorganisms that beneficially effect the host animal'. Given the ongoing interaction between the intestinal microbiota in aquatic animals, the term "probiotic" is described as a live microbial supplement that imparts advantageous effects, such as (i) altering the microbial community associated with the host or its surroundings, (ii) optimizing feed utilization or augmenting its nutritional content, bolstering the host's defense against diseases, or (iii) elevating the quality of its surrounding environment (Verschuere et al. 2000) [8,9]. In broad terms, probiotics in aquaculture refer to live and/or inactive microbial supplements or water additives. These may be in the form of single or multiple strains, or in combination with prebiotics or other immunostimulants. Their administration aims to improve the quality of rearing water, boost the physiological and immune responses of aquatic animals, and decrease reliance on chemicals and antibiotics in aquaculture [9]. In 1986, probiotics were initially employed to assess their capacity to enhance the growth of aquatic organisms [77]. Subsequently, their application extended to enhancing water quality and managing bacterial infections. Now a days, there is documented evidence that probiotics have been shown to enhance stress tolerance, promote reproduction, and enhance nutritional digestion.

Guidelines for Choosing Probiotics in Aquaculture

The main objective to the use of probiotics is to maintain good relationship between friendly and pathogenic microorganism in the Gastrointestinal track and skin mucus of fish and shell fish as well as maintain the surrounding environment such as water quality and soil quality. So, a good probiotic should have some unique characteristics.

According to Fuller (1986) [4] following characteristics should meet to selection a good probiotic in aquaculture-

- The chosen strain should demonstrate a positive impact on the host animal, such as enhanced growth or disease resistance.

- It must be non-pathogenic and non-toxic.
- The strain should exist in the form of viable cells, preferably in substantial quantities.
- It should exhibit the ability to survive and metabolize in the gut environment, including resistance to low pH and organic acids.
- Stability is essential, ensuring the strain remains viable for extended periods under storage and field conditions.

Probiotic strains

Currently there are plenty of commercial probiotic products are available in the market that are prepared by various bacterial strains, yeast, micro-algae etc.

Gram-positive bacteria exhibit greater effectiveness compared to gram-negative bacteria among bacterial species.

- Gram positive bacterial strains

Gram-positive lactic acid bacteria were primarily referred to as "probiotics," with specific examples coming from the genera *Bifidobacterium, Lactobacillus, and Streptococcus* [6,14] beside these *Bacillus, Carnobacterium, Enterococcus*, *Micrococcus* etc.

- Gram negative bacterial strain

Although gram-negative bacteria mainly pathogenic bacteria but some gram-negative bacterial strains show beneficial to host animal those are-

Aeromonas hydrophila, Alteromonas sp, Pseudomonas fluorescens

Vibrio alginolyticus, Vibrio fluvialis etc.

- Yeast

Phaffia rhodozyma, Saccharomyces cerevisiae, Debaryomyces hansenii

- Micro algae

Tetraselmis suecica

Examples of probiotics recognized as biological control agents in aquaculture

As per Janardana Redddy's research in 2015 [15], *Carnobacterium divergens* sourced from the intestines of Atlantic salmon is applied to Atlantic cod fry. *Bacillus megaterium*, *B. subtilis*, *B. polymyxa*, *B. licheniformis*, and similar strains are utilized for channel catfish. *Bacillus circulans*, extracted from the intestine of *Labeo rohita*, is employed for *Labeo rohita*. Additionally, *Vibrio hepatarius*, *Vibrio* sp, *Bacillus* sp, *Saccharomyces cerevisiae*, *Phaffia rhodozyma*, and others are used for *P. vannamei*.

Dosages:

A probiotic should be taken at the recommended dosage for that species in order to have the greatest possible benefit. The type of probiotics, the physiological state of fish, the conditions of growing, and the particular purpose of the application all influence the right dosage of probiotics Hey (2015) [9]. As stated by Bagheri et al. (2008) [74], supplementing diets with B. subtilis and B. licheniformis at a concentration of 10^9 CFU/g improved the performance of rainbow trout fry, including factors such as feed conversion ratio (FCR), specific growth rate, weight gain, and protein efficiency ratio. According to Sahandi et al. (2019), feeding probiotic Bifidobacterium strains at 1×10^7 CFU/g to rainbow trout resulted in increased growth performance, but not with 3×10^7 CFU/g. [75]. This shows that increased growth is not always a direct outcome of higher probiotic content in diet. In aquafeeds, dosage is a crucial factor to consider when adding functional nutrients [68]. As per the findings of Gourab et al. (2020) [76], providing Pabda catfish, *Ompok pabda*, with dietary commercial probiotics exceeding 0.2% results in a reduction in weight gain. According to this study, a concentration more than or equal to that may disrupt the fish's body physiology overall and cause problems with the metabolism of fat and carbohydrates. [68].

Probiotics, both singular and combined

Probiotics are available in various forms, either administered alone or in combinations, including multi-strain probiotics incorporating plant and yeast extracts (Shaibe Hossain, 2018). While the majority of research in aquaculture has focused on the use of probiotics in isolation, combining probiotics has proven to be more advantageous [68]. Multi-strain probiotics exhibit activity against diverse aquaculture animals and increased sensitivity to infections

(Pannu et al., 2014) [69]. These multi-strain probiotics have demonstrated positive effects on the development and survival of rohu during the hatchling and fry phases but not at other stages (Jha et al., 2015) [70]. According to Hamka et al. (2020), the administration of probiotics Bacillus megaterium PTB 1.4 and Pediococcus pentosaceus E2211 together in feed yielded superior results in catfish compared to the application of each probiotic alone [71]. In the case of Nile tilapia (Oerochromis niloticus), a higher survival rate (58.33%) was observed when Lactobacillus plantarum N11 and Bacillus velezensis H3.1 were used in combination, as opposed to individual applications (Lactobacillus plantarum 54.17% and Bacillus velezensis 41.67%) (Doan et al., 2018) [72]. Furthermore, the combination of the probiotic Bacillus coagulans and the botanical extract Mentha piperita exhibited enhanced growth performance, nutrient retention, and immunity in Catla catla when compared to their separate administration (Bhatnagar & Saluja, 2019). [73]

Probiotics - Mechanisms of Action

Although not completely understood, the modes of action of microorganisms employed as probiotics are characterized as:

a) **Competition for occupancy of binding sites:** often referred to as "competitive exclusion," in which probiotic bacteria attach themselves to the intestinal mucosa's binding sites to create a physical barrier that keeps harmful bacteria from connecting. [15].

b) **Production of antibacterial substances:** Probiotic bacteria synthesize antibacterial substances, including bacteriocins and hydrogen peroxide, primarily targeting pathogenic bacteria. Moreover, they produce organic acids that lower the pH of the gastrointestinal tract, inhibiting the growth of various pathogens and the proliferation of specific Lactobacillus species [15].

c) **Competition for nutrients:** Probiotics limit the quantity of nutrients by completing the nutritional absorption process with hazardous microorganisms. One of the things that prevents harmful bacteria from growing is the scarcity of nutrients that they may consume [15].

d) **Stimulation of immune system:** By boosting the generation of antibodies, activating macrophages, T-cell proliferation, and interferon, some probiotic microorganisms have been directly associated with the stimulation of the immune response. [15].

Types of probiotics used in Aquaculture

Probiotics mainly divided into 3 category water probiotic, soil probiotic, feed probiotic.

1. **Water probiotic-**

"The water probioics contain multiple strains of bacteria like *Bacillus acidophilus, B. subtilis B. lecheniformis, Nitrobacter sp, Aerobacter and Sacharomyces cerevisiae*. Application of probiotic through water of tanks and ponds may also have an effect on fish health by improving several qualities of water, since they modify the bacteria composition of the water and sediments (Ashraf, 2000; Venkateswara, 2007) [20,21]. When probiotics are applied in culture water they multiply and over grow the pathogenic organism present in the water. Beside this Venkateswara (2007) [21] reported that probiotic bacteria are generally called bacteria which can improve the water quality of aquaculture and inhibit the pathogens in water thereby increasing production" [16]. They also increase the primary productivity of the pond.

If we see the application process then is is very simple than other probiotics. Required quantity of water probiotic mix with pond water and then apply all over the pond water surface. Before using the water probiotic, we should ensure that all aerator of pond is switched on. Generally, water probiotics is use at morning time.

2. **Soil probiotic-**

During the culture period, organic substances, un-eaten food particles, excreta are accumulated at the bottom and make sludge, these increase the ammonia, hydrogen sulfide, nitrites and other toxic gases. Bacteria like *Rhodobacter, Rhodococcus, Nitrobactor, Nitrosomonas*. Plant like *Yucca schidigera* are used to control ammonia, hydrogen sulfide, nitrites and other toxic gases.

If we see the application process then it is not simple like water probiotics because our main target is to improve soil quality and breakdown the organic substances that are accumulated at the bottom of pond , so if we use soil probiotic directly to the water then it quickly dissolve with the pond water and its effect on pond bottom will be less for that reason required amount of soil probiotic is mixed with the white sand and let them for dry and after 15 to 16 minutes this sand and probiotics mix will be apply throughout the pond .The main principle behind this is the sand particle quickly settle down at the pond bottom so the soil probiotics can start their work on bottom very efficiently.

Figure 1: Mixture of soil probiotic and white sand

3. Feed or gut probiotics-

Probiotics are added to diet together with a binder (egg or cod liver oil), and the majority of commercial preparations include either Sacharomyces cerevisiae or Lactobacillus sp (Abidi, 2003) [16,17]. Numerous health advantages have been revealed by Cercato (2000) regarding the regular use of probiotics in fish feed in the UK and other European nations [16]. FAO and WHO recommendations state that probiotic organisms used in food must be able to withstand exposure to bile and gastric acids in order to survive travels through the gut (Senok et al 2005) [16,18]. Additionally, they must be safe, effective, and able to persist during the product's shelf life. They must also be able to multiply and colonize the digestive system (Senok et al, 2005) [16,18].

Probiotics that have been added to animal feed include yeasts like Saccharomyces cerevisiae, herbs, and extracted substrates like azadirachtin. Bacterial species that have been added include Lactobacillus spp., Enterococcus faecium, Bifidobacterium thermophilum, Streptomyces spp., Micrococcus spp., and Pseudomonas fluorescens. [19].

Probiotics function as a microbial dietary supplement that enhances nutritional and physiological advantages while lowering systemic and mucosal immunity and boosting host health. By promoting digestive enzymes and preserving the equilibrium of intestinal microorganisms, they boost the feed efficiency for fish and shrimp, which in turn improves the animals' ability to absorb and use nutrients and, eventually, survive and thrive [1].

The application process of gut probiotic is very simple. The main target of use gut probiotic is improved the intestine or gut and improve the feed intake and digestibility. The required amounts of gut probiotics mixed with feed and a binder that is given to the culture species and then let them for some time to dry and after some times this probiotic contain feed is given to the culture species.

Figure 2: Mixture of gut probiotic and feed

ROLE OF PROBIOTICS IN AQUACULTURE

1. GROWTH PROMOTER

As previously discussed, probiotic-supplemented feed facilitates easier digestion by promoting the production of digestive enzymes like amylase, lipases, and proteases. This results in the provision of essential nutrients such as amino acids, fatty acids, and vitamins. Probiotics enhance nutrient absorption and utilization, ultimately contributing to overall animal growth. Some

believe that probiotics improve the taste for aquaculture species. Rotifers play a crucial role as the initial live feed for the larvae of many aquacultured species due to their smaller size and accessibility for larvae. Utilizing lactic acid bacteria, including Lactococcus casei, Pediococcus acidilactici, and Lactobacillus lactis, Planas et al. [22] achieved optimal results in enhancing the development of the rotifer Brachionus plicatilis [6]. In an attempt to utilize probiotic bacteria as a growth promoter in tilapia (Oreochromis niloticus), Yassir et al. (2002) [23] discovered that the probiotic Micrococcus luteus yielded the best feed conversion ratio and the highest growth performance. Consequently, M. luteus shows potential for use as a growth enhancer in fish aquaculture. Lactic acid bacteria were found by Noh et al. (1994) to act as growth promoters for juvenile carp but not for sea bass [16]. Additionally, Enterococcus facium has been employed to enhance growth when added to fish diets (Bogut et al., 2000). According to Irianto and Austin (2002) [24], probiotics can increase appetite and improve nutrition by breaking down indigestible components, generating vitamins, and detoxifying chemicals in the food [16]. In studies involving ornamental fish such as swordtail (Xiphophorus helleri, X. maculatus) and guppy (Poecilia reticulata, P. sphenops), the inclusion of Bacillus subtilis and Streptomyces in the feed significantly increased the growth and survival of Xiphophorus and Poecilia after 90 and 50 days of administration, respectively [6, 25, 26].

2. INHIBITION OF PATHOGEN

As I have mentioned, antibiotics have long been employed in aquaculture as a disease preventive measure. But due to various problem occurred, now probiotics is only alternative way to prevent diseases. Now a days every aquaculture product is available in market all's are 'antibiotic free products' because many countries restricted the utilization of antibiotics in organisms intended for human consumption. Pathogenic bacteria are susceptible to chemical compounds released by probiotic organisms that have bactericidal (kill the bacteria) or bacteriostatic (stop the development of pathogenic bacteria) effects [6]. Typically, the antibacterial effect is attributed to the production of antibiotics, bacteriocins, siderophores, enzymes (such as lysozymes and proteases), and/or hydrogen peroxide, along with the modulation of intestinal pH through the generation of organic acids [37]. Taoka et al. [38] demonstrated the administration of viable probiotics to tilapia (Oreochromis niloticus), resulting in enhanced resistance to Edwardsiella tarda infection. This enhancement was observed through an increase in nonspecific immune response indicators, including lysozyme activity, neutrophil migration, and bactericidal activity [6]. Probiotics such as Bacillus cereus, Paenibacillus polymyxa, and Pseudomonas sp. PS-102 have been investigated concerning shrimp to evaluate their potential as biocontrol agents against diseases caused by various Vibrio species [6,39,40]. Gómez et al. [41] reported that probiotics containing Vibrio alginolyticus strains have demonstrated improvements in the growth and survival of white shrimp (Litopenaeus vannamei). In Ecuadorian shrimp hatcheries, the use of probiotics has led to a 35% increase in output, in stark contrast to a 94% decline in production observed when antimicrobials are employed [6].

3. IMPROVEMENT OF WATER QUALITY

Fish producers are advised to maintain elevated levels of probiotics in their production ponds throughout the growth season to mitigate the accumulation of soluble organic carbon [6]. This practice also contributes to sustaining a balance in phytoplankton production [7]. Probiotics possess the potential to enhance the ecological environment by mitigating the levels of detrimental gases such as NH^3, NO^2, H_2S, and methane. They achieve this by regulating pathogenic microflora, facilitating the decomposition of undesirable organic materials, increasing the abundance of aquatic organisms, elevating the nutritional content available to aquatic hosts, and fortifying their

immunity in the culture water, as highlighted in the findings of Venkateswara (2007) [21]. Several studies have demonstrated that the addition of probiotics, especially Bacillus sp., positively impacts water quality (Verschuere et al., 2000) [57]. This is likely attributed to the superior efficiency of this type of bacterium in converting organic materials to CO_2 compared to gram-negative bacteria [6].

4. STRESS TOLERANCE

Due to high density and also during handling and transportation of fish and shell fish get stressed and also for environmental fluctuation like pH, temperature, Dissolve Oxygen, Salinity, ammonia etc. they get stressed and stress is the primary reason for any disease. Therefore, by lowering plasma glucose cortisol levels, probiotic administration dramatically increases aquaculture species' ability to withstand stress. As cortisol functions as a stress hormone, the evaluation of stress tolerance in fish exposed to ammonia revealed that those fed with the probiotic Lactobacillus plantarum exhibited a lower increase in cortisol levels compared to those fed a control diet. (Nguyen Van Nguyen, 2018). The defense against hypoxic stress in Nile tilapia is greatly enhanced when *Aspergillus oryzae* is given as a probiotic (Dawood et al., 2019). [62].

Conclusion

Probiotics are now the only available treatment option for diseases instead of antibiotics and chemical disinfectants, which have been linked to environmental contamination and safety concerns for both aquatic and human food. Probiotics can be used to create favourable environment for the culture species to increase their immunity as well as health. To enhance the effectiveness of use of probiotics on a particular species, selection of a perfect probiotic strain is a very important. Only a good probiotic strain with optimum dose can give positive impact on particular host species. Probiotic can be used as singly or in combination with multi-strain probiotics such as probiotic with yeast extract and probiotic with plant extract. Most of the study on probiotic based on the single strain probiotic but it is proved that multi-strain probiotic is more effective. Currently there are plenty probiotics product available in the market and they are made from the various bacterial strain, yeast and plant extract; among the bacterial strain gram positive bacterial strain is mostly used although some gram-negative bacterial strain such as *Aeromonas hydrophila, Alteromonas sp, Pseudomonas fluorescens, Vibrio alginolyticus, Vibrio fluvialis* etc. are used. Among the alls application method of probiotics, use as feed additives is the most effective but the most useful approach is to utilize them as water additives. Probiotics not only used for disease control but also used for growth promoter, water quality improvement, improve digestibility and increase stress tolerance of aquaculture species. As we know that probiotics can enhance the digestive enzyme activity in gastrointestinal track, so it increases the food digestibility and nutrients absorption and hence results in better feed utilization. Feeding accounts for 60–70% of operating costs in intensive aquaculture techniques. By improving feed consumption and growth performance, probiotic usage can help control feeding costs [68].

Based on the preceding discussion, it can be inferred that probiotics serve as excellent additives in aquaculture, promoting immunity, digestibility, and overall enhancing sustainable aquaculture production. Through this review article, I encourage aqua farmers and other stakeholders in aquaculture management to consider the use of probiotics as an alternative to antibiotics.

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