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Aquatic probiotics: A potential solution for eatable fishes

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Abstract
The increasing demand for sustainable and environmentally friendly aquaculture practices has intensified the search for natural solutions to enhance fish health and productivity. Aquatic probiotics, comprising beneficial microorganisms such as bacteria, yeast and fungi have emerged as a promising strategy to improve the overall health and resilience of aquatic species. This research explores the role of aquatic probiotics in promoting disease resistance, enhancing nutrient absorption and modulating the gut microbiota to support optimal growth rates in fisheries. The mechanisms through which probiotics exert their benefits including competitive exclusion of pathogens immune system modulation and the production of antimicrobial substances, are discussed in detail. Additionally, the application of probiotics in diverse aquaculture systems ranging from freshwater to marine environments is examined highlighting their potential to reduce reliance on antibiotics and chemical treatments. Challenges related to the formulation, delivery and sustainability of probiotic use in aquaculture are also addressed. The findings underscore the potential of aquatic probiotics as a sustainable solution for healthy fisheries, paving the way for more resilient aquaculture industries in the face of growing environmental and economic pressures.

Keywords: Aquatic probiotics, Nutrient absorption, Growth rates, antimicrobial substances and Aquatic species

Introduction

The increasing demand for aquaculture production today is accompanied by many challenges, such as diseases and epizootics, improvement of broodstock and domestication, development of compliant pellets and feeding mechanisms, hatchery and rearing technologies, water quality management by increasing intensification and commercialization of aquaculture production. Epizootic Ulcerative Syndrome (EUS) is a seasonal epizootic disorder that affects various species cultivated in freshwater and brackish water fish, with a complicated etiology of infection. The fungus of the genus Aphanomyces is the cause of EUS, and according to current epidemiological data, the disease can be transmitted by water and, in some cases, the movement of fish without sufficient quarantine and health certification. Of all the problems facing farmers, disease outbreaks are a major challenge factor hindering the economic and social development of the aquaculture sector in many countries (Tuan *et al*., 2013) [21]. Over the past decades and even today, antibiotics have often been used to control and prevent disease, improve growth, and aid feed efficiency performance.

Probiotics and antibiotics have quite different mechanisms of action, and most antibiotics can only treat diseases but do not necessarily overcome the basic problems that occur. The FAO/OIE/WHO (The Food and Agriculture Organization of the United Nations/Office International des Epizooties/World Health Organization) expert meeting on antimicrobial resistance in its application in aquaculture in 2017 concluded that two main hazards could be caused by antimicrobials, namely antimicrobial residues and antimicrobial resistance. While probiotics have numerous modes of action, they play an essential role in maintaining the health of aquatic organisms (Banerjee and Ray, 2017; Schar *et al*., 2020) [16, 17].

Probiotics can be used to enhance growth, improve feed utilization, strengthen immune function, and improve water quality in aquaculture (Tabassum *et al*., 2021) [18]. Generally, there are three methods used in the application of probiotics to aquatic animals, and these include the addition via supplemented pellets, addition directly to the water column in which the fish live, and addition directly via injection to the host. According to Lauzon *et al*. (2014) [19], the best way to give probiotics to cod farming is by adding them directly to the water column, which is the only method that can be applied to all stages of fish. One is bound to encounter several limitations when probiotics are added and fed via supplemented pellet/food, which live in the early larval stage of fish since the fish's digestive tract at that stage is not matured enough to aid in proper digestion. As for the method of addition directly via injection to the host, it is feared that it will increase stress, affecting fish development; thus, the most recommended method is when added directly to the water column (Sveinsdottir *et al*., 2009) [20].

Methodology

1. Selection of Probiotic Strains

A range of beneficial microorganisms was selected based on their documented probiotic potential in aquaculture. These included strains of *Lactobacillus spp.*, *Bacillus spp.*, *Saccharomyces cerevisiae* (yeast).1. Non-pathogenicity to aquatic species.2. Ability to survive in aquatic environments.3. Capability to produce antimicrobial compounds.4. Gut colonization ability and strains were obtained from PSG Institute of Medical Sciences & Research (PSG IMSR) Coimbatore, TamilNadu. India certified microbial culture collections and isolated from the gut of healthy fish species and aquatic environments.

2. Experimental Design

Controlled laboratory and pond-based experiments were Conducted to assess the impact of probiotics on fish health and growth. Experimental groups included: Control group (no probiotics) Probiotic-treated groups (with varying concentrations and combinations). Aquatic species such as Tilapia (*Oreochromis niloticus*), Carp (*Cyprinus carpio*) and Shrimp (*Litopenaeus vannamei*) were used in the study.

Fig 1: Tilapia (*Oreochromis niloticus*)

Fig 2: Carp (*Cyprinus carpio*)

Fig 3: Shrimp (*Litopenaeus vannamei*)

3. Probiotic Administration

Probiotics were administered via

- **Feed supplementation:** Mixing probiotics into commercial fish feed
- **Water inoculation:** Adding probiotics directly to the aquaculture water Dosage and frequency of administration followed manufacturer guidelines and previous literature (e.g., 10^6-10^8 CFU/g of feed).

4. Water Quality Monitoring

Parameters such as pH, dissolved oxygen, ammonia, nitrite, and temperature were monitored regularly using standard aquaculture water testing kits to ensure optimal environmental conditions throughout the study.

5. Growth and Health Assessments

- **Growth Performance:** Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), and weight gain were calculated.
- **Disease Resistance:** Fish were exposed to known pathogens (*Aeromonas hydrophila*, *Vibrio spp.*) under controlled conditions to evaluate disease resistance.
- **Immune Response:** Hematological (e.g., total leukocyte count) and biochemical (e.g., lysozyme activity, phagocytic activity) parameters were measured.

6. Statistical Analysis

All data were statistically analyzed using ANOVA and post-hoc tests (Tukey's HSD) to determine significant differences between control and treatment groups. A p-value < 0.05 was considered statistically significant.

Table 1: Screening and Selection of Potential Aquatic Probiotic Strains

Strain Code	Microorganism	Source	Non-Pathogenicity	Survivability in Aquatic Conditions	Gut Colonization Ability	Selected for Study
B1	Bacillus subtilis	PSG IMSR Culture Collection	Yes	High	High	✓
L1	Lactobacillus plantarum	Gut of healthy Oreochromis niloticus	Yes	Moderate	High	✓
S1	Saccharomyces cerevisiae	PSG IMSR Culture Collection	Yes	High	Moderate	✓

Table 2: Summary of Experimental Design for Probiotic Application in Aquatic Species

Species	Scientific Name	Experimental Setting	Group Code	Treatment Description	Replicates	Duration
Tilapia	Oreochromis niloticus	Controlled Lab Tanks	T1	Control (no probiotics)	3	60 days
			T2	Feed + Bacillus spp. (10^7 CFU/g feed)	3	60 days
			T3	Feed + Lactobacillus spp. + Yeast	3	60 days
Carp	Cyprinus carpio	Semi-controlled Pond Setup	C1	Control (no probiotics)	3	60 days
			C2	Feed + Bacillus spp. (10^7 CFU/g feed)	3	60 days
			C3	Feed + Combination (Bacillus + Yeast)	3	60 days
Shrimp	Litopenaeus vannamei	Laboratory Aquaria	S1	Control (no probiotics)	3	45 days
			S2	Water + Lactobacillus spp. (10^8 CFU/mL water)	3	45 days
			S3	Feed + Saccharomyces cerevisiae	3	45 days

Table 3: Probiotic Administration Methods, Dosages, and Frequency

Species	Group Code	Administration Method	Probiotic Type	Dosage	Frequency	Mode
Tilapia (O. niloticus)	T1	-	None (Control)	-	-	-
	T2	Feed supplementation	Bacillus subtilis	1×10^7 CFU/g of feed	Once daily	Mixed in feed
	T3	Feed supplementation	Lactobacillus plantarum + Yeast	1×10^8 CFU/g combined	Once daily	Mixed in feed
Carp (C. carpio)	C1	-	None (Control)	-	-	-
	C2	Feed supplementation	Bacillus spp.	1×10^7 CFU/g of feed	Once daily	Mixed in feed
	C3	Feed supplementation	Bacillus + Saccharomyces cerevisiae	5×10^7 CFU/g (combined)	Once daily	Mixed in feed
Shrimp (L. vannamei)	S1	-	None (Control)	-	-	-
	S2	Water inoculation	Lactobacillus spp.	1×10^8 CFU/mL of water	Every 48 hours	Added to tank
	S3	Feed supplementation	S. cerevisiae	1×10^7 CFU/g of feed	Once daily	Mixed in feed

Table 4: Summary of Water Quality Parameters During the Experimental Period

Species / Group	pH (Range)	Dissolved Oxygen (DO) (mg/L)	Ammonia (NH_3) (mg/L)	Nitrite (NO_2^-) (mg/L)	Temperature (°C)
Tilapia - T1 (Control)	7.2 - 7.6	5.8 - 6.5	0.15 - 0.20	0.02 - 0.05	27 - 28
Tilapia - T2, T3	7.3 - 7.8	6.0 - 6.8	0.10 - 0.18	0.01 - 0.04	27 - 29
Carp - C1 (Control)	7.1 - 7.4	5.5 - 6.3	0.18 - 0.22	0.03 - 0.06	25 - 27
Carp - C2, C3	7.2 - 7.6	6.2 - 6.9	0.12 - 0.19	0.02 - 0.05	25 - 27
Shrimp - S1 (Control)	7.4 - 7.8	5.6 - 6.1	0.20 - 0.25	0.03 - 0.06	28 - 30
Shrimp - S2, S3	7.5 - 8.0	6.0 - 6.6	0.12 - 0.18	0.02 - 0.04	28 - 30

Table 5: Effects of Probiotic Treatments on Growth, Disease Resistance, and Immune Parameters

Species / Group	Weight Gain (g)	Specific Growth Rate (SGR %/day)	Feed Conversion Ratio (FCR)	Survival Post-Pathogen Challenge (%)	Total Leukocyte Count (cells/mm³)	Lysozyme Activity (U/mL)	Phagocytic Activity (%)
Tilapia - T1 (Control)	35.2 ± 2.5	1.10 ± 0.05	2.1 ± 0.1	60 ± 4	9,500 ± 300	3.2 ± 0.2	22 ± 2
Tilapia - T2	45.7 ± 2.1	1.45 ± 0.03	1.6 ± 0.1	78 ± 3	11,200 ± 250	4.8 ± 0.3	32 ± 2
Tilapia - T3	50.3 ± 2.0	1.60 ± 0.04	1.4 ± 0.1	85 ± 2	12,000 ± 270	5.1 ± 0.4	35 ± 2
Carp - C1 (Control)	40.1 ± 1.8	1.05 ± 0.06	2.3 ± 0.2	58 ± 3	8,800 ± 320	2.9 ± 0.2	20 ± 3
Carp - C2	48.5 ± 1.9	1.40 ± 0.05	1.7 ± 0.1	74 ± 3	10,600 ± 290	4.5 ± 0.3	30 ± 2
Carp - C3	53.0 ± 2.1	1.55 ± 0.03	1.5 ± 0.1	81 ± 2	11,800 ± 310	5.0 ± 0.3	34 ± 2
Shrimp - S1 (Control)	5.2 ± 0.3	0.90 ± 0.02	2.4 ± 0.2	65 ± 3	6,200 ± 180	2.5 ± 0.1	18 ± 2
Shrimp - S2	6.8 ± 0.4	1.20 ± 0.03	1.8 ± 0.1	80 ± 2	7,400 ± 150	3.8 ± 0.2	26 ± 2
Shrimp - S3	7.5 ± 0.3	1.35 ± 0.03	1.6 ± 0.1	86 ± 2	8,000 ± 170	4.2 ± 0.2	30 ± 2

Results

1. Selection of Probiotic Strains

Three promising probiotic strains were selected for the study based on key criteria including non-pathogenicity, survivability in aquatic conditions, and gut colonization ability (Table 1). *Bacillus subtilis* (B1) and *Saccharomyces cerevisiae* (S1) were sourced from PSG IMSR's certified culture collection, while *Lactobacillus plantarum* (L1) was isolated from the gut of healthy *Oreochromis niloticus*. All selected strains demonstrated strong probiotic traits, with B1 and L1 exhibiting high survivability and gut colonization potential.

2. Experimental Design

Experimental trials were conducted using three aquatic species: *O. niloticus* (Tilapia), *C. carpio* (Carp), and *L. vannamei* (Shrimp), across controlled laboratory and pond-based setups. Each species was divided into control and probiotic-treated groups with triplicate replicates. Probiotic treatments varied by species and administration method (Figure;1,2 and 3;Table 2).

3. Probiotic Administration

Probiotics were administered via feed or water depending on the species and treatment group (Table 3). Tilapia and Carp received feed-based supplementation with single or combined strains, while Shrimp were treated through both water inoculation and feed inclusion. Dosages ranged between 10^7 and 10^8 CFU/g of feed or mL of water, administered daily or every 48 hours as appropriate.

4. Water Quality Monitoring

Throughout the experimental period, all water quality parameters remained within optimal ranges for each species, ensuring that variations in performance were attributable to probiotic effects and not environmental stress (Table 4). Notably, probiotic-treated groups showed slightly improved water parameters, particularly in reduced ammonia and

nitrite concentrations, indicating better nutrient cycling and microbial balance in culture environments.

5. Growth and Health Performance

Growth Metrics

Probiotic-treated groups exhibited significantly improved growth performance compared to controls (Table 5). In Tilapia, weight gain increased from 35.2 g in the control group (T1) to 50.3 g in the T3 group (Lactobacillus + Yeast). Specific Growth Rate (SGR) improved from 1.10%/day (T1) to 1.60%/day (T3), while Feed Conversion Ratio (FCR) improved from 2.1 to 1.4. Similar trends were observed in Carp and Shrimp, with the highest growth observed in multi-strain treatments.

Disease Resistance

Survival rates post-pathogen challenge with *Aeromonas hydrophila* and *Vibrio spp.* were significantly higher in probiotic groups. Tilapia survival increased from 60% (T1) to 85% (T3), and Shrimp from 65% (S1) to 86% (S3). These results indicate enhanced disease resistance in probiotic-treated groups.

Immune Response

Probiotic supplementation resulted in notable enhancement of immune parameters. Total leukocyte count, lysozyme activity, and phagocytic activity were all significantly elevated in treated groups compared to controls. In Carp, leukocyte counts increased from 8,800 cells/mm³ (C1) to 11,800 cells/mm³ (C3), while lysozyme activity rose from 2.9 U/mL to 5.0 U/mL. Shrimp treated with *S. cerevisiae* (S3) exhibited the highest immune stimulation, with phagocytic activity reaching 30%.

Discussion

Subedi and Shrestha (2020) [22] concluded that despite having a beneficial effect on aquaculture management, probiotics are still very limited in inclusive research and studies. The use of probiotics still has various obstacles, including the inability of aquatic species strains to produce on a large scale and the unpreparedness of the industry to process probiotic products for the needs of aquatic organisms; hence, the use of terrestrial probiotics is more frequent. Moreover, the lack of knowledge and understanding of the methods of administration and the benefits of probiotics occurs among fish farmers. To overcome this limitation, further research is needed on the mechanism of action of probiotics, their impact on microbial communities in the aquatic environment, and their potential ecological risks. In this case, collaboration between aquaculturists, fish nutritionists, and microbiologists is very important. By overcoming these limitations, probiotics can be used more widely and effectively in aquaculture to increase production sustainably.

Some other obstacles, such as the difficulty of isolation and functional verification of probiotics isolated from the organs of host organism (intestines, liver, stomach, oral mucosa, mucus-secreting glands, etc.) are very difficult so this will severely limit the effective application of probiotics to aquatic organisms. In addition, very limited works have been done *Bacillus* species derived from the host intestine in regulating mucosal immunity and intestinal microbiota, making it difficult to explore superior strains.

Furthermore, the interaction of probiotics with culture media is also very influential; several parameters such as pH level and temperature, can affect the effectiveness of probiotic strains. Most bacteria have an optimal pH range, so if bacteria are placed in a medium that is too acidic or alkaline, their viability and efficacy will be reduced. Alkalinity is a buffering agent to resist dramatic changes in pH levels through the interaction between carbonate-bicarbonate with neutralizing acids or acids and bases (Makori *et al*., 2017) [23]. The survival and function of probiotics are also affected by the fluctuations that occur in alkalinity. Another factor that affects the performance of probiotics is temperature, which can affect the metabolic activity of probiotics. Dissolved oxygen levels are one of the most important factors in the growth environment of probiotics, but its effect depends on the type of probiotic used and the type of fermentation or culture process applied. Anjum *et al*. (2022) [24] reported that high temperatures can cause a decrease in the viability and stability of probiotics, either during the storage period or in the digestive tract of organisms.

It can be seen that native species that originate from a specific environment or ecosystem are being explored for their potential in aquaculture. It is necessary to perform more effective trials to find out in detail and direct the effectiveness and mechanism of action of native species in their function as disease prevention and increase the growth of aquaculture animals. Understanding the potential use of probiotics against microbial communities and the potential ecological risks that will be posed is not fully known, so further research is still urgently needed. Several other supporting factors also affect the effectiveness of probiotic action, such as the type of strain candidate that must be evaluated according to global standards, the suitability of the dose needed with the capacity of the cultivated organism, and the application method used in its application.

Conclusion

The present study demonstrates the significant potential of aquatic probiotics *Bacillus subtilis*, *Lactobacillus plantarum*, and *Saccharomyces cerevisiae* in enhancing the health, growth performance, and disease resistance of key aquaculture species including Tilapia (*Oreochromis niloticus*), Carp (*Cyprinus carpio*), and Shrimp (*Litopenaeus vannamei*). Probiotic administration, whether through feed or water, led to improved growth metrics such as weight gain, specific growth rate (SGR), and feed conversion ratio (FCR). Additionally, probiotic-treated groups exhibited enhanced immune responses and higher survival rates against common aquatic pathogens. Water quality parameters were also more stable in probiotic-treated systems, indicating their positive role in maintaining optimal environmental conditions and microbial balance. The combined application of bacterial and yeast strains showed superior results compared to single-strain treatments, underscoring the synergistic benefits of multi-strain probiotic formulations.

Overall, this research supports the incorporation of probiotics as a sustainable and effective alternative to antibiotics and chemical treatments in aquaculture farms. Their consistent performance across species and systems highlights their adaptability and value in promoting resilient, environmentally friendly, and economically viable fisheries. Further long-term field studies and formulation optimization will be essential for large-scale application and commercial success.

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