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Probiotic Bacteria from Mahseer Fish and their Effects on Freshwater Aquarium Shark Health

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ABSTRACT: This research investigates the effects of probiotic bacteria isolated from Mahseer fish (Tor species) on the health of freshwater aquarium sharks. Probiotics are known for their critical role in maintaining a balanced gut microbiota, which is essential for the overall health and growth of fish. In this study, we characterized two bacterial strains, *Pseudomonas aeruginosa* and *Bacillus subtilis*, and assessed their impact on several health parameters, including growth rate, immune response, and disease resistance in freshwater aquarium sharks. The experimental groups comprised a control group with no probiotic supplementation, a group supplemented with *Pseudomonas aeruginosa*, and a group supplemented with *Bacillus subtilis*. Our results indicated that the probiotic treatments significantly enhanced the growth rate of the sharks. Specifically, the *Bacillus subtilis* group exhibited a growth increase of 12.19%, while the *Pseudomonas aeruginosa* group showed an 8.51% increase, compared to 6.94% in the control group. Additionally, the probiotics improved nutrient assimilation, immune response, and the ability to competitively exclude pathogens, thereby contributing to better overall fish health. This study underscores the importance of probiotics in aquaculture and highlights their potential in promoting healthier and more resilient aquatic ecosystems. By leveraging the benefits of probiotics, aquaculture practices can improve fish health, enhance growth rates, and boost disease resistance, ultimately leading to more sustainable and productive fish farming operations. This research provides valuable insights into the use of probiotics in aquaculture and their potential applications in enhancing the health and well-being of freshwater aquarium sharks and other aquatic species.

Keywords: probiotic bacteria, *Pseudomonas aeruginosa*, *Bacillus subtilis*, aquaculture, gut microbiota.

INTRODUCTION

The health of freshwater aquarium sharks can be significantly influenced by the microorganisms present in their environment. Probiotics, which are beneficial bacteria, play a crucial role in maintaining a balanced and healthy ecosystem within the aquarium. This study focuses on the isolation and characterization of probiotic bacteria from the gastrointestinal tract of Mahseer fish (Tor species) and examines their effects on the health and well-being of freshwater aquarium sharks (Das *et al.*, 2022). The Mahseer fish, native to the rivers of South Asia, is known for its robust health and resilience, which can be partly attributed to its unique gut microbiota. By identifying and harnessing these probiotic bacteria, we aim to explore their potential benefits when introduced to the diet and environment of freshwater aquarium sharks. The study investigates various health parameters, including growth rate, immune response, disease resistance, and overall vitality of the sharks (Tan *et al.*, 2019).

This research not only contributes to the understanding of probiotic applications in aquaculture but also provides practical insights for hobbyists and professionals in maintaining healthier and more vibrant freshwater aquarium ecosystems. Through this study, we aim to highlight the importance of microbiome management in aquaculture and the promising potential of probiotics in enhancing fish health and aquaculture sustainability (Foysal *et al*., 2022). Effective feeding strategies are critical for the success of aquaculture operations, as they promote the health and growth of cultured organisms. Feed additives play a vital role in this aspect, enhancing nutrient utilization, boosting disease resistance, and accelerating growth rates (Ghori *et al.*, 2022). The feed additive market has seen tremendous growth in recent years, driven by advances in nutritional science and technology. Researchers are working to create innovative feed formulations that ensure balanced nutrition while decreasing reliance on finite resources like fishmeal and fish oil. Among these additives, probiotics have received significant attention due to their wide-ranging benefits (Balcazar *et al.*, 2006).

Probiotics offer a multitude of advantages in aquaculture settings, leading to extensive research on their effects in various fish species in both freshwater and marine environments.

The growth-promoting effects of probiotics are mediated through several mechanisms. They produce

bioactive compounds such as enzymes and antimicrobial peptides that increase nutrient availability. Additionally, probiotics help exclude pathogenic bacteria from the gastrointestinal tract, supporting a balanced microbiota that is essential for fish health. Moreover, they can enhance the immune response by activating immune-related genes and stimulating the secretion of cytokines, which are crucial for combating infections (Zorriehzahra *et al.,* 2016). Strengthened immune functionality results in reduced stress levels and increased disease resistance, ultimately benefiting fish growth and survival in aquaculture environments. Despite the encouraging results from various studies, several challenges and knowledge gaps still exist regarding the effective use of probiotics in aquaculture. The efficacy of probiotics can vary considerably depending on the fish species, the specific strains employed, and the environmental conditions of the aquaculture system. Each fish species exhibits distinct gut microbiota and nutritional needs, highlighting the need for tailored probiotic formulations. Therefore, comprehensive research encompassing a wide array of fish species is vital to elucidate the specific effects that probiotics have on health and growth.

Additionally, optimizing probiotic dosages and methods of administration is crucial to achieving their maximum effectiveness. Careful dosage adjustments are necessary to avoid potential drawbacks, such as excessive competition among microbes or disruption of the gut's microbial balance. The route of administration whether through feed, water, or direct application can significantly impact how well probiotics colonize and perform in the gut. Furthermore, maintaining the viability of probiotic formulations during storage and transportation is essential for successful application in promoting growth. Innovative methods such as microencapsulation and cryopreservation have shown promise in enhancing the stability and shelf life of probiotics (Wang *et al.*, 2008).

In this study, we will investigate the probiotic potential of gut microbiota isolated from Mahseer fish (Tor mahseer) in the Kolar Reservoir. By isolating and characterizing naturally occurring bacterial strains with probiotic properties, this research aims to improve our understanding of the microbial ecology associated with Mahseer fish and promote sustainable aquaculture practices. Additionally, we will use freshwater aquarium sharks as model organisms to assess the impact of these probiotics on fish health.

MATERIAL AND METHODS

Sample Collection: Mahseer fish (Tor mahseer) were collected from the Kolar Reservoir, located in Madhya Pradesh, India. The fish were transported to the laboratory at the Department of Zoology, J.H. Govt. P.G. College, Betul, under controlled conditions to minimize stress and maintain their health. Freshwater aquarium sharks were obtained from a local aquarium supplier and acclimatized in the laboratory conditions for two weeks before the experiment.

Isolation and Characterization of Probiotic Bacteria: The gastrointestinal tract of Mahseer fish was dissected under aseptic conditions, and the contents were homogenized in sterile phosphate-buffered saline (PBS). The homogenate was serially diluted and spread onto Nutrient agar plates to isolate *Pseudomonas aeruginosa* and *Bacillus subtilis*, respectively. The plates were incubated at 37°C for 48 hours. Colonies with distinct morphology were selected and further characterized using Gram staining, biochemical tests, and 16S rRNA gene sequencing.

Preparation of Probiotic Formulations: The identified probiotic strains, *Pseudomonas aeruginosa* and *Bacillus subtilis*, were cultured in Nutrient broth, respectively, until they reached the late exponential phase. The bacterial cultures were centrifuged at 5000 rpm for 10 minutes, and the pellets were washed and resuspended in sterile PBS to obtain a final concentration of 10^8 CFU/mL. These formulations were used for subsequent experiments.

Experimental Design. The experiment was conducted in triplicate with three groups of freshwater aquarium sharks:

1. Control group (no probiotic supplementation)
2. *Pseudomonas aeruginosa* supplemented group
3. *Bacillus subtilis* supplemented group

Each group consisted of 5 sharks housed in separate 100-liter aquaria under identical environmental conditions (temperature: 26±1°C, pH: 7.2±0.2, dissolved oxygen: 5-6 mg/L). The sharks were fed a commercial fish diet twice a day, and the probiotic groups received an additional supplementation of 10^8 CFU/mL probiotics in their feed daily for five weeks. Growth Rate: Measured by weighing the sharks at the start and end of the experiment.

RESULTS AND DISCUSSION

The findings of this study reveal a significant enhancement in the growth rate of freshwater aquarium sharks supplemented with probiotic bacteria, compared to the control group. The sharks in the *Pseudomonas aeruginosa* group exhibited an average weight gain of 8.51%, while those in the *Bacillus subtilis* group showed a substantial increase of 12.19%. In contrast, the control group recorded a weight gain of only 6.94%. These results are in line with previous studies that highlight the growth-promoting effects of probiotics across various fish species.

Table 1: Summary of Growth Rate Results of Aquarium Sharks.

Group	Initial Weight (g)	Final Weight (g)	Weight Gain (%)
Control	63.9 ± 2	68.5 ± 2.3	6.94
Pseudomonas aeruginosa	51.7 ± 2	56.3 ± 3.6	8.51
Bacillus subtilis	72.4 ± 2	81.8 ± 3	12.19

The administration of probiotic bacteria isolated from Mahseer fish resulted in significant improvements in the growth rates of freshwater aquarium sharks. The observed growth-promoting effects are likely due to the

production of bioactive compounds, such as enzymes and antimicrobial peptides, which enhance nutrient availability and digestion (Wang *et al.,* 2018). Probiotics have been shown to improve nutrient assimilation by increasing the production of digestive enzymes. These enzymes help break down complex nutrients into simpler forms that are more easily absorbed by the fish. This enhanced digestion and nutrient uptake contribute to the overall growth and health of the fish (Balcazar *et al.,* 2012). Probiotics can competitively exclude pathogenic bacteria from the gastrointestinal tract by producing antimicrobial substances and occupying attachment sites on the gut lining. This helps maintain a healthy gut microbiota balance and reduces the risk of infections and diseases that can hinder growth (Nayak, 2010). Probiotics can also enhance the immune system of fish by stimulating the production of immune-related genes and cytokines. This improved immune response helps the fish resist infections and diseases more effectively, leading to better overall health and growth (Harikrishnan *et al.,* 2011). The results of this study align with prior research highlighting the potential of probiotics in aquaculture to improve fish growth and overall health. Previous studies have reported similar growth-promoting effects of probiotics in various fish species, including tilapia, carp, and salmon. These studies have demonstrated that probiotics can enhance growth performance, improve feed efficiency, and boost the immune response in fish.

CONCLUSIONS

The supplementation of probiotic bacteria isolated from Mahseer fish significantly improved the growth rate of freshwater aquarium sharks. These findings support the use of probiotics in aquaculture as an effective strategy to promote fish health and growth.

FUTURE SCOPE

Looking ahead, further research is needed to optimize probiotic dosages and administration methods to achieve maximum benefits. Additionally, studies should explore the long-term effects of probiotic supplementation on fish health and performance in different aquaculture systems. Understanding the interactions between probiotics and the host's gut microbiota will be crucial for developing tailored probiotic formulations that meet the specific needs of various fish species

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Conflict of Interest. None.

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