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Fungal diversity and its ecological role in probiotic-enhanced aquaculture ponds

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Abstract: Two aquaculture ponds of freshwater prawn (*Macrobrachium rosenbergii*) near Vishnuvakkam, Tamil Nadu, India, were studied to analyse how probiotics affect fungi and how they together contribute to improve soil condition, development of culture and ecosystem balance. One pond received no additives; the other was treated with regular doses of beneficial microbes. In the probiotic-enhanced pond, fungal species diversity increased to 35 species, whereas the untreated water hosted only 28, increasing diversity by a quarter. We observed, also using standard statistical controls, that the number of fungi increased on average by 365.45% ± 17.736% under the influence of probiotics. Among fungal groups, *Aspergillus* dominated the control pond; the probiotic setting encouraged a wider mix of fungi. Better silt levels - reaching 35.09% - alongside 29.59% sand appeared alongside this richer community. Nutrient access improved under these conditions, supporting stronger prawn growth. Survival stayed total in the treated group, where growth measured 35.54, nearly double the control's 19.37, and output climbed to 372 kg against just 237 kg without intervention. Financial outcomes followed the same trend: returns outweighed costs by 1.785 times, far above the control's 1.355. Profit landed at INR 2,06,758, dwarfing the INR 57,971 seen elsewhere. Fungi, shaped by probiotics, clearly shape system performance; they are not merely present but pivotal in lasting aquaculture setups.

INTRODUCTION

Fungi rank high in ecological flexibility across the planet. Because they tolerate wide pH ranges they establish easily in varied habitats. While found in oxygen-rich settings, some flourish where air is limited. These traits let them dominate early stages of freshwater community development (Manoharachary & Ramarao 1983). Far from being mere residents, fungal populations drive change within fish farming waters. Through breaking down dead material, nutrients shift forms under their influence. Soil texture improves when their networks spread underground.

Manoharachary & Ramarao (1983) found 47 species of fungi living in just two muddy freshwater ponds in Hyderabad - evidence that small water bodies can host dense fungal communities. Although limited to one region, their work highlighted biodiversity potential tucked inside quiet wetlands. Flowing waters tell a different story; Singh & Wadhwani (1989) showed this clearly when they compared fungi in moving rivers versus still pools. Where water moves slowly or fast appears to guide which fungi settle where. Because movement alters conditions beneath the surface, it also shifts what grows below.

Further studies have broadened the understanding of fungal life from other regions. In Nigeria, Okaemo & Olufemi (1997) mapped fungi in tilapia farming waters; and Okpokwasilli, Olufemi & Okaemo (1998) explored similar communities in catfish aquaculture systems. Indian researchers contributed findings too - Girivasan,

Kumar & Sathish (1998) reported on fungus species living in peat-based earths. Then came discoveries from hidden places: Koilraj, Marimuthu & Muthulakshmi (1999) pulled distinct fungal biomes out of cavern ecosystems in 1999, showing survival where nourishment is scarce and sunlight absent.

Among the most recent studies, Paulraj (2002a) identified twelve species of mesophilic fungi alongside seven thermophilic ones within *Macrobrachium rosenbergii* farming systems, offering one of the first fungal inventories in such environments. Shifting focus slightly, this work probes deeper: instead of just listing species, it explores whether introducing probiotics alters these fungal networks, then traces any shifts toward changes in pond ecology or harvest outcomes.

MATERIALS AND METHODS

Pond Location

The *Macrobrachium rosenbergii* culture farm used for this study is located in Vishnuvakkam, approximately 56 km from Chennai, in the Thiruvallur District of Tamil Nadu, India. The farm comprises two ponds: a control pond and a probiotic experimental pond, each covering an area of 0.603 hectares (length 298 m, width 213 m). Both ponds have a depth of approximately 1.5 m. The control pond is separated from the probiotic experimental pond by a bund measuring 80-95 cm in width, and all other sides of the ponds are similarly bordered. The ponds are surrounded by agricultural fields and feature a sluice gate (2 m × 1.5 m) for water drainage. Three screens with mesh sizes of 0.5, 1.0, and 1.5 cm are installed at the sluice gate to prevent the escape of animals during water drainage. Additionally, two 8 inch diameter emergency pipes with valves facilitate water release during the rainy season. To prevent cannibalism, shelters (such as country tiles and coconut leaves) were placed within the ponds.

Postlarval Stocking and Acclimatization

Postlarvae (60,000) were sourced from Aqua Nova hatchery (P) Ltd., located in Kannathur, Chennai, approximately 106 km from the culture farm. Five hundred healthy and active postlarvae (PL-15), with a mean length of 12.8 ± 1.1 mm and a mean weight of 1.2 ± 0.2 mg, were packed in polythene bags (40 × 80 cm) containing two liters of water, which were inflated with oxygen and sealed with rubber bands, Brine shrimp nauplii were added as food during transportation.

The post-larvae were transported after sunset using a van. Upon arrival, the polythene bags were placed in the ponds for about one hour to acclimatize. The bags were then opened with minimal disturbance, allowing pond water to gradually enter. The postlarvae were slowly released into both ponds, resulting in a stocking density of 1.3 individuals/m^2.

Soil Fungal Analysis

Mesophilic fungi were isolated from soil samples using different culture media at varying temperatures. For this study, a Czapek-Dox-Agar (CDA) medium was employed for mesophilic fungi isolation. The composition of the CDA medium per 1000 ml of distilled water is as follows:

Sodium nitrate: 2.0 g
Potassium dihydrogen phosphate: 1.0 g
Magnesium sulfate: 0.5 g
Potassium chloride: 0.5 g
Ferrous sulfate: 0.01 g
Agar: 20.0 g
Sucrose: 30.0 g

One gram of soil sample was thoroughly dispersed in 10 ml of sterile distilled water to create a stock solution. Sequential dilutions were prepared: 1 ml of the stock solution was transferred to 9 ml of sterile water, mixed well and this process was repeated. From this dilution, 1 ml was pipetted into sterile petri plates containing antibiotic-amended CDA medium (10^3 dilutions), with streptomycin sulfate used to inhibit bacterial growth.

The Petri plates were incubated at 29 ± 1°C and 21± 1°C for one week, with six replicates maintained for each soil sample. Fungi were stained with lacto-phenol cotton blue and observed under a light microscope.

Identification was performed using standard manuals (Cooney & Emerson 1964; Gilman 1967; Barnett & Hunter 1972; Onions, Bridge & Sutton 1981). Percentage contribution and colony-forming units (CFU) were calculated using the following formulas:

Percentage Contribution (PC):

$$\frac{\text{Total no. of colonies of a species}}{\text{Total no. of colonies of all species}} \times 100$$

Colony Forming Unit (CFU):

$$\frac{\text{Average no. of colonies / plates}}{\text{Volume plated}} \text{ dilution factor}$$

Harvesting and Growth Assessment

The total length (cm) and body weight (g) of harvested prawns in both ponds were measured four times monthly during the harvest period. Growth metrics, including specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), benefit-cost ratio (BCR), and feed efficiency (FE), were calculated according to the methods outlined by Sweilum (2006):

Specific Growth Rate (SGR):

$$\frac{\text{(Final weight - Initial weight)}}{\text{Culture period in days}} \times 100 \text{ days}$$

Feed Conversion Ratio (FCR):

$$\frac{\text{Quantity of feed consumed}}{\text{Total weight gained}}$$

Protein Efficiency Ratio (PER):

$$\frac{\text{Weight gain (g) x No. of prawns}}{\text{Protein intake}}$$

Feed Efficiency (FE):

$$\frac{\text{Total weight gain (g)}}{\text{Quantity of food consumed}}$$

Harvesting Process

The harvest occurred on the 119th day of culture, with partial harvests performed in subsequent months. before harvesting, the water level was lowered to 0.5 m. The complete harvest was conducted over four days (December 15-20, 2018) between 5:00 am - 10:30 am and 3:00 pm - 6:30 pm, using drag nets and hand-picking to ensure 100% harvest efficiency. Fish populations were also collected. Post-harvest, the animals were weighed, sorted by size, and packed in ice. Stunted prawns were segregated and cultured in a separate pond for further growth analysis.

Economic Analysis

Harvested prawns were sold in the Chennai export market, while fish were sold in the local market. Costs associated with seed, feed, fertilizers, power, labour, harvesting, and trial netting were recorded and compared for both control and probiotic experimental ponds. Operational costs, net income, and profits were calculated, excluding the cost of pond leasing. Production costs were based on wholesale market prices for inputs from the 2018-2019 periods.

Statistical Analysis

Statistical analysis was performed on data regarding soil texture, water parameters, fungal populations, and plankton counts using the t-test (Systat version 10.0). Dissolved oxygen (DO), pH, and temperature were analyzed using correlation, regression, and ANOVA at a 5% significance level. Growth relationships (positive/negative) between the control and experimental pond-cultured prawns were assessed using SPSS Inc. (2010).

RESULTS (FUNGAL DIVERSITY AS THE PRIMARY ECOLOGICAL DIFFERENTIATOR)

Comparative Fungal Genera Richness

Among results, one stands out clearly: fungal variety differed sharply across the two ponds. In the probiotic-treated water body, researchers identified 35 separate fungal groups; meanwhile, the untreated counterpart hosted just 28 - marking a gain of 25% in classification count due to added microbial supplements. Measurements showed fungal levels in the treated pond reached an average of 365.45%, with a margin of error at ±17.736%, notably surpassing those in the standard pond under statistical testing ($P < 0.05$). Such a rise in fungus numbers suggests that introduced beneficial microbes - especially strains within the *Bacillus* family - helped shape underground conditions favouring greater fungal settlement.

Dominant Fungal Genus: *Aspergillus* P. Micheli ex Haller

Despite its strong presence in both environments, *Aspergillus* showed reduced dominance in the probiotic pond where new fungal groups appeared alongside it. The control pond, hosted only a few species making *Aspergillus* stand out clearly. This shift suggests external inputs helped broaden microbial range. Because these fungi play key roles in organic breakdown and nutrient cycling, such changes may influence ecosystem function directly. Breaking down tough organics like cellulose, starch, or proteins depends on enzyme activity.

Besides the shift in numbers, one pond showed greater fungal variety than the other. With probiotics, it hosted 35 separate fungal groups; without them, just 28 - meaning a quarter more types appeared where supplements were used. Fungal levels there reached 365.45% ±17.736%, clearly exceeding the second site, confirmed by statistical testing at P less than 0.05. That rise points to beneficial bacteria, especially *Bacillus* strains, shaping underground conditions favouring fungi.

Fungal Diversity and Soil Texture: A Bidirectional Relationship

The soil in the probiotic pond was found to be different when examined - its makeup carried 35.09% silt and 29.59% sand, levels that exceeded those found in the untreated plots. Because fungi worked beneath the surface, tiny threads wove through grains, locking bits together into stable clusters. Where these networks grew denser, moisture stayed longer, held within microscopic pockets. Complexity emerged not from size but variety: broader species of fungi built more intricate webs. With each new strand, the ground gained strength, shaped by unseen branching.

Beyond microbial shifts, chemical markers tell a clear story: nitrogen levels rose notably where fungi thrived (probiotic: 41.09 ± 1.423; control: 29.454 ± 1.485). Phosphorus followed a similar trend, higher in treated conditions (4.654 ± 0.228 compared to 3.945 ± 0.166). Potash displayed an even starker contrast, reaching 129.272 ± 8.543 against 105.909 ± 8.182. These jumps trace back to active fungal processes boosting elemental release. As nutrients increase below, life above benefits just as much - the prawn population gains support through enriched ground chemistry.

Fungal Contributions to Prawn Growth and Pond Productivity

While fungi do not directly feed the prawns, their contributions to the trophic web of the pond are profound. Fungi start the breakdown of dead leaves, leftover food, and waste, returning essential elements to the water. Following this process, tiny animals like zooplankton feed on the fungal growth, bridging energy flow toward *Macrobrachium rosenbergii* through natural consumption patterns. Some fungi can suppress waterborne diseases through competition for space and nutrients, thereby limiting the establishment and spread of pathogenic fungi such as *Chytridiomyces* (Paulraj 2012). Where fungal threads form dense layers,

oxygen moves better through mud, helping bottom-dwelling shrimp find food. These changes happen gradually, shaped by how species interact beneath the surface.

The outcome of this enriched fungal ecology was a Specific Growth Rate (SGR) of 35.54 in the probiotic pond versus 19.37 in the control — an 83.5% improvement — alongside 100% survival rate and a total yield of 372 kg compared to 237 kg in the control.

DISCUSSION (RETHINKING AQUACULTURE THROUGH A FUNGAL LENS)

Among ponds treated with probiotics, a 25% increase in fungal genera stand out. Ecosystem resilience often reflects fungal diversification. For example, in the event of heat peaks or excess waste, the system with 35 genera adapts more smoothly than its counterpart which contains only 28. Stability in prawn habitats improves when fungi multiply, as they support the balance through their layered presence.

In both ponds, *Aspergillus* predominates; a pattern which is also echoed in other studies (Paulraj 2002b; Manoharachary & Ramarao 1983; Okaemo & Olufemi 1997). Found everywhere in tropical pond soils, these fungi adapt quickly due to broad metabolic abilities. Their status as leading organisms points clearly toward central involvement in breaking down organic material within such environments.

Soil nutrient gains - especially nitrogen, phosphorus and potassium - run alongside each other suggesting that fungi are key to breaking down organic matter within the probiotic pond. Instead of passive bystanders, these organisms likely drove nutrient recycling by transforming complex materials into usable forms. Moreover, evidence from Wang, Zhang & Liu (2005), together with findings from Rengpipat, Ratanachai & Khamput (1998), points to microbial life as a driving force behind sediment nutrition shifts. Behind the scenes, tiny biological networks appear responsible for freeing locked-up resources. Rather than acting alone, microbes functioned as a linked system enabling sustained availability of vital elements.

Looking at the numbers, the probiotic pond showed a benefit-cost ratio of 1.785, whereas the control sat at 1.355. Net earnings reached Rs. 2,06,758 in contrast to Rs. 57,971 without treatment. Although these improvements are often tied to probiotics generally, they depend heavily on the support provided by fungal activity within the system. Such benefits emerge alongside shifts driven by microbial interactions made possible through probiotic use.

CONCLUSION

Fungi play a silent but vital role in probiotic fish farming. Evidence shows that adding probiotics to ponds where *Macrobrachium rosenbergii* is raised doesn't just shift bacterial life - it triggers wider changes among fungi, boosting the number of fungal species by one quarter while also raising overall fungus levels noticeably ($P < 0.05$).

What stands out is how *Aspergillus* dominates in both settings, forming the core of fungal activity and keeping basic nutrient flows running. However, resilience emerges differently - where the probiotic pond hosts 35 genera, interactions grow deeper, supporting stronger prawn development, better soil structure, and higher profits over time.

These findings position fungal diversity as a key indicator of aquaculture pond health and advocate for mycological monitoring as a standard component of sustainable aquaculture management. Future research should investigate specific fungal taxa responsible for biocontrol and nutrient transformation functions within probiotic ponds, and explore targeted fungal inoculants as complements to established probiotic regimes.

Fungi are not background organisms in aquaculture — they are foundational architects of pond ecology.

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Conflict of Interest

The authors have declared no conflict of interest.

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