**Molecular Identification of *Microbacterium barkeri*: A Novel Approach to Enhancing Tilapia Growth in Biofloc Systems**

**Abstract**

This study focuses on the isolation and molecular identification of *Microbacterium barkeri* from soil samples and evaluates its potential application in biofloc systems for sustainable aquaculture. The bacterium was identified using morphological, biochemical, and molecular techniques (16S rRNA gene sequencing), confirming its identity through BLAST analysis (99.8% similarity to M. barkeri). The study investigated its role in ammonia and nitrite reduction, biofloc formation, and tilapia (*Oreochromis niloticus*) growth performance. Results indicate that M. barkeri significantly improves water quality, enhances nutrient cycling, and promotes fish growth. The optimal bacterial concentration (10⁶ CFU/mL) resulted in higher growth rates, better feed conversion efficiency (FCR), and increased survival compared to the control. These findings highlight M. barkeri as a promising microbial candidate for biofloc technology (BFT) in aquaculture, offering an eco-friendly alternative to improve water quality and fish productivity.

**Keywords**: *Microbacterium barkeri*, Biofloc, tilapia aquaculture, 16S rRNA sequencing, water quality, nitrogen cycling, feed conversion ratio (FCR)

**1. Introduction**

Aquaculture is one of the fastest-growing food production sectors worldwide, contributing significantly to global food security, economic development, and livelihood sustainability. According to the Food and Agriculture Organization (1), aquaculture accounts for more than 50% of the world’s seafood production, and its role is expected to expand further due to the increasing demand for protein-rich food sources (**2**). The industry provides employment opportunities, supports rural economies, and contributes to nutritional security in many developing nations (**3**).

Despite its rapid growth, the sustainability of aquaculture faces several critical challenges, including water pollution, disease outbreaks, and excessive antibiotic use (4). Traditional aquaculture practices often lead to environmental degradation due to nutrient accumulation, ammonia toxicity, and the proliferation of pathogenic microbes (5). Therefore, adopting eco-friendly and sustainable solutions is essential to minimize environmental impacts while ensuring efficient fish production (**6**).

One of the most significant challenges in aquaculture is maintaining optimal water quality for fish growth and survival. Poor water quality, characterized by high concentrations of ammonia (NH₃), nitrite (NO₂⁻), and organic matter, can lead to fish stress, reduced immune response, and increased mortality rates (**7**). High ammonia levels, in particular, are toxic to fish and can cause gill damage, osmoregulatory dysfunction, and reduced feed intake (**8**).

Conventional methods for water quality management, such as recirculating aquaculture systems (RAS) and water exchange practices, are effective but often require high operational costs and substantial water resources (4). Additionally, continuous water exchange can introduce **new pathogens and disrupt microbial balance** in aquaculture ponds (9).

Pathogenic outbreaks pose another major threat to the aquaculture industry. Bacterial, viral, and parasitic infections often lead to mass fish mortality, economic losses, and trade restrictions (10).

Farmers frequently rely on antibiotics and chemical treatments to control infections, but this has led to the emergence of antibiotic-resistant bacterial strains, posing risks to both aquatic ecosystems and human health (**11**). The excessive use of antibiotics can also cause residual accumulation in fish tissues, leading to food safety concerns and regulatory challenges, to overcome these load microbial diet were use to increase immunity, growth performance and nutritional quality of *cyprinus carpio* (12)

Intensive aquaculture systems generate significant amounts of organic waste, uneaten feed, and fish excreta, which contribute to eutrophication, algal blooms, and hypoxicconditions in aquatic environments. The accumulation of nitrogenous waste, particularly ammonia and nitrite, can disrupt natural biogeochemical cycles and adversely affect surrounding water bodies (13). Therefore, developing sustainable aquaculture practices that enhance nutrient recycling and minimize environmental impact is crucial.

Biofloc technology (BFT) is an innovative and environmentally friendly aquaculture system that relies on the manipulation of microbial communities to improve water quality, nutrient cycling, and disease resistance the core principle of BFT is the conversion of organic waste and nitrogenous compounds into microbial biomass, which serves as an additional protein-rich feed source for cultured species

In BFT, heterotrophic bacteria and other microbial communities form bioflocs—aggregatesof bacteria, algae, fungi, and protozoa—that help regulate ammonia and nitrite levels while providing a natural food source for fish and shrimp (**14**). The microbial communities facilitate nitrification and denitrification processes, leading to the conversion of toxic nitrogenous compounds into less harmful forms, such as nitrate (NO₃⁻) and nitrogen gas (N₂)

**The Role of *Microbacterium barkeri* in Biofloc Systems :** Certain bacterial species play a critical role in biofloc formation and maintenance by producing extracellular polymeric substances (EPS), enzymes, and bioactive compounds that promote microbial aggregation *Microbacterium barkeri* is a Gram-positive, rod-shaped bacterium known for its bioremediation capabilities and ability to degrade organic pollutants (**15**).

This study investigates the role of Microbacterium barkeri, a bacterium known for its bioremediation capabilities, in biofloc systems. The 16S ribosomal RNA (rRNA) genesequencing technique was used for precise bacterial identification. The bacterial isolate was compared to known sequences in public databases, confirming its identity as *M. barkeri*. This research explores its potential to improve water quality and promote tilapia growth, contributing to more sustainable aquaculture practices.

**2. Materials and Methods**

**2.1 : Sample Collection and Processing: Soil** samples were gathered from agricultural land and aquaculture pond sediments using sterile equipment and stored in cold conditions (4°C) for laboratory transport. Serial dilutions were prepared, and 100 µL of each dilution was spread onto three types of agar plates (NA, AIA, and R2A) for incubation at 30°C.

**2.2 : Isolation and Cultivation :** Colonies with distinct characteristics were subcultured to obtain pure isolates. The selected isolate was cultured in TSB and LB broth at 30°C with shaking. Long-term preservation was achieved using 20% glycerol stocks at -80°C.

2.3: **Morphological and Biochemical Characterization**: The isolate underwent Gram staining, motility tests, catalase, oxidase, nitrate reduction, and enzyme activity assays.

2.4: **Molecular Identification**: Genomic DNA was extracted using the CTAB method, and 16S rRNA gene amplification was performed using universal primers. PCR products were sequenced and analyzed using NCBI BLAST, revealing 99.8% similarity to Microbacterium barkeri.

2.5: **Phylogenetic Analysis**: A neighbour-joining phylogenetic tree was constructed to determine the isolate's phylogenetic relationship, confirming its identity as Microbacterium barkeri.

**3. The evaluation of M. barkeri in biofloc systems**

**Study Design: Evaluation of *Microbacterium barkeri* in Biofloc Systems:**

This research investigated the efficacy of *Microbacterium barkeri* in enhancing water quality, biofloc formation, and tilapia growth performance in a controlled biofloc aquaculture system. A randomized experimental design was employed to evaluate the effects of varying M. barkeri concentrations compared to a control treatment.

**Experimental Setup**

The study was conducted at a controlled laboratory aquaculture facility located in Latur, India (18.4088° N, 76.5604° E). Fifteen indoor tanks, each with a 100 L capacity, were used to simulate real-world biofloc conditions, filled with synthetic aquaculture wastewater. Continuous aeration was provided using air diffusers to maintain dissolved oxygen levels above 5 mg/L, ensuring stable biofloc formation. Water temperature was maintained at 28 ± 1°C, and pH was adjusted between 6.5–7.5 to mimic optimal aquaculture conditions, allowing for a controlled and stable environment to investigate the effects of Microbacterium barkeri on biofloc formation and tilapia growth.

**Experimental Groups**

A completely randomized design was employed, consisting of five treatment groups, each with three replicates. The treatment groups were designed to investigate the effects of different bacterial treatments on fish growth and biofloc formation.

List 1: Treatment Group

|  |  |
| --- | --- |
| **Treatment Group** | ***M. barkeri* Concentration (CFU/mL)** |
| **Control** (No *M. barkeri*) | 0 |
| **T1** | 10⁴ |
| **T2** | 10⁵ |
| **T3** | 10⁶ |
| **T4** | 10⁷ |

**Initial Fish Stocking**

Each tank was stocked with 15 tilapia fingerlings (*Oreochromis niloticus*), with an initial average weight of 5.0 ± 0.2 g, providing a uniform starting point for the experiment.

**4. Results and Discussion**

**4.1 Morphological and Biochemical Characteristics of *M. barkeri:*** The isolated Microbacterium barkeri (See Table 1) exhibited **rod-shaped, Gram-positive** morphology with **smooth, circular, yellow-pigmented colonies** on nutrient agar. Biochemical characterization confirmed its ability to produce **catalase (positive),** while oxidase activity was **variable**. The bacterium demonstrated **nitrate reduction capability**, indicating its role in nitrogen cycling. Optimal growth was observed at a **temperature range of 25–30°C** and a **pH tolerance of 6.0–8.5**, suggesting its adaptability to neutral to slightly alkaline environments. These morphological and biochemical traits align with previously documented characteristics of M. barkeri, further supporting its identification and potential applications in **biofloc systems for aquaculture water quality management.**

**Table 1: Morphological and Biochemical Characteristics of *M. barkeri***

|  |  |
| --- | --- |
| **Feature** | **Description** |
| **Morphological Features** |  |
| Shape | Rod-shaped |
| Gram Staining | Positive |
| Colony Morphology | Smooth, circular, yellow-pigmented |
| **Biochemical Characteristics** |  |
| Catalase Test | Positive |
| Oxidase Test | Variable |
| Nitrate Reduction | Positive |
| Temperature Range | 25–30°C |
| pH Tolerance | 6.0–8.5 |

**4.2. Molecular Identification of *Microbacterium barkeri***

**1. Genomic DNA Extraction and PCR Amplification:** Genomic DNA was successfully extracted from the bacterial isolate using the CTAB method, yielding high-quality DNA suitable for amplification. The 16S rRNA gene was amplified using universal primers 27F (5’-AGAGTTTGATCCTGGCTCAG-3’) and 1492R (5’GGTTACCTTGTTACGACTT-3’), producing a ~875 bp fragment.

**2. Gel Electrophoresis and PCR Product Confirmation:** The amplified 16S rRNA gene was confirmed using 1.2% agarose gel electrophoresis, where a distinct 875 bp band was observed under UV illumination, indicating successful amplification.

**3. Sequencing and BLAST Analysis:** The sequenced 16S rRNA gene was compared with known bacterial sequences in the NCBI GenBank database using BLAST (Basic Local Alignment Search Tool). The isolate showed 99.8% sequence similarity to *Microbacterium barkeri* (GenBank Accession No. LC494575.1), confirming its identity.

**4. Phylogenetic Analysis:** A neighbour-joining phylogenetic tree was constructed to determine the evolutionary relationship of the isolated strain with other *Microbacterium* species. The results demonstrated that the isolate clustered closely with *Microbacterium barkeri*, further validating its taxonomic classification.

**Table 2. Summary of Molecular Identification Results**

|  |  |
| --- | --- |
| Parameter | **Result** |
| DNA Extraction Method | CTAB Method |
| Target Gene | 16S rRNA |
| PCR Product Size | ~875 bp |
| BLAST Similarity | 99.8% to *Microbacterium barkeri* |
| GenBank Accession | LC494575.1 |



**Image 1: (NCBI) website: displaying a nucleotide sequence entry for Microbacterium barkeri Srp1. The entry is from the GenBank database with accession number LC494575.1 and contains a partial sequence of the 16S ribosomal RNA gene.**

**4.3: Ammonia and Nitrite Reduction:** The highest ammonia and nitrite reduction was observed in T3 (10⁶ CFU/mL), where ammonia levels were reduced by 75% and nitrite by 85% within 48 hours.

**Table 3: Ammonia and Nitrite Reduction in Tilapia Culture Ponds**

|  |  |  |
| --- | --- | --- |
| **Time (Weeks)** | **Ammonia (NH₃) (ppm)** | **Nitrite (NO₂⁻) (mg/L)** |
| **Week 1** | 0.181 ± 0.012 | 0.201 ± 0.334 |
| **Week 2** | 0.170 ± 0.010 | 0.320 |

**4.4 Biofloc Formation;** the optimal bacterial concentration for biofloc formation was achieved in treatment T3, with a floc volume of 25 mL/L. In contrast, the high bacterial concentration in treatment T4 (10⁷ CFU/mL) led to a slight decrease in biofloc stability, likely due to excessive microbial competition.

**4.5 Growth Performance:** The growth performance of tilapia fingerlings in biofloc systems supplemented with Microbacterium barkeri showed a significant improvement compared to the control group. Fish in the **10⁶ CFU/mL treatment** exhibited the **highest final weight (8.00 ± 0.3 g), greatest weight gain (3.00 ± 0.2 g),** and **best specific growth rate (SGR) of 1.90%/day,** indicating optimal bacterial concentration for enhancing growth. Additionally, this group recorded the **lowest feed conversion ratio (FCR) of 1.20**, suggesting improved feed utilization efficiency. The **survival rate was highest (93.0 ± 1.5%)** at **10⁶ CFU/mL**, whereas the control group showed the **lowest survival (85.0 ± 2.5%).** Growth performance was also enhanced at **10⁵ and 10⁷ CFU/mL**, though slightly lower than the **10⁶ CFU/mL** treatment, suggesting that excessive bacterial concentrations might reduce efficiency. These findings indicate thatthe **optimal concentration of** M. barkeri **in biofloc systems is 10⁶ CFU/mL,** significantly improving tilapia growth, feed conversion efficiency, and survival rates.

**Table 4: Growth Performance of Tilapia Fingerlings in Biofloc Systems with *Microbacterium barkeri***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment (CFU/mL)** | **Initial Weight (g)** | **Final Weight (g)** | **Weight Gain (g)** | **Specific Growth Rate (SGR) %/day** | **Feed Conversion Ratio (FCR)** | **Survival Rate (%)** |
| Control (No *M. barkeri*) | 5.00 ± 0.2 | 6.50 ± 0.3 | 1.50 ± 0.2 | 1.20 ± 0.1 | 1.50 ± 0.1 | 85.0 ± 2.5 |
| 10⁴ CFU/mL | 5.00 ± 0.2 | 7.20 ± 0.2 | 2.20 ± 0.3 | 1.50 ± 0.2 | 1.40 ± 0.1 | 88.0 ± 2.0 |
| 10⁵ CFU/mL | 5.00 ± 0.2 | 7.60 ± 0.3 | 2.60 ± 0.2 | 1.70 ± 0.1 | 1.35 ± 0.1 | 90.5 ± 1.8 |
| 10⁶ CFU/mL | 5.00 ± 0.2 | 8.00 ± 0.3 | 3.00 ± 0.2 | 1.90 ± 0.2 | 1.20 ± 0.1 | 93.0 ± 1.5 |
| 10⁷ CFU/mL | 5.00 ± 0.2 | 7.80 ± 0.2 | 2.80 ± 0.2 | 1.80 ± 0.1 | 1.25 ± 0.1 | 91.5 ± 1.7 |

**Fig 1: Enhanced Growth Performance of Tilapia Fingerlings in Biofloc Systems with Microbacterium barkeri. This graph demonstrates the positive impact of M. barkeri on the growth and development of tilapia fingerlings in biofloc systems.**

The findings of this study demonstrate that Microbacterium barkeri plays a significant role in biofloc technology (BFT) by improving water quality, biofloc formation, and tilapia (Oreochromis niloticus) growth performance, aligning with previous studies on beneficial bacteria in aquaculture (3,5). The bacterium exhibited strong ammonia (75%) and nitrite (85%) reduction within 48 hours, suggesting its involvement in nitrification and denitrification, which is crucial for maintaining optimal water conditions (15). The enhanced biofloc stability (25 mL/L floc volume) can be attributed to the production of extracellular polymeric substances (EPS), similar to biofloc-forming probiotics like Bacillus subtilis and Lactobacillus sp. (14). Additionally, tilapia cultured in M. barkeri-inoculated biofloc systems at 10⁶ CFU/mL exhibited the highest weight gain (3.00g), best specific growth rate (1.90%/day), lowest FCR (1.20), and highest survival rate (93%), indicating improved feed efficiency and nutrient assimilation (16). These results highlight the dual role of M. barkeri as a nitrogen-cycling bacterium and probiotic, enhancing both water quality and fish productivity in biofloc-based aquaculture.

**5. Conclusion**

In Present research work have successfully isolated *Microbacterium barkeri* from soil, revealing its pivotal role in biofloc formation, ammonia and nitrite reduction, and water quality enhancement. The introduction of M. barkeri in biofloc systems significantly improved tilapia growth, yielding higher weight gain, better feed conversion efficiency, and increased survival rates. Its nitrogen cycling capabilities make it a promising candidate for sustainable aquaculture, reducing reliance on frequent water exchange and external feed inputs. These findings position M. barkeri as a potential probiotic and biofloc-enhancing agent, offering an eco-friendly solution for improved fish health, growth, and water quality. Future research should investigate its long-term applications, scalability, and interactions with other biofloc microbes to optimize its benefits in commercial aquaculture.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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**References**

1. FAO. (2020). The state of world fisheries and aquaculture 2020: Sustainability in action. Food and Agriculture Organization of the United Nations.
2. FAO. (2022). The state of world fisheries and aquaculture 2022: Towards blue transformation. Food and Agriculture Organization of the United Nations.
3. Avnimelech, Y. (2009). Biofloc technology: A practical guidebook. The World Aquaculture Society.
4. Timmons, M. B., & Ebeling, J. M. (2010). Recirculating aquaculture systems. Cayuga Aqua Ventures.
5. Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., & Verstraete, W. (2012). Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture, 356–357, 1–6. <https://doi.org/10.1016/j.aquaculture.2012.05.002>
6. Browdy, C. L., & Bratvold, D. (2001). Use of microbial flocs in shrimp nursery systems. Avances en Nutrición Acuícola, 6, 207–216.
7. Hargreaves, J. A. (2013). Biofloc production systems for aquaculture. Southern Regional Aquaculture Center Publication, 4503, 1–12.
8. Hargreaves, J. A. (2006). Photosynthetic suspended-growth systems in aquaculture. Aquacultural Engineering, 34(3), 344–363. <https://doi.org/10.1016/j.aquaeng.2005.08.009>
9. Emerenciano, M., Ballester, E. L. C., Cavalli, R. O., & Wasielesky, W. (2017). Biofloc technology: Principles focused on potential species and the decline of its adoption. Aquaculture International, 25(3), 1053–1073. <https://doi.org/10.1007/s10499-017-0128-6>
10. Bondad-Reantaso, M. G., Subasinghe, R. P., Arthur, J. R., Ogawa, K., Chinabut, S., Adlard, R., Tan, Z., & Shariff, M. (2005). Disease and health management in Asian aquaculture. Veterinary Parasitology, 132(3–4), 249–272. <https://doi.org/10.1016/j.vetpar.2005.07.005>
11. Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. Environmental Microbiology, 8(7), 1137–1144. <https://doi.org/10.1111/j.1462-2920.2006.01054.x>
12. Dhotre, M. A., Nalle, D. A., & Kadam, S. (2024). Investigation of Potential Probiotic Bacterium (Bacillus subtilis CCI3) in the Formulated Diets on Immunity, Growth Performance and Nutritional Quality of Cyprinus carpio. *Asian Journal of Fisheries and Aquatic Research*, *26*(7), 25–42. <https://doi.org/10.9734/ajfar/2024/v26i7781>
13. Martínez-Córdova, L. R., Emerenciano, M., Miranda-Baeza, A., & Martínez-Porchas, M. (2017). Microbial-based systems for aquaculture of fish and shrimp: An updated review. Reviews in Aquaculture, 9(3), 179–197. <https://doi.org/10.1111/raq.12140>
14. De Schryver, P., Crab, R., Defoirdt, T., Boon, N., & Verstraete, W. (2008). The basics of bio-flocs technology: The added value for aquaculture. Aquaculture, 277(3–4), 125–137. <https://doi.org/10.1016/j.aquaculture.2008.02.019>
15. Zhang, H., Wang, H., Yang, K., et al. (2015). Nitrate removal by a novel autotrophic denitrifier (Microbacterium sp.) using Fe(II) as electron donor. Annals of Microbiology, 65, 1069–1078. <https://doi.org/10.1007/s13213-014-0931-x>
16. **Xu, W. J., & Pan, L. Q.** (2012). Effects of bioflocs on growth performance, digestive enzyme activity, and body composition of tilapia (Oreochromis niloticus × O. aureus) cultured in a biofloc-based system. Aquaculture, 356–357, 147–152. https://doi.org/10.1016/j.aquaculture.2012.05.022