ISOLATION AND ANTAGONISTIC EVALUATION OF POTENTIAL PROBIOTIC BACTERIA FROM NILE TILAPIA-BASIL IN AN AQUAPONIC SYSTEMS

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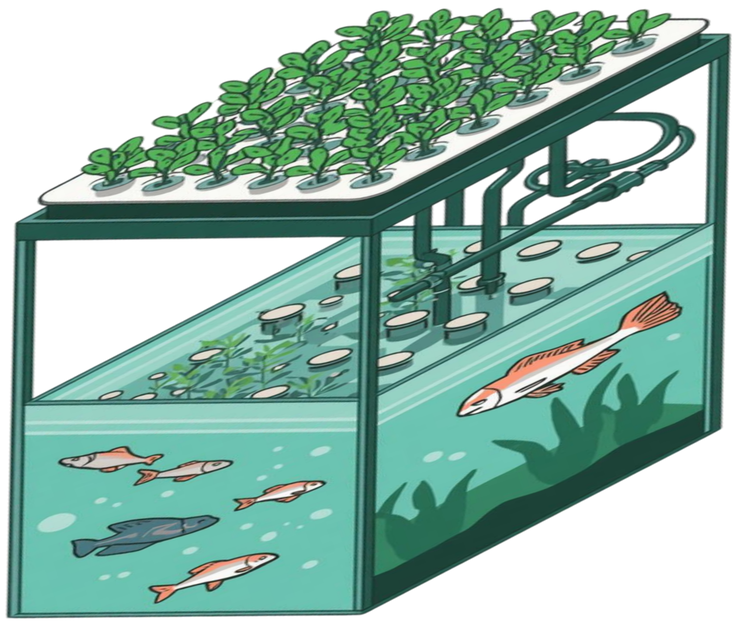
Introduction

The potential of probiotic candidates has been the subject of evaluation across a range of environments, including semi-intensive systems, river-based floating cages, farm cultures, and natural lakes (Chantharasophon et al., 2011; Chemlal-Kherraz et al., 2012). Recent studies have demonstrated an increasing number of investigations focusing on the utilisation of probiotics within aquaponic systems (Jossefa et al., 2024; Rudoy et al., 2025).

The objective of this study was to isolate and identify bacteria with the potential to be classified as probiotics from water samples collected from an aquaponic system that was cultivating tilapia and basil. Furthermore, the antagonistic effects of the probiotics that were identified as candidates against fish pathogens, including *Aeromonas hydrophila*, *Aeromonas caviae* and *Lactococcus garvieae*, were evaluated



Material and method

Tank water samples from the aquaponic system were collected into sterile 250 mL Erlenmeyer flasks. The samples were serially diluted (1:9, 1:99, and 1:999) using sterile distilled water. From each dilution, 200 µL aliquots were spread onto Tryptic Soy Agar (TSA) plates using sterile Drigalski spatulas, and all procedures were performed in triplicate. The inoculated plates were incubated at 22 °C for 24–48 hours. Following incubation, bacterial colonies were evaluated based on their morphological characteristics, including color, shape, and margin.

For MALDI-TOF MS analysis, a single colony from each purified isolate was transferred onto a 96-spot polished steel UV-absorbing MALDI-TOF target plate.

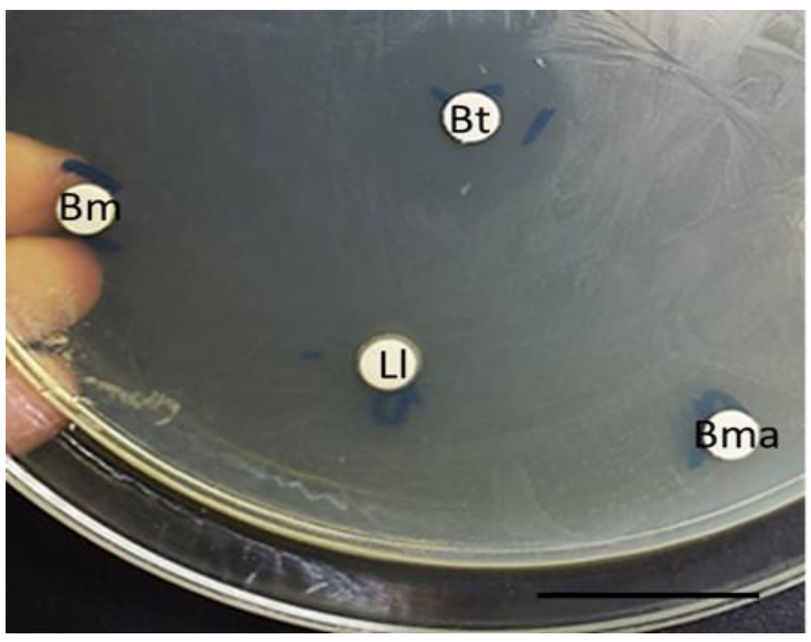


Figure: Antagonistic activity of candidate probiotic bacteria against *L. garvieae* determined by the disc diffusion method. No inhibition was observed *B. mycoides* (Bm)*, B. marisflavi* (Bma)*,* and *L. lactis* (Ll) while *B. thuringiensis* (Bt) showed a 2.3 cm inhibition zone against *L. garvieae.* (Bar:2.5 cm)

Results

The antagonistic activity of candidate probiotic bacteria *(B. mycoides*, *B. marisflavi*, *B. thuringiensis*, and *L. lactis*) isolated from aquaponic system water was evaluated against fish pathogens using the disc diffusion method. The results showed that none of the tested probiotic candidates produced inhibition zones against *A. hydrophila* or *A. caviae*, indicating a lack of antibacterial activity against these pathogens.

In contrast, *B. thuringiensis* exhibited a clear inhibition zone with a diameter of 2.3 cm against *L. garvieae*, demonstrating a strong antagonistic effect. No inhibition zone was observed in the negative control, confirming that the antibacterial activity was associated with metabolites present in the cell-free supernatants (Figure).

Furthermore, the positive control, oxytetracycline, produced inhibition zones of 2.1 cm, 1.8 cm, and 1.7 cm against *A. hydrophila*, *A. caviae*, and *L. garvieae*, respectively. Overall, these findings indicate that among the tested probiotic candidates, *B. thuringiensis* exhibits selective antibacterial activity with notable effectiveness against *L. garvieae*.

In conclusion, the findings suggest that aquaponic systems cultivating basil harbor beneficial microbial communities, and *B. thuringiensis* in particular shows promise as a probiotic candidate for controlling *L. garvieae* and improving aquaculture health management.

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