

Research Report

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In-Vitro Probiotic Characterization and Molecular Identification of Autochthonous *Bacillus* spp. from *Oreochromis niloticus*

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Abstract The escalating global aquaculture production necessitates sustainable alternatives to traditional antibiotics due to concerns regarding antimicrobial resistance and environmental contamination. This study investigated the probiotic potential and molecular identity of autochthonous bacterial isolates from the gastrointestinal tract of *Oreochromis niloticus* (Nile tilapia). Microbial populations from the upper and lower intestinal segments were enumerated and characterized using morphological, biochemical, and molecular methods. Results revealed significant spatial variation in bacterial distribution along the gastrointestinal tract, with total bacterial abundance significantly higher ($p < 0.05$) in the lower intestine than in the upper intestine. Among the bacterial groups identified, *Bacillus* spp. showed strong probiotic potential due to their high abundance, spore-forming capacity, and tolerance to gastrointestinal stress conditions. The isolates were Gram-positive, non-hemolytic, and demonstrated strong survival under acidic conditions (pH 2.5~7) and bile salt concentrations up to 1%. Antimicrobial activity assays revealed significant inhibition of pathogenic bacteria including *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus*, with inhibition zones reaching 34 mm. Molecular characterization using 16S rRNA gene sequencing confirmed the isolates as *Bacillus clausii*. Phylogenetic analysis showed high genetic similarity with previously reported *Bacillus clausii* strains in the NCBI database. These findings suggest that autochthonous *Bacillus clausii* isolated from *O. niloticus* possess desirable probiotic characteristics and may serve as promising candidates for functional probiotic applications in sustainable aquaculture.

Keywords Probiotics; *Bacillus clausii*; Nile tilapia; Gut microbiota; Antimicrobial activity; Aquaculture biotechnology

1 Introduction

Global aquaculture production has expanded rapidly over the past few decades, providing a significant proportion of the world's animal protein supply (FAO, 2014). Among cultured species, *Oreochromis niloticus* (Nile tilapia) is one of the most economically important fish species globally, being the most widely cultured fish species in Africa and the second most cultured species in Nigeria (Cavalcante et al., 2020). Its importance is due to its fast growth, adaptability to diverse environmental conditions, and high consumer demand.

However, intensification of aquaculture practices has led to increased disease outbreaks caused by bacterial pathogens such as *Aeromonas*, *Vibrio*, and *Edwardsiella* species. Traditionally, antibiotics have been used to control such infections, but their widespread application has resulted in antimicrobial resistance, environmental contamination, and food safety concerns (Cabello, 2006; Defoirdt et al., 2011).

Consequently, probiotics have gained increasing attention as environmentally friendly alternatives to antibiotics. Probiotics are defined as live microorganisms that confer health benefits to the host when administered in adequate amounts (FAO/WHO, 2002). In aquaculture, probiotics have been shown to improve nutrient utilization, enhance immune responses, and suppress pathogenic bacteria (Nayak, 2010; Ringø et al., 2018).

Host-associated or autochthonous probiotics are particularly promising because they originate from the host microbiome and are therefore better adapted to colonize the gastrointestinal environment. The gut microbiota of fish plays essential roles in digestion, nutrient metabolism, immune regulation, and pathogen exclusion (Gatesoupe, 1999; Ringø and Olsen, 2014).