

Among potential probiotic microorganisms, *Bacillus* species are widely studied due to their ability to produce antimicrobial compounds and extracellular enzymes, as well as their capacity to form spores that enhance survival under harsh environmental conditions (Cutting, 2011; Zorriehzahra et al., 2016).

Despite the increasing interest in probiotic development for aquaculture research in rainbow Trout (Ruholla et al., 2021), Catfish (Akanmu et al., 2016; Setufe et al., 2021), Nile Tilapia (Mohammadi et al., 2022), information on autochthonous probiotic bacteria associated with *O. niloticus* remains limited in many aquaculture systems (Cavalcante et al., 2020).

Therefore, the present study aimed to characterize the gut microbial population of *Oreochromis niloticus*, evaluate the in vitro probiotic properties of bacterial isolates, and identify promising probiotic strains using molecular techniques.

2 Materials and Methods

2.1 Sample collection and gut dissection

Healthy Nile tilapia were obtained and dissected under sterile conditions. The gastrointestinal tract was separated into upper and lower intestinal segments to investigate the spatial distribution of gut microbiota.

2.2 Bacterial isolation and enumeration

Gut contents were homogenized, serially diluted, and plated on nutrient agar plates. Colony forming units (CFU g⁻¹) were calculated after incubation. Isolation and enumeration of gut microbiota were performed following standard microbiological procedures described by Cappuccino and Welsh (2017) and Madigan et al. (2018).

2.3 Morphological and biochemical characterization

The isolates were characterized based on the following morphological and biochemical tests:

- 1 Gram staining was performed according to the classical method described by Beveridge (2001).
- 2 Endospore staining was conducted using the Schaeffer-Fulton staining technique (Schaeffer and Fulton, 1933).
- 3 Catalase activity was evaluated according to MacFaddin (2000).
- 4 Hemolytic activity was determined on blood agar following Verschuere et al. (2000).
- 5 These tests were used to evaluate the safety and probiotic potential of the bacterial isolates.

2.4 Acid tolerance test

Bacterial isolates were exposed to pH levels of 2.5, 3.0, 3.5, 4.0, and 7.0. Growth and survival were recorded. Acid tolerance assays were performed following methods described by Hyronimus et al. (2000) and Charteris *et al.* (1998).

2.5 Bile salt tolerance test

Bile tolerance was assessed using media containing different bile salt concentrations (0.2%, 0.4%, 0.6%, and 1.0%). Bile tolerance was evaluated according to the procedure described by Gilliland *et al.* (1984).

2.6 Antimicrobial activity assay

Antagonistic activity was evaluated against the following pathogenic bacteria:

- 1 *Escherichia coli*
- 2 *Salmonella typhi*
- 3 *Staphylococcus aureus*

The diameter of inhibition zones was measured in millimeters.

Antimicrobial activity was determined using the agar well diffusion method described by Tagg and McGiven (1971).