

2.7 Molecular identification

Genomic DNA was extracted and amplified using universal 16S rRNA primers. PCR products were sequenced and analyzed using BLAST analysis. Genomic DNA was extracted using a modified CTAB protocol according to Saraniya and Jeevaratnam (2012).

2.8 Statistical analysis

All experiments were conducted in triplicate and data were expressed as mean $\pm$ standard deviation (SD). Statistical analyses were performed using one-way analysis of variance (ANOVA) to determine significant differences among treatments.

When significant differences were detected ($p < 0.05$), means were separated using Duncan's Multiple Range Test (DMRT). Superscript letters were used to denote statistical differences between treatments.

For microbial enumeration data, bacterial counts were $\log_{10}$ transformed prior to analysis to ensure normal distribution and homogeneity of variance.

The statistical model applied was

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

Where:

- 1 Y_{ij} = observed value
- 2 μ = overall mean
- 3 T_i = treatment effect
- 4 ϵ_{ij} = experimental error

Significance was declared at $p < 0.05$.

All analyses were conducted using SPSS version 25.0 (IBM Corp., USA).

3 Results

3.1 Distribution of gut microbiota

The distribution of bacterial populations along the intestinal tract of *Oreochromis niloticus* is presented (Figure 1). The results indicate that the lower gut harboured consistently higher bacterial loads compared to the upper gut across all bacterial groups, including total heterotrophic bacteria, cocci, and *Bacillus* spp. This pattern may be associated with the more stable physicochemical conditions and nutrient availability in the distal intestine, which favor microbial proliferation and colonization.

Table 1 Relative abundance of bacterial groups in the gastrointestinal tract

Bacterial Group	Representative Genera	Upper Gut (CFU g ⁻¹)	Lower Gut (CFU g ⁻¹)	Probiotic Potential
<i>Helicobacter</i> / <i>Fusobacterium</i>	Opportunistic commensals	1.75×10^{1a}	6.65×10^{6b}	No
Cocci bacteria	Staphylococcus, Streptococcus	1.14×10^{6a}	3.05×10^{6b}	Variable
<i>Bacillus</i> spp.	Spore-forming rods	1.14×10^{6a}	3.05×10^{6b}	Yes

Different superscripts indicate significant differences ($p < 0.05$).

3.2 Morphological and biochemical characterization

3.2.1 Gram staining

Gram staining result presented shows both *Bacillus* spp. and Cocci bacteria to be gram positive while *Helicobacter* spp and *Fusobacterium* spp to be gram negative (Table 2).

3.2.2 Spore test

Table 3 shows that only *Bacillus* spp. is capable of sporulation, while the other isolates; Cocci bacteria, *Helicobacter* spp. and *Fusobacterium* spp. are non-spore forming bacteria.