

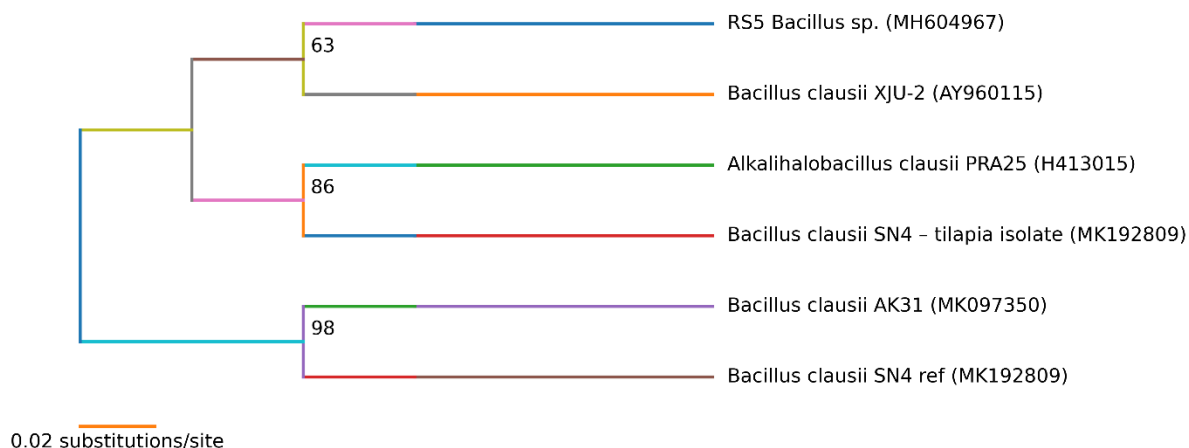


Figure 3 Phylogenetic tree based on 16S rRNA gene sequences

Image caption: The phylogenetic tree shows the relationship between the isolated strain *Bacillus clausii* SN4 from *Oreochromis niloticus* and closely related *Bacillus* species retrieved from GenBank. The tree was constructed using the Neighbor-Joining method, and bootstrap values (%) based on 1000 replicates are indicated at branch nodes. The scale bar represents 0.02 nucleotide substitutions per site.

4 Discussion

4.1 Gastrointestinal microbial distribution in *Oreochromis niloticus*

In the present study, microbial abundance was significantly higher in the lower intestine compared with the upper intestine (Table 1 and Figure 1). This pattern is consistent with the typical spatial organization of microbial communities in the gastrointestinal tract of teleost fish. The distal intestine generally provides more favorable conditions for microbial colonization due to lower oxygen concentrations, slower digesta transit, and greater availability of fermentable substrates. These environmental conditions promote the growth of facultative and anaerobic microorganisms that participate in fermentation processes and nutrient metabolism. Similar spatial distribution patterns have been reported in several fish species, including tilapia, salmonids, and carp, where the distal gut harbors higher microbial densities and greater microbial diversity (Nayak, 2010; Ringø et al., 2016). The higher bacterial counts observed in the lower intestine in this study therefore suggest that this region represents a major site for microbial metabolic activity and host–microbe interactions.

4.2 Probiotic potential of *Bacillus* species

Among the bacterial isolates obtained from the gastrointestinal tract of *O. niloticus*, members of the genus *Bacillus* demonstrated promising probiotic characteristics. *Bacillus* species are widely recognized as beneficial probiotic candidates in aquaculture due to several advantageous physiological traits, including their ability to form endospores, produce antimicrobial metabolites, and secrete extracellular digestive enzymes.

The spore-forming ability observed in the isolates as presented in Table 3 is particularly significant for probiotic applications. Endospores exhibit remarkable resistance to environmental stressors such as heat, desiccation, and acidic conditions. This resilience improves the stability of probiotic formulations during feed processing and storage while ensuring survival during passage through the gastrointestinal tract (Cutting, 2011; Hong et al., 2005). As a result, *Bacillus*-based probiotics are commonly incorporated into aquaculture feeds to improve digestive efficiency and disease resistance.

4.3 Safety assessment of the probiotic candidates

The safety evaluation showed that the isolates exhibited no hemolytic activity on blood agar, as shown in Table 4, indicating that they are non-hemolytic and therefore unlikely to be pathogenic. Hemolysis is commonly used as an indicator of bacterial virulence, and the absence of hemolytic activity is considered an important safety criterion