

for probiotic candidates (Verschuere et al., 2000). The non-hemolytic behavior observed in this study therefore supports the suitability of these isolates for further probiotic development. Catalase activity was positive in *Bacillus* spp., indicating the ability of the isolates to produce catalase enzymes that degrade hydrogen peroxide into water and oxygen.

4.4 Acid and bile tolerance as probiotic selection criteria

Tolerance to acidic and bile environments represents a fundamental requirement for probiotic microorganisms, as they must survive the harsh conditions of the stomach and intestine before exerting beneficial effects within the host.

In the present study, the bacterial isolates demonstrated considerable survival across a wide pH range (2.5~7.0) table 5, with *Bacillus* isolates showing significantly higher tolerance compared to cocci bacteria. The ability to withstand acidic environments suggests that these isolates possess physiological adaptations that maintain cellular stability under gastric stress. Similar acid tolerance characteristics have been reported for *Bacillus clausii* strains used in commercial probiotic preparations, further supporting the robustness of this species under gastrointestinal conditions (Hong et al., 2005).

The isolates in this study exhibited tolerance to bile concentrations of up to 1% table 6, with *Bacillus* isolates showing superior tolerance compared to cocci bacteria. Such tolerance indicates that the isolates are capable of surviving within the intestinal environment where bile salts are present during digestion. Previous studies have suggested that bile tolerance in probiotic bacteria may be associated with mechanisms such as bile salt hydrolase activity and membrane modifications that protect cells from bile-induced stress (Gilliland et al., 1984). Therefore, the acid and bile tolerance exhibited by the isolates strongly supports their probiotic potential.

4.5 Antimicrobial activity against fish pathogens

The antimicrobial activity of *Bacillus* species is well documented and is often attributed to the production of bioactive compounds such as bacteriocins, lipopeptides (including surfactin, iturin, and fengycin), and organic acids (Zhou et al., 2010; Ringø et al., 2018). These antimicrobial substances inhibit pathogenic microorganisms through mechanisms such as membrane disruption, competitive exclusion, and nutrient competition. The strong inhibitory activity of *Bacillus* species against *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* as observed in this study is presented in table 7 and figure 2 therefore suggests that the isolates may contribute to maintaining microbial balance within the gastrointestinal tract and preventing pathogen colonization in *O. niloticus*.

4.6 Molecular identification and phylogenetic analysis

Molecular characterization using 16S rRNA gene sequencing identified the isolates as *Bacillus clausii*. This species is widely recognized for its probiotic properties and has been extensively used in both human and veterinary probiotic formulations. *Bacillus clausii* is known for its remarkable resilience to environmental stress and its ability to modulate host immune responses.

Previous studies have demonstrated that *B. clausii* can enhance innate immune responses, stabilize intestinal microbiota, and improve resistance to bacterial infections in aquaculture species (Ringø et al., 2018). The identification of this species within the gastrointestinal tract of *O. niloticus* therefore suggests that it may play a natural role in maintaining gut microbial homeostasis.

Phylogenetic analysis further confirmed the taxonomic identity of the isolate by demonstrating close genetic relationships with previously reported *Bacillus clausii* strains in the NCBI database. The high bootstrap support values (86%~98%) observed in the phylogenetic tree indicate strong confidence in the clustering of the isolate within the *B. clausii* lineage.

4.7 Significance of autochthonous probiotics in aquaculture

The identification of autochthonous probiotic microorganisms is particularly advantageous for aquaculture