

applications because host-associated bacteria are more likely to colonize and persist within the gastrointestinal tract. Unlike allochthonous probiotics introduced from external sources, autochthonous strains are naturally adapted to the host's intestinal environment, which enhances their colonization efficiency and functional stability (Gatesoupe, 1999; Ringø and Olsen, 2014). The isolation of *Bacillus clausii*-like strain of *O. niloticus* therefore represents a promising step toward developing host-specific probiotic formulations that could improve fish health and aquaculture productivity.

4.8 Limitations and future research directions

Despite the promising findings obtained in this study, several limitations should be acknowledged. First, the probiotic properties of the isolates were evaluated only through in vitro assays, which may not fully reflect their performance within the complex biological environment of the fish gastrointestinal tract. Second, the study focused primarily on basic probiotic characteristics such as acid tolerance, bile tolerance, and antimicrobial activity, while other important functional properties—including enzyme production, adhesion ability, and immunomodulatory effects—were not investigated.

Future research should therefore focus on in vivo validation of the probiotic potential of the identified *Bacillus clausii* strain. Controlled feeding trials should be conducted to evaluate its effects on growth performance, feed conversion efficiency, immune responses, and disease resistance in *O. niloticus*. Additionally, advanced molecular approaches such as metagenomics and transcriptomics could provide deeper insights into the interactions between the probiotic strain, native gut microbiota, and host immune pathways.

4.9 Implications for sustainable aquaculture

Overall, the findings of this study contribute to the growing body of knowledge on fish gut microbiota and probiotic development for aquaculture. The isolation and characterization of *Bacillus clausii* from the gastrointestinal tract of *Oreochromis niloticus* highlight the potential of host-associated microorganisms as sustainable alternatives to antibiotics in aquaculture production systems. By promoting microbial balance, enhancing digestive efficiency, and suppressing pathogenic bacteria, such probiotics may play an important role in improving fish health, increasing aquaculture productivity, and supporting environmentally sustainable aquaculture practices.

Authors Contribution

O.A. Onada was responsible for conceptualization, methodology development, investigation, data curation, laboratory experiments, formal analysis, and writing the original draft. E.K. Ajani and E.F. Osho contributed to supervision, project administration, methodology validation, result interpretation, and manuscript review and editing. All authors have read and approved the final version of the manuscript.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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