ISOLATION AND CHARACTERIZATION OF HOST-ASSOCIATED *BACILLUS SPP.* AS PROBIOTICS FOR ENHANCING FISH GROWTH AND WATER QUALITY

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ABSTRACT

Incorporating *Bacillus* probiotics into feed can yield additional benefits, including enhanced growth and production in aquaculture. However, study did not receive much attention on enriched feed with host-associated *Bacillus* spp. isolated from the fish gut. Therefore, this study was conducted to isolate *Bacillus* spp. from the tilapia gut and to evaluate their probiotic potential for the growth of Nile tilapia (*Oreochromis niloticus)* and water quality parameters. Bacterial strains were isolated from tilapia guts using *Bacillus* selective media and then were identified by MALDI-TOF mass spectrometry and 16S rRNA gene sequencing. Probiotic potential was evaluated based on pH (2.5) and bile (0.5%) tolerance, extracellular enzyme production, and antibiotic susceptibility profiling. A total of 16 bacterial strains were isolated; of them, two were identified as *Bacillus pumilus* and *Bacillus subtilis.* Both survived in low pH and high bile concentration; showed extracellular protease and amylase activity; and had high susceptibility to most of the selected antibiotics. A 60-day *in vivo* experiment was conducted under three treatments: control (without probiotics); T2H (*B. pumilus*); and T2B (*B. subtilis*). A significant difference ($p < 0.05$) was observed among three treatments regarding weight gain (WG; g), average daily gain (ADG; g/day), and specific growth rate (SGR; %/day). Remarkably, the highest WG, ADG, and SGR values were estimated in T2B compared to T2H and control. Additionally, T2B exhibited significantly ($p < 0.05$) increased nitrate level compared to T2H and control. This study indicated that host-associated *B. pumilus* and *B. subtilis* could be the potential probiotics for *O. niloticus*.

KEYWORDS: Probiotics, *Bacillus pumilus, Bacillus subtilis*, Nile tilapia

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Introduction

Fish are considered one of the prime animal protein sources, which can meet the rising global demand of aquatic foods, provide nutrition, food security and livelihoods (FAO, 2024). In Bangladesh, fish contributed 60% of animal protein intake, which largely depends on aquaculture production (DoF, 2024). Sustainable aquaculture therefore requires healthy fish seeds, a good culture environment, and most importantly, economically feasible and high-quality feed (Amenyogbe et al., 2021). Application of a high-quality feed not only enhances growth, survival and productivity but also minimizes aquaculture pollution caused by feed wastage and nutrient loading (Guo et al., 2020; Kong et al., 2020). On the other hand, low-quality fish feed reduces production efficiency through the occurrence of harmful accumulation in the water, deteriorating water quality, increasing stress and disease risk, impairing growth, and potentially causing mass mortality (Ghori et al., 2022; Lieke et al., 2020). As aquaculture production is constrained by high feed costs, which account for about 60-75% of total production expenses, improving feed quality is also essential for reducing the cost-benefit ratio, enhancing profitability and ensuring sustainable aquaculture (Mamun-Ur-Rashid et al., 2013). In this context, dietary probiotics have emerged as promising quality feed additive that can guarantee aquaculture's long-term viability and profitability (Hossain et al., 2023).

Probiotics are live microorganisms which, when administered in adequate amounts, confer health benefits on the host (FAO, 2001). A probiotic must meet the criteria of being tolerant of low to high pH as well as bile salt concentrations (>2.5%), lacking antibiotic resistance genes, providing extracellular digestive enzymes and so on (Simon et al., 2021). The host-associated probiotics in fish has often been isolated from the gut of fish, notably known as autochthonous probiotics having the ability to better colonize than the microbiota that already resides in the gut. They significantly enhanced the growth, productivity and overall health status of fish and rearing environment (Van Doan et al., 2020; Hlordzi et al., 2020). In Bangladesh, most of the imported commercial probiotics are terrestrial or non-fish originated, likely being concerned for their effectiveness and viability when applying in culture system (Hossain et al., 2023; Ninawe & Selvin, 2009). *Bacillus*, host-associated probiotics, are widely used in aquaculture due to their endospore formation, which enables resistance to harsh environmental conditions, including high temperatures during feed processing, acidic gastric condition, and bile exposure in the intestine, making them viable aqua-feed additives (Van Doan et al., 2020; Kuebutornye et al., 2020). Several species, *B. subtilis*, *B. pumilus*, *B. clausii*, *B. velezensis*, *B. safensis*, *B. cereus*, *B. coagulans*, *B. licheniformis*, *B. amyloliquefaciens*, *B. mojavensis*, and *B. megaterium* have been commonly applied in aquaculture (Wu et al., 2021; Soltani et al., 2019; Yi et al., 2018).

Nile tilapia (*Oreochromis niloticus*) is one of the most widely cultured aquaculture species globally (FAO, 2024). In Bangladesh, its production has expanded rapidly, and the country currently ranks 4th in the world and 3rd in Asia in tilapia production (DoF, 2024). Its hardiness, quick growth, high nutritional value, tolerance to diverse environments and handling conditions, short production cycle, low feed requirement, and easy marketing due to the absence of lateral musculature spines make it an ideal species for both small-scale and large-scale aquaculture (Shija et al., 2023; Moesch et al., 2016; Ogello et al., 2014). Although many studies showed probiotic strains of *Bacillus* were viable for tilapia culture (Ferdoush et al., 2025; Yu et al., 2025; Shija et al., 2025; Dighiesh et al., 2024; Etyemez Buyukdeveci et al., 2023; Hassaan et al., 2021; Asha et al., 2024; Munni et al., 2023; Hossain et al., 2022; Zabidi et al., 2021), comprehensive assessment of host-associated *Bacillus* probiotics in Bangladesh remains limited. Therefore, this study aimed to isolate and identify *Bacillus* spp. from the gastrointestinal tract of *O. niloticus* and then evaluate their probiotic potential through laboratory screening along with a field trial on growth performance and water quality parameters. The experiment was carried out in a clear-water culture system to better understand their practical applicability in aquaculture.

Materials and Methods

Isolation and *MALDI-TOF Identification*

A total of 12 healthy fish with an average body weight of approximately 150 g were collected from three ponds of the Fish Seed Production Farm, Kurigram Sadar, Kurigram (Figure 1). The gut of each fish was aseptically dissected under sterile conditions. For gut bacteria isolation, 1 g of intestine was homogenized with 9 ml of sterile distilled water. The homogenate was serially diluted with normal saline solution (NSS; 0.85% NaCl), and the aliquot was spread onto *Bacillus* selective agar plates. The plates were incubated for 24 h at 37°C under aerobic condition (Kavitha et al., 2018). Morphologically different *Bacillus*-like colonies were picked up, and repeated streaking was done in fresh Tryptic Soy Agar media to obtain pure colonies. Each single isolate was inoculated in LB (Luria-Bertani) broth and incubated at 37°C with constant shaking at 150 rpm (Kuebutornye et al., 2020). The isolates were identified by MALDI-TOF mass spectrometry (MALDI Bio-typer Sirius RUO System, Bruker Daltonics GmbH & Co. KG, Bremen, Germany) at the Animal Biotechnology Division, National Institute of Biotechnology (NIB), and stored at -40°C in LB broth supplemented with 30% glycerol. Based on the confidence scores of the best matches, *Bacillus* isolates corresponding to previously reported probiotic strains were selected for further study.

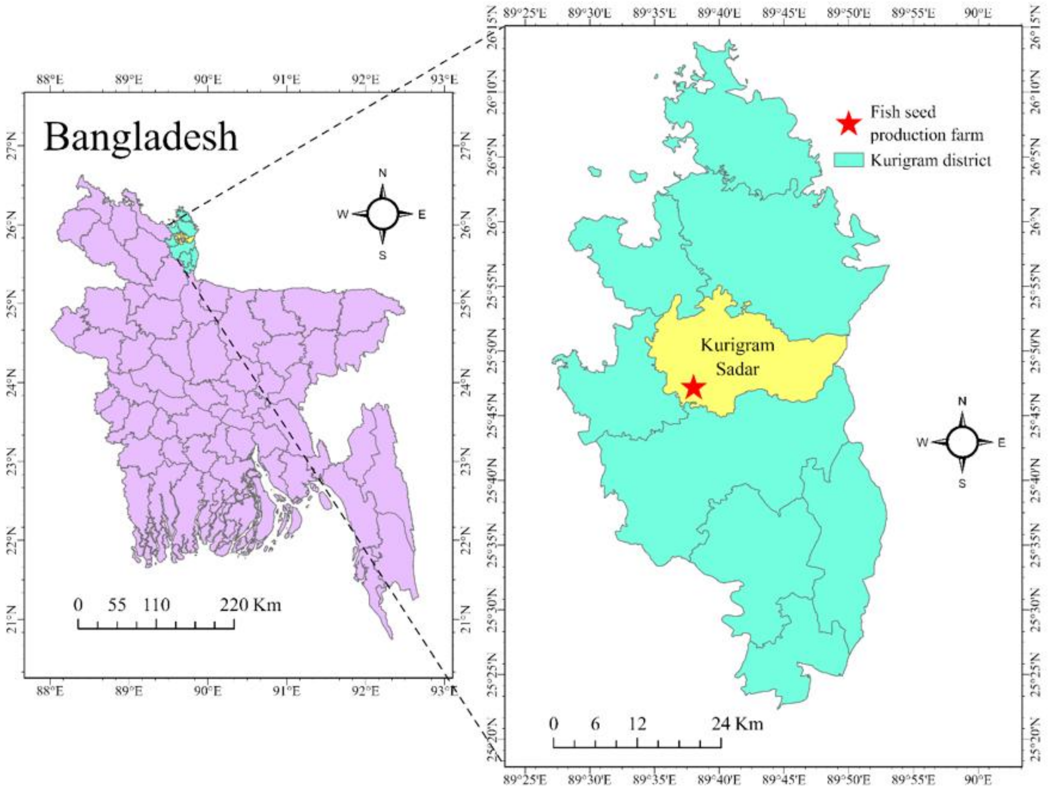

Figure 1. Geographical location of sampling site used to isolate and identify potential host-associated probiotic bacteria.

pH and Bile Tolerance

pH and bile tolerance of probable probiotic *Bacillus* species were assessed following the method of gastric juice tolerance described by Kavitha et al. (2018) and Kuebutornye et al. (2020), respectively, with slight modifications. For pH tolerance, a fresh overnight culture of bacteria was inoculated into LB broth adjusted to pH 2.5. The pH was adjusted with a 37% concentration of HCl and 5N NaOH. The inoculated broth was incubated for 0 h and 3 h at 37°C and spread onto Tryptic Soy Agar plate. The growth of bacteria was monitored by counting viable colonies following an overnight incubation at 37°C. For bile resistance, a 24 h bacterial culture was centrifuged at 8000 rpm for 15 min, washed twice with PBS solution (pH 7.4), and resuspended in PBS solution (pH 7.4) containing 0.5% bile salts. Growth was monitored by measuring absorbance using a spectrophotometer (U-2910 spectrophotometer, HITACHI, Tokyo, Japan) at 600 nm after 2 h of incubation at 37°C with continuous shaking (150 rpm).

Extracellular Enzyme Activity

The extracellular amylase and protease activities of the selected isolates were screened according to Reda et al. (2018). The amylase activity was determined by cultivation on starch agar (1%). The isolates were incubated overnight in Tryptic Soy Broth (TSB), followed by spotting onto the agar plates and incubated at 37°C for 24 h, and then the bacterial growth was flooded with Lugol's iodine (1%). For protease activity, the isolates were spotted on Tryptic Soy Agar plates with 3% skimmed milk (10%). Colonies characterized by amylase and protease activity were surrounded by a clear zone.

Antibiotic Susceptibility Assay

The antibiotic susceptibility of the selected isolates was evaluated using the disc diffusion method with commercially available antibiotic discs (Liofilchem, Italy), following the procedures described by Kavitha et al. (2018), Patel et al. (2009) and Thankappan et al. (2015). The antibiotic discs used in this study were azithromycin, tetracycline, erythromycin, ceftriaxone, ciprofloxacin, gentamicin, amoxicillin, cefepime, ampicillin, levofloxacin, oxytetracycline, and chloramphenicol. Mueller-Hinton agar plates were uniformly inoculated with each selected isolate, after which the antibiotic discs were aseptically placed on the agar surface. The plates were incubated at 37°C for 24 h, and antibiotic susceptibility was determined by measuring the diameter of the inhibition zones (mm) surrounding each disc.

Bacterial 16S rRNA Identification

Genomic DNA was extracted using the phenol-chloroform method with slight modifications to the standard protocol described by Sudha et al. (2019). The purity of DNA was checked by calculating the ratio of absorbance at 260nm/280nm. The values of 1.8 generally indicate 100% purity. A set of universal primers (27F: AGAGTTTGATCATGGCTCAG and 1492R: TACGGTTACCTTGTTACGACTT) was used to amplify the 16S rRNA gene. The PCR reaction volume was 25µl, containing 1µl of template DNA, 10.5µl of nuclease-free water, 0.5µl of each primer, and 12.5µl of 1× Taq Master Mix. The PCR amplification was initiated with denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 60 s, and extension at 72°C for 2 min, finishing by holding the reaction mixture at 72°C for 10 min. The PCR products were detected by agarose gel (1% w/v) electrophoresis stained with RedSafe™ (iNtRON Biotechnology, Korea) and compared with 1Kb DNA ladder (New England Biolabs, UK) on the gel documentation system (Azure Biosystems C200, USA). The PCR products were purified and sent for sequencing at the Molecular Biotechnology Division of NIB. After purification, the PCR products were subjected to Sanger's dideoxy sequencing using the ABI 3500 Genetic Analyzer, exploiting the BigDye® Terminator v3.1 cycle sequencing kit.

Molecular Phylogeny Analysis

After sequencing, the collected ab1 file of nucleotide sequences was trimmed and assembled using "BioEdit v7.7.1" software. The assembled sequences (contigs) in FASTA format were blasted into BLASTNs (Basic Local Alignment Search Tool for Nucleotides) against the NCBI database. Similarities >99% among the sequences were considered significant for species-level bacterial identification. The best-matched sequence files were downloaded from the NCBI database. Multiple sequence alignment was performed using the MAFFT v7 (https://mafft.cbrc.jp/alignment/server/) online server (Katoh and Standley, 2013), and a phylogenetic tree from the resultant MSA data was constructed where the neighbor-joining method (NJ) method and general time reversible (GTR) model with 1000 bootstrap replicates were applied. The constructed tree was edited using MEGA12 (Kumar et al., 2024), The 16S rRNA gene sequences of the isolates were submitted to NCBI to obtain accession numbers.

Feed Preparation

The experimental feed was prepared according to Zokaeifar et al. (2012) with minor modifications. The selected isolates, T2H and T2B, were grown in LB broth using a shaking incubator at 37°C for 24 h. The cultures were then centrifuged at 3000 g for 10 min at 4°C and, after discarding the supernatant, the pelleted bacteria were washed two times with sterile NSS and resuspended in skimmed milk. The cell densities of the suspensions were calculated using a spectrophotometer at 600 nm and also correlated to the colony-forming unit (CFU) using the spread-plate technique. These suspensions were kept at 4°C until used. A commercial tilapia nursery feed (Nourish Feed Ltd., Bhaluka, Mymensingh, Bangladesh) containing 35.0-40.0% crude protein, 5.0-8.0% crude lipid, and ≤10.0% moisture was used as the basal diet for bacterial strain supplementation. Molasses was added to the suspension of the selected bacterial isolates before the suspension was sprayed onto the commercial feed. The feed was prepared to achieve a final bacterial concentration of approximately 10^{10} CFU/g (Rengpipat et al., 1998). The amount of the selected isolates in the feed was determined using the spread-plate technique. Briefly, 1 g of feed was randomly sampled and serially 10-fold diluted in PBS (pH 7.2), and 100 µl of each dilution was spread onto Tryptic Soy Agar in order to estimate the bacterial concentration (CFU/g).

Experimental Setup

A total of 1000 *O. niloticus* fry with an average weight of 0.2475±0.06 g was purchased from Mesharsh Chairman Agro-Fisheries, Lalmohon, Bhola. These were carefully transported to the Fisheries Biotechnology Division, NIB. The fish were randomly stocked into nine plastic tanks (each filled with 20 liters of water) and acclimatized for ten days in the ambient environmental conditions. The fish were fed ad libitum with a control diet. The behavior and apparent health status of each fish were monitored. After acclimatization, the tanks were divided into three groups, namely, control (only basal diets), T2H and T2B. Twenty fish were randomly stocked in each tank for each treatment in triplicate (Lara-Flores et al., 2003). The fish were fed the experimental diets to apparent satiation three times daily for 60 days. To maintain probiotic viability, the probiotic-supplemented feed was freshly prepared twice weekly and stored at 4°C until use (Llewellyn et al., 2018; Chomová et al., 2023). Adequate aeration was provided for all treatments to maintain saturated dissolved oxygen. Unutilized feed and feces were siphoned off to maintain good water quality. The tanks were cleaned every two days to reduce the potential risk of bacterial growth and ammonia toxicity (Islam et al., 2021).

Growth Performances

The weight of fish in all treatments was recorded in the beginning and at the end of the experiment. All fish were determined for growth rate using the mathematical growth model according to Dighiesh et al. (2024) as follows:

Weight gain (WG; g) = final body weight (FBW, g) − initial body weight (IBW, g)

Average daily gain (ADG; g/day) = [(FBW - IBW) / duration (days)]

Specific growth rate (SGR; %/day): = 100 × [(ln FBW - ln IBW) / duration (days)]

Survival rate (%) = 100 × (final number of fish / initial number of fish)

Water Quality Parameters

The water was directly supplied from the groundwater, and it was treated with $CaCO_3$ before use. At the end of the experiment, water samples from all tanks were collected into a beaker in the laboratory. Physico-chemical analysis of water samples was carried out to determine the total ammonia (NH_3), nitrite (NO_2^-), and nitrate (NO_3^-) using a test kit (API, USA). pH (HI-2002, HANNA Instruments, USA), temperature, dissolved oxygen (DO), total dissolved solids (TDS), and electrical conductivity (EC) were measured using a Multi-Parameter Meter (Thermo Scientific™ Eutech™ CyberScan PCD 650, Singapore**)** according to Dighiesh et al. (2024).

Statistical Analysis

All data were analyzed using the SPSS version 26.0 software (IBM SPSS Statistics, New York, USA) for Windows. Prior to analysis, data were checked for normality and homogeneity of variances using Levine's test. One-way analysis of variance (ANOVA) and Tukey's HSD post hoc test were used to determine significant variation ($p < 0.05$) of the parameters studied among treatments.

Results

Isolation and MALDI-TOF Identification

A total of 16 bacterial isolates were recovered from the gut of tilapia fish and of them, two isolates, T2H and T2B, were selected regarding their MALDI-TOF identification score of 1.83 and 2.04, respectively. T2H had the best matches with *Bacillus pumilus*, while T2B showed the closest similarity with *Bacillus subtilis*. Their colony morphology is summarized in Table 1 and colony appearance is presented in Figure 2(a, b). The observed morphology also characterized as *Bacillus* spp. using MALDI-TOF.

Table 1. Colony morphology of the selected two isolates cultured onto Tryptic Soy Agar plate.

Isolates	Shape	Surface	Texture	Size	Color	Elevation	Margin
T2H	Irregular	Smooth	Moist	Small	Creamy white	Crateriform	Undulate
T2B	Circular	Wrinkled	Dry	Large	Dull white	Flat	Filamentous

pH and Bile Tolerance

The selected strains gave promising tolerance results to pH and bile-mediated stress (Table 2). Survivability was monitored by counting the number of colony-forming units following 0 h, and 3 h of exposure to pH 2.5 adjusted LB broth The T2H and T2B showed tolerance rates of 81.25% and 65.85%, respectively, after overnight incubation. Additionally, after 2 h of incubation in PBS containing 0.5% bile salts, optical density measurements at 600 nm showed minimal reduction in growth, with OD 600 values retaining 85-91% of initial readings across strains.

Table 2. pH and bile tolerance analysis of two *Bacillus* strains, T2H and T2B in terms of CFU/ml.

Name of the strains	pH tolerance			Bile tolerance		
	Viability of Bacteria in CFU/ml ($\times 10^6$)		% of survivability after 3 h	OD at 600 nm		% of survivability after 2 h
	0 h	3 h		0 h	2 h	
T2H	1.597 ± 0.025	1.307 ± 0.021	81.84	0.822 ± 0.001	0.712 ± 0.001	86.59
T2B	4.093 ± 0.110	2.700 ± 0.020	65.96	1.016 ± 0.005	0.918 ± 0.008	90.35

Values are given as mean ± SD of four separate experiments.

Extracellular Enzyme Activity

Both *Bacillus* strains, T2H and T2B, exhibited protease activity on skimmed milk agar plates (10%), as evidenced by a distinct clear zone of 12 mm and 11 mm surrounding the growth, respectively (Figure 2c). The T2B produced a clear zone of 20 mm on starch agar plates (1%) after flooding with Lugol's iodine (Figure 2d), confirming amylase activity. In contrast, the T2H did not exhibit amylase activity, with no clear zone observed around the colony; instead, it showed blue-black staining (brown color remains in colony).

Antibiotic Susceptibility Assay

The results of antibiotic susceptibility test of the selected bacterial strains are shown in Table 3. Both strains were highly susceptible (more than 10 mm of zone of inhibition) to most of the antibiotics Viz; azithromycin, tetracycline, erythromycin, ceftriaxone, ciprofloxacin, gentamicin, amoxicillin, levofloxacin, oxytetracycline, and chloramphenicol. Both strains were found to be resistant to cefepime, while only T2B showed intermediate susceptibility to ampicillin. The strains, T2B and T2H showed susceptibility with zone diameters ranging from 12 to 44 mm and 14 to 36 mm, respectively.

Table 3. Zone of inhibition (mm) for antibiotic susceptibility of two *Bacillus* strains, T2H and T2B.

Antibiotic (µg)	T2H	T2B
Azithromycin (15 µg)	21(S)	34(S)
Erythromycin (15 µg)	23(S)	40(S)
Ciprofloxacin (5 µg)	24(S)	44(S)
Ceftriaxone (30 µg)	23(S)	38(S)
Tetracycline (30 µg)	25(S)	20(S)
Gentamicin (10 µg)	25(S)	34(S)
Cefepime (30 µg)	0(R)	0(R)
Ampicillin (2 µg)	18(S)	10(I)
Amoxicillin (30 µg)	14(S)	12(S)
Levofloxacin (5 µg)	36(S)	42(S)
Oxytetracycline (30 µg)	32(S)	23(S)
Chloramphenicol (30 µg)	21(S)	40(S)

S: Susceptible (>10 mm); I: Intermediate (6-10 mm); R: Resistant (<6 mm or no zone)

Growth Performances

The results of growth performances, including weight gain (WG; g), average daily gain (ADG; g/day), specific growth rate (SGR; %/day), and survival rate, are presented in Figure 3. The highest WG, ADG, and SGR were observed in fish in the T2B group, and it was significantly ($p < 0.05$) different compared to fish in the control group. But no significant differences ($p > 0.05$) regarding these values were recorded either between the T2H and T2B groups or between the T2H and control groups. Additionally, there was no dietary effect found on the survivability ($p > 0.05$) of the fish among treatments.

Water Quality Parameters

As the study was conducted under controlled conditions for a limited duration, the observed variations reflect treatment effects rather than monthly changes. All the measured water quality parameters remained within the productive range throughout the trial (Table 4). No significant difference ($p > 0.05$) was observed in terms of temperature, DO, pH, TDS, EC and ammonia level; meanwhile, a significant difference ($p < 0.05$) was observed in nitrite and nitrate levels. The T2B group showed significantly higher nitrite levels than the control, but significantly similar to T2H group. Again, the T2B and T2H group showed significantly increased nitrate compared to the control group.

Bacterial Identification Based on 16S rRNA Gene

From BLAST results, it was found that the 16S rRNA partial gene sequences of the isolates T2H and T2B had 99% sequence homology with *Bacillus pumilus* and *Bacillus subtilis*, respectively. The 16S rRNA gene sequences of T2H and T2B were submitted to NCBI GenBank, and accession numbers were obtained. Thus, the isolates T2H and T2B were designated as *Bacillus pumilus* strain T2H (PZ311998) and *Bacillus subtilis* strain T2B (PZ311999), respectively, and they are publicly available in the NCBI database.

Phylogenetic Identification

From the phylogenetic tree, it was clearly found that T2H and T2B were clustered with their best matches and showed separately in two groups with one outgroup (Figure 4). These two isolates were also distinctly separated from each other. Phylogenetic analysis also ensures that T2H and T2B belong to

Bacillus pumilus and *Bacillus subtilis*, respectively. *Priestia aryabhattai*, formerly known as *Bacillus megaterium* can be easily differentiated from members of the *B. cereus* and *B. subtilis* groups based on morphological, biochemical and molecular characteristics (Vos et al., 2009), thus used as an outgroup of this analysis. Bootstrap values ranging from 87% to 100% strongly supported the phylogenetic tree. The major clades of the two isolates received 100% bootstrap support. It indicated a high level of confidence in their taxonomic placement. The 87% bootstrap value within the *B. pumilus* clade also represents reliable statistical support for the inferred relationship.

Table 4. Water quality parameters during the culture of Nile tilapia fed with *Bacillus* strains, T2H and T2B in the diet for 60 days.

Parameters	Treatments		
	Control	T2H	T2B
Temperature (°C)	29.28±0.62	29.07±0.28	29.35±0.54
Dissolved Oxygen (DO; mg/L)	9.23±1.33	9.74±0.22	9.28±0.34
pH	7.74±0.08	7.65±0.07	7.68±0.19
Total Dissolved Solid (TDS; ppm)	124.92±15.28	116.28±0.99	107.20±1.30
Electrical Conductivity (EC; µS/cm)	132.40±17.23	125.70±0.46	119.38±0.56
Ammonia	0.25±0.07	0.25±0.00	0.25±0.00
Nitrite	0.54 ± 0.19^{b}	0.83 ± 0.14^{ab}	1.08 ± 0.14^{a}
Nitrate	12.50 ± 6.6^{c}	29.17 ± 21.26^{b}	73.33 ± 11.55^{a}

Values are presented as mean ± SD. The mean values in the same row with different lowercase superscripts (a,b,c) are significant differences ($p < 0.05$).

Discussion

Host-associated probiotics have been shown to support host growth and immunity, as well as to alter a disrupted host microbiome and intestinal morphology for better nutrient absorption (Kuebutornye et al., 2020). Van Doan et al. (2020) reviewed that host-associated probiotics are the future of sustainable aquaculture. The gut of *O. niloticus* harbors diverse bacteria that could be a more effective resource for indigenous probiotics development than any allochthonous probiotic in tilapia production. This study was conducted to evaluate in vitro

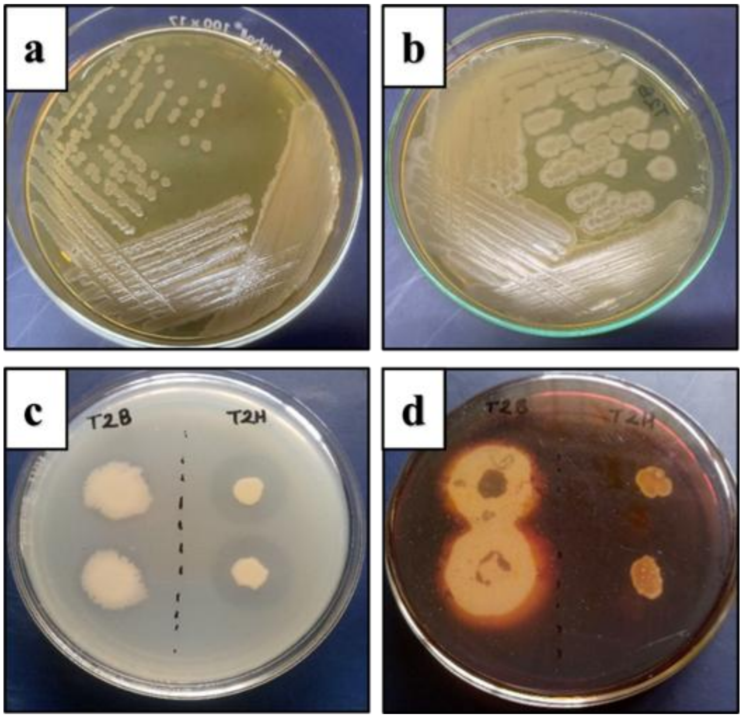

Figure 2. Colony appearances of the two isolates on Tryptic Soy Agar plates: (a) T2H; (b) T2B. Extracellular enzyme activity of the two isolates: (c) Protease activity; (d) Amylase activity.

probiotic potential, *i.e.*, pH and bile tolerance, extracellular amylase, and protease production, and antibiotic susceptibility of two *Bacillus* species identified as *B. pumilus* and *B. subtilis* isolating from the gut of *O. niloticus,* along with assessing their effects on growth, and water quality of Nile tilapia rearing environment over two months. Both *B. pumilus* and *B. subtilis* are well-recognized host-associated probiotics in tilapia aquaculture (Van Doan et al., 2020).

The ability of the isolate to tolerate and grow in the low pH environment of gastric juice, and high bile concentration in the stomach passage time, and adherence on the fish gut epithelium cell is a key probiotic feature to be noted (Kavitha et al., 2018). In this study, both probiotic candidates have showed viability at pH 2.5 and 0.5% bile concentration. Such results were in congruence with other studies, which had confirmed the low pH and high bile tolerance of fish gut-isolated *L. lactis*, *E. faecalis*, *B. pumilus*, *B. subtilis*, *L. plantarum*, *B. amyloliquefaciens*, and *B. velezensis* (Eamsakul et al., 2025; Devi et al., 2024; Kuebutornye et al., 2020; Kavitha et al., 2018; Reda et al., 2018; Sahoo et al., 2015). The *in vitro* assays confirmed that both strains are capable of acid and bile tolerance, indicating potential for gut colonization.

Extracellular amylase and protease are secreted enzymes that can convert complex carbohydrates into absorbable sugar and peptide bonds in protein nutrients into amino acids, thereby facilitating the digestibility and absorbability of nutrients in aquatic animals. Both strains in this study showed protease activity with a clear zone, while T2B only showed amylase activity. There are a number of published reports on the ability of *Bacillus* spp. to produce extracellular enzymes Viz: amylolytic, and proteolytic (Eamsakul et al., 2025; Nakharuthai et al., 2023; Kavitha et al., 2018; X. F. Liu et al., 2015).

The absence of transmissible antibiotic-resistant genes in the potential probiotic ensures their safe use in aquaculture, along with in human consumption (Panigrahi & Azad, 2007). The innate antibiotic-resistance of probiotics could be helpful in restoring beneficial microbiota in the gut after proper antibiotic treatments. The study revealed that both strains were sensitive to all tested antibiotics except cefepime and ampicillin. Similar findings were earlier reported in several studies (Devi et al., 2024; Nakharuthai et al., 2023; Kuebutornye et al., 2020; Kavitha et al., 2018; Thankappan et al., 2015). Regarding cefepime, both strains were resistance. However, a quantitative antibiotic susceptibility test will ensure whether the resistance is intrinsic or acquired. Host-associated probiotics administration stabilized cultured water quality by lowering the accumulation of harmful metabolites from uneaten feed, improving microbial diversity, and the nitrification process (Mahfuz et al., 2025).

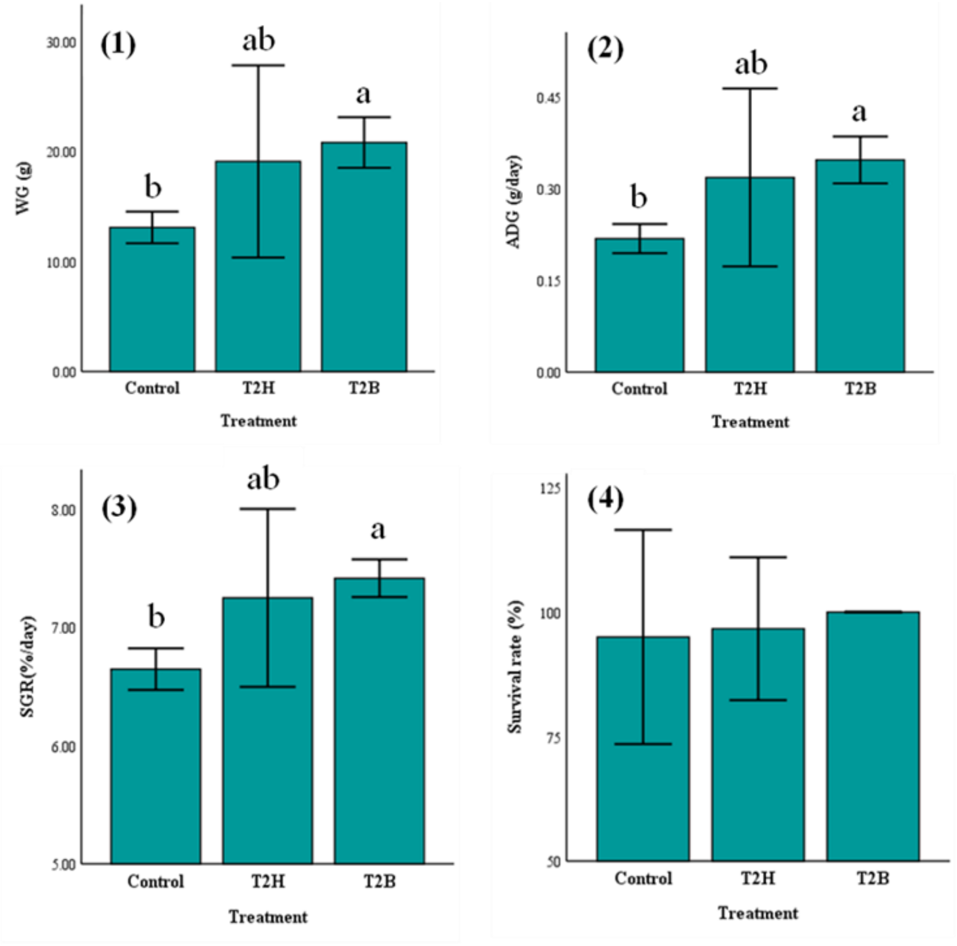

Figure 3. Growth performances, and survival rate of Nile tilapia fed with *Bacillus* strains, T2H and T2B in diet for 60 days. Bar with lowercase letters (a, b) denote significant difference ($p < 0.05$). Value at the top-left of each graph 1, 2, 3, 4 represents weight gain (WG), average daily gain (ADG), specific growth rate (SGR), and survival rate, respectively.

In this study, an increased nitrate level was found in the T2H and T2B group compared to the control. This modification may contribute to improving water quality, and hence, fish health and performance. Similar results were depicted in several experiment with *Bacillus* spp. such as *B. subtilis*, *B. megaterium*, *B. pumilus, B. amyloliquifaciens* (Li et al., 2022; Wang et al., 2022; Zabidi et al., 2021; Zokaeifar et al., 2014). No effects of *Bacillus* mixture on ammonia, nitrite, and nitrate were found in the study by Dighiesh et al. (2024). However, the stable ammonia level and increased nitrite level across treatments in this experiment indicate no effects of T2H and T2B on ammonia production or its removal from culture system, and there may remain a partial imbalance in nitrification process which accumulates toxic nitrite. Additionally, probiotics in this study were provided via mixing with feed rather than being directly added to water, and there have no evidence of their abundance in rearing water. Therefore, the effects of host-associated *Bacillus* probiotics on water quality based on optimal dosage, and the bacterial population of rearing water deserve further research in a comparative approach, such as probiotics added to water or feed (Elsabagh et al., 2018). This may help to reduce ammonia production, minimize nitrite accumulation, confirm more balanced nitrification and ensure better culture environment.

The factors responsible for the improvement in growth performances are the diverse bioactive mechanisms of *B. subtilis*, including the release of extracellular enzymes, increased appetite, production of vitamins, breakdown of indigestible compounds, supporting gut health proliferation, production of short-chain fatty acids, and serving as additional energy sources (Yu et al., 2025). The results of this study demonstrated that *Bacillus* supplementation significantly enhanced key growth parameters, including WG, ADG, and SGR. At the end of the feeding trial, fish in T2B exhibited the highest WG, ADG, and SGR, followed by T2H and control. These findings are consistent with previous studies reporting similar effects of *B. subtilis*, *B. pumilus*, *B. amyloliquefaciens*, *B. mojavensis* in *O. niloticus* and *B. pumilus* in *Macrobrachium rosenbergii* (Yu et al., 2025; Shija et al., 2025; Atef et al., 2024; Dighiesh et al., 2024; Etyemez Buyukdeveci et al., 2023; Hassaan et al., 2021; S. Liu et al., 2020; Zhao et al., 2019). This confirms the broader role of *Bacillus* species in enhancing fish growth and productivity.

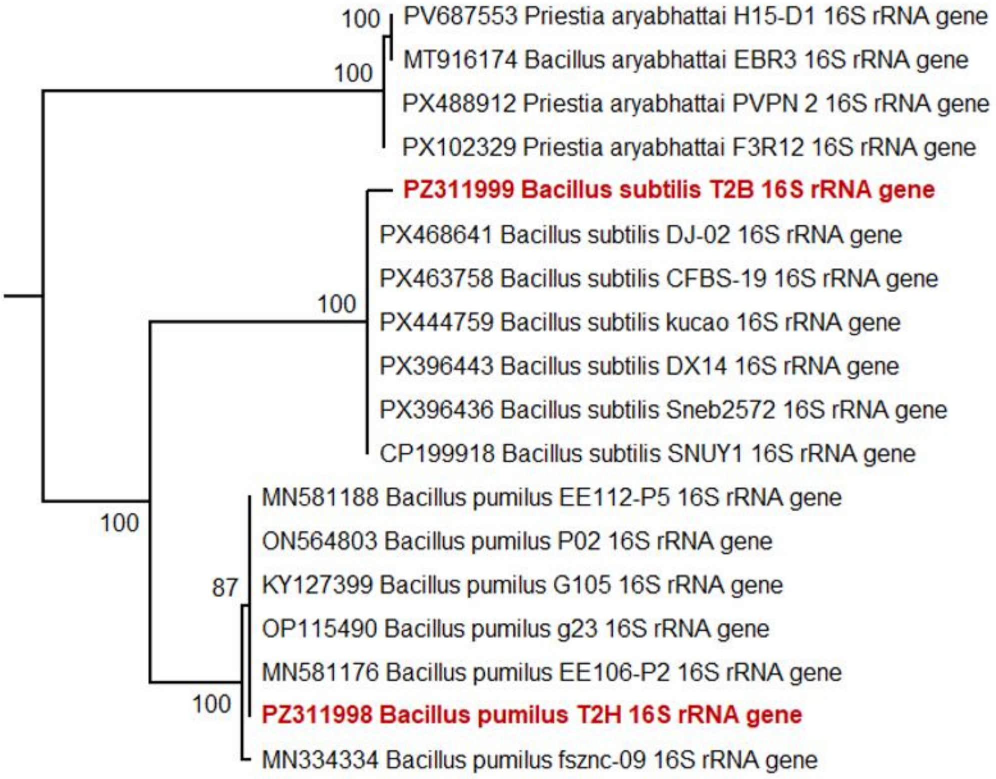

Figure 4. Phylogenetic tree constructed by the neighbor-joining method representing the relatedness of T2H and T2B to other *Bacillus* species with an outgroup *Priestia aryabhattai*. The number at the node indicates bootstrap value (out of 100) based on 1000 replicates.

Application of host-associated probiotics could outperform the commercial probiotics in terms of their better colonization ability in the gut, interact with the host's microbiome and minimal adverse effect on water quality (Mahfuz et al., 2025). A comparative study between commercial probiotics and host-associated probiotics, along with a formal economic evaluation, is required to determine the possible economic advantages of host-associated probiotics over commercial probiotics and to minimize dependency on imported products. Nonetheless, ongoing monitoring and functional evaluation are encouraged to minimize any possible risks concerning probiotic use. The improved water quality and increased growth parameters in this study suggest that host-associated *Bacillus* species, T2H and T2B, could be the potential probiotics in tilapia culture, which underscores the potential for developing locally adapted, cost-effective products.

Conclusion

The findings of the present study reveal that dietary provision of host-associated *B. pumilus* and *B. subtilis* could act as potential probiotics to enhance the growth performance of *O. niloticus* as well as keep the rearing environment suitable and safe. This study was conducted at the fry stage of Nile tilapia, as supplementation during this growth phase may significantly influence early development. However, further detailed research with these two *Bacillus* strains is recommended to study the effect of the host-associated probiotics on tilapia starting from the fry stage up to the market size by both continuous and non-continuous addition of probiotics at different periods of the experiment.

References

1. Amenyogbe, E., Huang, J.S., Chen, G. and Wang, W.Z. (2021), "Probiotic potential of indigenous (*Bacillus sp.* RCS1, *Pantoea agglomerans* RCS2, and *Bacillus cereus* strain RCS3) isolated from cobia fish (*Rachycentron canadum*) and their antagonistic effects on the growth of pathogenic *Vibrio alginolyticus*, *Vibrio harveyi*, *Streptococcus iniae*, and *Streptococcus agalactiae*", *Frontiers in Marine Science*, Vol. 8 pp. 672213.
2. Asha, A.A., Haque, M.M., Hossain, M.K., Hasan, M.M., Bashar, A., Hasan, M.Z., Shohan, M.H., Farin, N.N., Schneider, P. and Bablee, A.L. (2024), "Effects of commercial probiotics on the growth performance, intestinal microbiota and intestinal histomorphology of Nile Tilapia (*Oreochromis niloticus*) reared in biofloc technology (BFT)", *Biology*, Vol. 13 No. 5 pp. 299.
3. Atef, S., Ahmed, O.M., Said, M.M. and Abo-Al-Ela, H.G. (2024), "Dietary *Bacillus* species modulate lipid metabolism-related parameters, growth, water quality, and bacterial load in Nile tilapia (*Oreochromis niloticus*)", *Animal Feed Science and Technology*, Vol. 310 pp. 115943.
4. Buyukdeveci, M.E., Cengizler, I., Balcázar, J.L. and Demirkale, I. (2023), "Effects of two host-associated probiotics *Bacillus mojavensis* B191 and *Bacillus subtilis* MRS11 on growth performance, intestinal morphology, expression of immune-related genes and disease resistance of Nile tilapia (*Oreochromis niloticus*) against *Streptococcus iniae*", *Developmental & Comparative Immunology*, Vol. 138 pp. 104553.
5. Chomová, N., Pavloková, S., Sondorová, M., Mudroňová, D., Fečkaninová, A., Popelka, P., Koščová, J., Žitňan, R., & Franc, A. (2023), "Development and evaluation of a fish feed mixture containing the probiotic *Lactiplantibacillus plantarum* prepared using an innovative pellet coating method", *Frontiers in Veterinary Science*, **10**, 1196884.
6. Devi, W.M., Irungbam, S., Kumar, M., Choudhury, T.G. and Kamilya, D. (2023), "Probiotic potential of *Bacillus pumilus* COFAHE_Pro08 and *Lysinibacillus macroides* COFAHE_Pro06 isolated from the intestine of *Labeo catla*", *Turkish Journal of Fisheries and Aquatic Sciences*, Vol. 24 No. 1.
7. Dighiesh, H.S., Alharbi, N.A., Awlya, O.F., Alhassani, W.E., Hassoubah, S.A., Albaqami, N.M., Aljahdali, N., Abd El-Aziz, Y.M., Eissa, E.S.H., Munir, M.B. and Sakr, S.E.S. (2024), "Dietary multi-strains *Bacillus* spp. enhanced growth performance, blood metabolites, digestive tissues histology, gene expression of Oreochromis niloticus, and resistance to *Aspergillus flavus* infection", *Aquaculture International*, Vol. 32 No. 6 pp.7065-7086.
8. DOF. (2024), "Yearbook of Fisheries Statistics of Bangladesh, 2023-24", *Fisheries Resources Survey System (FRSS), Department of Fisheries; Ministry of Fisheries and Livestock*, Vol. 41 pp.140.
9. Eamsakul, S., Konrian, R. and Suanyuk, N. (2025), "Isolation, characterization, and identification of *Bacillus* spp. strains from the digestive tract of mad carp (*Leptobarbus hoevenii*) and their potential probiotic properties", *ASEAN Journal of Scientific and Technological Reports*, Vol. 28 No. 4 pp. e258486-e258486.
10. Elsabagh, M., Mohamed, R., Moustafa, E.M., Hamza, A., Farrag, F., Decamp, O., Dawood, M.A. and Eltholth, M. (2018), "Assessing the impact of *Bacillus* strains mixture probiotic on water quality, growth performance, blood profile and intestinal morphology of Nile tilapia, *Oreochromis niloticus*", *Aquaculture nutrition*, Vol. 24 No. 6 pp. 1613-1622.
11. FAO. (2001), "Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria", Cordoba, Argentina.
12. FAO. (2024), "The State of World Fisheries and Aquaculture 2024. Blue Transformation in action", Rome.
13. Ferdoush, J., Hossain, A., Haque, M.I.M., Hosen, M.I., Mamun, A.A., Rahman, M.M., Shafiujjaman, M. and Mandal, S.C. (2025), "Probiotic supplementation improved the growth, haematology and immune response of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758)", *Dhaka University Journal of Biological Sciences*, Vol. 34 No. 2 pp.149-162.
14. Ghori, I., Tubassam, M., Ahmad, T., Zuberi, A. and Imran, M. (2022), "Gut microbiome modulation mediated by probiotics: Positive impact on growth and health status of *Labeo rohita*", *Frontiers in physiology*, Vol 13 pp.949559.
15. Guo, J., Huang, Y., Salze, G., Roy, L.A. and Davis, D.A. (2020), "Use of plant-based protein concentrates as replacement for fishmeal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*) reared under high stocking density and low salinity conditions", *Aquaculture Nutrition*, Vol. 26 No. 2 pp.225-232.
16. Hassaan, M.S., Mohammady, E.Y., Soaudy, M.R., Elashry, M.A., Moustafa, M.M., Wassel, M.A., El-Garhy, H.A., El-Haroun, E.R. and Elsaied, H.E. (2021), "Synergistic effects of *Bacillus pumilus* and exogenous protease on Nile tilapia (*Oreochromis niloticus*) growth, gut microbes, immune response and gene expression fed plant protein diet", *Animal Feed Science and Technology*, Vol. 275 pp. 114892.
17. Hlordzi, V., Kuebutornye, F.K., Afriyie, G., Abarike, E.D., Lu, Y., Chi, S. and Anokyewaa, M.A. (2020), "The use of *Bacillus* species in maintenance of water quality in aquaculture: A review", *Aquaculture reports*, Vol. 18 pp. 100503.
18. Hossain, M.K., Islam, S.M., Rafiquzzaman, S.M., Nuruzzaman, M., Hossain, M.T. and Shahjahan, M. (2022), "Multi-species probiotics enhance growth of Nile tilapia (*Oreochromis niloticus*) through upgrading gut, liver and

muscle health", *Aquaculture Research*, Vol. 53 No. 16 pp. 5710-5719.

19. Hossain, M.K., Shahjahan, M., Kari, Z.A. and Téllez-Isaías, G. (2023), "Trends in the use of probiotics in aquaculture of Bangladesh—present state, problems, and prospects", *Aquaculture Research*, Vol. 2023 No. 1 pp. 5566980.
20. Islam, S.M., Rohani, M.F. and Shahjahan, M. (2021), "Probiotic yeast enhances growth performance of Nile tilapia (*Oreochromis niloticus*) through morphological modifications of intestine", *Aquaculture Reports*, Vol. 21 pp. 100800.
21. Katoh, K., Rozewicki, J. and Yamada, K.D., 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in bioinformatics*, *20*(4), pp.1160-1166.
22. Kavitha, M., Raja, M. and Perumal, P. (2018), "Evaluation of probiotic potential of *Bacillus* spp. isolated from the digestive tract of freshwater fish *Labeo calbasu* (Hamilton, 1822)", *Aquaculture Reports*, Vol 11 pp. 59-69.
23. Kong, W., Huang, S., Yang, Z., Shi, F., Feng, Y. and Khatoon, Z. (2020), "Fish feed quality is a key factor in impacting aquaculture water environment: evidence from incubator experiments" *Scientific reports*, Vol. 10 No. 1 pp. 187.
24. Kuebutornye, F.K., Lu, Y., Abarike, E.D., Wang, Z., Li, Y. and Sakyi, M.E. (2020), "In vitro assessment of the probiotic characteristics of three *Bacillus* species from the gut of Nile tilapia, *Oreochromis niloticus*", *Probiotics and antimicrobial proteins*, Vol. 12 No. 2 pp. 412-424.
25. Kumar, S., Stecher, G., Suleski, M., Sanderford, M., Sharma, S. and Tamura, K. (2024), "MEGA12: molecular evolutionary genetic analysis version 12 for adaptive and green computing", *Molecular biology and evolution*, Vol. 41 No. 12 pp. msae263.
26. Lara-Flores, M., Olvera-Novoa, M. A., Guzmán-Méndez, B. E. and López-Madrid, W. (2003), "Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*)", *Aquaculture*, 216(1–4), 193–201.
27. Li, X., Wang, T., Fu, B. and Mu, X. (2022), "Improvement of aquaculture water quality by mixed *Bacillus* and its effects on microbial community structure", *Environmental Science and Pollution Research*, Vol. 29 No. 46 pp. 69731-69742.
28. Lieke, T., Meinelt, T., Hoseinifar, S.H., Pan, B., Straus, D.L. and Steinberg, C.E. (2020), "Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases", *Reviews in Aquaculture*, Vol. 12 No. 2 pp. 943-965.
29. Liu, S., Wang, S., Cai, Y., Li, E., Ren, Z., Wu, Y., Guo, W., Sun, Y. and Zhou, Y. (2020), "Beneficial effects of a host gut-derived probiotic, *Bacillus pumilus*, on the growth, non-specific immune response and disease resistance of juvenile golden pompano, *Trachinotus ovatus*", *Aquaculture*, Vol. 514 pp. 734446.
30. Liu, X.F., Li, Y., Li, J.R., Cai, L.Y., Li, X.X., Chen, J.R. and Lyu, S.X. (2015), "Isolation and characterisation of Bacillus spp. antagonistic to *Vibrio parahaemolyticus* for use as probiotics in aquaculture", *World Journal of Microbiology and Biotechnology*, Vol. 31 No. 5 pp. 795-803.
31. Llewellyn, M.S., Boutin, S., Hoseinifar, S.H. and Derome, N. (2018), "Isolation and characterization of native probiotics for fish farming", *BMC Microbiology*, **18**, 119. https://doi.org/10.1186/s12866-018-1260-2
32. Mahfuz, M.A., Nobony, T.A., Jilani, A.K., Hasan, A., Rifat, M.N.I. and Haider, M.N. (2025), "Isolation and Validation of Host-Derived Probiotics From the Giant Freshwater Prawn (*Macrobrachium rosenbergii*): Impacts on Water Quality and Growth Performance", *Aquaculture Nutrition*, Vol. 2025 No. 1 pp. 9156848.
33. Mamun-Ur-Rashid, M., Belton, B., Phillips, M. and Rosentrater, K.A. (2013), "Improving aquaculture feed in Bangladesh: From feed ingredients to farmer profit to safe consumption", WorldFish, Penang, Malaysia. Working Paper: 2013-34.
34. Moesch, A., Meurer, F., Zadinelo, I.V., Carneiro, W.F., da Silva, L.C.R. and dos Santos, L.D. (2016), "Growth, body composition and hepatopancreas morphology of Nile tilapia fingerlings fed crude glycerol as a replacement for maize in diets", *Animal feed science and technology*, Vol. 219 pp. 122-131.
35. Munni, M.J., Akther, K.R., Ahmed, S., Hossain, M.A. and Roy, N.C. (2023), "Effects of probiotics, prebiotics, and synbiotics as an alternative to antibiotics on growth and blood profile of Nile tilapia (*Oreochromis niloticus*)", *Aquaculture Research*, Vol. 2023 No. 1 p. 2798279.
36. Nakharuthai, C., Boonanuntanasarn, S., Kaewda, J. and Manassila, P. (2023), "Isolation of potential probiotic *Bacillus* spp. from the intestine of Nile tilapia to construct recombinant probiotic expressing CC chemokine and its effectiveness on innate immune responses in Nile tilapia" *Animals*, Vol. 13 No. 6 pp. 986.
37. Ninawe, A.S. and Selvin, J. (2009), "Probiotics in shrimp aquaculture: avenues and challenges", *Critical reviews in microbiology*, Vol. 35 No. 1 pp. 43-66.
38. Ogello, E.O., Munguti, J.M., Sakakura, Y. and Hagiwara, A. (2014), "Complete replacement of fish meal in the diet of Nile tilapia (*Oreochromis niloticus L.*) grow-out with alternative protein sources. A review", *International Journal of Advanced Research*, Vol. 2 No. 8 pp. 962-978.
39. Panigrahi, A. and Azad, I.S. (2007), "Microbial intervention for better fish health in aquaculture: the Indian scenario", *Fish physiology and biochemistry*, Vol. 33 No. 4 pp. 429-440.
40. Patel, A.K., Ahire, J.J., Pawar, S.P., Chaudhari, B.L. and Chincholkar, S.B. (2009), "Comparative accounts of probiotic characteristics of *Bacillus* spp. isolated from food wastes" *Food Research International*, Vol. 42 No. 4 pp. 505-510.
41. Reda, R.M., Selim, K.M., El-Sayed, H.M. and El-Hady, M.A. (2018), "In vitro selection and identification of potential probiotics isolated from the gastrointestinal tract of Nile tilapia, *Oreochromis niloticus*", *Probiotics and antimicrobial proteins*, Vol. 10 No. 4 pp. 692-703.
42. Rengpipat, S., Phianphak, W., Piyatiratitivorakul, S. and Menasveta, P. (1998), "Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth", *Aquaculture*, Vol. 167 No. 3-4 pp. 301-313.
43. Sahoo, T.K., Jena, P.K., Nagar, N., Patel, A.K. and Seshadri, S. (2015), "In vitro evaluation of probiotic properties of lactic acid bacteria from the gut of *Labeo rohita* and *Catla catla*", *Probiotics and antimicrobial proteins*, Vol. 7 No. 2 pp. 126-136.
44. Shija, V.M., Amoah, K. and Cai, J. (2023), "Effect of bacillus probiotics on the immunological responses of Nile tilapia (*Oreochromis niloticus*): a review", *Fishes*, Vol. 8 No. 7 pp. 366.

45. Shija, V.M., Zhimin, J., Chen, H., Amoah, K., Li, Y., Ng'onga, L., Ndandala, C.B., Zhong, Y., Masanja, F., Huang, J. and Cai, J. (2025), "Effects of dietary inclusion of *Bacillus amyloliquefaciens* AV5 on growth performance, antioxidant activity, innate immune, and hematological responses in Nile tilapia (*Oreochromis niloticus*) reared at low and high stocking densities", *Fish & shellfish immunology*, Vol. 156 pp. 110042.
46. Simón, R., Docando, F., Nuñez-Ortiz, N., Tafalla, C. and Díaz-Rosales, P. (2021), "Mechanisms used by probiotics to confer pathogen resistance to teleost fish", *Frontiers in immunology*, Vol. 12 pp. 653025.
47. Soltani, M., Ghosh, K., Hoseinifar, S.H., Kumar, V., Lymbery, A.J., Roy, S. and Ringø, E. (2019), "Genus *Bacillus*, promising probiotics in aquaculture: aquatic animal origin, bio-active components, bioremediation and efficacy in fish and shellfish", *Reviews in Fisheries Science & Aquaculture*, Vol. 27 No. 3 pp. 331-379.
48. Sudha, A., Bhavan, P.S., Udayasurian, R., Manjula, T., Kalpana, R. and Karthik, M. (2019), "Molecular Identification of Gut Microflora of the Prawn *Macrobrachium rosenbergii* Fed With Probiotic Bacterium *Bacillus licheniformis* Supplemented Diet", *Haya: The Saudi Journal of Life Sciences*, Vol. 4 No. 9 pp. 303-317.
49. Thankappan, B., Ramesh, D., Ramkumar, S., Natarajaseenivasan, K. and Anbarasu, K. (2015), "Characterization of *Bacillus* spp. from the gastrointestinal tract of *Labeo rohita*—towards to identify novel probiotics against fish pathogens", *Applied biochemistry and biotechnology*, Vol. 175 No. 1 pp. 340-353.
50. Van Doan, H., Hoseinifar, S.H., Ringø, E., Ángeles Esteban, M., Dadar, M., Dawood, M.A. and Faggio, C. (2020), "Host-associated probiotics: a key factor in sustainable aquaculture", *Reviews in fisheries science & aquaculture*, Vol. 28 No. 1 pp. 16-42.
51. Vos, P., Garrity, G., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.H. and Whitman, W.B. eds. (2009), "Bergey's manual of systematic bacteriology: Volume 3: The Firmicutes", *New York: Springer New York.* pp. 21-127.
52. Wang, M., Liu, Y., Luo, K., Li, T., Liu, Q. and Tian, X. (2022), "Effects of Bacillus pumilus BP-171 and carbon sources on the growth performance of shrimp, water quality and bacterial community in *Penaeus vannamei* culture system", *Water*, Vol. 14 No. 24 pp. 4037.
53. Wu, P.S., Liu, C.H. and Hu, S.Y. (2021), "Probiotic *Bacillus safensis* NPUST1 administration improves growth performance, gut microbiota, and innate immunity against *Streptococcus iniae* in Nile tilapia (*Oreochromis niloticus*)", *Microorganisms*, Vol. 9 No. 12 pp.2494.
54. Yi, Y., Zhang, Z., Zhao, F., Liu, H., Yu, L., Zha, J. and Wang, G. (2018), "Probiotic potential of *Bacillus velezensis* JW: antimicrobial activity against fish pathogenic bacteria and immune enhancement effects on *Carassius auratus*", *Fish & shellfish immunology*, Vol. 78, pp. 322-330.
55. Yu, H., Nazir, S., Ijaz, F., Zahid, M.U., Mushtaq, M., Khan, M., Rahman, A. and Rahman, M.A.U. (2025), "Dietary supplementation of *Bacillus subtilis* as probiotic influenced the growth performance, hematological parameters, immune function, antioxidant status, and digestive enzyme activity of Nile tilapia fingerlings (*Oreochromis niloticus*)" *Animals*, Vol. 15 No. 9 pp. 1256.
56. Zabidi, A., Yusoff, F.M., Amin, N., Yaminudin, N.J.M., Puvanasundram, P. and Karim, M.M.A. (2021), "Effects of probiotics on growth, survival, water quality and disease resistance of red hybrid tilapia (*Oreochromis* spp.) fingerlings in a biofloc system" *Animals*, Vol. 11 No. 12 pp. 3514.
57. Zhao, C., Zhu, J., Hu, J., Dong, X., Sun, L., Zhang, X. and Miao, S. (2019), "Effects of dietary Bacillus pumilus on growth performance, innate immunity and digestive enzymes of giant freshwater prawns (*Macrobrachium rosenbergii*)" *Aquaculture Nutrition*, Vol. 25 No. 3 pp. 712-720.
58. Zokaeifar, H., Babaei, N., Saad, C.R., Kamarudin, M.S., Sijam, K. and Balcazar, J.L. (2014), "Administration of *Bacillus subtilis* strains in the rearing water enhances the water quality, growth performance, immune response, and resistance against *Vibrio harveyi* infection in juvenile white shrimp, *Litopenaeus vannamei*", *Fish & shellfish immunology*, Vol. 36 No. 1 pp. 68-74.
59. Zokaeifar, H., Balcázar, J.L., Saad, C.R., Kamarudin, M.S., Sijam, K., Arshad, A. and Nejat, N. (2012), "Effects of *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, *Litopenaeus vannamei*", *Fish & shellfish immunology*, Vol. 33 No. 4 pp. 683-689.