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Potential probiotic characteristics of *Bacillus* sp. originated from intensive snakehead fish (*Channa striata*) raising ponds in Vinh Long Province, Vietnam

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Abstract. *Chi NTY, Minh NLK, Thi QVC. 2025. Potential probiotic characteristics of* Bacillus *sp. originated from intensive snakehead fish* (Channa striata) *raising ponds in Vinh Long Province, Vietnam. Biodiversitas 26: 85-93.* Internal white spot disease caused by *Aeromonas schubertii* in snakehead fish (*Channa striata*) has become increasingly common and leading to significant economic losses to fish farmers. This study aimed to isolate and screen *Bacillus* with potential probiotic characteristics from intensively cultured snakehead fish in Vinh Long province. The results isolated 16 out of 34 *Bacillus* strains from pond water, sludge, and the intestine samples of snakehead fish were antagonistic against *A. schubertii* using the diffusion well method. In the study, strain BC2LM1 exhibited the highest antibacterial activity and was identified as *Bacillus* based on morphological, physiological, and biochemical characteristics, and 16S rRNA gene sequencing results. Strain BC2LM1 in this study was able to survive in media with pH ranging from 2.0 to 4.0 after 6 h of incubation. In particular, it tolerated bile salts at a concentration of 0.5% after 9 h of inoculation. The strain also demonstrated the ability to produce extracellular enzymes, including cellulase, amylase, and protease. Moreover, BC2LM1 was found to be susceptible to ampicillin/sulbactam, clindamycin, doxycycline, tetracycline, erythromycin, and levofloxacin. These findings show the potential application of *Bacillus* to control *A. schubertii* infections in intensively cultured snakehead fish in the Mekong Delta.

Keywords: *Aeromonas schubertii, Bacillus,* internal white spot disease, Mekong Delta, snakehead fish

INTRODUCTION

The freshwater snakehead fish (*Channa striata* (Bloch, 1793)), belonging to the Channidae family and the Perciformes order (Nakkrasae et al. 2015), is characterized by fast growth, good adaptation to harsh environmental conditions, and is a food with high economic value with delicious meat quality and promising farming subjects (Lâm et al. 2018). Furthermore, its meat is known to boost immunity and serve as a post-operative treatment (Marimuthu et al. 2009; Rahman et al. 2018). Consequently, it is popularly farmed in several provinces of the Mekong Delta, like An Giang, Tra Vinh, Dong Thap, and Vinh Long (Bình 2020). However, in the rearing process, to achieve high yields and profits, farmers often adopt high stocking densities during cultivation (Thịnh et al. 2020). This practice increases the risk of disease outbreaks, causing significant losses and making disease prevention and treatment increasingly challenging (Tahir et al. 2024). According to Thịnh et al. (2020), visceral white-scale disease caused by *Aeromonas schubertii* (Oanh and Nghĩa 2016) had the highest frequency in the two provinces of An Giang and Tra Vinh.

Aeromonas schubertii belongs to the group of Gram-negative bacteria, is short rod-shaped, and can be found in soil, freshwater, and saltwater environments (Fernández-Bravo and Figueras 2020; Tahir et al. 2024). First identified as a pathogen, this bacterium significantly damaged the industrial snakehead fish farming industry in China, affecting two snakehead species, *Ophiocephalus argus* (Cantor, 1842), and *C. maculata*, as well as a hybrid between *C. maculata* and *C. argus*, resulting in up to 45% mortality (Liu and Li 2012). Many studies have also reported its presence in other aquatic animals such as frogs, shrimps, and mussels (Latif-Eugenín et al. 2016) and its first appearance in tilapia (Ren et al. 2018). In Malaysia, recently, Tahir et al. (2024) first reported infection by *A. schubertii* in snakehead fish *C. striata*. In Vietnam, this bacterial species was first detected in snakehead fish in An Giang (Oanh and Nghĩa 2016). Later, Dung et al. (2019) reported its presence in farmed snakehead fish in Dong Thap, An Giang, Dong Nai, and Tra Vinh Provinces. Visceral white spot diseases caused by *A. schubertii* have now appeared on farmed snakehead fish in all the Mekong Delta Provinces.

Up to now, the prevention and treatment of internal white spot disease caused by *A. schubertii* have primarily relied on bactericidal chemicals and antibiotics (Thịnh and Phú 2020). However, improper antibiotic use has led to the development of antibiotic resistance strains of *A. schubertii* in aquaculture (Châu et al. 2018; Pham Thi et al. 2023; Thi et al. 2023). In addition, antibiotic residues in food can also affect consumer health (Merhi et al. 2023; Bacanlı 2024). Therefore, biological measures, especially beneficial bacteria capable of inhibiting pathogenic bacteria, are being investigated in many nations worldwide (Sionek et al. 2023). Numerous studies have shown the antibacterial activity of *Bacillus* on aquatic animals like shrimp (*Penaeus monodon* (Fabricius, 1798) and *Litopenaeus vannamei* (Boone, 1931)) (Temario et al. 2022; Proespraiwong et al. 2023),

freshwater, and saltwater fish (Chen et al. 2016; Kavitha et al. 2018). Similarly, in Vietnam, the antibacterial activity of *Bacillus* against bacterial pathogens was found in many previous reports, such as shrimp and fish (Phung et al. 2020; Hạnh et al. 2021; Hậu et al. 2022; Cường et al. 2023). However, to date, the inhibitory activity of *Bacillus* against *A. schubertii* has not been reported in Vietnam. This study was conducted to identify and isolate *Bacillus* with possible probiotic qualities from extensive snakehead fish farming ponds in Vinh Long Province. The research results are the scientific basis for further studies in the production of probiotics to control *A. schubertii* and towards developing a sustainable aquaculture industry in the future.

MATERIALS AND METHODS

Sampling method

Healthy snakehead fish, water, and sludge samples were collected for *Bacillus* isolation. Water and sediment samples were collected in 3 locations in the same pond. After being combined, these samples were transported to the lab for bacterial isolation. The snakehead fish chosen for bacterial isolation were in good health (no external or internal signs of disease). Two to three fish were collected from each pond, and their body weights ranged from 200-600 g/fish. The fish were stored in a tank and transported to the laboratory for bacterial isolation. All samples originated from intensive fish farms in Tra On District, Vinh Long Province, Vietnam.

Bacillus isolation

Bacillus isolation from the fish gut was performed according to Santos et al. (2021). Before bacterial isolation, fish samples (Figures 1.A-1.B) were washed many times with sterile distilled water and disinfected externally with 70% ethanol. Then, the fish were dissected, and the fish intestines (approximately 25 g/fish) were cut into small pieces (4-6 mm). The intestinal fragments were placed in physiological saline (0.85% NaCl) solution and ground. The intestinal fluid samples were enriched in nutrient broth (NB) medium (HiMedia, India) and boiled at 80°C for 20 min. Next, the samples were diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions. Finally, one hundred microliters of each diluent were spread on Nutrient Agar (NA) medium (HiMedia, India), and the plates were incubated at 37°C for 24-48 h. Meanwhile, pond water (10 mL) and sludge samples (10 g) were enriched in NB medium (Truong et al. 2021; Jeon et al. 2022). The samples were then diluted and isolated similarly to the one described above. Pure bacteria were examined for basic morphological, physiological, and biochemical features such as Gram staining, spore staining, motility, oxidase, and catalase reactions (Wulff et al. 2002).

Probiotic characteristics of *Bacillus*

Antagonistic activity of isolated Bacillus strains *against* A. schubertii

The diffusion well approach was employed to test the antibacterial activity of isolated *Bacillus* strains against *A. schubertii* (Palacios-Rodriguez et al. 2024). First, strain As11 (causing visceral white spot disease on snakehead fish used as an indicator) (Thi et al. 2023) was grown in 5 mL of Luria Bertani (LB) medium (HiMedia, India) on a shaker for 24 h at room temperature. The enriched solution was centrifuged at 10.000 rpm for 5 min to collect bacterial biomass. Bacterial solutions were prepared at 10^8 CFU/mL by comparison with a standard McFarland 0.5 solution and spread on Tryptic Soy Agar (TSA) medium (HiMedia, India). At the same time, *Bacillus* isolates were grown in NB medium on a shaker at 120 rpm. The bacterial solution was centrifuged at 10.000 rpm for 5 min, and the supernatant was collected. Eighty microliters of the supernatant were added to the wells. After 24 h of incubation at 37°C, an inhibition zone around the well shows the antibacterial activity of *Bacillus* against *A. schubertii*. The inhibition zone diameter (d) was measured (mm) and is defined as follows: no inhibition (d: 0 mm); weak inhibition (d: 1-5 mm); moderate inhibition (d: 6-10 mm); strong inhibition (d>10 mm) (Ran et al. 2012).

Hemolytic ability

The hemolysis test of *Bacillus* isolates was tested using a blood agar base (HiMedia, India) containing 5% sheep blood (Dabiré et al. 2022). Following a 24-h incubation period at 37°C, the strains' hemolytic activity was assessed and categorized according to the colony's obvious lysis of red blood cells. Alpha (α), beta hemolysis (β), and gamma (γ) appear as a clear, green color with no clear zone around the colonies. γ-hemolytic strains were considered safe.

Acid resistance

Acid tolerance of the bacterial strain was performed according to Khochamit et al. (2015). After preparing bacterial solutions with a density of 10^8 CFU/mL, they were added to NB medium adjusted to pH 2.0 and 3.0 by 1M HCl solution. The mixture was then incubated at 37°C while being shaken 200 rpm. The samples were spread onto TSA medium and incubated at 37°C for 24 h to determine cell counts after 1 and 3 h. Acid tolerance is calculated based on the number of viable cells remaining after the test period.

Bile salt tolerance

Similarly, bile salt tolerance of the bacterial strain was performed according to Khochamit et al. (2015). Bacteria were spread onto TSA medium supplemented with 0.3% and 0.5% bile salts and incubated at 37°C. The results were obtained after incubating the plates for 1 and 3 h at 37°C. The number of viable cells that survived after the test period was used to calculate bile salt resistance.

Extracellular enzyme activity

The ability to degrade cellulose, protein, lipid, and starch was performed as described by Khochamit et al. (2015). The bacterial suspension was centrifuged at 12.000 rpm at 4°C for 15 min, and the supernatant was collected. Sixty microliters of the supernatant were added into wells (diameter 6 mm) on NA medium supplemented with 1% Carboxymethylcellulose (CMC), 0.5% casein, 1% tween 20, and 1% starch to detect cellulase, protease, amylase,

and lipase. After 24 h incubating at room temperature, the cellulolytic, proteolytic, amylolytic, and lipolytic activities were determined by flooding with Congo red, Trichloroacetic Acid (TCA), and lugol's solution.

Antibiotic susceptibility

The Kirby-Bauer disk diffusion method (Bauer et al. 1966) was used to assess the antimicrobial resistance of *A. schubertii* on Mueller Hinton Agar (MHA) (HiMedia, India) using 20 antibiotics (Nam Khoa, Vietnam), including ampicillin (AMP/10 µg), chloramphenicol (CHL/30 µg), ceftazidime (CTX/30 µg), penicillin (PEN/5 µg), ampicillin/ sulbactam (AMS/10/10 µg), clindamycin (CLI/2 µg), doxycycline (DOX/30 µg), tetracycline (TET/30 µg), erythromycin (ERY/15 µg), levofloxacin (LEV/5 µg), sulfamethoxazole/ trimethoprim (SXT/1,25/23,75 µg), ciprofloxacin (CIP/5 µg), gentamicin (GEN/10µg), kanamycin (KAN/30 µg), neomycin (NEO/30 µg), nalidixic acid (NAL/30 µg), norfloxacin (NOR/5 µg), ofloxacin (OFL/5 µg), streptomycin (STR/10 µg), and vancomycin (VAN, 30 µg). An MHA plate was spread by single bacterial colonies suspended in 0.85% saline solution, whose turbidity matched the McFarland 0.5. The plates were then incubated at 30°C for 24 h. Based on the inhibition zone diameter (mm), the isolates were classified as Susceptible (S) and Resistant (R) according to the Clinical and Laboratory Standards Institute's interpretative standards recommendations (CLSI 2020).

Bacterial identification

The 16S rRNA fragment of the bacterial isolate was amplified by PCR reaction with primer pairs 27F: 5'-AGAGTTTGATCMTGCTCAG-3' and 1492R: 5'-TACGGYTACCTTGTTACGACTT-3' (Heuer et al. 1997). Following the manufacturer's instructions, isolated bacterial DNA was extracted using the TopPURE® Genomic DNA Extraction Kit (ABT, Vietnam). PCR reaction components include: 12.5 µL iStandard iVAPCR Master Mix (Thermo Scientific, USA); 9.5 µL double distilled water; 0.5 µL primer 27F (25 pmol); 0.5 µL primer 1492R (25 pmol); and 2 µL of sample DNA. The thermal cycle to perform the PCR reaction includes stages: initial denaturation at 94°C for 5 min, then performing 30 cycles including denaturation at 94°C for 1 min, primer annealing at 63°C for 1 min, extension at 72°C for 2 min, and final extension at 72°C for 10 min. *Bacillus* strain with the strongest resistance to *A. schubertii* was selected for sequencing at DNA Sequencing Company (Vietnam).

Data analysis

The sequence similarity of bacterial strains was compared with sequences on the National Center for Biotechnology Information (NCBI) database using the BLASTn program. The bacterial sequences were compared with each other (multialignment) using the program CLUTAL W. The phylogenetic tree showing the genetic relationships between bacterial strains was built using MEGA5 software based on the neighbor-joining algorithm with a bootstrap value of 1.000 repetitions (Tamura et al. 2011).

RESULTS AND DISCUSSION

***Bacillus* isolation**

A total of 34 bacterial strains were isolated from 38 samples (consisting of 15 fish samples, 12 water samples, and 11 sediment samples) on the NA medium. In general, all strains of *Bacillus* were present in 3 sample types from intensive snakehead fish ponds in Tra On District, Vinh Long Province. The number of isolated strains from the sludges was 16, with the highest proportion being 47.06%. The number of isolated strains from snakehead fish's intestines was 10 strains (29.41%), and 8 strains originated from pond water, with the lowest rate of 25.53% (Table 1).

Morphological, physiological, and biochemical characteristics of *Bacillus*

The results showed that all 34 isolates had colony morphological characteristics such as circular colonies, irregular margins, raised elevation, yellow-gray or milky white, and wrinkled surfaces (Figure 1.C). They were all Gram-positive bacteria (Figure 1.D), motile, and spore-forming (Figure 1.E). Additionally, the findings revealed that 100% of the strains were oxidase and catalase-positive (Figure 1.F). The API 20E kit identification showed that strain BC2LM1 was positive for arginine, citrate, tryptophan deaminase, Voges-Proskauer, and gelatinase and negative for ortho-nitrophenyl galactosidase, lysine, ornithine, H_2S, urease, indole, glucose, mannitol, rhamnose, saccharose, melibiose, and amygdalin (Figure 1.G, Table 2).

Probiotic characteristics of *Bacillus*

Antibacterial activity of Bacillus *strains*

Of the total 34 bacterial strains isolated, 16 strains (47.06%) were capable of antagonizing *A. schubertii*, and 18 strains (52.94%) exhibited no inhibition (Figure 2). Of the eleven strains (68.75%) with strong antagonistic ability, three strains (18.75%) were with moderate resistance, and two strains (12.50%) showed weak resistance (Table 3).

Hemolytic ability

The results showed that strain BC2LM1 did not have a transparent or green area around the colony on the blood agar medium. Therefore, strain BC2LM1 in this study did not have hemolytic or γ-type hemolytic activity.

Table 1. Presumptive *Bacillus* strains isolated from pond water, sludge, and snakehead fish's intestines in Vinh Long Province

Isolation sources	Number of samples	Number of bacterial isolates
Sediments	11	16
Fish intestines	15	10
Pond water	12	8
Total	38	34

Acid resistance

The results showed that strain BC2LM1 was unable to tolerate acid at pH 1.0 and 1.5 after 6 h of incubation (Table 4). However, this bacterial strain could survive in media with pH values ranging from 2.0 to 4.0 after 6 h of culture.

Bile salts tolerance

The findings demonstrated that strain BC2LM1 was able to tolerate bile salts at a concentration of 0.1% and 0.3% after 24 hours of incubation. In particular, strain BC2LM1 was also able to tolerate bile salts at a concentration of 0.5% after 9 h of culture (Table 5).

Extracellular enzyme activity

The results showed that strain BC2LM1 was capable of producing cellulase, amylase, and protease enzymes (Figure 3), while this bacterial strain did not produce lipase.

Antibiotic susceptibility

The results showed that strain BC2LM1 was sensitive to ampicillin/sulbactam, clindamycin, doxycycline, tetracycline, erythromycin, and levofloxacin. Meanwhile, this bacterial strain was resistant to sulfamethoxazole/trimethoprim, ciprofloxacin, gentamicin, kanamycin, neomycin, nalidixic acid, norfloxacin, ofloxacin, streptomycin, and vancomycin (Table 6).

Table 2. The morphological, physiological, and biochemical characteristics of isolated bacterial strains

Bacterial characteristics	Isolate BC2LM2	BC2N	*Bacillus* spp.*
Gram staining	Positive	Positive	Positive
Cell shape	Short-rod	Long-rod	Short-rod
Spore staining	+	+	+
Motility	+	+	+
Oxidase	+	+	+
Catalase	+	+	+
Ortho-nitrophenyl galactosidase	-	-	-
Arginine	+	+	+
Lysine	-	-	-
Ornithine	-	-	-
Citrate	+	+	+
H_2S production	-	-	-
Urease	-	-	+
Tryptophane	+	+	-
Indole	-	-	-
Voges-Proskauer	+	+	+
Gelatin	+	+	+
Glucose	-	-	-
Manniton	-	-	-
Inositol	-	-	-
Sorbitol	-	-	-
Rhamnose	-	-	-
Saccharose	-	-	-
Melibiose	-	-	-
Amygdalin	-	-	-
Arabinose	-	-	-

Notes: (+): Positive; (-): Negative; * Phượng et al. (2018)

Table 3. Resistance to *A. schubertii* of isolated strains *Bacillus* sp.

Isolated strains	*A. schubertii* strain As11	Isolated strains	*A. schubertii* strain As11
C5	++	B33.1	-
CN7	+++	B35.2	-
CN3	+++	B1.3	-
C3Đ	+	BMT	-
C3.1	+++	BA	+++
C4	++	B2	-
CN1	++	B3	-
CN6	-	B1.4	-
CT1	-	B1.1	-
CK1	-	NC2LM1	+++
BC5M2	+++	NC2NM2	+++
BC5M1	+++	N3.2	-
BC2N	+++	N5.1	-
BC2LM1	+++	N5.2	-
B3.2	-	N1.2	-
B33.2	-	TN2.1	+++
B3.4	-	TN2	+

Notes: -: No inhibition (d = 0 mm); +: Weak inhibition (d = 1-5 mm); ++: Moderate inhibition (d = 6-10 mm); +++: Strong inhibition (d>10 mm)

Table 4. Number of bacterial cells (CFU/mL) that survived after strain BC2LM1 was treated with low pH

pH value	1.0	1.5	2.0	2.5	3.0	3.5	4.0
1 h	0	0	>200	>200	>200	>200	>200
3 h	0	0	142	128	>200	>200	>200
6 h	0	0	5	16	>200	>200	>200
9 h	0	0	0	0	112	>200	>200
12 h	0	0	0	0	0	>200	>200
24 h	0	0	0	0	0	0	0

Table 5. Number of bacterial cells (CFU/mL) that survived after strain BC2LM1 was treated with different bile salts

Bile balt (%) (hour)	0.1	0.3	0.5
1	>200	>200	>200
3	>200	>200	>200
6	>200	>200	164
9	>200	>200	58
12	>200	>200	0
24	>200	>200	0

Table 6. Antibiotic susceptibility of isolate BC2LM2

Antibiotics	Isolate BC2LM2	Antibiotics	Isolate BC2LM2
Ampicillin	I	Kanamycin	R
Ampicillin/Sulbactam	S	Levofloxacin	S
Sulfamethoxazole/ Trimethoprim	R	Neomycin	R
Ciprofloxacin	R	Nalidixic acid	R
Chloramphenicol	I	Norfloxacin	R
Clindamycin	S	Ofloxacin	R
Ceftazidime	R	Penicillin	I
Doxycycline	S	Streptomycin	R
Erythromycin	S	Tetracycline	S
Gentamicin	R	Vancomycin	R

Note: S: Sensitivity; I: Intermediate; R: Resistance

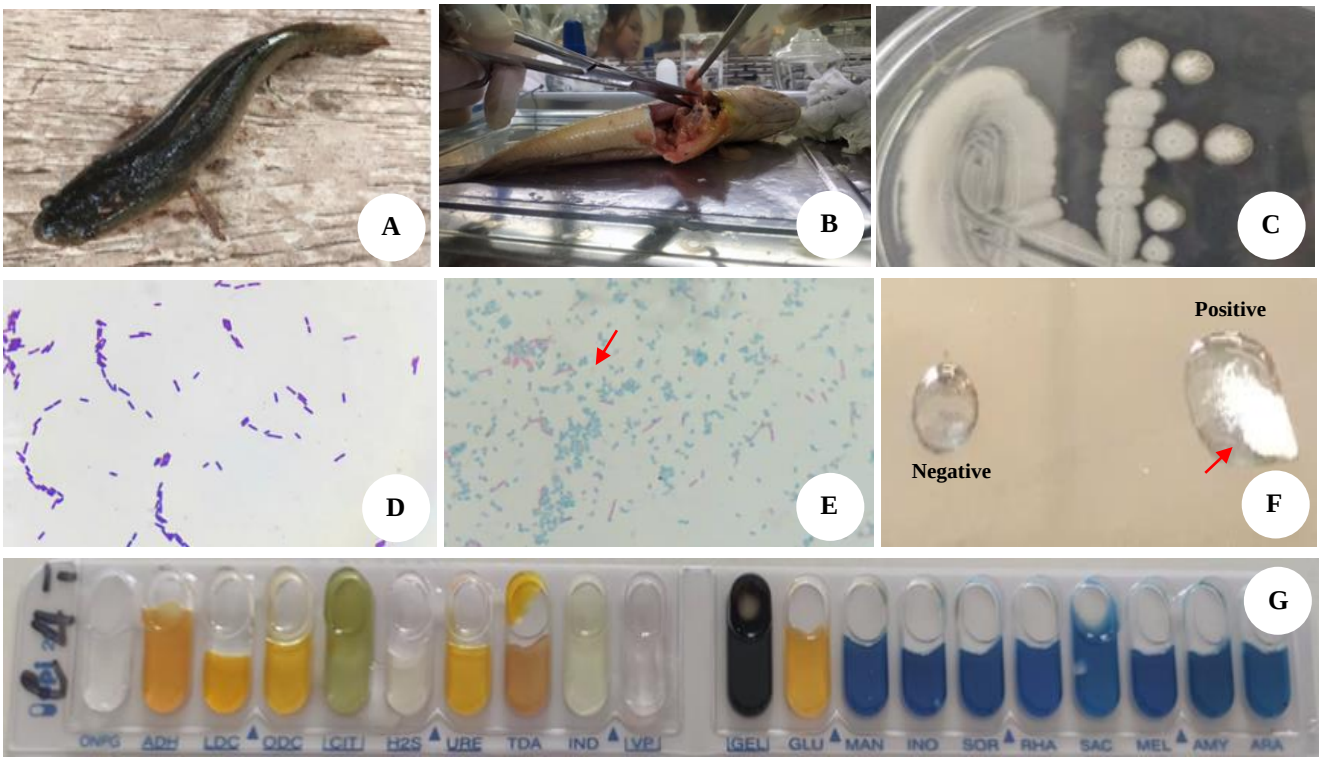

Figure 1. Colony morphological characteristics of *Bacillus* on NA medium. A, B: Healthy snakehead fish with normal signs (no external or internal signs of disease); C: Bacterial strain BC2LM1 with round shape, raised elevation, irregular edge, and wrinkled colonies after 24 hours of incubation; D. Gram staining (100X); E. Spore staining (red arrow, 100X); F. Catalase positive reaction (bubble gas, red arrow); G. Identification of strain BC2LM1 by API 20E kit

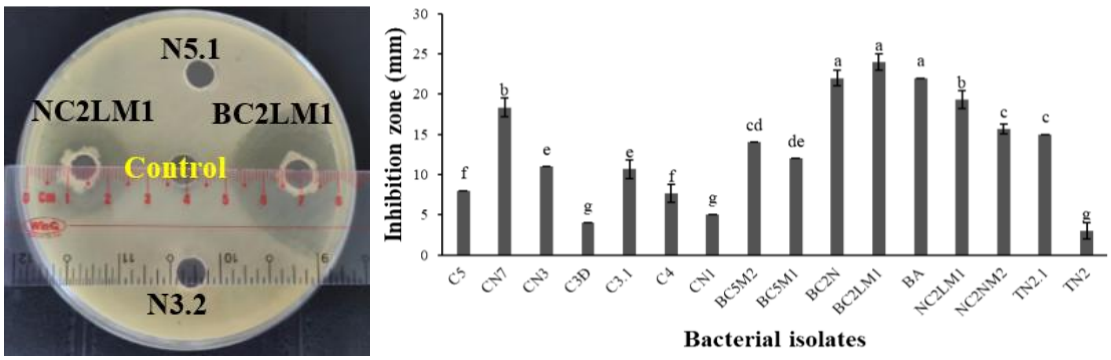

Figure 2. Antibacterial activity of isolated *Bacillus* strains against *A. schubertii* (significant differences ($p<0.5$) are shown by bars with distinct letters)

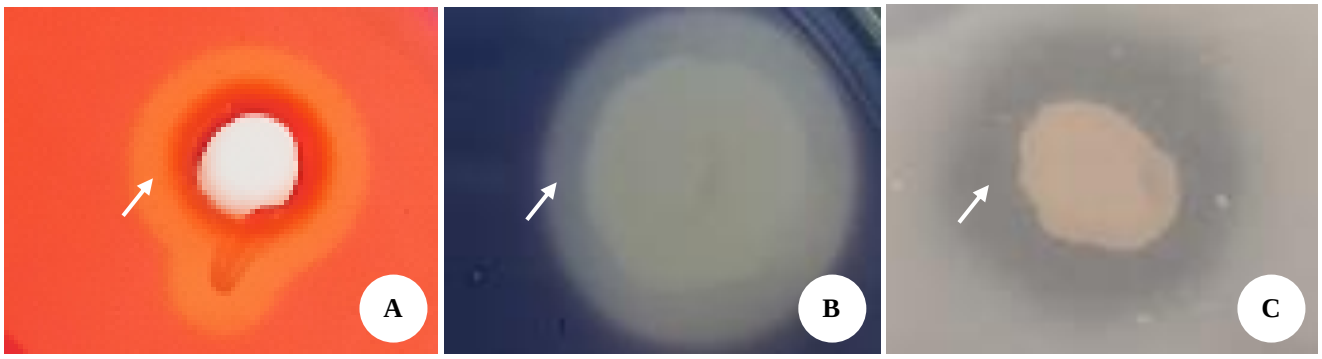

Figure 3. Cellulase, amylase, and protease activity of isolated strains. A. Cellulase activity; B. Amylase activity; C. Protease activity

Identification of *Bacillus* by PCR and sequencing of 16S rRNA genes

The electrophoresis results revealed that all 16S rRNA fragments of 10 representative *Bacillus* strains (high antagonistic activity against *A. schubertii*) were amplified with a DNA band at 1.500 bp position (Figure 4). Strain BC2LM1 showed 100% similarity with *Bacillus* species on the NCBI database. Moreover, the phylogenetic analysis revealed that strain BC2LM1 was clustered into the same branch with *Bacillus* species on GenBank (Figure 5). Therefore, based on PCR and gene sequencing combined with morphological, physiological, and biochemical characteristics, strain BC2LM1 was reconfirmed to belong to the *Bacillus* genus.

Discussion

The current study isolated and identified the strain BC2LM1 as belonging to the *Bacillus* genus based on morphological, physiological, and biochemical characteristics, PCR, and 16S rRNA gene sequencing results. This research was similar to previous studies indicating the common presence of *Bacillus* in different environments (Maughan and Van der Auwera 2011; Golnari et al. 2024). Besides lactic acid bacteria, Bacillus is also considered a group of potential probiotic microorganisms. The effectiveness of a probiotic product is that when introduced into the digestive tract, it will help increase food metabolism, increase immunity, and inhibit the growth of pathogenic microorganisms, helping to balance the body by intestinal microflora and limit gastrointestinal diseases (Torres-Maravilla et al. 2024). In the current investigation, the findings showed that 16 bacterial isolates have inhibitory activity against *A. schubertii*. Among them, strain BC2LM1 in this study was identified as *Bacillus*, which showed the strongest inhibitory activity. *Bacillus* exhibited antagonistic activity against many Gram-negative and Gram-positive bacterial microbes, as reported by many authors (Xie et al. 2009). In aquaculture, research by Kavitha et al. (2018) showed that *Bacillus* spp. originated from the digestive tract of freshwater fish, *Labeo calbasu*, and exhibited inhibitory activity against the fish pathogens *A. hydrophila* (KX756709), *Acinetobacter junii* (KX756708), *A. tandoii* (KX775222), *A. veronii* (KX688046), *Pseudomonas stutzeri* (KX721473), and *Acinetobacter* sp. (KX775221). Another study by Jiang et al. (2023) showed that B. HLJ1 and B. C1 strains identified as *B. subtilis* and *B. pumilus* had strong antimicrobial activity on *V. parahaemolyticus* when collected from aquaculture water. However, the inhibitory activity of strain BC2LM1 was lower than that of *Bacillus* strains reported by Cao et al. (2019), who demonstrated that strain BvL03 derived from sediment samples of fishponds had outstanding antibacterial activity against a lot of fish pathogenic bacteria, especially *Aeromonas*, consisting of *A. veronii*, *A. hydrophila*, *A. sobria*, and *A. caviae*. This result may be due to differences in bacterial strains and environmental conditions. Therefore, further studies need to be performed to clarify this issue, such as evaluating the effects of pH, temperature, nitrogen, and carbon sources on the antibacterial activity of strain BC2LM1.

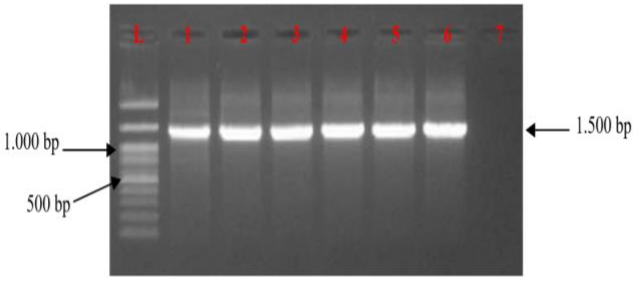

Figure 4. Amplification of the 16S rRNA gene segment of isolated *Bacillus* strains from snakehead fish, pond water, and sludges in Vinh Long province. L: 100 bp plus-DNA standard ladder; Lanes 1-6: Bacterial strains BC5M1, BC5M2, BC2N, BC2LM1, NC2NM2, and TN2.1, respectively; Lane 7: Negative control

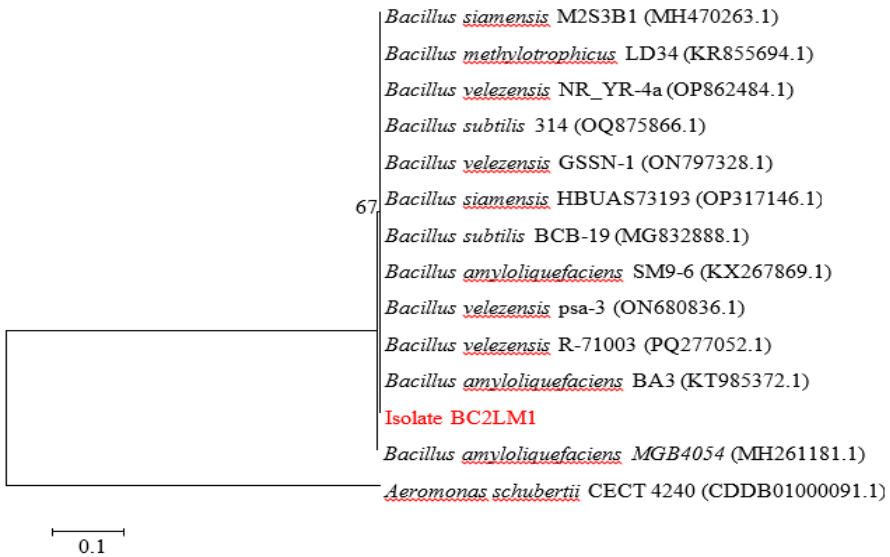

Figure 5. The phylogenetic tree displays strain BC2LM1 clustered into the same branches with *Bacillus* on the NCBI database (using *Aeromonas schubertii* CECT 4240 (CDDB01000091.1) as an outgroup). The numbers on the branches represent bootstrap values of 1.000 replicates

Hemolytic activity is considered a virulence factor, is an important selection criterion, and is recommended to be mandatory to evaluate the safety of strains with probiotic characteristics before investigating other probiotic properties to ensure that bacterial strains are not potentially toxic (Peres et al. 2014). In this study, strain BC2LM1 did not show hemolytic activity. This result was in line with previous reports showing that *Bacillus* isolated from aquatic animals also lack hemolytic activity (Banerjee et al. 2017; Kavitha et al. 2018).

For instance, Amoah et al. (2021) showed that GPSAK2 and GPSAK9 strains originating from the digestive tract of hybrid grouper were identified as *B. tequilensis* GPSAK2 and *B. subtilis* GPSAK9 were γ-hemolytic. However, most of the isolated *Bacillus* strains showed β-hemolytic activity (Dabiré et al. 2022). The absence of hemolytic activity in strain BC2LM1 suggests that this strain is unlikely to cause disease in animals, making it a promising candidate for probiotic applications.

Another important and necessary characteristic for selecting bacterial strains to produce probiotics is their ability to survive under low pH condition (FAO/WHO 2002). According to some previous studies, bacterial strains with probiotic potential must have low pH tolerance to help them overcome the acidic environment of the stomach (Yousuf et al. 2023; Anyairo et al. 2024). The pH in the small intestine of animals usually has a low pH; for fish, it is usually between 2.0 and 3.0. In this study, strain BC2LM1 demonstrated the ability to tolerate low pH levels, ranging from pH 2.0 to pH 4.0, for a longer time (3 h). This finding was consistent with some earlier research that demonstrated *Bacillus* could resist low pH (Guo et al. 2016; Kuebutornye et al. 2019). Research by Hou et al. (2024) showed *B. subtilis*, isolated from darkbarbel catfish (*Pelteobagrus fulvidraco*), could withstand harsh conditions such as low pH. Similarly, *Bacillus* strains from the study by Anyairo et al. (2024) revealed that six *Bacillus* species derived from Miang, a fermented tea in north Thailand, and can withstand pH 2.0 and 3.0 for 3 hours. Additionally, our findings were also supported by Yousuf et al. (2023), who demonstrated that two strains of *B. paramycoides*, PBG9D and BCS10, from *Channa punctata* and *Channa striata*, showed tolerance to acidic and alkaline pH (2.0, 3.0, 4.0, 7.0, and 9.0).

Another challenge to microbial survival in the fish gastrointestinal tract is the presence of bile in the small intestine. Bile has the effect of destroying microorganisms thanks to the action of destroying microbial cell membranes. Probiotic microorganisms only exert beneficial effects on the host when they settle and exist in the small intestine, an environment containing bile salts. A bile concentration of 0.3% is often used to select bile-resistant probiotic strains because this concentration is considered the average bile concentration in the fish intestine (Balcázar et al. 2008). In this current study, strain BC2LM1 showed the ability to grow at 0.3% bile concentration after 3-6 h. These findings were in line with the results obtained by Nakharuthai et al. (2023), who revealed that *Bacillus* isolates from the intestine of nile tilapia could grow in an LB medium containing 0.5-2% bile salts for 6 h. A similar finding from Jlidi et al. (2022) also showed that *Bacillus* strains from sardine and shrimp intestines could withstand 0.5%-5% bile concentrations after a 3-h exposure, where strain CJ3 had the lowest tolerance to 5% bile salt, whereas S17 had the maximum tolerance.

Extracellular enzymes are crucial for aiding food digestion, facilitating easy absorption, and promoting healthy weight gain in animals. As a result, one crucial factor in choosing bacterial strains for probiotics is their capacity to create extracellular enzymes. Amylase and protease enzymes are important for probiotics to have a digestive effect because these enzymes can produce a variety of amino acids, sugars, organic acids, and low molecular compounds. In the current study, this investigation showed that strain BC2LM1 was capable of producing cellulase, amylase, and protease enzymes, while this strain did not produce lipase. This finding was in line with some earlier studies that revealed that *Bacillus* strains were able to produce extracellular enzymes like cellulase, amylase, lipase, and protease (Thurlow et al. 2019; Jiang et al. 2023; Anyairo et al. 2024). According to Elsadek et al. (2023), *Bacillus* isolates obtained from *Rhynchocypris lagowskii*'s intestines generated lipase, amylase, and protease. A recent study by Shokrak et al. (2024) revealed that *B. rugosus* NM007 from soil samples and fish samples (nile tilapia (*Oreochromis niloticus*), and sardine) exhibited the ability to produce the main digestive enzymes, such as lipase, amylase, and protease.

In addition to hemolytic tests, antibiotic sensitivity was performed to ensure the bacterial isolate was safe for aquaculture applications. This study showed that strain BC2LM1 was sensitive to ampicillin/sulbactam, clindamycin, doxycycline, tetracycline, erythromycin, and levofloxacin. These findings were consistent with earlier research showing that *Bacillus* isolates were susceptible to chloramphenicol, ciprofloxacin, erythromycin, and vancomycin (Kavitha et al. 2018). Research by Nakharuthai et al. (2023) revealed that *Bacillus* isolate B29 was susceptible to neomycin, ampicillin, erythromycin, oxytetracycline, ciprofloxacin, enrofloxacin, sulfamethoxazole, nalidixic acid, amoxicillin, and tetracycline. This work was also strongly supported by a report by Shokrak et al. (2024) that demonstrated that *B. rugosus* NM007 was susceptible to 16 antibiotics like cefoperazone, cefaclor, clarithromycin, imipenem, cefdinir, vancomycin, ofloxacin, pefloxacin, chloramphenicol, nitrofurantoin, moxifloxacin, tobramycin, nalidixic acid, tetracycline, streptomycin, and gentamicin, except this isolate only resistance to penicillin. However, strain BC2LM1 was resistant to many antibiotics, including sulfamethoxazole/ trimethoprim, ciprofloxacin, ceftazidime, gentamicin, kanamycin, neomycin, nalidixic acid, norfloxacin, ofloxacin, streptomycin, and vancomycin. This investigation was in line with an earlier study showing that *Bacillus* strain FC3 obtained from the digestive tract of freshwater fish, *L. calbasu*, was found to be resistant to ciprofloxacin, amoxicillin, and ampicillin (Kavitha et al. 2018). Hence, when using this strain to make probiotics, care should be taken because antibiotic-resistant bacteria can pass their resistance genes to other bacterial species (Jian et al. 2021).

In conclusion, the current findings demonstrated that *Bacillus* strains from pond water, sediment samples, and intestines of snakehead fish showed antibacterial activity against *A. schubertii*. Strain BC2LM1 was determined to be *Bacillus* based on morphological, physiological, and biochemical traits and the results of PCR and 16S rRNA gene sequencing. After 6 h of culture, strain BC2LM1 in this study was able to survive in media with a pH between 2.0 and 4.0. In particular, strain BC2LM1 could withstand bile salts at a concentration of 0.5% after 9 h of culture. The results showed that strain BC2LM1 could produce cellulase, amylase, and protease enzymes. Additionally, this strain exhibited susceptibility to erythromycin, clindamycin, tetracycline, doxycycline, ampicillin/sulbactam, and levofloxacin. The study's results show the potential of strain BC2LM1 as a probiotic in intensive snakehead fish farming.

REFERENCES

Amoah K, Dong X-H, Tan B-P, Zhang S, Kuebutornye FKA, Chi S-Y, Yang Q-H, Liu H-Y, Zhang H-T, Yang Y-Z. 2021. In vitro assessment of the safety and potential probiotic characteristics of three *Bacillus* strains isolated from the intestine of hybrid grouper (*Epinephelus fuscoguttatus*♀ × *Epinephelus lanceolatus*♂). Front Vet Sci 8: 675962. DOI: 10.3389/fvets.2021.675962.

Anyairo CS, Unban K, Wongputtisin P, Rojtinnakorn J, Shetty K, Khanongnuch C. 2024. *Bacillus* spp. isolated from Miang as potential probiotics in nile tilapia culture-in vitro research. Microorganisms 12 (8): 1687. DOI: 10.3390/microorganisms12081687.

Bacanlı MG. 2024. The two faces of antibiotics: An overview of the effects of antibiotic residues in foodstuffs. Arch Toxicol 98 (6): 1717-1725. DOI: 10.1007/s00204-024-03760-z.

Balcázar JL, Vendrell D, de Blas I, Ruiz-Zarzuela I, Muzquiz JL, Girones O. 2008. Characterization of probiotic properties of lactic acid bacteria isolated from intestinal microbiota of fish. Aquaculture 278 (1-4): 188-191. DOI: 10.1016/j.aquaculture.2008.03.014.

Banerjee G, Nandi A, Ray AK. 2017. Assessment of hemolytic activity, enzyme production and bacteriocin characterization of *Bacillus subtilis* LR1 isolated from the gastrointestinal tract of fish. Arch Microbiol 199 (1): 115-124. DOI: 10.1007/s00203-016-1283-8.

Bình B. 2020. Potential from snakehead fish farming. https:// thuysanvietnam.com.vn/tiem-nang-tu-nuoi-ca-loc/ [18 November 2024]

Bauer AW, Kirby WM, Sherris JC, Turck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45 (4): 493-496. DOI: 10.1093/ajcp/45.4_ts.493.

Cao L, Pan L, Gong L et al. 2019. Interaction of a novel *Bacillus velezensis* (BvL03) against *Aeromonas hydrophila* in vitro and in vivo in grass carp. Appl Microbiol Biotechnol 103 (21-22): 8987-8999. DOI: 10.1007/s00253-019-10096-7.

Châu ĐTM, Mai LH, Dung TT. 2018. Antimicrobial susceptibility of bacterial pathogen causing internal white spot disease in snakehead fish (*Channa striata*) in Tra Vinh Province. CTU J Sci 54: 108-115. DOI: 10.22144/ctu.jsi.2018.043. [Vietnamese]

Chen Y, Li J, Xiao P, Zhu W, Mo Z. 2016. The ability of marine *Bacillus* spp. isolated from fish gastrointestinal tract and culture pond sediment to inhibit growth of aquatic pathogenic bacteria. Iran J Fish Sci 15 (2): 701-714.

CLSI. 2020. Performance standards for antimicrobial susceptibility testing for bacteria isolated from aquatic animals. In: CLSI supplement VET04 (Third Edition). Clinical and Laboratory Standards Institute, Wayne, Pennsylvania.

Dabiré Y, Somda NS, Somda MK, Mogmenga I, Traoré AK, Ezeogu LI, Traoré AS, Ugwuanyi JO, Dicko MH. 2022. Molecular identification and safety assessment of *Bacillus* strains isolated from Burkinabe traditional condiment "soumbala". Ann Microbiol 72: 10. DOI: 10.1186/s13213-022-01664-w.

Oanh ĐTH, Nghĩa NT. 2016. Determination of causative agent of Bacillary necrosis disease in snakehead fish (*Channa striata*) cultured in the Mekong Delta. J Agric Rural Develop 1: 82-89. DOI: 10.22144/ctujos.2024.428. [Vietnamese]

Dung TT, Trung NB, Khôi LM. 2019. Isolation, identification and characterization of *Aeromonas schubertii* causing internal white spot on snakehead fish (*Channa striata*) in the Mekong Delta of Vietnam. CTU J Sci 55: 69-78. DOI: 10.22144/ctu.jvn.2019.049. [Vietnamese]

Elsadek MM, Wang S, Wu Z, Wang J, Wang X, Zhang Y, Yu M, Guo Z, Wang Q, Wang G, Chen Y, Zhang D. 2023. Characterization of *Bacillus* spp. isolated from the intestines of *Rhynchocypris lagowskii* as a potential probiotic and their effects on fish pathogens. Microb Pathog 180: 106163. DOI: 10.1016/j.micpath.2023.106163.

FAO/WHO. 2002. Guidelines for the evaluation of probiotics in food. Report of a Joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. Available at: ftp://ftp.fao.org/es/esn/food/wgreport2.pdf. [20 November 2024]

Fernández-Bravo A, Figueras MJ. 2020. An update on the genus *Aeromonas*: Taxonomy, epidemiology, and pathogenicity. Microorganisms 8 (1): 129. DOI: 10.3390/microorganisms8010129.

Golnari M, Bahrami N, Milanian Z, Khorasgani MR, Asadollahi MA, Shafiei R, Fatemi SS-A. 2024. Isolation and characterization of novel *Bacillus* strains with superior probiotic potential: Comparative analysis and safety evaluation. Sci Rep 14 (1): 1457. DOI: 10.1038/s41598-024-51823-z.

Guo X, Chen D-D, Peng K-S, Cui Z-W, Zhang X-J, Li S, Zhang Y-A. 2016. Identification and characterization of *Bacillus subtilis* from grass carp (*Ctenopharynodon idellus*) for use as probiotic additives in aquatic feed. Fish Shellfish Immunol 52: 74-84. DOI: 10.1016/j.fsi.2016.03.017.

Heuer H, Krsek M, Baker P, Smalla K, Wellington EM. 1997. Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. Appl Environ Microbiol 63: 3233-3241. DOI: 10.1128/aem.63.8.3233-3241.1997.

Hou T, Tang Z, Wang Z, Li C. 2024. Evaluation of the potential probiotic *Bacillus subtilis* isolated from darkbarbel catfish (*Pelteobagrus fulvidraco*) on growth performance, serum immunity, and disease resistance of *Aeromonas hydrophila*. J Fish Biol 2024: 1-11. DOI: 10.1111/jfb.15925.

Jeon HJ, Song JW, Lee C, Kim B, Park SY, Kim JH, Han JE, Park JH. 2022. Antibacterial activity of *Bacillus* strains against acute hepatopancreatic necrosis disease-causing *Vibrio campbellii* in Pacific white leg shrimp. Fishes 7 (5): 287. DOI: 10.3390/fishes7050287.

Jian Z, Zeng L, Xu T, Sun S, Yan S, Yang L, Huang Y, Jia J, Dou T. 2021. Antibiotic resistance genes in bacteria: Occurrence, spread, and control. J Basic Microbiol 61 (12): 1049-1070. DOI: 10.1002/jobm.202100201.

Jiang N, Hong B, Luo K, Li Y, Fu H, Wang J. 2023. Isolation of *Bacillus subtilis* and *Bacillus pumilus* with anti-*Vibrio parahaemolyticus* activity and identification of the anti-*Vibrio parahaemolyticus* substance. Microorganisms 11 (7): 1667. DOI: 10.3390/microorganisms11071667.

Jlidi M, Akremi I, Ibrahim AH, Brabra W, Ali MB, Ali MB. 2022. Probiotic properties of *Bacillus* strains isolated from the gastrointestinal tract against pathogenic Vibriosis. Front Mar Sci 9: 884244. DOI: 10.3389/fmars.2022.884244.

Kavitha M, Raja M, Perumal P. 2018. Evaluation of probiotic potential of *Bacillus* spp. isolated from the digestive tract of freshwater fish *Labeo calbasu* (Hamilton, 1822). Aquac Rep 11: 59-69. DOI: 10.1016/j.aqrep.2018.07.001.

Khochamit N, Siripornadulsil S, Sukon P, Siripornadulsil W. 2015. Antibacterial activity and genotypic-phenotypic characteristics of bacteriocin-producing *Bacillus subtilis* KKU213: Potential as a probiotic strain. Microbiol Res 170: 36-50. DOI: 10.1016/j.micres.2014.09.004.

Kuebutornye FKA, Lu Y, Abarike ED, Wang Z, Li Y, Sakyi ME. 2019. In vitro assessment of the probiotic characteristics of three *Bacillus* species from the gut of nile tilapia, *Oreochromis niloticus*. Probiotics Antimicrob Proteins 12: 412-424. DOI: 10.1007/s12602-019-09562-5.

Lâm MT, Đức PM, Hiền TTT. 2018. Effects of Vitamin C on growth and immune parameters of snakehead culture in Hapa. J Vietnam Agric Sci Technol 89 (4): 109-114. [Vietnamese]

Latif-Eugenín F, Beaz-Hidalgo R, Figueras MJ. 2016. First record of the rare species *Aeromonas schubertii* from mussels: Phenotypic and genetic reevaluation of the species and a review of the literature. Arch Microbiol 198 (4): 333-345. DOI: 10.1007/s00203-016-1189-5.

Hạnh LLP, Mai NHC, Linh TNP, Hậu LV, Quân NĐ, Thảo NHP. 2021. Antagonistic activity of *Bacillus amyloliquefaciens* B894 against *Edwardsiella ictaluri* and *Aeromonas hydrophila*. Vietnam J Agri Sci 1 (122): 113-119. [Vietnamese]

Cường LT, Thư NTA, Cầm VH. 2023. Isolation and selection of *Bacillus* spp. with antimicrobial property against *Vibrio parahaemolyticus* causing Acute hepatopancreatic necrosis disease in marine shrimp. J Fisher Sci Technol - Nha Trang University 01: 002-012. [Vietnamese]

Hậu LV, Mai PTH, Hạnh LLP, Thảo NHP. 2022. Screening antagonistic *Bacillus* spp. strains against *Streptococcus agalactiae* causing popeye and hemorrhage in tilapia (*Oreochromis* sp.). Univ Agric For Hue Univ 6 (1): 2751-2761. DOI: 10.46826/huaf-jasat.v6n1y2022.796. [Vietnamese]

Liu JY, Li AH. 2012. First case of *Aeromonas schubertii* infection in the freshwater cultured snakehead fish, *Ophiocephalus argus* (Cantor) in China. J Fish Dis 35 (5): 335-342. DOI: 10.1111/j.1365-2761.2012.01350.x.

Marimuthu K, Arokiara AJ, Haniffa MA. 2009. Effect of diet quality on seed production of the spotted snakehead *Channa punctatus* (Bloch). Am-Eurasian J Sustain Agric 3 (3): 344-347.

Maughan H, Van der Auwera G. 2011. *Bacillus* taxonomy in the genomic era finds phenotypes to be essential though often misleading. Infect Genet Evol 11 (5): 789-797. DOI: 10.1016/j.meegid.2011.02.001.

Merhi A, El Khatib S, Haddad J, Hassan HF. 2023. A review of the antibiotic residues in food in the Arab countries. Appl Food Res 3 (2): 100332. DOI: 10.1016/j.afres.2023.100332.

Nakharuthai C, Boonanuntanasarn S, Kaewda J, 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 13 (6): 986. DOI: 10.3390/ani13060986.

Nakkrasae L, Wisetdee K, Charoenphandhu N. 2015. Osmoregulatory adaptations of freshwater air-breathing snakehead fish (*Channa striata*) after exposure to brackish water. J Comp Physiol B 185 (5): 527-537. DOI: 10.1007/s00360-015-0902-z.

Palacios-Rodriguez AP, Espinoza-Culupú A, Durán Y, Sánchez-Rojas T. 2024. Antimicrobial activity of *Bacillus amyloliquefaciens* BS4 against Gram-negative pathogenic bacteria. Antibiotics 13 (4): 304. DOI: 10.3390/antibiotics13040304.

Peres CM, Alves M, Hernandez-Mendoza A, Moreira L, Silva S, Bronze MR, Vilas-Boas L, Peres C, Malcata FX. 2014. Novel isolates of Lactobacilli from fermented Portuguese olive as potential probiotics. LWT Food Sci Technol 59: 234-246. DOI: 10.1016/j.lwt.2014.03.003.

Pham Thi HH, Kim D-H, Quach Van CT, Nguyen PT, Nguyen TL. 2023. Prevalence and antibiotic resistance of *Aeromonas schubertii* causing internal white spot disease on snakehead fish, *Channa striata*, in the Mekong Delta, Vietnam. J World Aquac Soc 54 (5): 1260-1276. DOI: 10.1111/jwas.12954.

Phung LT, Phuong TTM, Phung NK, Nicolas M. 2020. Isolation of *Bacillus* strains inhibition disease resistance of acute hepatopancreatic necrosis disease on the shrimp in Can Gio, Vietnam. IOP Conf Ser: Mater Sci Eng 736: 062003. DOI: 10.1088/1757-899X/736/6/062003.

Phượng VH, Hậu VT, Ngọc NTH, Phước LH, Tuấn NH, Lộc NH, Thủy LTB. 2018. Evaluation of antagonism properties of *Bacillus licheniformis* (B1) against *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease (AHPND) on shrimp *in-vitro* condition. CTU J Sci 54: 91-100. DOI: 10.22144/ctu.jsi.2018.041. [Vietnamese]

Proespraiwong P, Mavichak R, Imaizumi K, Hirono I, Unajak S. 2023. Evaluation of *Bacillus* spp. as potent probiotics with reduction in AHPND-related mortality and facilitating growth performance of Pacific white shrimp (*Litopenaeus vannamei*) farms. Microorganisms 11 (9): 2176. DOI: 10.3390/microorganisms11092176.

Rahman MA, Molla MHR, Sarker MK, Chowdhury SH, Shaikh MM. 2018. Snakehead fish (*Channa striata*) and its biochemical properties for therapeutics and health benefits. SF J Biotechnol Biomed Eng 1 (1): 1005.

Ran C, Carrias A, Williams MA, Capps N, Dan BCT, Newton JC, Kloepper JW, Ooi EL, Browdy CL, Terhune JS, Liles MR. 2012. Identification of *Bacillus* strains for biological control of catfish pathogens. PLoS One 7: e45793. DOI: 10.1371/journal.pone.0045793.

Ren Z, Cai Y, Wang S, Liu S, Li A, Xiong Y, Tang J, Sun Y, Guo W, Zhou Y. 2018. First case of *Aeromonas schubertii* infection in brackish water wild nile tilapia, *Oreochromis niloticus*, in China. Aquaculture 501: 247-254. DOI: 10.1016/j.aquaculture.2018.11.036.

Santos RA, Oliva-Teles A, Pousão-Ferreira P, Jerusik R, Saavedra MJ, Enes P, Serra CR. 2021. Isolation and characterization of fish-gut *Bacillus* spp. as source of natural antimicrobial compounds to fight aquaculture bacterial diseases. Mar Biotechnol 23 (2): 276-293. DOI: 10.1007/s10126-021-10022-x.

Shokrak NM, Khairi N, Hazrin-Chong NH, Mohamed RA, Abdella B. 2024. Isolation, characterization, and assessment of *Bacillus* rugosus potential as a new probiotic for aquaculture applications. Sci Rep 14: 25019. DOI: 10.1038/s41598-024-74534-x.

Sionek B, Szydłowska A, Zielińska D, Neffe-Skocińska K, Kołożyn-Krajewska D. 2023. Beneficial bacteria isolated from food in relation to the next generation of probiotics. Microorganisms 11 (7): 1714. DOI: 10.3390/microorganisms11071714.

Tahir NDM, Matori MF, Gan HM. 2024. First report of *Aeromonas schubertii* infection in striped Snakehead *Channa striata* (Bloch, 1793) fingerlings in Malaysia. J Medik Veteriner 7 (1): 33-40. DOI: 10.20473/jmv.vol7.iss1.2024.33-40.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28 (10): 2731-2739. DOI: 10.1093/molbev/msr121.

Temario EE, Baure JG, Mameloco EJG, Cadiz RE, Traifalgar RFM. 2022. Inhibitory activity of probiotic *Bacillus subtilis* BF12 against *Vibrio parahaemolyticus* infection and its growth-promoting effects on juvenile *Penaeus monodon*. Intl J Aquat Biol 10 (1): 32-44.

Thi QVC, Trung NB, Dung TT, Thuy NP. 2023. The antibiotic susceptibility of *Aeromonas schubertii* isolated from snakehead in An Giang province of the Mekong Delta. Vet Integr Sci 22 (2): 609-629. DOI: 10.12982/VIS.2024.042.

Thịnh NQ, Maita M, Phú TM. 2020. Status of diseases, drugs and chemicals use in snakehead (*Channa striata*) culture in An Giang and Tra Vinh provinces. CTU J Sci 56 (1): 179-184. DOI: 10.22144/ctu.jsi.2020.020. [Vietnamese]

Thurlow CM, Williams MA, Carrias A, Ran C, Newman M, Tweedi J, Allison E, Jescovitch LN, Wilson AE, Terhune JS, Liles MR. 2019. *Bacillus velezensis* AP193 exerts probiotic effects in channel catfish (*Ictalurus punctatus*) and reduces aquaculture pond eutrophication. Aquaculture 503: 347-356. DOI: 10.1016/j.aquaculture.2018.11.051.

Torres-Maravilla E, Parra M, Maisey K, Vargas RA, Cabezas-Cruz A, Gonzalez A, Tello M, Bermúdez-Humarán LG. 2024. Importance of probiotics in fish aquaculture: Towards the identification and design of novel probiotics. Microorganisms 12 (3): 626. DOI: 10.3390/microorganisms12030626.

Truong QP, Phan TCT, Vu HH, Pham TTN, Huynh TG, Vu NU. 2021. Isolation of potential probiotic *Bacillus subtilis* CM3.1 and its effects on water quality and growth performance of the whiteleg shrimp *Litopenaeus vannamei* in the Mekong Delta, Vietnam. AACL Bioflux 14 (6): 3347-3357.

Wulff EG, Mguni CM, Mansfeld-Giese K, Fels J, Lübeck M, Hockenhull J. 2002. Biochemical and molecular characterization of *Bacillus amyloliquefaciens*, *B. subtilis* and *B. pumilus* isolates with distinct antagonistic potential against *Xanthomonas campestris* pv. *campestris*. Plant Pathol 51 (5): 574-584. DOI: 10.1046/j.1365-3059.2002.00753.x.

Xie J, Zhang R, Shang C, Guo Y. 2009. Isolation and characterization of a bacteriocin produced by an isolated *Bacillus subtilis* LFB112 that exhibits antimicrobial activity against domestic animal pathogens. Afr J Biotechnol 8 (20): 5611-5619.

Yousuf S, Jamal MT, Al-Farawati RK, Al-Mur BA, Singh R. 2023. Evaluation of *Bacillus paramycoides* strains isolated from *Channa* fish sp. on growth performance of *Labeo rohita* fingerlings challenged by fish pathogen *Aeromonas hydrophila* MTCC 12301. Microorganisms 11 (4): 842. DOI: 10.3390/microorganisms11040842.