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ARTICLE

Isolation and Identification of the Ammonium and Nitrite Oxidizing Bacterial Strains for Nitrogen Treatment in Shrimp Pond in Quang Ninh Province, Vietnam

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ABSTRACT

The objective of this study was to isolate and identify the nitrifying bacteria from wastewater in the aquaculture system at Quang Ninh province and initially evaluate their ability to treat nitrogen in water and determine the strain of bacteria and identify the strain of bacteria. Nitrifying bacteria were isolated by serial dilution and agar plates and then purified by agar slant until axenic colonies were obtained. The study has successfully isolated 3 bacterial strains capable of ammonium oxidation (AOB-1, AOB-2, AOB-3) and 3 bacterial strains with nitrite oxidation ability (NOB-1, NOB-2, NOB-3) from shrimp farming wastewater in Quang Ninh province, Vietnam. These strains demonstrate potential for ammonium and nitrite oxidation, making them applicable for nitrogen treatment in sewage. Strains with nitrite oxidation ability showed a potential high capability for nitrogen removal from water. The decoding of the 16S rRNA gene sequences, compared using Blastn (NCBI) indicated similarities with *Bacillus subtilis* (AOB-1), *Pantoea agglomerans* strain JCM1236 16S RNA (AOB-2), Enterobacter hormaechei strain ES1 16S RNA (AOB-3), *Arthrobacter nicotianae* (NOB-1), *Acinetobacter lactucae* strain JVAP01 16S RNA (NOB-2) and *Enterobacter asburiae* (NOB-3). Therefore, the bacteria that contained 6 strains can be considered to apply for wastewater treatment in shrimp farming.

Keywords: Bacteria isolation; Identification; Nitrification; Eutrophication; Ammonium oxidizing; Nitrite oxidizing

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ARTICLE INFO
Received: 14 June 2024 | Revised: 24 June 2024 | Accepted: 30 June 2024 | Published Online: 20 July 2024
DOI: https://doi.org/10.30564/jees.v6i2.6749

CITATION
Tham, N.T., Giang, P.Q., Oanh, D.Q., et al., 2024. Isolation and Identification of the Ammonium and Nitrite Oxidizing Bacterial Strains for Nitrogen Treatment in Shrimp Pond in Quang Ninh Province, Vietnam. Journal of Environmental & Earth Sciences. 6(2): 187–199. DOI: https://doi.org/10.30564/jees.v6i2.6749

1. Introduction

Water pollution has emerged as a critical global concern. This pollution originates from industrial and agricultural activities, as well as human behavior. Nitrogen is a major pollutant, in the form of ammonia, nitrate, and nitrite, is prevalent in various water systems, harming aquatic life and disrupting ecosystems by causing eutrophication [1–3]. In aquaculture, nitrogen excess in ponds due to farming activities hinders the growth and productivity of aquatic species. While shrimp farming has initially succeeded in Asian countries, it has encountered problems such as disease outbreaks and environmental degradation, reducing shrimp farming area and yield. This decline is mainly due to rapid aquaculture expansion focused on increasing cultivation areas and production volume without addressing waste treatment [4–6]. To address these challenges, countries have invested in research to revitalize aquaculture by managing and protecting the farming environment. Over the decades, various physical, chemical, and biological methods have been developed to treat wastewater and water in aquaculture ponds. A promising alternative is using microorganisms to reduce nitrogen levels in water, an approach that is economically efficient, easy to implement, safe, effective, and environmentally friendly. One such strategy is the nitrate reduction process, conducted by denitrifying microorganisms, which are gram-negative, non-spore-forming bacteria in spherical, rod-shaped, or spiral forms [2,7–10].

Therefore, the identification of specific microorganisms is crucial for determining their potential in biological wastewater treatment [11,12]. The crucial role of bacteria in the mechanisms of nitrogen transformation processes in aquatic environments has been studied by Bock (1992), Moriarty (1997), and Van de Graaf et al. (1995) [5,10]. Hargreaves (1998) examined nitrogen biogeochemistry in aquaculture ponds, influenced by the biological transformation of nitrogen from fertilizers and feeds. Excessive nitrogen use can deteriorate water quality due to toxic compounds like ammonia and nitrite. Major ammonia sources include fish excreta and sediment runoff. Sediments interact with water, regulating nitrogen biochemical processes. They act as ammonia sources and reservoirs for nitrite and nitrate, with the potential for nitrogen removal through denitrification, though limited by oxygen penetration depth [13]. Lin (2007) isolated a novel nitrate-forming *Bacillus sp.* from a membrane bioreactor treating synthetic wastewater. The *Bacillus sp.* LY showed 80% nitrogen removal efficiency and 71.7% COD removal efficiency after 24 days. It can use organic carbon as a denitrification source and reduce nitrate presence, suggesting its potential for biological nitrogen removal [14]. Sahu et al. (2008) highlighted aquaculture's rapid growth, especially in Asia, contributing to 90% of global production. However, disease outbreaks hinder growth, affecting economic and socio-economic aspects. Disease control methods include traditional, synthetic chemicals, and antibiotics, but these have negative effects, such as residual chemical accumulation [6].

The objective of this study was to isolate and identify the nitrifying bacteria from wastewater in the aquaculture system at Quang Ninh province and initially evaluate their ability to treat nitrogen in water and identify the strain of bacteria.

2. Materials and methods

2.1 Sampling method

Wastewater samples were collected from shrimp farming ponds in Quang Ninh province, exhibiting a pH range of 7 to 8 and a salinity level near 25 parts per thousand. The sampling equipment was sterilized through autoclaving and drying. A total of 100 ml of the surface level of wastewater was collected and mixed from 3 to 4 different locations in each shrimp pond. These samples were combined thoroughly in sterilized conical flasks. The homogenized sample was agitated, and 10 ml aliquots were used for the isolation of ammonium-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). The samples were dissolved in 90 mL of sterile liquid mineral medium in sterile conical flasks. The liquid mineral medium for AOB included 0.1 g $MgSO_4 \cdot 7H_2O$, 13.5

g Na_2HPO_4, 0.7 g KH_2PO_4, 0.01 g anhydrous $FeCl_3$, 0.18 g $CaCl_2 \cdot 2H_2O$, 0.5 g $(NH_4)_2SO_4$, and 0.5 g $NaHCO_3$, based on a modified protocol from Spieck and Bock (2005). For NOB, ammonium sulfate was replaced with 0.5 g of $NaNO_2$ [8]. The solutions were prepared in 1000 mL of distilled water, with the final pH adjusted to 7.5. The mixture was stirred with a sterile glass rod, shaken at 120 rpm for 30 minutes at room temperature, vortexed for 30 seconds, and allowed to settle, yielding a 1:10 dilution.

Isolation of native nitrifying bacterial consortia

Native nitrifying bacterial consortia were isolated and purified based on observed nitrifying bacterial counts. This process involved reseeding in a minimal mineral agar medium composed of 0.66 g $K_2HPO_4 \cdot 3H_2O$, 1 g NH_4Cl, 2 g Na_2SO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, and 15 g of agar per 1000 mL of distilled water, with a final pH of 7.5. Cultures were maintained under conditions identical to those for nitrifying bacterial counts, with necessary reseeding to achieve pure strains on solid minimal mineral agar medium. Pure strains from the native consortia were cultivated in inclined tubes with minimal mineral agar to ensure sufficient growth for full identification and viability preservation [15].

To isolate microbial strains, a serial dilution method was employed. Each 1 ml sample of the AOB or NOB suspensions (diluted 1:10) was mixed into test tubes containing 9 ml of sterile liquid mineral medium to create a 1:100 dilution. This process continued to produce subsequent dilutions of 1:1000 and 1:10000, adjusting based on microorganism density to achieve isolated colonies. For suitable dilutions, 0.1 ml of each suspension was spread on Petri dishes containing a sterile medium with the same liquid mineral composition plus 15 g of agar. These petri dishes were incubated at room temperature, and post-bacterial growth, pure colonies were isolated and preserved for further analysis. For higher dilution concentrations (10^{-5} or 10^{-6}), 0.1 ml of soil suspension was spread on a solid medium in Petri dishes. The inoculation loop was sterilized by immersion in alcohol and flaming, cooled, and used to evenly spread the suspension over the agar surface. Plates were incubated at 30–37 °C and colonies were observed with a magnifying glass after 1–2 days.

To obtain pure strains, sterilized inocula were used to transfer biomass from each colony into test tubes with inclined minimal mineral agar medium, followed by incubation at appropriate temperatures. The purity of microorganisms in each tube was then verified.

Checking the inoculum

The growth of microorganisms on a solid medium was observed to ensure purity. Bacterial morphology was assessed during the isolation process by colony counter, colonies with uniform surface and color were retained as pure, while non-homogeneous inocula were discarded.

2.2 Culturing method

Nitrate-oxidizing bacteria were cultured in a closed system using 500 ml conical flasks, which were sealed to prevent light exposure and shaken at 120 rpm. This experiment was performed in triplicate. Ammonium-oxidizing bacteria (AOB) were cultured for 30 days, while nitrate-oxidizing bacteria (NOB) were cultured for 22 days in minimal mineral media. Samples of 30 ml were taken from the culture every 8 days for analysis [15].

The cultured media underwent incremental dilution and were cultivated in experimental tubes containing a liquid sterile mineral medium to achieve pure bacterial cultures. Bacterial suspensions were collected for DNA extraction using the trickle spreading method. Each isolated bacterium was selected and transferred onto Petri dishes containing agar-enriched sterile mineral medium by depositing 0.1 ml of diluted bacterial suspension onto the agar surface. The Petri dishes were then inverted and incubated at 37 °C. Bacterial growth on the agar plates was monitored, and pure cultures were subsequently isolated.

2.3 Testing the treatment of nitrogen-polluted wastewater in shrimp pond

Preparing bacteria strains

The isolated bacterial strains of ammonium-oxidizing bacteria (AOB) were cultured for 30 days, and bacterial strains capable of oxidizing nitrate (NOB) were cultured for 22 days in minimal mineral media. Then, the treatment of nitrogen-polluted wastewater in shrimp ponds was conducted on a laboratory scale. The bacteria were transferred to culture for 5 days in wastewater samples contained in sterilized conical flasks collected from the outdoor aquaculture area, shaken at 120 rpm in the dark, at room temperature. Each isolate was cultured in a wastewater medium with 3 replicates and 1 control. After 3 days, the water samples in the control and the water samples supplemented with isolated bacterial strains were analyzed for ammonium and nitrite content. The determination of ammonium and nitrate was conducted manually using spectrometry according to ISO 7150/1: 1984 (E) and ISO 7890/3: 1988 (E), respectively. The ammonium/nitrite concentration in wastewater was calculated using the following equation (1):

$$X = (y - b)/a \quad (1)$$

where:

x is ammonium/nitrite concentration (mg/l)

y is the measured optical density value

a and b are the coefficients from the standard curve

The construction of a standard curve for the determination of ammonium

Before measuring the ammonium content using spectrophotometric methods according to ISO 7150/1: 1984 (E), the ammonium standard solution was prepared. 3.819 g ± 0.004 g of ammonium chloride was dissolved in approximately 800 ml of water in a 1000 ml volumetric flask. Then, it was diluted with water to the mark to obtain a solution with a concentration (ρN) of 1000 mg/l. A pipette was used to transfer 100 ml of the standard solution (ρN = 1000 mg/l) to a 1000 ml volumetric flask. It was then diluted with water to the mark to obtain a solution with ρN = 100 mg/l. 1 ml of the ammonium nitrate standard solution (ρN = 100 mg/l) was transferred to a 100 ml volumetric flask using a pipette and then diluted with water to the mark to obtain a solution with ρN = 1 mg/l. A series of points was created as shown in **Table 1**.

Table 1. Sample points created for constructing the ammonium standard curve.

Sample	1	2	3	4	5	6
Standard solution (ammonium nitrogen) ρ_N = 1 mg/l	0	0.25	1.25	2.5	6.25	12.5
Distilled water (ml)	25	24.75	23.75	22.5	18.75	12.5
Concentration (g/l)	0	0.01	0.05	0.1	0.25	0.5

The construction of a standard curve for the determination of ammonium

Before measuring the nitrite content using spectrophotometric methods according to ISO 7890/3: 1988 (E), the nitrite standard solution was prepared. 0.4922 g ± 0.0002 g of sodium nitrite (dried at 105 °C for at least 2 hours) was dissolved in approximately 750 ml of water. The entire solution was transferred to a 1000 ml volumetric flask and diluted with water to the mark to obtain a nitrite standard solution with a concentration (ρN) of 100 mg/l. Then, 10 ml of the standard nitrite solution (ρN = 100 mg/l) was transferred into a 1000 ml volumetric flask and diluted with distilled water to the mark. A series of points was created as shown in **Table 2**.

Table 2. Standard curve for nitrite concentration measurement

Sample	1	2	3	4	5	6
Standard solution ammonium nitrite ρ_N = 1 mg/l (ml)	0	0.625	1.25	2.5	6.25	12.5
Distilled water (ml)	25	24.375	23.75	22.5	18.75	12.5
Concentration (g/l)	0	0.025	0.05	0.1	0.25	0.5

2.4 Analytical methods

Analytical methods is using standard deviation (STDEV.P) and statistical analysis methods of Microsoft Excel software with confidence $P < 0.05$. The

results presented include the mean ± standard error of the ammonium and nitrite data from each strain to determine whether the effect was due to bacterial action or due to time.

2.5 Identification of bacterial strains using molecular biology methods

The total Bacterial DNA Extraction Method was used by the PSP®Spin Stool DNA extraction kit following the manufacturer's protocol (Invitek GmbH, Berlin, Germany). The bacterial cell samples were added directly to PCRs to amplify the 16S rRNA genes. Accurate identification of bacterial species based on the nucleotide sequence of the 16S rRNA gene using Sanger sequencing and built phylogenetic tree, compared using Blastn (NCBI) to determine the species of selected bacteria [16,17].

3. Results

3.1 Results of bacterial isolation from shrimp farm wastewater

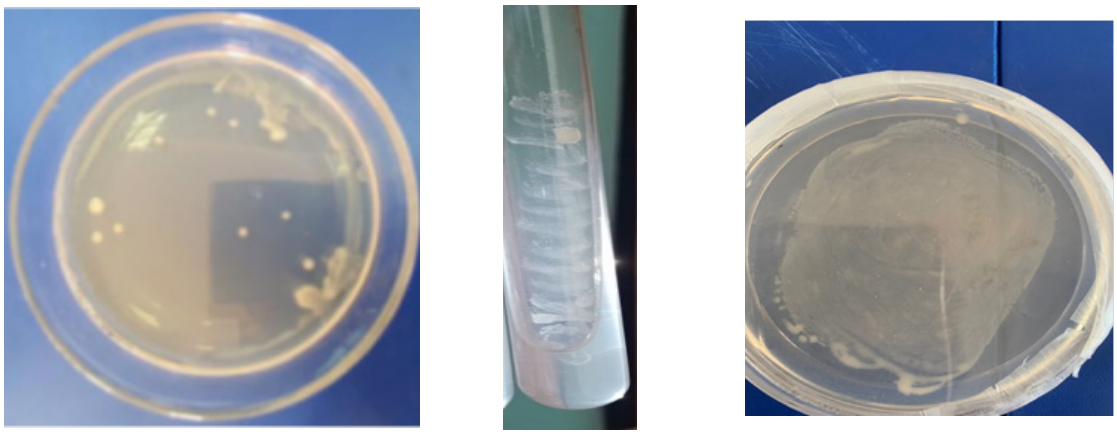
After obtaining a diluted suspension with a dilution factor of 1:10, a consecutive dilution method was employed to isolate bacterial strains. Taking 1 ml of the suspension for each bacterial type (diluted at 1:10) and adding it to Falcon tubes containing 9 ml of AOB and NOB liquid mineral solution, we obtained a diluted suspension with a dilution factor of 1:100. Continuing this process, subsequent dilution factors of 1:1000, 1:10000, and so on were achieved. Depending on the quantity of bacteria in the water, dilution was performed to an appropriate concentration to create distinct bacterial colonies. Using a sample with a suitable dilution concentration, 0.1 ml of the diluted suspension was drawn and spread onto Petri dishes containing sterile mineral medium supplemented with 15 g agar.

Through the trickle spreading method, the Petri dishes were then inverted and placed in an incubator at an appropriate temperature (30–37 °C). After 1–2 days, bacterial colonies on the agar plates were observed, and the results obtained were shown in **Figure 1**.

(a) Bacterial strains isolated from wastewater in shrimp farms on a bacterial culture medium (addition of 15g agar).

(b) Bacterial strains were purified and cultured on slanted agar (supplementation of 15g agar).

(c) Bacterial suspensions were collected to extract DNA by the trickle spreading method.



(a) (b) (c)

Figure 1. Bacterial strains isolated from shrimp farm wastewater in Quang Ninh on bacterial culture medium.

Upon observing the morphology of the isolated bacteria, it is noted that the bacterial colonies appear milky white, exhibiting a round, flat, and intact shape with sizes ranging from 3 to 10 mm. After the bacteria have proliferated on the agar plates, bacterial colonies are observed, and pure cultures are separated. The process involves collecting and selecting pure strains: Using sterilized inoculation loops, a small amount of biomass from each individual bacterial colony is transferred and streaked onto Petri dishes containing AOB and NOB culture media (supplemented with agar), and then placed in an incubator at an appropriate temperature. After a cultivation period, the purity of each bacterial strain is examined on the agar plates.

The results demonstrate the uniform and consistent growth of bacteria on the specialized agar medium, indicating the pure and uncontaminated nature of the isolated strains, which are subsequently retained for further study. Three pure bacterial strains capable of ammonium oxidation on AOB medium were denoted as AOB-1, AOB-2, and AOB-3, respectively. Three pure bacterial strains with nitrite oxidation ability from shrimp farming wastewater in Quang Ninh province, Vietnam on NOB medium were denoted as NOB-1, NOB-2, and NOB-3, respectively. These strains were then selected to identify and apply biological tests to remove nitrogen from wastewater.

Ammonium or nitrite-contaminated samples were diluted according to the standard curve construction ratio in a 100 ml volumetric flask, then added to a triangular flask with an additional 30 ml of nitrite bacterial culture medium. The flasks were shaken for 5 days at a speed of 120 rounds per minute at room temperature.

3.2 Results of testing the treatment of nitrogen-polluted wastewater in shrimp pond on the laboratory scale

Results of standard curve construction for determination of ammonium and nitrite

Measuring the optical density (OD) using a UV-VIS spectrophotometer was used to establish a standard curve for the determination of ammonium at a wavelength of 655 nm. The results obtained are shown in **Figure 2**.

Measuring the optical density (OD) using a UV-VIS spectrophotometer was used to establish a standard curve for the determination of nitrite at a wavelength of 540 nm. The obtained results are shown in **Figure 3**.

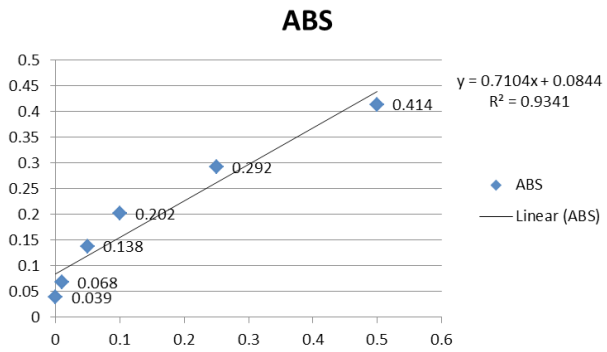


Figure 2. Standard curve for ammonium concentration measurement.

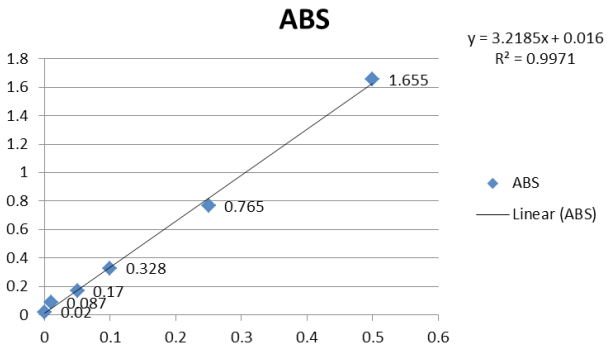


Figure 3. Standard curve for measuring nitrite concentration.

Results of the determination of ammonium and nitrite concentration in wastewater

Three pure bacterial strains (NOB-1, NOB-2, and NOB-3) with nitrite oxidation ability and three pure bacterial strains (AOB-1, AOB-2, and AOB-3) with ammonium oxidation ability from shrimp farming wastewater in Quang Ninh province, Vietnam, were selected and cultured in a closed system using 500 ml conical flasks. These flasks were sealed to prevent light exposure and shaken at 120 rpm. Ammonium-oxidizing bacteria (AOB) were cultured for 30 days, while nitrate-oxidizing bacteria (NOB) were cultured for 22 days in minimal mineral media.

This experiment was performed in triplicate to identify and apply biological tests to remove nitrogen in wastewater. Samples of 30 ml were taken from the culture every 8 days for analysis. They were then supplemented with ammonium and shaken at a speed of 120 revolutions per minute at room temperature for 5 days.

The determination of ammonium was conducted manually using spectrometry according to ISO 7150/1: 1984 (E), and the nitrogen removal capability in wastewater was calculated using equation (1) . The results for the determination of ammonium in wastewater are shown in **Table 3**.

Table 3. The determination of ammonium and nitrite concentration.

Bacterial solution/Control	The concentration of amonium (mg/l)	The concentration of nitrite (mg/l)
Control	3.8 ± 0.11	0.47 ± 0.02
AOB-1	2.69 ± 0.56	-
AOB-2	2.08 ± 0.18	-
AOB-3	2.23 ± 0.07	-
NOB-1	-	0.38 ± 0.02
NOB-2	-	0.41 ± 0.04
NOB-3	-	0.43 ± 0.04

The results indicated that the sample supplemented with bacteria exhibited reduced concentrations of both ammonia and nitrite compared to the sample without bacterial supplementation (Control). The ammonium concentration decreased from 1.21 to 1.72 mg/l, while the nitrite concentration decreased from 0.04 to 0.11 mg/l. This implies that bacterial supplementation effectively lowers ammonium and nitrite levels in wastewater. However, there were varying nitrogen processing capabilities among different bacterial strains, with no significant differences observed between them.

3.3 Molecular biological identification results of bacterial strains

Bacterial strains were isolated and selected. From each isolation site, the largest, roundest, smoothest, and opaque white colonies were chosen. These bacterial colonies were cultured on fresh agar plates supplemented with 10 µl of sterilized AOB or NOB liquid mineral solution. Once the colonies covered the plates uniformly, a small, sterilized steel spoon was used to collect the bacterial colonies from the surface of the agar plates. These colonies were then placed in Eppendorf tubes and identified as bacterial strains. The bacterial sample obtained is shown in **Figure 4**.

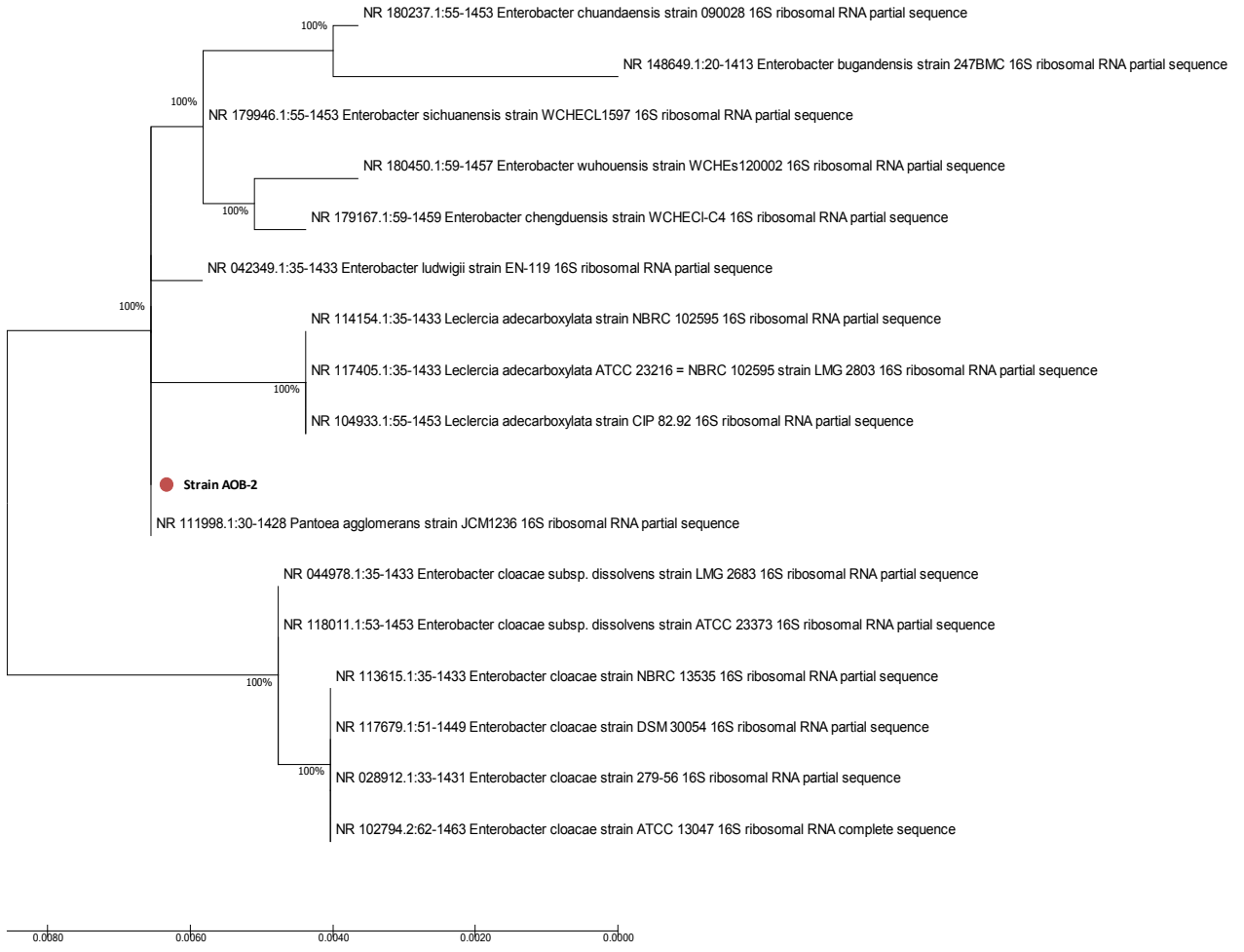
Figure 4. Bacterial samples for identification.

The decoding results of the 16S rRNA gene sequences, were compared using Blastn (NCBI) for similarity with *Bacillus subtilis* (AOB-1), *Arthrobacter nicotianae* (NOB-1), and *Enterobacter asburiae* (NOB-3). The identification of other bacteria strains is shown in **Figures 5**, **6 and 7**.

Accurate bacterial species identification was achieved by sequencing the 16S rRNA gene using Sanger sequencing. The resulting nucleotide sequences were used to construct phylogenetic trees and were compared against reference sequences using Blastn (NCBI) for species determination. The analysis, illustrated in Figures 5, 6, and 7, identified the bacterial strains as *Pantoea agglomerans* strain JCM1236 (AOB-2), *Acinetobacter lactucae* strain JVAP01 (NOB-2), and *Enterobacter hormaechei* strain ES1 (AOB-3).

4. Discussion

The bacteria *Bacillus subtilis*, *Arthrobacter nicotianae*, and *Enterobacter asburiae* have been identified with the capability of oxidizing ammonia and nitrite by Verstraete 1972, Badrah 2021, Xu 2023 [18–20]. The decoding of these gene sequences provides valuable information about the genetic composition of the bacteria and their potential roles in ammonia and nitrite oxidation processes.



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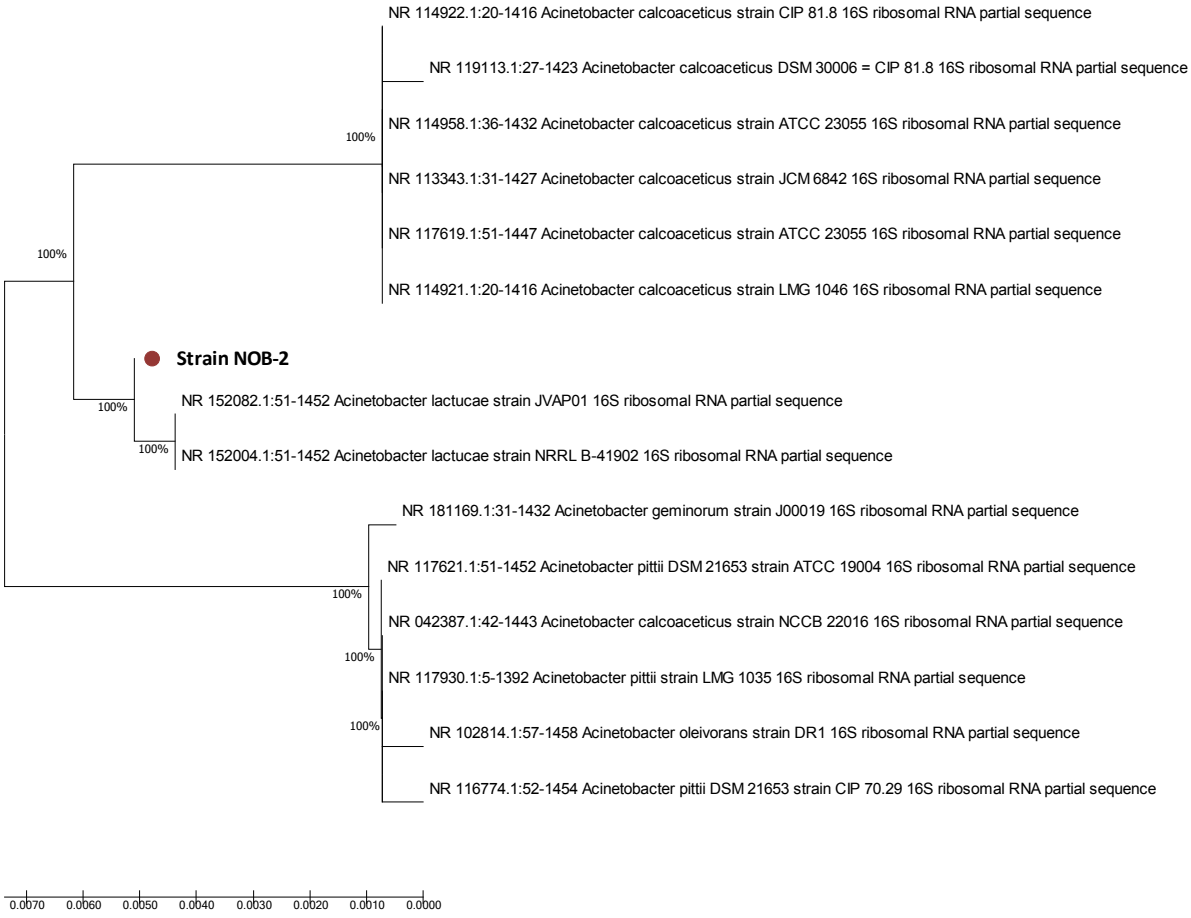
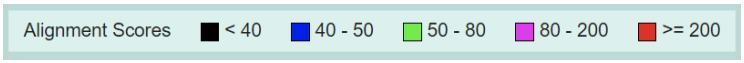
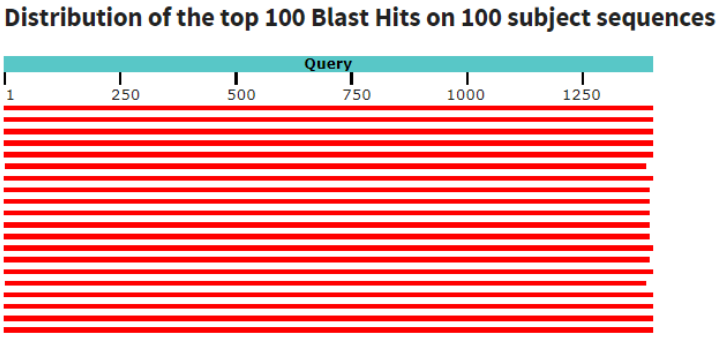
Pantoea agglomerans strain JCM1236 16S ribosomal RNA, partial sequence	Pantoea agglomerans	2547	2547	100%	0.0	99.36%	1449	NR_111998.1
Enterobacter ludwigii strain EN-119 16S ribosomal RNA, partial sequence	Enterobacter ludwigii	2542	2542	100%	0.0	99.29%	1513	NR_042349.1
Enterobacter sichuanensis strain WCHECL1597 16S ribosomal RNA, partial sequence	Enterobacter sichuanensis	2540	2540	100%	0.0	99.29%	1528	NR_179946.1
Leclercia adecarboxylata ATCC 23216 = NBRC 102595 strain LMG 2803 16S ribosomal RNA, partial se...	Leclercia adecarboxylata A...	2531	2531	100%	0.0	99.29%	1506	NR_117405.1
Leclercia adecarboxylata strain NBRC 102595 16S ribosomal RNA, partial sequence	Leclercia adecarboxylata	2531	2531	100%	0.0	99.14%	1465	NR_114154.1

Figure 5. Phylogenetic tree and comparison using Blastn (NCBI) indicating similarities respectively with *Pantoea agglomerans* strain JCM1236 16S RNA.





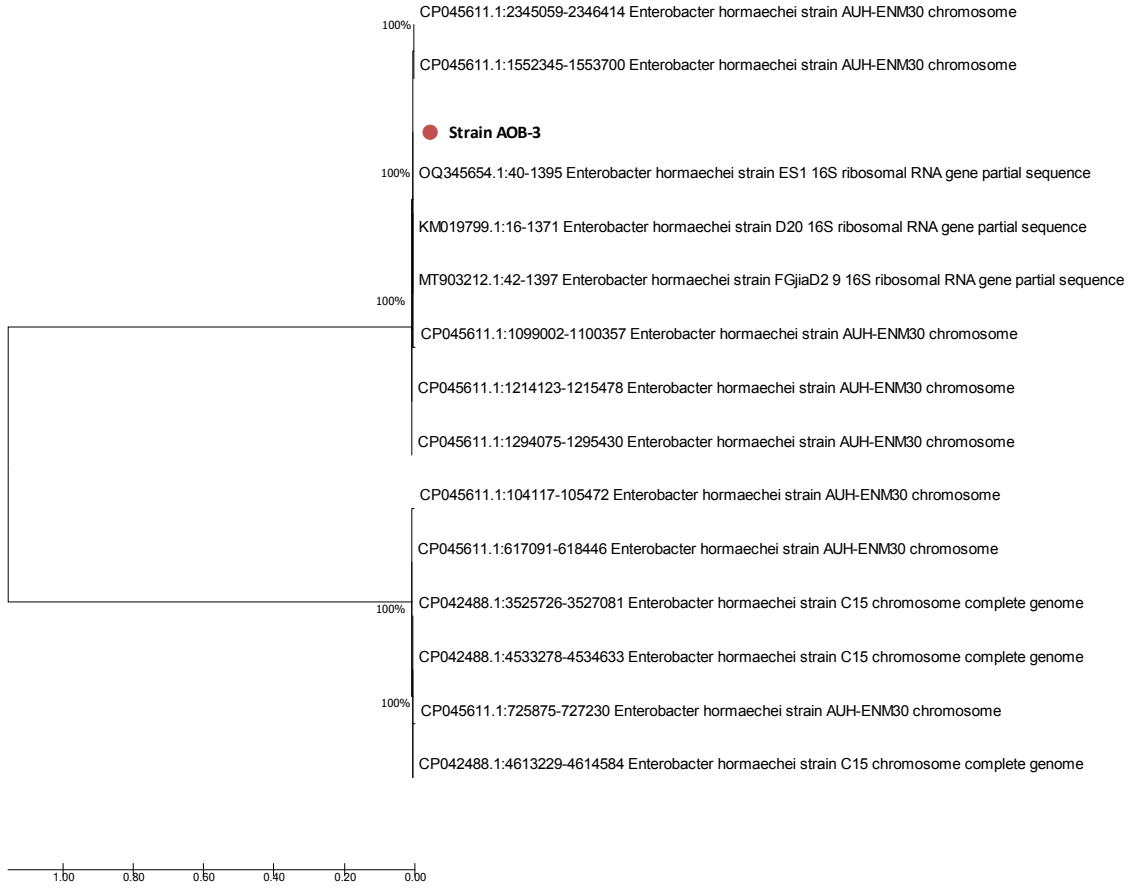


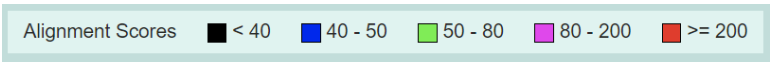
Sequences producing significant alignments Download Select columns Show 100

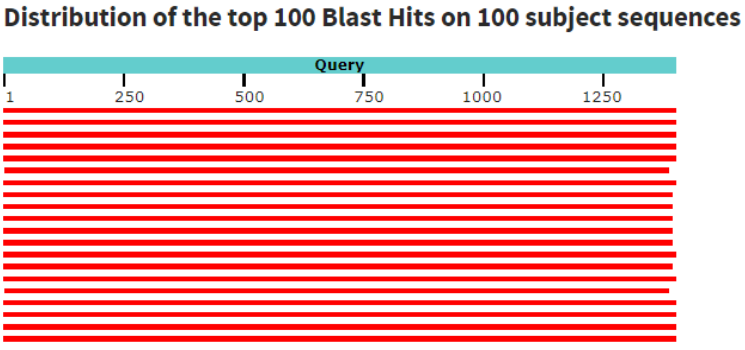
select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Acinetobacter lactucae strain JVAP01 16S ribosomal RNA, partial sequence	Acinetobacter lactucae	2579	2579	99%	0.0	99.86%	1526	NR_152082.1
Acinetobacter lactucae strain NRRL B-41902 16S ribosomal RNA, partial sequence	Acinetobacter lactucae	2579	2579	99%	0.0	99.86%	1526	NR_152004.1
Acinetobacter geminorum strain J00019 16S ribosomal RNA, partial sequence	Acinetobacter geminorum	2540	2540	99%	0.0	99.36%	1460	NR_181169.1
Acinetobacter pittii DSM 21653 strain ATCC 19004 16S ribosomal RNA, partial sequence	Acinetobacter pittii DSM 21653	2534	2534	99%	0.0	99.29%	1530	NR_117621.1
Acinetobacter calcoaceticus strain NCCB 22016 16S ribosomal RNA, partial sequence	Acinetobacter calcoaceticus	2534	2534	99%	0.0	99.29%	1516	NR_042387.1

Figure 6. Phylogenetic tree and comparison using Blastn (NCBI) indicating similarities respectively with *Acinetobacter lactucae* strain JVAP01 16S RNA.







Sequences producing significant alignments Download Select columns Show 100

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Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Enterobacter hormaechei strain ES1 16S ribosomal RNA gene, partial sequence	Enterobacter hor…	2484	2484	100%	0.0	99.63%	1431	OQ345654.1
Enterobacter hormaechei strain D20 16S ribosomal RNA gene, partial sequence	Enterobacter hor…	2484	2484	100%	0.0	99.63%	1391	KM019799.1
Enterobacter hormaechei strain FGjiaD2_9 16S ribosomal RNA gene, partial sequence	Enterobacter hor…	2484	2484	100%	0.0	99.63%	1441	MT903212.1
Enterobacter hormaechei strain AUH-ENM30 chromosome	Enterobacter hor…	2479	19734	100%	0.0	99.56%	4869611	CP045611.1
Enterobacter hormaechei strain C15 chromosome, complete genome	Enterobacter hor…	2479	19795	100%	0.0	99.56%	4780403	CP042488.1

Figure 7. Phylogenetic tree and comparison using Blastn (NCBI) indicating similarities respectively with *Enterobacter hormaechei* strain ES1 16S RNA.

Nitrogen cycle and bacterial species identification research on the nitrogen cycle involving bacterial species has been extensively reported through studies by various author groups such as Papen et al., 1989; Bock, E., 1992; Van de Graaf et al., 1995; Lin et al., 2007; Yang et al., 2011; Rodriguez et al., 2017 [8,10,12,14,15,21]. Bacterial strains involved in these studies include *Streptomyces sp., Pseudomonas putida, Sphingomonas sp., Aeromonas sp.,* and *Bacillus spp.* Studies focusing on the role of microorganisms in the nitrogen cycle within aquaculture have revealed that the microbial-mediated oxidation of ammonium is a crucial process, particularly through the Anammox process under anaerobic conditions. Microorganisms, especially in aquaculture ponds, play a pivotal role in influencing productivity, nutrient cycles, feed quality, water quality, disease control, and overall environmental impact, as demonstrated by Moriarty et al., 1997, and Hargreaves et al., 1998 [5,13].

Furthermore, research by Verstraete and colleagues in 1972, Badrad et al. in 2021, and Xu et al. in 2023 has identified specific bacterial species, such as *Bacillus subtilis, Arthrobacter nicotianae,* and *Enterobacter asburiae*, with the ability to oxidize ammonium and nitrite in aquatic environments [18–20].

Although the bacterial identification results from the isolation and selection in the shrimp pond in Quang Ninh province did not pinpoint new bacterial species, they did successfully identify the names of indigenous bacterial species capable of nitrogen processing in the aquatic environment.

5. Conclusions

The study successfully isolated three bacterial strains capable of ammonium oxidation (AOB-1, AOB-2, AOB-3) and three bacterial strains with nitrite oxidation ability (NOB-1, NOB-2, NOB-3) from shrimp farming wastewater samples at a shrimp farm in Quang Ninh province, Vietnam. These strains demonstrate potential for ammonium and nitrite oxidation, making them applicable for nitrogen treatment in water. The 16S rRNA gene sequences of the three ammonium-oxidizing bacterial strains (AOB-1, AOB-2, AOB-3) and the three nitrite-oxidizing bacterial strains (NOB-1, NOB-2, NOB-3) were decoded and compared using Blastn (NCBI), indicating similarities respectively with *Bacillus subtilis* (AOB-1), *Pantoea agglomerans* strain JCM1236 16S RNA (AOB-2), Enterobacter hormaechei strain ES1 16S RNA (AOB-3), *Arthrobacter nicotianae* (NOB-1), *Acinetobacter lactucae* strain JVAP01 16S RNA (NOB-2) and *Enterobacter asburiae* (NOB-3). These findings aim to further enhance the understanding and practical application of the isolated bacterial strains in addressing nitrogen pollution in various water sources.

Author Contributions

Nguyen Thi Tham (First author): Conception and design of the study, data collection, data analysis and interpretation, drafting of the manuscript, and critical revision of the manuscript for important intellectual content.

Pham Quy Giang: Conception and design of the study, data collection, data analysis and interpretation, drafting of the manuscript, and critical revision of the manuscript for important intellectual content.

Dinh Quynh Oanh: Provided critical reagents and materials, and critical revision of the manuscript.

Diep Thi Thu Thuy: Experimental procedures, data collection, and initial data analysis.

Hoang Thi Bich Hong: Data analysis and interpretation, manuscript drafting, and contributed to the manuscript revision process.

Pham Thi Huong: Provided critical reagents and materials, experimental procedures, and contribution to manuscript drafting.

Conflict of Interest

The authors declare no conflict of interest. All research was conducted independently, and there are no financial or personal relationships that could have influenced the work reported in this paper.

Data Availability

The data that support the findings of this study

are available from the corresponding author upon reasonable request.

Funding

This research was funded by Halong University.

Acknowledgments

The authors would like to thank Ha Long University for the financial support to conduct this research.

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