

Isolation and selection of *Nitrobacter* spp. from shrimp ponds in Hue City

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Abstract. Nitrite (NO_2^-) is a critical toxic intermediate in the nitrogen cycle and represents a major water quality concern in shrimp aquaculture. Elevated nitrite concentrations can severely compromise shrimp health, productivity, and survival. This study aimed to isolate and identify *Nitrobacter* spp. with potential application in the bioremediation of nitrite-contaminated aquaculture systems. Seven *Nitrobacter* spp. strains were successfully isolated from sediment samples collected from shrimp ponds. The isolates exhibited consistent biochemical characteristics, among which PH11 showed a higher growth rate *in vitro* than the other isolates. Furthermore, this strain demonstrated efficient nitrite removal at NaCl concentrations of 0–2.5% and within a pH range of 6.5–8.0. Collectively, these findings suggest that *Nitrobacter* sp. PH11 is a highly promising candidate for nitrite remediation in freshwater or low-salinity aquaculture systems. Overall, a thorough understanding of nitrite toxicity and properly managing nitrogen cycling in aquatic environments are essential for promoting the health, performance, and sustainable production of cultured species.

Keywords: ammonia oxidation, nitrifying bacteria, *Nitrobacter* bacteria, nitrite

1 Introduction

High-yield shrimp farming has expanded rapidly in recent years, representing a major advancement in the aquaculture sector. In 2025, national shrimp production reached approximately 1.38 million metric tons. Shrimp has become a key export commodity, generating approximately USD 4.6 billion in export revenues and accounting for nearly 41% of the total export value of aquatic products.

However, the increasing intensification of shrimp farming—particularly the practice of elevating stocking densities to maximise productivity—has resulted in significant environmental challenges. These include the accumulation of organic matter derived from uneaten feed, pharmaceuticals, chemicals, antibiotics, shrimp excreta, and the decomposition

of plant and animal residues, as well as leachates from pond embankments. Such accumulation adversely affects sediment quality and contributes to the deterioration of water quality in aquaculture systems [1–4].

In intensive aquaculture systems, farmers commonly increase feeding rates to shorten culture duration and enhance the commercial value of shrimp. Commercial shrimp feeds typically contain 30–40% protein; however, only approximately 20–25% of this protein is effectively assimilated by shrimp, while the remainder settles to the pond bottom and is regarded as organic waste. Previous studies have indicated that during digestion and excretion, consuming 1 kg of feed can generate approximately 50 g of $\text{NH}_3\text{-N}$ [4, 5].

Consequently, the mineralisation of excess organic matter promotes the accumulation of

NH_4^+ , which is subsequently transformed primarily into NO_2^- and, to a lesser extent, NO_3^- . The nitrification process, which converts NH_4^+ to NO_3^- , plays a critical role in mitigating the formation of toxic nitrogenous compounds such as NH_3 and NO_2^- in aquaculture environments. Nitrite (NO_2^-) is an intermediate in the oxidation of ammonium to nitrate, and its accumulation in aquatic systems is highly toxic to aquatic organisms. Elevated nitrite concentrations can disrupt multiple physiological functions, including respiration, osmoregulation, endocrine regulation, growth rate, and moulting cycles [1, 6].

The toxicity of nitrite to fish and crustaceans is strongly influenced by environmental parameters such as salinity, pH, temperature, and dissolved oxygen, as well as biological factors, including species, age, size, and individual sensitivity [7, 8].

In crustaceans, elevated nitrite concentrations in the aquatic environment can have severe physiological consequences. Nitrite reacts with Cu^{2+} in haemocyanin, the primary oxygen-transport protein in shrimp hemolymph, forming methaemoglobin and causing the hemolymph to turn brown. This transformation reduces the oxygen-binding and transport capacity of haemocyanin, leading to functional hypoxia in shrimp even when dissolved oxygen levels in the water remain high [9].

The application of probiotics containing nitrifying bacteria, such as *Nitrosomonas* and *Nitrobacter*, is considered an effective and increasingly recommended strategy for managing water quality and organic waste in aquaculture systems [10]. This biological approach offers significant potential for controlling toxic nitrogenous compounds in intensive shrimp ponds, thereby enhancing animal health, reducing environmental pollution, and supporting the sustainable development of aquaculture. As a result, microbial-based interventions aimed at

regulating and transforming NO_2^- have been widely studied and applied [1, 11–13].

According to Dang Dinh Kim et al. [14], the addition of microbial products to aquaculture systems significantly improves physicochemical and biological parameters, contributing to the growing adoption of beneficial microorganisms in aquaculture practices. Currently, a wide range of commercial probiotics is available, typically containing bacterial genera such as *Bacillus*, *Pseudomonas*, *Lactobacillus*, *Nitrosomonas*, and *Nitrobacter*. These microorganisms are capable of degrading proteins into polypeptides, amino acids, and NH_3 . However, under conditions characteristic of shrimp aquaculture, including high salinity, nutrient-rich water, and elevated ammonia and nitrite concentrations, bacteria of the genus *Nitrobacter* demonstrate superior and more stable nitrite-oxidising capacity than other groups [11, 12].

Therefore, the isolation and characterisation of *Nitrobacter* strains with high nitrite-removal efficiency from shrimp pond sediments are essential for providing a scientific basis for the development and application of effective probiotic formulations in aquaculture.

2 Materials and methods

2.1 Sediment sampling method

Sediment sampling was collected across 25 commercial *Litopenaeus vannamei* ponds distributed in four distinct localities in Hue City: the communes of Vinh Loc and Phu Vang, Phong Quang Ward, and Hoa Chau Ward. All samples were obtained immediately after harvest.

A total of eight sampling events were conducted from October 26, 2024, to August 15, 2025. All analytical procedures were performed at the Laboratory of the Faculty of Fisheries,

University of Agriculture and Forestry, Hue University.

Sediment collection followed a diagonal sampling method at five points within each pond. Subsamples were combined into a single composite sample for analysis for each pond. All samples were collected from earthen ponds. Sampling was carried out when the pond bottom was under stable conditions, with minimal disturbance from farming activities.

Sediment was collected using sterile 50 mL Falcon tubes. Prior to sampling, the thin surface layer of sediment was removed, and approximately 100 g of underlying sediment was collected. The samples were immediately preserved on ice in the field, transported to the laboratory within 3–5 hours, stored at 4 °C, and processed within 2 hours after arrival [10].

2.2 Bacterial cultivation and isolation methods

Bacterial isolation was conducted by using a minimal mineral medium with the following composition (per litre of distilled water): 0.3 g KNO₂, 0.175 g K₂HPO₄, 0.175 g MgSO₄, 0.5 g NaCl, 1.0 g KHCO₃, and 35 µg FeSO₄·7H₂O, adjusted to pH 7 [13].

Initially, 1 g of sediment was enriched in 250 mL Erlenmeyer flasks containing the mineral medium and incubated on a rotary shaker at 120 rpm and 30 °C for 6 days. Nitrite (NO₂⁻) concentrations in the culture medium were determined on days 2, 4, and 6 with a Hanna HI97708 photometer, following the manufacturer's instructions.

The samples demonstrating nitrite-reducing activity were serially diluted and spread onto solid mineral medium supplemented with 2% agar. The plates were incubated at 30 °C for 5–7 days. Colonies were preliminarily identified as putative *Nitrobacter* based on morphological characteristics, including colony shape, colour, and size.

Representative, well-isolated, and morphologically distinct colonies were selected and purified by streaking onto Tryptic Soy Agar (TSA, HiMedia, India). The plates were incubated at 30 °C for 24–48 hours; after that, the colony morphology of the pure cultures was examined and recorded.

2.3 Gram staining and biochemical characterisation

Pure colonies were selected from a TSA medium, followed by Gram staining according to the method of Gram [15] by using a Gram staining kit supplied by Nam Khoa Biotek Co., Ltd., Vietnam. Subsequently, the bacterial strains were subjected to biochemical characterisation by using the API 20E identification system (bioMérieux, France).

2.4 Bacterial identification with PCR methods

DNA extraction was performed according to the method described by Boon et al. [16], with the PEKA-02 DNA extraction kit (Visitech, Vietnam) following the manufacturer's instructions. Briefly, the bacterial suspension obtained after overnight incubation was centrifuged at 13,000 rpm for 5 minutes. The pellet was then resuspended in a 200 µL PSB buffer, followed by the addition of 20 µL of proteinase K and 200 µL of CL buffer. The mixture was homogenised by vortexing and incubated at 72 °C for 10 minutes. Subsequently, the sample was sequentially treated with 96–100% ethanol and WB buffer, and centrifuged at 13,000 rpm for 1 minute. Finally, genomic DNA was eluted by centrifugation at 13,000 rpm for one minute after incubation with a 50 µL EB buffer for 1 minute at ambient temperature. The quality of the extracted DNA was assessed according to Tran Nhan Dung [17].

Target gene amplification was performed via PCR with the primer pair PNTG 1F (5'-TTTTTTGAGATTGCTAG-3') and PNTG 2R (5'-

CTAAACTCAAAGGAATTTGA-3'), which are specific primers designed to amplify a fragment of the *16S rRNA* gene of *Nitrobacter* spp. [18]. The PCR cycling conditions consisted of an initial denaturation at 95 °C for 10 minutes, followed by 35 cycles of denaturation at 95 °C for one minute, annealing at 50 °C for one minute, and extension at 72 °C for one minute, with a final extension at 72 °C for 10 minutes [18].

Electrophoresis was carried out in a 0.5× TAE buffer with a 1.5% agarose gel (Promega, USA). The molecular weight of the amplified products was determined by comparing with a 200 bp DNA ladder (Tiangen, China).

2.5 Assessment of NO₂⁻ transformation capacity of selected *Nitrobacter* strain

The experiment was conducted in three treatments in a minimal mineral medium supplemented with different concentrations of KNO₂ (250, 125, and 62.5 ppm). The cultures were prepared in sterile 50 mL Falcon tubes, with an initial bacterial density of 2 × 10⁶ CFU/mL, determined with a UV-Vis spectrophotometer (U-2900, Hitachi, Japan).

The control treatment contained 250 ppm KNO₂ without inoculation with the *Nitrobacter* sp. strain exhibiting the highest nitrite-reducing activity. Each treatment was performed in triplicate. The experiment was incubated at 30 °C for 24 hours. Subsequently, the residual NO₂⁻ concentration was determined with a Hanna HI97708 nitrite meter (Vietnam) according to the manufacturer's instructions.

2.6 Effect of pH and salinity on NO₂⁻ transformation capacity of selected *Nitrobacter* strain

The experiment evaluating the effect of pH on the NO₂⁻ transformation capacity of the selected *Nitrobacter* strain was conducted with eight

treatments corresponding to different pH values (5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0). The cultures were prepared in sterile 50 mL Falcon tubes containing 20 mL of minimal mineral medium supplemented with KNO₂ at a concentration of 62.5 ppm. The pH of the medium was adjusted with 1 N NaOH or 1 N HCl. Each treatment was performed in triplicate. The experiment was incubated at 30 °C for 24 hours; after that, the residual NO₂⁻ concentration was measured and recorded.

A similar experimental design was applied to evaluate the effect of salinity on the NO₂⁻ transformation capacity of the PH11 strain. In this case, NaCl concentrations were adjusted to 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5%, while maintaining the medium at pH 7.0. All other experimental conditions were kept consistent with the pH experiment.

2.7 Statistical analysis

The nitrite removal capacity of strain PH11 across treatments was analysed by using one-way analysis of variance (one-way ANOVA) performed with SPSS software version 20.0 followed by Tukey's Post hoc test.

3 Results and discussion

3.1 Results of sample collection and isolation

Forty samples were collected from four different locations. The sampling results are presented in Table 1, showing *Nitrobacter*-like bacterial isolates were obtained in 5 out of 8 sampling events. In Phong Quang Ward, two isolates (PH11 and PH12) were obtained; in Hoa Chau, two isolates (RC11 and RC12) were recovered; in Vinh Loc Commune, two isolates (VH11 and VH21) were obtained; in Phu Vang Commune, one isolate (PT11) was recovered.

Table 1. Results of bacterial isolation

Sampling time	Sampling location	Number of samples collected	Isolation results
26/10/2024	Vinh Loc	5	No isolated
26/11/2024	Phong Quang	5	No isolated
20/3/2025	Phong Quang, Phu Vang	5	PH11, PT11
20/4/2025	Hoa Chau	5	RC11
18/5/2025	Phong Quang	5	PH12
10/6/2025	Hoa Chau	5	RC12
20/7/2025	Vinh Loc	5	VH11; VH21
15/8/2025	Vinh Loc	5	No isolated

3.2 Results of *Nitrobacter* isolation

The results of these experiments show that seven bacterial strains were isolated, and all of them exhibited relatively similar colony morphology

and pigmentation. All isolates formed yellow colonies on the TSA medium and consisted of short, rod-shaped cells; all seven strains were Gram-negative bacteria (Table 2, Fig. 1).

Table 2. Results of bacterial isolation

Sample location	Sample code	Colony shape	Colony margin	Colony color	Gram	Cell mor.
Phong Quang	PH11	Circular	Circular convex	Pale yellow	-	Short rod
	PH12	Circular	Circular convex	Pale yellow	-	Short rod
Hoa Chau	RC11	Circular	Circular convex	Pale yellow	-	Short rod
	RC12	Circular	Circular convex	Pale yellow	-	Short rod
Vinh Loc	VH11	Circular	Circular convex	Pale yellow	-	Short rod
	VH21	Circular	Circular convex	Pale yellow	-	Short rod
Phu Vang	PT11	Circular	Circular convex	Pale yellow	-	Short rod

Note: Mor.: morphology

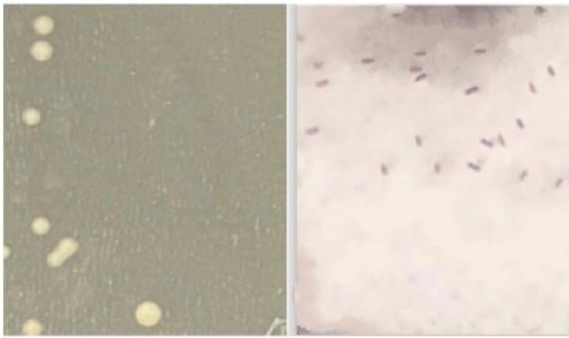


Fig. 1. Colonies (left) on TSA medium and cell morphology (right) of PH11 strain (×100)

3.3 Results of PCR identification

The PCR identification results for the *Nitrobacter* strains are presented in Fig. 2. The PCR products from all samples exhibited bands with approximately 400 bp when compared with the DNA ladder. The agarose gel electrophoresis of PCR products from the isolates PH11, PH12, RC11, RC12, VH11, VH21, and PT11 revealed distinct bands consistent with those of the positive control, *Nitrobacter winogradskyi* (ATCC 25381). These findings preliminarily indicate that all obtained isolates belong to the genus *Nitrobacter*.

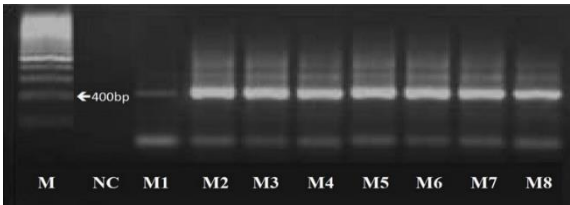


Fig. 2. Amplified PCR products of isolated bacterial strains. M: 200 bp DNA ladder; NC: negative control; M1: positive control (*Nitrobacter winogradskyi*, ATCC 25381); M2: PH11; M3: PH12; M4: RC11; M5: RC12; M6: VH11; M7: VH21; M8: PT11

This result is supported by Hoang Phu Hiep et al. [19], who reported that *Nitrobacter winogradskyi*, together with *Nitrosomonas europaea*, is among the most representative species identified

in the water samples collected from shrimp ponds in Quang Ninh province and Hai Phong City.

3.4 Biochemical characterisation of the selected bacterial isolates

The isolated bacterial strains exhibited a high degree of biochemical homogeneity, with all isolates testing positive for β -galactosidase, lysine decarboxylase, and nitrite detection assays (Table 3). The integrated analyses of colony morphology, cellular architecture, biochemical characteristics, and PCR-based identification consistently revealed defining features characteristic of the genus *Nitrobacter*, in concordance with previously reported findings by Hoang Phu Hiep et al. [19] and Bothe et al. [20].

Among the isolates, strain PH11, obtained from Phong Quang, demonstrated the highest nitrite-oxidising activity after 48 h of cultivation in a minimal mineral medium (data not shown). This strain also exhibited a comparatively enhanced growth performance, reaching an optical density (OD₆₀₀) of 1.2, exceeding that of the other isolates. Based on its superior physiological and biochemical performance, we selected strain PH11 for subsequent experimental investigations.

Table 3. Results of biochemical characterisation of selected bacterial isolates

Biochemical parameters	Bacterial strains						
	PH11	PH12	RC11	RC12	VH11	VH21	PT11
ONPG (β -galactosidase)	+	–	+	+	+	+	+
ADH (arginine dihydrolase)	–	–	–	–	–	–	–
LDC (lysine decarboxylase)	+	+	+	–	–	+	+
ODC (ornithine decarboxylase)	–	–	–	–	–	–	–
CIT (citrate)	+	+	+	+	+	+	+
H ₂ S (Hydrogen sulfide)	–	–	–	–	–	–	–
URE (enzyme urease)	–	+	–	–	–	–	–

Biochemical parameters	Bacterial strains						
	PH11	PH12	RC11	RC12	VH11	VH21	PT11
TDA (enzyme tryptophan deaminase)	–	–	–	–	–	–	–
IDN (indole test)	–	–	–	–	–	–	–
VP (Voges-Proskauer)	–	–	–	–	–	–	–
GEL (enzyme gelatinase)	+	+	+	+	+	+	+
GLU (glucose)	–	–	–	–	–	–	–
MAN (D-mannose)	–	–	–	–	–	–	–
INO (inositol)	–	–	–	–	–	–	–
SOR (sorbitol)	–	–	–	–	–	–	–
RHA (rhamnose)	–	–	–	–	–	–	–
SAC (sucrose)	–	+	–	–	–	–	–
MEL (melibiose)	–	–	–	–	–	–	–
AMY (amygdalin)	–	–	–	–	–	–	–
ARA (arabinose)	–	–	–	–	–	–	–
OX (oxidase test)	+	+	+	+	+	+	+
NO ₂ (nitrite reduction)	+	+	+	+	+	+	+
OD ₆₀₀ after 72 h of cultivation	1.2	0.4	0.4	0.7	0.6	0.5	0.5

Notes: (+): positive result; (–) negative result

3.5 Evaluation of the NO₂[–] transformation capacity of strain PH11

The *Nitrobacter* PH11 bacterium strain was able to remove 100% of the nitrite in the culture medium after 24 hours of cultivation at a concentration of 62.5 ppm. At higher nitrite concentrations, the nitrite removal ability of this bacterium gradually decreased: PH11 was able to remove 28% of the nitrite content in the medium at a concentration of 125 ppm and 20% at a concentration of 250 ppm (Table 4).

The recommended threshold for nitrite (NO₂[–]) in aquaculture ponds is ≤ 2 mg·L^{–1} [21]. However, the toxicity of nitrite toward cultured shrimp is strongly modulated by chloride concentration in the water; consequently, nitrite exhibits lower toxicity in saline environments than

in freshwater systems. For instance, the LC₅₀ value after 24 h for the giant freshwater prawn *Macrobrachium rosenbergii* is 36.19 mg·L^{–1} [8], whereas the corresponding LC₅₀ for *Penaeus monodon* (postlarval stage) is 61.87 mg·L^{–1} [22]. In addition, susceptibility to nitrite toxicity varies with developmental stage, with larger shrimp exhibiting greater tolerance to nitrite exposure [23].

In fish, elevated environmental nitrite levels facilitate diffusion of nitrite from plasma into erythrocytes, where it oxidises the ferrous iron (Fe²⁺) in hemoglobin to ferric iron (Fe³⁺) [24]. The resulting oxidised form, known as methemoglobin or ferrihemoglobin, is incapable of reversible oxygen binding. The formation of methemoglobin consequently reduces the blood’s oxygen-carrying capacity, leading to hypoxic conditions in fish. A characteristic clinical sign of elevated

methemoglobin levels is the brown discolouration of blood and gill tissues [24].

The permissible concentration of NO_2^- in aquaculture systems is less than $10 \text{ mg}\cdot\text{L}^{-1}$, with an optimal level of less than $2 \text{ mg}\cdot\text{L}^{-1}$ [21, 25].

Table 4. Nitrite (NO_2^-) reduction performance of strain PH11 at different nitrite concentrations

Concentration of KNO_2 (ppm)	Control	Treatment 1	Treatment 2	Treatment 3
Initial concentration	250	250	125	62.5
After 24 h of incubation	250 ± 0^a	200 ± 16^b	90 ± 10^c	0 ± 0^d

Notes: The results are presented as the mean of three replicates $\pm$ standard deviation; different superscript letters (a, b, c, d) within the same row indicate statistically significant differences at the 95% confidence level.

3.6 Effects of pH on NO_2^- transformation capacity of PH11

The results of these experiments are presented in Table 5. It can be seen that the pH values ranging from 6.5 to 8.0 did not significantly affect the nitrite transformation activity of strain PH11. In contrast, acidic (pH: 5.5–6.0) and alkaline (pH: 8.5–9.0) conditions resulted in a marked reduction in the

nitrite transformation efficiency. Notably, the residual NO_2^- concentration after 24 h of incubation at pH 5.5 was higher than that observed at pH 9.0, suggesting that strain PH11 exhibits greater physiological adaptability under alkaline conditions than under acidic environments. This may be attributed to more robust, adaptive mechanisms in alkaline media or to the higher stability of nitrite-transforming enzymes at elevated pH than at low pH.

Table 5. Effects of pH on NO_2^- transformation capacity of strain PH11

pH	Initial concentration of NO_2^- (ppm)	Concentration of NO_2^- (ppm) after 24 h
5.5	62.5	18.7 ± 1.1^a
6.0	62.5	18.0 ± 0.0^a
6.5	62.5	0 ± 0.0^c
7.0	62.5	0 ± 0.0^c
7.5	62.5	0 ± 0.0^c
8.0	62.5	0 ± 0.0^c
8.5	62.5	8.5 ± 0.0^b
9.0	62.5	8.7 ± 1.1^b

Notes: The results are presented as the mean of three replicates $\pm$ standard deviation. Different superscript letters (a, b, c) within the same column indicate statistically significant differences at the 95% confidence level.

pH is one of the critical environmental parameters that directly affects microbial viability and metabolic activity through multiple physiological processes, including enzyme activity, membrane integrity and function, and the regulation of intracellular pH homeostasis [26]. In

the present study, the pH values between 6.5 and 8.0 were identified as the optimal range for nitrite transformation by strain PH11, suggesting that key enzymes involved in nitrite metabolism (e.g., nitrite reductase) likely exhibit maximal catalytic efficiency and stability within this pH window.

According to García-Heredia [27], the bacterial cell membrane plays a central role in cellular biosynthesis, partly through the presence of extracellular enzymes that regulate substrate fluidity and coordinate biosynthetic processes. Alterations in membrane properties, including structural organisation, fluidity, or lipid composition, can significantly affect bacterial growth dynamics and overall cellular functionality. Accordingly, the enhanced NO_2^- transformation observed in strain PH11 within the pH range of 6.5–8.0 may be attributed to optimal membrane stability and more efficient biosynthetic activity under these conditions. Furthermore, strain PH11 is likely a neutrophilic bacterium [28], and thus exposure to either acidic or alkaline environments may impose physiological stress on cellular metabolic processes. Under acidic conditions, extracellular protons can diffuse across the cell membrane, disrupting membrane stability and diminishing the proton motive force. Conversely, extreme pH conditions can adversely affect substrate transport and diffusion across the membrane and compromise overall cellular structural integrity [29].

3.7 Effect of salinity on NO_2^- transformation capacity of strain PH11

The NO_2^- transformation efficiency of strain PH11 at NaCl concentrations ranging from 0 to 2.5% was markedly higher than that observed at elevated salinity levels of 3.0% and 3.5% (Table 6).

After 24 h of incubation, strain PH11 achieved complete (100%) NO_2^- transformation at NaCl concentrations ranging from 0 to 2.5%. In contrast, at elevated salinity levels of 3.0% and 3.5% NaCl, the NO_2^- transformation efficiencies decreased markedly to 41 and 37%, respectively. These findings indicate that a salinity range of 0–2.5% NaCl likely represents the optimal condition for the metabolic activity of strain PH11. This range may provide favourable osmotic conditions that support cellular homeostasis, enzymatic functionality, and the biochemical pathways involved in nitrite transformation. Accordingly, strain PH11 shows strong application potential in NO_2^- remediation in freshwater or low-salinity environments (<2.5% NaCl).

Table 6. Effect of salinity on NO_2^- transformation capacity of strain PH11

NaCl (%)	Initial concentration of NO_2^- (ppm)	Concentration of NO_2^- (ppm) after 24 h
0	62.5	0 ± 0.0^c
0.5	62.5	0 ± 0.0^c
1	62.5	0 ± 0.0^c
1.5	62.5	0 ± 0.0^c
2	62.5	0 ± 0.0^c
2.5	62.5	0 ± 0.0^c
3	62.5	36.7 ± 2.8^b
3.5	62.5	39.3 ± 1.1^a

Notes: The results are presented as the mean of three replicates $\pm$ standard deviation; different superscript letters (a, b, c) within the same column indicate statistically significant differences at the 95% confidence level.

In addition to pH, salinity is a critical environmental factor influencing bacterial physiology. Elevated salt concentrations can

impose osmotic stress on microbial cells, adversely affecting their viability and metabolic performance. Specifically, increased external

salinity can lead to cellular dehydration, compromising membrane integrity and structural stability. Consequently, cells must expend additional energy to maintain osmotic balance, potentially diverting resources away from essential metabolic processes, including nitrite transformation. Furthermore, high salinity may alter the conformation of cellular proteins and enzymes, leading to denaturation or loss of catalytic activity [26, 30].

In aquaculture systems, the toxicity of nitrite is well known to be strongly dependent on the salinity of the aquatic environment. The mortality of aquatic organisms in marine systems typically occurs at nitrite concentrations approximately 50–100 times as high as those observed in freshwater environments [31]. According to Mazik et al. [32], CaCl_2 is more effective than NaCl in mitigating nitrite toxicity in *Lates calcarifer*. Therefore, our findings are reasonable and supported by previous studies.

4 Conclusions

In summary, nitrite is a significant toxicant in aquaculture species, primarily impairing oxygen transport and inducing a range of physiological disturbances, including disruption of osmoregulatory processes, moulting cycles in crustaceans, and endocrine functions. Therefore, a comprehensive understanding of the factors influencing nitrite toxicity, along with effective management of the nitrogen cycle in aquatic systems, is essential for improving the health and sustainability of cultured organisms.

Seven *Nitrobacter* spp. strains were successfully isolated, all of which exhibited consistent biochemical characteristics. Among these isolates, the most efficient nitrite-transforming strain, *Nitrobacter* PH11, was selected for further investigation. Notably, strain *Nitrobacter* PH11 demonstrated superior NO_2^-

transformation efficiency in the minimal mineral medium, particularly within a NaCl concentration range of 0–2.5% and a pH range of 6.5–8.0 after 24 h of incubation.

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References

1. Ha TL, Anh PT, Thanh NT. Isolation and selection of nitrifying bacterial strains for application in water pollution treatment. *Journal of Science and Technology*. 2007;45(3):95-100.
2. Ge J, Zhang Y, Heo YJ, Park SJ. Advanced design and synthesis of composite photocatalysts for the remediation of wastewater: A review. *Catalysts*. 2019;9(2):122.
3. Tampangallo BR, Idra Jaya Asaad A, Undu MC, Khairana Kadriah IA, Anshary H. Bacterial diversity in superintensive vannamei shrimp aquaculture wastewater treatment plants in Barru and Takalar Regencies. *IOP Conference Series: Earth and Environmental Science*. 2020;564(1):012007.
4. Iber BT, Kasan NA. Recent advances in Shrimp aquaculture wastewater management. *Heliyon*. 2021;7(11):e08283.
5. Iber BT, Okomoda VT, Rozaimah SA, Kasan NA. Eco-friendly approaches to aquaculture wastewater treatment: Assessment of natural coagulants vis-a-vis chitosan. *Bioresource Technology Reports*. 2021;15:100702.
6. Yusoff FM, Banerjee S, Khatoon H, Shariff M. Biological approaches in management of nitrogenous compounds in aquaculture systems. *Dynamic biochemistry, process biotechnology and molecular biology*. 2011;5(1):21-31.
7. Kroupova H, Machova J, Svobodova Z. Nitrite influence on fish: a review. *Veterinarni medicína-praha-*. 2005;50(11):461-471.
8. Huong DTT, Thu CCM. The effects of nitrite on the molting cycle and growth of freshwater prawn

- (*Macrobrachium rosenbergii*). CTU Journal of Science. 2012;21b:19-28.
9. Robles-Porchas GR, Gollas-Galván T, Martínez-Porchas M, Martínez-Cordova LR, Miranda-Baeza A, Vargas-Albores F. The nitrification process for nitrogen removal in biofloc system aquaculture. *Reviews in Aquaculture*. 2020;12(4):2228-49.
 10. Kim JK, Park KJ, Cho KS, Nam SW, Park TJ, Bajpai R. Aerobic nitrification–denitrification by heterotrophic *Bacillus* strains. *Bioresource Technology*. 2005;96(17):1897-906.
 11. Nogueira R, Melo LF. Competition between *Nitrospira* spp. and *Nitrobacter* spp. in nitrite-oxidising bioreactors. *Biotechnology and bioengineering*. 2006;95(1):169-75.
 12. Ma S, Zhang D, Zhang W, Wang Y. Ammonia stimulates growth and nitrite-oxidising activity of *Nitrobacter winogradskyi*. *Biotechnology & Biotechnological Equipment*. 2014;28(1):27-32.
 13. Aleem MI, Alexander M. Nutrition and physiology of *Nitrobacter agilis*. *Applied Microbiology*. 1960;8(2):80-4.
 14. Kim DD, Nam NV, Chi LT. Investigation and assessment of the current status of medicines, chemicals, and biological preparations used in aquaculture to propose management solutions. Summary report. Research Institute for Aquaculture No.1; 2006.
 15. Gram C. The differential staining of Schizomycetes in tissue sections and in dried preparations. *Fortschritte der Medicin*. 1884;2(6):185-9.
 16. Boon N, De Windt W, Verstraete W, Top EM. Evaluation of nested PCR–DGGE (denaturing gradient gel electrophoresis) with group-specific 16S rRNA primers for the analysis of bacterial communities from different wastewater treatment plants. *FEMS microbiology ecology*. 2002;39(2):101-12.
 17. Dung TN. *Molecular biology practical handbook*. Can Tho: Can Tho University Publishing House; 2011. 175 p.
 18. Degrange V, Bardin R. Detection and counting of *Nitrobacter* populations in soil by PCR. *Applied and Environmental Microbiology*. 1995;61(6):2093-8.
 19. Hiep HP, Minh TTN, Nga HTT. Isolation and selection of indigenous high-activity ammonia-oxidising bacteria in Quang Ninh and Hai Phong. *TNU Journal of Science and Technology*. 2021;226(14):30-36.
 20. Bothe H, Ferguson S, Newton WE, editors. *Biology of the Nitrogen Cycle: COST edition*. Elsevier; 2006.
 21. Vu TQ, Ut VN. *Water Quality for Aquaculture* (Original work by Claude E. Boyd, Auburn University, Alabama 36849, USA). Can Tho: Department of Fisheries Management and Exploitation, Can Tho University. 2014. 63 p.
 22. Chen JC, Chin TS. Acute toxicity of nitrite to tiger prawn, *Penaeus monodon*, larvae. *Aquaculture*. 1988;69(3-4):253-62.
 23. Lin YC, Chen JC. Acute toxicity of nitrite on *Litopenaeus vannamei* (Boone) juveniles at different salinity levels. *Aquaculture*. 2003;224(1-4):193-201.
 24. US Department of Health and Human Services. Agency for Toxic Substances and Disease Registry-ATSDR. US Department of Health and Human Services.
 25. Boyd CE, Tucker CS. *Pond aquaculture water quality management*. Springer Science & Business Media; 2012.
 26. Gale EF. Factors influencing the enzymic activities of bacteria. *Bacteriological reviews*. 1943;7(3):139-73.
 27. García-Heredia A. Plasma membrane-cell wall feedback in bacteria. *Journal of Bacteriology*. 2023;205(3):e00433-22.
 28. Baker-Austin C, Dopson M. Life in acid: pH homeostasis in acidophiles. *Trends in microbiology*. 2007;15(4):165-71.
 29. Jin Q, Kirk MF. pH as a primary control in environmental microbiology: 2. Kinetic perspective. *Frontiers in Environmental Science*. 2018;6:101.
 30. Morrissey EM, Gillespie JL, Morina JC, Franklin RB. Salinity affects microbial activity and soil organic matter content in tidal wetlands. *Global change biology*. 2014;20(4):1351-62.
 31. Crawford RE, Allen GH. Seawater inhibition of nitrite toxicity to chinook salmon. *Transactions of the American Fisheries Society*. 1977;106(1):105-9.
 32. Mazik PM, Hinman ML, Winkelmann DA, Klaine SJ, Simco BA, Parker NC. Influence of nitrite and chloride concentrations on survival and hematological profiles of striped bass. *Transactions of the American Fisheries Society*. 1991;120(2):247-54.