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Identification of *Nitrobacter* isolates and their bacteriophages from swiftlet house environments: implications for nitrate control in edible bird nests

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Abstract

This study aimed to identify and characterize nitrifying bacteria and their associated bacteriophages from swiftlet house environments as a preliminary step toward developing biological control strategies for nitrate contamination in edible bird nests,a food safety concern due to the excessive nitrite and nitrate accumulation. A total of 80 environmental samples were collected between October and November 2024 from four swiftlet houses in Central Kalimantan, Indonesia, including 20 samples each from feces, pond water, soil, and edible bird nests (*Aerodramus fuciphagus*). Phenotypic characterization (culture, Gram staining, and biochemical tests) and genotypic identification (PCR and *16S rRNA* gene sequencing) were performed. Bacteriophages were screened using spot tests and plaque assays. The study resulted in the isolation and identification of 15 *Nitrobacter* isolates. Molecular analysis confirmed all isolates belonged to the *Nitrobacter* genus. One isolate, *Nitrobacter* AP6, was selected as a representative host for phage screening due to its consistent growth and plaque clarity. Spot tests revealed four bacteriophages capable of lysing *Nitrobacter* AP6. These findings demonstrate the feasibility of isolating nitrifying bacteria and phages from swiftlet environments and provide a foundation for future biocontrol development, though further *in vitro* and *in situ* validation is required to assess effectiveness and safety.

Introduction

Edible bird nests, produced mainly by *Aerodramus fuciphagus*, are highly valued delicacies in East and Southeast Asia, renowned for their nutritional and medicinal properties.[1] However, concerns over food safety and quality have intensified due to the nitrate contamination in edible bird nests,[2] which can originate from various environmental sources within swiftlet house environments, such as fecal accumulation, contaminated water, and microbial activity in the soil. Elevated nitrate levels not only pose public health risks but also threaten the export potential of edible bird nests, particularly to countries with stringent food safety regulations.[3]

One of the primary biological contributors to nitrate accumulation is nitrification, a microbial process driven by autotrophic nitrifying bacteria.[4] In this process, ammonia is oxidized to nitrite by *Nitrosomonas* spp., and subsequently to nitrate by *Nitrobacter* spp. The swiftlet farming environment, characterized by high ammonium input from feces, moist conditions, and organic-rich substrates, creates a favorable niche for these bacteria to thrive, increasing the likelihood of nitrate buildup in the nests.[5] Controlling this microbial source of contamination is essential for ensuring the safety and economic value of edible bird nests. Traditional approaches, such as chemical treatments or environmental modifications, often carry ecological risks, offer limited long-term efficacy, and may not be permitted in food-related contexts.[6] In contrast, biological strategies, particularly those involving bacteriophages, offer selective, environmentally friendly, and food-safe alternatives. Bacteriophages (phages) are viruses infecting and lysing specific bacterial hosts without harming other organisms.[7] Their application has been successfully demonstrated in agriculture,[8] aquaculture,[9] and food industries for controlling bacterial pathogens.[10] Given their specificity and self-limiting nature, bacteriophages hold potential for targeting nitrifying bacteria, like *Nitrobacter*, in swiftlet house environments, thereby mitigating nitrate accumulation in a natural and sustainable manner.

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Despite this potential, research on isolating nitrifying bacteria and associated bacteriophages from swiftlet houses is limited. Most existing studies focus narrowly on nitrite contamination in edible bird nests or general environmental hygiene,[11] without addressing the microbial pathways responsible for nitrate formation. Furthermore, whereas bacteriophages have been thoroughly investigated for their efficacy against pathogenic bacteria,[12] their application for managing *Nitrobacter* populations in the context of swiftlet farming remains largely unexplored. To our knowledge, there is currently a lack of published data regarding *Nitrobacter*-specific phages in this setting.

Understanding the diversity of nitrifying bacteria in swiftlet environments and identifying effective phages against them are essential for developing a targeted, eco-friendly nitrate control strategy. This study aimed to isolate and characterize nitrifying bacteria, particularly *Nitrobacter* spp., and their associated bacteriophages from swiftlet house environments in Central Kalimantan, Indonesia. The goal is to explore the feasibility of using bacteriophages as a biological control tool to reduce nitrate contamination in edible bird nests, enhancing food safety and supporting sustainable swiftlet farming practices from a veterinary public health perspective.

Materials and Methods

Sample collection. A total of 80 environmental samples were collected from four purposively selected swiftlet houses located in Kota Besi (≈ 2.05 °S and 113.12 °E), Palangkaraya (≈ 2.21 °S and 113.91 °E), Jabiren (≈ 2.49 °S and 113.84 °E), and Buntok (≈ 1.72 °S and 114.85 °E), Central Kalimantan, Indonesia. These sites were selected based on accessibility, owner permission, and prior nitrite-level data reported by Ningrum *et al*.[13] In each swiftlet house, 20 samples were collected, consisting of feces (n = 5), pond water (n = 5), soil (n = 5), and edible birds nests (n = 5). Five discrete samples of each type (*e.g.*, feces, pond water, soil, and edible bird's nest) were collected from separate locations within each house. The sampling took place between October and November 2024, typically during the wet season. All samples were collected between 11:00 AM and 01:00 PM local time at ambient temperatures ranging from 26.00 - 30.00 ˚C, with relative humidity between 75.00 - 85.00%. Approximately 10.00 cm depth from the surface was sampled using sterile techniques for pond water and soil samples, following the method described by the previous study.[14] Each sample was aseptically placed into a sterile container and labeled. All samples were immediately stored at 4.00 ˚C and transported to the laboratory within 12 hr of collection for further processing. Samples were processed within 2 hr upon arrival at the laboratory to minimize microbial community changes.

Nitrifying bacteria isolation. A total of 25.00 g of each solid sample and 25.00 mL of each liquid sample were processed according to the methodology outlined by the previous study.[15] The selective formulation favors the growth of chemolithoautotrophic nitrifying bacteria by limiting available organic nutrients while providing ammonium and inorganic carbon sources essential for nitrification. The enrichment process was repeated twice at 7-day intervals using a modified Alexander medium composed of 13.50 g of KH_2PO_4 (Sigma-Aldrich, St. Louis, USA), 0.70 g of K_2HPO_4 (Sigma-Aldrich), 0.10 g of $MgCl_2{\cdot}6H_2O$ (Sigma-Aldrich), 0.18 g of $CaCl_2{\cdot}2H_2O$ (Sigma-Aldrich), 0.10 g of NH_4Cl (Sigma-Aldrich) as a sole nitrogen source), 0.20 g of ethylenediaminetetraacetic acid (Sigma-Aldrich), 0.50 g of Na_2CO_3 (Sigma-Aldrich) as a sole carbon source), 0.18 g of $FeCl_3{\cdot}6H_2O$ (Supelco, Bellefonte, USA), 0.50 g of D(+)-glucose (Millipore, Burlington, USA), and 13.00 g of nutrient broth (Himedia, Mumbai, India) in 1,000 mL of aquadest. The pH of the enrichment medium was measured and adjusted to 7.00 ± 0.20 at the beginning of each enrichment cycle. The medium was sterilized at 121˚C for 30 min before use. Each 25.00 g of solid or 25.00 mL of liquid sample was added to 225 mL of the sterile enrichment medium. The cultures were incubated at 29.00 ˚C for seven days with continuous shaking at 130 rpm to promote the growth of nitrifying bacteria. For the second enrichment, 225 mL of freshly prepared medium (pH: 7.00 ± 0.20) was added to 25.00 mL of the first enrichment culture and the cultures were incubated at 29.00 ˚C for 7 days at 130 rpm. Incubation was carried out at 29.00 ˚C for 7 days, reflecting the typical environmental temperature of swiftlet houses in Central Kalimantan (28.00 - 32.00 ˚C), Indonesia, and aligning with the reported optimal growth conditions for nitrifying bacteria.[14] This incubation period was sufficient for the enrichment of slow-growing nitrifying bacteria under selective conditions.

Bacterial growth on plate agar. For plating, 100 µL of each Stage two enrichment was spread onto the surface of modified Alexander medium, containing 13.50 g KH_2PO_4, 0.70 g K_2HPO_4, 0.10 g $MgCl_2{\cdot}6H_2O$, 0.18 g $CaCl_2{\cdot}2H_2O$, 0.10 g NH_4Cl (as a sole nitrogen source), 0.20 g ethylene-diaminetetraacetic acid, 0.50 g Na_2CO_3 (as a sole carbon source), 0.18 g $FeCl_3{\cdot}6H_2O$, 0.50 g D(+)-glucose (low levels to minimize stimulation of fast-growing heterotrophs), and 15.00 g bacteriological agar (Oxoid, Basingstoke, UK) in 1,000 mL of distilled water. The plates were incubated at 29.00 ˚C for 7 days, inverted, and enclosed in sealed plastic containers with moist paper towels to maintain humidity.[15] Colonies appeared white, smooth, and convex were selected from nitrifying bacteria selective media for Gram staining and subsequently confirmed as nitrifying bacteria through biochemical and molecular methods.

Phenotypic characterization. The selected bacterial colonies were first subjected to Gram staining to determine their Gram reaction and morphological shape.

Colonies being Gram-negative and rod-shaped were further evaluated through a series of biochemical tests, including the urease, Simmons citrate agar, sulfide-indole-motility, triple sugar iron agar, and catalase tests. Interpretation criteria for the biochemical tests were as follows: In the sulfide-indole-motility test, hydrogen sulfide production was indicated by blackening of the medium, indole production was confirmed by a red ring after the addition of Kovac's reagent (Oxoid), and motility was observed as diffuse growth away from the stab line. A positive urease test was identified by a pink color change due to the increased alkalinity from ammonia production. Citrate utilization was indicated by growth on Simmons citrate agar accompanied by a color shift from green to blue. In the triple sugar iron agar test, fermentation of glucose and/or lactose was shown by yellowing of the slant and/or butt, while black precipitate indicated hydrogen sulfide production. Catalase activity was confirmed by bubble formation upon the addition of 3.00% hydrogen peroxide (Merck, Darmstadt, Germany). *Nitrobacter winogradskyi* (ATCC 25391) was used as a positive control. These biochemical tests were used for preliminary screening. Only isolates showing consistent profiles with known *Nitrobacter* characteristics were selected for molecular confirmation.

Bacterial DNA extraction, *16S rDNA* amplification, and sequencing. Genomic DNA from selected bacterial isolates was extracted using the Geneaid™ DNA Isolation Kit (Geneaid, New Taipei City, Taiwan) following the manufacturer's protocol. The DNA concentration and purity, expressed as the absorbance ratio at 260 nm and 280 nm (A_{260}/A_{280}), were measured using NanoDrop™ 2000 spectrophotometer (ThermoFisher Scientific, Waltham, USA) and only samples with an A_{260}/A_{280} ratio between 1.80 and 2.00 were used for subsequent analyses. The *16S rRNA* gene was amplified using universal bacterial primers, including 27-F (AGAGTTTGATCMTGGCTCAG) and 1492-R (TACGGYTACCTTGTTACGACTT), as described by the previous study.[16] Each 25.00 µL PCR reaction included 1.00 µL of each primer (10.00 pmol), 50.00 ng of DNA template, 12.50 µL of GoTaq® Master Mix (Promega Corp., Madison, USA), and nuclease-free water. The thermal cycling parameters were as follows: Initial denaturation at 95.00 ˚C for 5 min, 30 cycles, including denaturation at 95.00 ˚C for 1 min, annealing at 50.00 ˚C for 45 sec, and extension at 72.00 ˚C for 2 min, concluded with a final extension at 72.00 ˚C for 8 min. The PCR results were analyzed using agarose gel electrophoresis and sequenced using Sanger sequencing (1st BASE, Selangor, Malaysia).

Phylogenetic analysis. The resulting *16S rDNA* sequences were compared with known sequences in the GenBank® database. Sequence alignment and phylogenetic tree construction were performed using DNASTAR Lasergene (version 7.0; DNASTAR Inc., Madison, USA). Sequence analysis was carried out using the SeqMan tools, and phylogenetic trees were constructed using the MegAlign tool (DNASTAR) with the Neighbor-Joining method and 1,000 bootstrap replicates, based on Clustal W Multiple Sequence Alignment Program (UCD, Dublin, Ireland), following the method described by a the previous study.[17]

Bacteriophage isolation. Bacteriophage enrichment followed the modified method of the previous study.[18] Both solid (edible birds nest, soil, and feces) and liquid samples were processed for phage isolation. Solid samples were suspended in 50.00 mL of sterile distilled water. Samples were centrifuged at 4,200 rpm for 10 min at 4.00 ˚C, and the resulting supernatants were sequentially filtered through 0.45 µm and 0.22 µm membranes at room temperature (~ 16.00 ˚C), ensuring minimal loss of phage viability. Phage enrichment was performed using 10.00 mL of double-strength nutrient broth (HiMedia), 40.00 µL of 1.00 M $CaCl_2$, 100 µL of *Nitrobacter* host culture, and 10.00 mL of filtered environmental sample. Nutrient broth was selected based on preliminary optimization trials, which showed improved phage propagation and clearer lytic activity compared to the minimal media, possibly due to the enhanced host bacterial growth in the richer environment. Although nutrient broth is not selective for nitrifiers, it supported sufficient growth of *Nitrobacter* to enable phage-host interaction. As a control, parallel enrichment cultures were prepared without environmental samples (nutrient broth + host bacteria + $CaCl_2$) to monitor for spontaneous lytic activity or contamination; no lysis zones were observed in these control plates. The mixture was incubated at 29.00 ˚C for 24 hr with gentle agitation at 50.00 rpm. After incubation, 5.00 mL of every mix was centrifuged at 10,000 rpm for 10 min. The supernatant was re-filtered through a 0.22 µm filter membrane and analyzed for phage presence utilizing the spot test method.

Spot test. Filtered samples (n = 16), derived from pooling 80 environmental samples (four sample types × four swiftlet houses), were serially diluted (1.00×10^{-1} to 1.00×10^{-8}) in phosphate-buffered saline (PBS; pH: 7.40). Each pooled sample represented a specific sample type (feces, pond water, soil, or edible birds nest) collected from one swiftlet house. For each assay, 100 µL of overnight bacterial culture (n = 15) was mixed with 4.00 mL of semi-solid agar (maintained at 55.00 ˚C) and poured onto nutrient agar plates to create a double layer. Following solidification, 10.00 µL of each phage dilution was applied to the bacterial lawn. Plates were incubated for 24 hr at 29.00 ˚C. The presence of distinct lytic zones (plaques) suggested possible bacteriophage activity, and positive samples followed further testing by plaque assay. A negative control was included in each assay by spotting 10.00 µL of sterile PBS onto the bacterial lawn to confirm that any observed lytic zones were due to the

bacteriophage activity and not media artifacts or desiccation effects. No clearing or inhibition was observed in the PBS-only controls.

Plaque assay. Filtered phage suspensions were serially diluted from 1.00× 10^{-1} to 1.00× 10^{-8} in PBS. For each dilution, 100 µL of overnight bacterial culture was mixed with 4.00 mL of semi-solid agar (maintained at 55.00 ˚C), and 500 µL of each phage dilution was added prior to plating. A larger volume of phage suspension (500 µL) was used to increase the likelihood of plaque formation on the slow-growing nitrifying bacteria. The mixtures were gently overlaid onto nutrient agar plates to create a double-layer format and incubated overnight at 29.00 ˚C. Plaques were counted only on plates containing 25 - 250 plaques for accuracy. Plaque-forming units (PFU) mL^{-1} were calculated using the standard following formula:

$$PFU\ mL^{-1} = \frac{number\ of\ plaques \times dilution\ factor}{volume\ of\ phage\ plated\ (mL)}$$

Plaque diameters were measured using a Jiusion 1,600× USB digital microscope (Jiusion Technology Co., Ltd., Shenzhen, China). Five well-isolated plaques from countable plates were randomly selected and measured for diameter. All plaque assays were performed in triplicate. The mean PFU counts and standard deviations were reported to ensure reproducibility and statistical reliability.

Results

Phenotypic characterization. Based on cultural characteristics and phenotypic tests, 15 isolates were identified as *Nitrobacter* sp. (Table 1). These isolates exhibited consistent colony morphology and biochemical profiles, being aligned with the characteristics of *Nitrobacter*. Strain designations, such as *Nitrobacter* sp. AP1 through AP15, are used throughout, where strain codes are not italicized.

Table 1. The results of biochemical tests of isolates evaluated in this study.

Phenotypical properties		Isolates															
		SB6	SB5	SB8	SB10	SP3	SP4	SP7	SJ8	SJ9	SJ3	TB4	TJ9	TP6	AP6	FB3	NB
Urease		(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	-	-	-	-	-	-
Citrate		+	+	+	+	+	+	+	+	+	+	(-)	(-)	(-)	+	(-)	+
Hydrogen sulfide production		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Motility		(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	-	(+)	-
Indole production		-	-	-	-	-	-	-	-	-	-	(+)	(+)	(+)	-	(+)	-
Fermentation of	**Glucose**	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	-	(+)	-
	Lactose	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	-	-	-
	Sucrose	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	-	-	-
	Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+: Positive result; -: Negative result; (+): Weak positive result; (-): Weak negative result; SB6, SB5, SB8, SB10, SP3, SP4, SP7, SJ8, SJ9, SJ3, TB4, TJ9, TP6, AP6, FB3: *Nitrobacter* sp.; NB: *Nitrobacter winogradskyi* ATCC 25391.

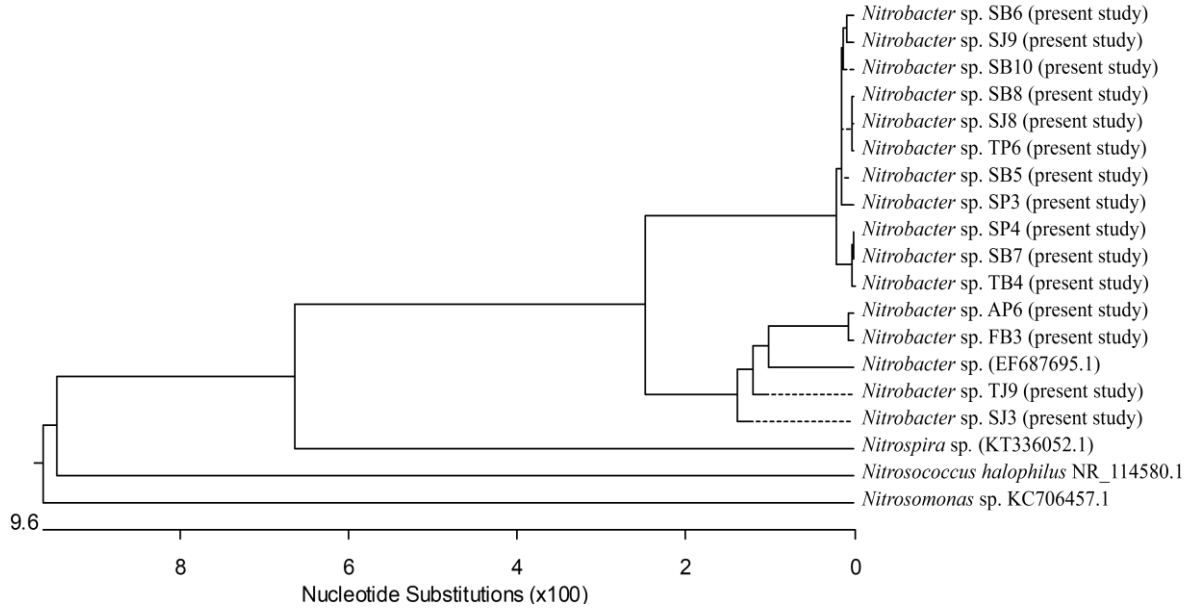

Fig. 1. Clustering analysis of the *16S rRNA* gene sequences of *Nitrobacter* sp. from this study and other important species of nitrifying bacteria obtained from NCBI GenBank®.

Phylogenetic analysis. The BLAST analysis of the *16S rRNA* gene sequences from the 15 isolates confirmed their classification within the genus *Nitrobacter*. Sequence identity ranged from 93.50 to 97.50% compared to the *N. winogradskyi* (Accession No. EF687695.1). A dendrogram constructed using the Neighbor-Joining method (Clustal W, 1,000 bootstrap replicates) illustrated the genetic relationships among the isolates (Fig. 1). Isolates with sequence identity ≥ 97.00% were considered presumptive *N. winogradskyi*, while isolates with identity between 93.50 - 96.90% were provisionally labeled as *Nitrobacter* sp., pending further whole-genome sequencing or additional molecular characterization.

Bacteriophage isolation and detection. A total of 240 spot assays (16 environmental samples × 15 host isolates) were performed using enriched filtrates and *Nitrobacter* sp. as hosts. Spot test analysis revealed that four of these assays (AP1 - AP6, FJ1 - AP6, FK1 - AP6, and TK1 - AP6) produced characteristic clear lytic zones against *Nitrobacter* sp. AP6 (Fig. 2), indicating the presence of lytic bacteriophages in samples from pond water, feces, and soil. No phage activity was observed in samples derived from edible bird nests.

Plaque assay confirmation. The four phage-positive filtrates identified by spot tests were further confirmed *via* plaque assay on *Nitrobacter* sp. AP6. Each yielded distinguishable lytic plaques with titers ranging from from 5.40×10^9 to 8.10×10^{10} PFU mL^{-1} (Table 2). Clear plaques were produced by isolates AP1 - AP6, FJ1 - AP6, and FK1 - AP6, suggesting strong lytic activity, whereas TK1 - AP6 yielded turbid plaques (Fig. 3), potentially indicating a delayed or incomplete lytic cycle.

Discussion

The present study provides the first investigation of nitrifying bacteria and their corresponding bacteriophages in swiftlet houses in Central Kalimantan, Indonesia. By phenotypic screening and *16S rRNA* gene analysis, 15 isolates were identified as belonging to the genus *Nitrobacter*, showing sequence identity between 93.50% and 97.50%. These bacteria are recognized as nitrite-oxidizing autotrophs in the α-Proteobacteria and are crucial to the second phase of nitrification, converting nitrite to nitrate.[19] The widespread presence of *Nitrobacter* spp. in our environmental samples is notable. Although

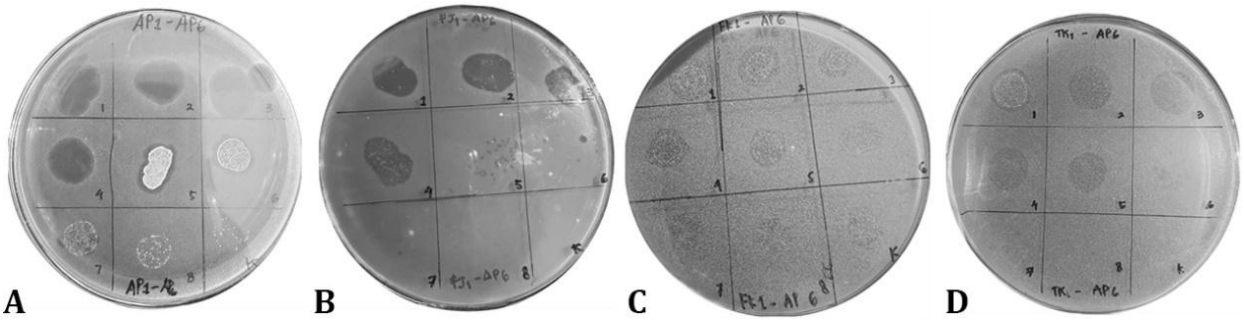

Fig. 2. The results of spot tests. **A)** AP1 - AP6; **B)** FJ1 - AP6; **C)** FK1 - AP6; and **D)** TK1 - AP6.

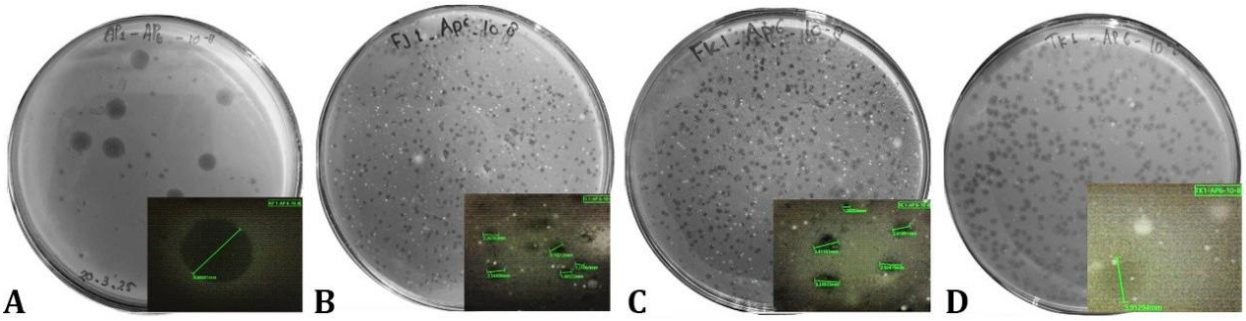

Fig. 3. The results of plaque assays. **A)** AP1 - AP6 (clear plaque); **B)** FJ1 - AP6 (clear plaque); **C)** FK1 - AP6 (clear plaque); and **D)** TK1 - AP6 (turbid plaque).

Table 2. The results of the plaque assay.

Plaque ID	Sample type	Number of plaque	Dilution factor	Plaque size (mm)	Titer (PFU mL^{-1})
AP1 - AP6	Pond water	27	10^{-8}	1.70 - 9.00	5.40×10^9
FJ1 - AP6	Feces	407	10^{-8}	1.30 - 3.30	8.10×10^{10}
FK1 - AP6	Feces	343	10^{-8}	1.00 - 3.60	6.70×10^{10}
TK1 - AP6	Soil	34	10^{-8}	1.80 - 7.60	6.80×10^9

PFU: Plaque-forming units.

Nitrosomonas spp. often dominate aquatic environments,[20] *Nitrobacter* has been reported as a dominating genus in terrestrial and semi-aquatic micro-habitats characterized by variable oxygen levels, including soil and sediment interfaces.[21,22] The selective enrichment technique used in this investigation, specifically the incubation temperature (29.00 ˚C), defined mineral composition, and aerobic shaking conditions, may have facilitated the proliferation of *Nitrobacter* over other bacteria.

All isolates displayed Gram-negative, rod- or pear-shaped morphology consistent with *Nitrobacter* descriptions.[23] The observed sequence identity below 98.00% suggests the potential presence of novel strains adapted to swiftlet house environments.[24] Prior studies have suggested that *Nitrobacter* possesses considerable genomic plasticity and the ability to adapt to nutrient-limited or fluctuating environments.[25] These findings underscore the need for future whole-genome sequencing to better characterize the isolates.

Importantly, we successfully isolated lytic bacteriophages capable of infecting *Nitrobacter* spp. Among 240 spot tests, only four showed lytic activity, all against the *Nitrobacter* sp. AP6 isolate. This low recovery rate (1.67%) aligns with prior observations that phages specific to nitrifiers are rarely isolated.[26] Nevertheless, the plaque assays confirmed that all four phage-positive samples could produce well-defined lytic plaques, with high titers ranging from 5.40×10^9 to 8.10×10^{10} PFU mL^{-1}. These high titers suggest robust phage replication and infectivity, even against slow-growing hosts, such as *Nitrobacter*.

Variation in plaque size and clarity suggests differing phage morphotypes or infection dynamics. For instance, turbid plaques produced by TK1 - AP6 may indicate incomplete lysis or temperate phage behavior, whereas clear plaques suggest strong lytic activity. Such differences have also been reported in phage studies targeting other slow-growing bacterial species.[27-29] Considering that our phages replicated well despite *Nitrobacter*'s slow metabolism and nutritional demands, this highlights their promise as biological control agents.

By lysing *Nitrobacter*, these phages can potentially disrupt the nitrite-to-nitrate conversion step in the nitrification pathway. This interruption may reduce nitrate accumulation in swiftlet houses, offering a targeted and environmentally sustainable strategy to manage nitrate contamination in edible bird nests. Unlike chemical treatments, phage-based methods offer specificity and preserve microbial balance while addressing food safety and regulatory concerns.[30,31]

Additional studies are required to validate phage stability under swiftlet house conditions (temperature, humidity, and ultraviolet exposure), evaluate the risk of resistance development, and assess interactions within complex microbial communities. Studies in aquaculture and wastewater treatment suggest that phage efficacy can be influenced by biofilm formation and microbial competition.[32] Understanding the dynamics inside the distinct environment of swiftlet houses will be essential for formulating successful phage-based biocontrol techniques.

Although bacteriophages have been widely investigated for controlling bacteria, like *Salmonella*, *Escherichia coli*, and *Pseudomonas*, in food systems and livestock settings, their application against nitrifying bacteria is still relatively unexplored. Previous studies largely focused on phages against *Nitrosomonas europaea* or *Nitrospira* species,[33-34] typically from wastewater sources. Our discovery of *Nitrobacter*-specific phages isolated from swiftlet houses is unique and contributes significantly to the wider utilization of phages in food systems.

Our approach aligns with recent advances in nitrifiers and phage ecology, such as those reported in bioreactor systems, showing phages can significantly influence nitrification rates.[35] The present study describes this idea more within the commercially applicable area of swiftlet farming, allowing microbiologists to study phages in swiftlet settings. Future studies should explore host range, environmental stability, and genomic features of these phages to fully assess their utility in phage application and nitrate mitigation.

This study successfully isolated and characterized *Nitrobacter* spp. and their lytic bacteriophages from swiftlet house environments. The findings suggest that nitrifying bacteria contribute to nitrate accumulation, and the phages targeting *Nitrobacter* offer a promising biocontrol solution. By disrupting nitrite oxidation, these phages could reduce nitrate buildup, improving the safety and export quality of edible bird nests. This work lays the foundation for phage-based nitrate control strategies in swiftlet farming from a veterinary public health perspective.

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Conflict of interest

The authors have no conflict of interest to declare.

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