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Antimicrobial activity of *Bacillus paramycoides* from Xiem duck against poultry pathogens

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Abstract. *Thuy NP, Tuu NT. 2025. Antimicrobial activity of* Bacillus paramycoides *from Xiem duck against poultry pathogens. Biodiversitas 26: 5567-5573.* Antimicrobial Resistance (AMR) in poultry production poses a significant threat, demanding the development of effective antibiotic alternatives. This study aimed to isolate and identify native *Bacillus* strains from the gastrointestinal tract of Vietnamese Xiem ducks (*Cairina moschata*) and screen them for antagonistic activity against key poultry pathogens. Using heat-shock isolation and agar well diffusion assays, 30 different *Bacillus* species were obtained from 50 healthy ducks. In vitro screening via an agar well diffusion assay showed that over 93% of these isolates possessed broad-spectrum antagonistic activity against *Escherichia coli*, *Salmonella enterica*, and *Staphylococcus aureus*. Among 30, isolate B10 exhibited the highest efficacy, creating significant inhibition zones against *E. coli* (≥10 mm), *S. enterica* (6-9 mm), and *S. aureus* (6-9 mm). Molecular analysis of the 16S rRNA gene identified isolate B10 as *Bacillus paramycoides*. This study reports the first identification of *B. paramycoides* from Xiem ducks, highlighting the importance of indigenous poultry as a reservoir for novel, host-derived probiotics. However, given its taxonomic affiliation with the *B. cereus* group, a rigorous safety evaluation, including genomic screening for virulence factors, is an essential prerequisite for its future development and application in sustainable poultry farming.

Keywords: Antimicrobial activity, *Bacillus paramycoides*, *Cairina moschata,* microbial biodiversity, poultry pathogens

INTRODUCTION

Duck farming is a vital component of the global poultry industry, providing a substantial source of protein and a significant economic livelihood. However, the productivity of this sector is persistently undermined by infectious bacterial diseases, which lead to significant economic losses through diminished growth rates, poor feed conversion, and high flock mortality (Hamed et al. 2024). Key pathogens responsible for these losses include Avian Pathogenic *Escherichia coli*, *Salmonella* species, and *Staphylococcus aureus* (AbdelRahman and Amer 2021). The challenge of controlling these infections is severely exacerbated by the global rise of Antimicrobial Resistance (AMR). The widespread use of antibiotics in poultry production has driven the selection of Multidrug-Resistant (MDR) bacteria, rendering many conventional treatments ineffective (Patil et al. 2021; Azizi et al. 2024). This not only jeopardizes animal welfare and farm sustainability but also poses a significant public health threat under the "One Health" framework, as duck farms can act as reservoirs for transferring resistance genes to human pathogens (Aguidissou et al. 2022; Msemakweli et al. 2024). For instance, recent surveillance has identified genes conferring resistance to last-resort antibiotics like fosfomycin (*fosA*) and linezolid (*optrA*) in *S. aureus* from duck farms, revealing a critical link in the AMR transmission chain (Hu et al. 2023; Li et al. 2024). This situation underscores the urgent need to develop effective, non-antibiotic alternatives for disease management in poultry.

In response to the AMR crisis, probiotics, live microorganisms that confer a health benefit to the host, have emerged as a leading sustainable strategy for improving poultry health and productivity (Plaza-Diaz et al. 2019). The benefits of probiotics are multifaceted, including the competitive exclusion of pathogens from intestinal binding sites, the production of potent antimicrobial compounds, and the beneficial modulation of the host's immune system (Krysiak et al. 2021). The adoption of probiotics aligns with the growing consumer demand for antibiotic-free food production (Rahimoon et al. 2023). Among the diverse range of probiotic candidates, spore-forming bacteria from the genus *Bacillus* offer distinct advantages for application in animal feed. Their ability to form highly resilient endospores allows them to withstand the high temperatures of commercial feed pelleting and survive the harsh acidic and enzymatic conditions of the upper gastrointestinal tract, ensuring the delivery of viable cells to the lower intestine, where they can exert their beneficial effects (Ramlucken et al. 2020). Once germinated, *Bacillus* species are known to produce a wide array of beneficial compounds, including enzymes that improve nutrient digestion and antimicrobial lipopeptides with broad-spectrum activity against key poultry pathogens (Hanim et al. 2021; Tran et al. 2022).

A crucial, yet largely overlooked, reservoir of microbial biodiversity for discovering novel probiotics exists within the gastrointestinal tracts of indigenous, locally-adapted poultry breeds. Probiotics sourced from the target host, known as autochthonous probiotics, are often superior

because they are co-evolved and pre-adapted to the specific gut environment, enhancing their persistence and efficacy (Duraisamy et al. 2022). However, probiotic research has predominantly focused on commercial chicken breeds, creating a significant knowledge gap concerning the gut microbiomes of native waterfowl (Mazanko et al. 2022). This gap is especially pertinent for hardy, indigenous breeds like the Vietnamese Xiem duck (*Cairina moschata* (Linnaeus, 1758)). Having adapted over generations to local environmental pressures and endemic pathogens, these ducks may harbor a unique gut microbiota containing novel *Bacillus* strains with specialized, highly effective antimicrobial capabilities.

Therefore, this study was designed to bridge this research gap by providing the first exploration of the untapped microbial biodiversity of the indigenous Xiem duck as a source for novel probiotic candidates. The primary objectives were to: (i) isolate and characterize native *Bacillus* strains from the gastrointestinal tract of healthy Xiem ducks; (ii) screen the isolates for in vitro antagonistic activity against the major poultry pathogens *Escherichia coli*, *Salmonella enterica*, and *Staphylococcus aureus*; and (iii) 16S rRNA gene sequencing was performed to identify the most potent strain and evaluate its potential as a novel, host-derived probiotic for sustainable poultry production.

MATERIALS AND METHODS

Ethical statement and sample collection

All animal handling and sampling procedures were conducted in strict accordance with the ethical guidelines established by the scientific board of Tra Vinh University and received formal approval (Approval No. 5887/QĐ-ĐHTV). Gastrointestinal (GI) samples were collected from 50 healthy, 12-week-old Xiem ducks (*Cairina moschata*), comprising 25 males and 25 females, sourced from a commercial farm in Vinh Long Province, Vietnam. Immediately following humane euthanasia, the entire GI tract of each animal was aseptically excised. Luminal contents were then collected from four distinct sections: the crop, gizzard, small intestine, and cecum. Samples were placed in sterile containers and transported on ice to the laboratory for immediate processing.

Isolation of *Bacillus* strains

To isolate spore-forming bacteria, one gram of luminal content from each GI section was homogenized in 9 mL of sterile Phosphate-Buffered Saline (PBS, pH 7.2). The resulting homogenate was subjected to a standard heat-shock treatment at 80°C for 15 minutes in a water bath. This step is designed to eliminate non-spore-forming vegetative cells and enrich for thermo-resistant *Bacillus* endospores (Hellany et al. 2024). The heat-treated suspension was then serially diluted (10^{-2} to 10^{-7} in PBS), and 100 µL aliquots from each dilution were spread-plated onto Nutrient Agar (NA). Each dilution was plated in triplicate. The plates were incubated aerobically at 37°C for 24-48 hours (Golnari et al. 2024). Colonies displaying morphologies characteristic of the *Bacillus* genus (large, opaque, irregular) were selected and sub-cultured on fresh NA plates to ensure purity (Al Azad et al. 2020).

Phenotypic and biochemical characterization

Pure isolates were identified as *Bacillus* based on microscopic examination, confirming a Gram-positive stain reaction and the presence of rod-shaped, endospore-forming cells (Tripathi et al. 2020). To confirm genus identification, a panel of standard biochemical tests was performed for each isolate in triplicate. These tests included catalase and urease activity, motility, citrate utilization, and the fermentation of glucose, sucrose, and lactose (Shen and Zhang 2022; Khushboo et al. 2023). For each assay, validated ATCC reference strains were included as positive and negative controls to ensure accuracy: *Staphylococcus aureus* ATCC 25923 (catalase-positive) and *Lactobacillus plantarum* ATCC 14917 (catalase-negative); *Proteus mirabilis* ATCC 12453 (urease-positive) and *Escherichia coli* ATCC 25922 (urease-negative); *Enterobacter aerogenes* ATCC 13048 (citrate-positive) and *E. coli* ATCC 25922 (citrate-negative). Only isolates confirmed as Gram-positive, spore-forming, and catalase-positive were retained for subsequent antimicrobial screening. All purified isolates were cryopreserved at -20°C in Luria-Bertani (LB) broth containing 30% (v/v) sterile glycerol.

Screening for antimicrobial activity

The antagonistic potential of isolates was assessed against three globally significant indicator pathogens using an agar well diffusion assay (El-Banna and Qaddoumi 2016). Standardized American Type Culture Collection (ATCC) strains, *E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *S. enterica* serovar Typhimurium ATCC 14028 were used to ensure reproducibility and comparability of results. To prepare a Cell-Free Supernatant (CFS), each *Bacillus* isolate was cultured in LB broth at 37°C for 24 hours with shaking, and then culture was centrifuged (10,000 rpm, 5 min, 4°C). To ensure that any observed inhibition was due to secreted antimicrobial compounds rather than acidity, the pH of the resulting CFS was neutralized to 7.0±0.2 using 1 M NaOH. For the assay, molten NA was seeded with an overnight culture of an indicator pathogen to a final concentration of approximately 1×10^6 CFU/mL and poured into sterile petri plates. Once solidified, 4 mm wells were cut into the agar and filled with 100 µL of the neutralized CFS. Ampicillin (10 µg) and sterile, neutralized LB broth served as positive and negative controls, respectively. After incubation at 37°C for 24 hours, antimicrobial activity was quantified by measuring the diameter of the zone of inhibition (in mm). Inhibition zones were categorized as: Weak (+, 2-5 mm); Moderate (++, 6-9 mm); Strong (+++, ≥10 mm); or Inactive (-, No inhibition) (Hatem et al. 2024). All antimicrobial screening assays were performed in triplicate.

Molecular identification by 16S rRNA gene sequencing

The single isolate demonstrating the most potent and broad-spectrum antimicrobial activity was selected for molecular identification. The primary selection criterion was the largest mean zone of inhibition when averaged across all three indicator pathogens from the triplicate assays. Genomic DNA was extracted from this top-performing isolate using a modified phenol-chloroform method (De et al. 2010). The 16S rRNA gene was amplified by PCR using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Swacita et al. 2022). The reaction was performed in a Veriti thermocycler with GoTaq® Green Master Mix (Promega, USA) under the following conditions: initial denaturation at 94°C for 3 min; 29 cycles of 94°C for 45s, 53°C for 60s, and 72°C for 90s; and a final extension at 72°C for 5 min. The presence of the expected ~1,500 bp amplicon was verified on a 2% agarose gel. The purified PCR product (QIAquick PCR Purification Kit, Qiagen, Germany) was subjected to Sanger sequencing (Next Gen Scientific Co., Ltd, Ho Chi Minh City, Vietnam).

Statistical and sequence analysis

All quantitative data from the antimicrobial assays were generated from three independent replicates and are presented as the mean±Standard Deviation (SD). Statistical analysis was conducted using SPSS software (v. 26.0). A one-way Analysis of Variance (ANOVA) was used to compare the mean inhibition zones among isolates. Where the ANOVA indicated a significant difference, Tukey's Honestly Significant Difference (HSD) post-hoc test was employed to identify specific differences between group means. As recommended, the full results of the ANOVA, including F-values and degrees of freedom, are reported in the Results section. For all tests, statistical significance was set at $p<0.05$. For sequence analysis, the raw 16S rRNA gene sequence was edited and assembled using BioEdit (v. 7.0). Species identification was performed by querying the NCBI database with the BLASTn algorithm, using a sequence identity and query coverage of ≥99% to a validated type strain as the threshold for definitive species assignment (Tamura et al. 2021).

RESULTS AND DISCUSSION

Isolation and phenotypic characterization of *Bacillus* isolates

Results of heat-shock treatment showed that a total of thirty distinct bacterial isolates were successfully recovered from gastrointestinal samples of healthy Xiem ducks. All 30 isolates were identified as members of the genus *Bacillus* based on a consistent and uniform profile of morphological, physiological, and biochemical characteristics (Table 1). On Nutrient Agar (NA), isolates typically formed milky-white, opaque, and circular colonies (Figure 1.A). Microscopic and biochemical analysis confirmed that all isolates were Gram-positive, motile, endospore-forming rods (Figure 1.B), positive for catalase test and citrate utilization but negative for urease. This consistent profile affirmed their classification within the *Bacillus* genus, validating them as suitable candidates for subsequent antimicrobial screening.

Table 1. Morphological, physiological, and biochemical characteristics of 30 *Bacillus* isolates isolated from Xiem ducks

Characteristics	Observations
Colony morphology	Circular, convex, milky-white, opaque; texture typically dry with entire or undulate margins
Cellular morphology	Gram-positive (+), rod-shaped, endospore-forming
Physiology	Motile (+)
Biochemical profile	Catalase (+), Urease (−), Citrate Utilization (+)
Carbohydrate fermentation	Glucose (+), Sucrose (+), Lactose (−)

Note: All 30 isolates displayed a uniform profile for the tests listed. +: Indicates a positive result, −: Indicates a negative result

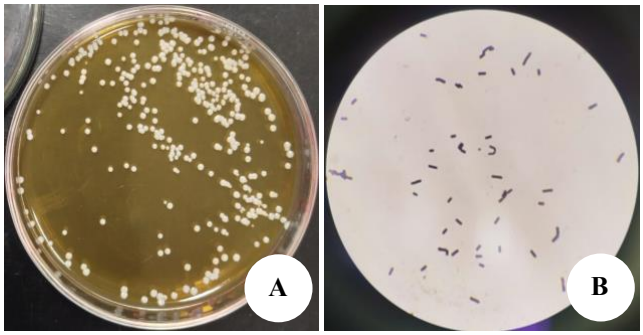

Figure 1. Representative morphology of isolate B10: A. Colony morphology on Nutrient Agar, showing characteristic circular, opaque, milky-white colonies, B. Gram stain revealing Gram-positive, rod-shaped cells

Figure 2. A representative agar well diffusion assay showing the zone of inhibition produced by the nCFS of isolate B10 against *Salmonella enterica*. B10: nCFS from *Bacillus paramycoides* B10, P: Positive control (Ampicillin, 10 µg), N: Negative control (sterile neutralized LB broth)

Table 2. Distribution of antimicrobial activity of 30 *Bacillus* isolates against key poultry pathogens

Indicator pathogens	Strong	Moderate	Weak	Inactive	Total active isolates (%)
Escherichia coli ATCC 25922	4	25	0	1	29 (96.7%)
Salmonella enterica ATCC 14028	0	29	0	1	29 (96.7%)
Staphylococcus aureus ATCC 25923	0	27	1	2	28 (93.3%)

Note: Data represent the number of isolates producing a specific inhibition zone size. Inhibition was categorized as: Strong (+++: ≥10 mm), Moderate (++: 6-9 mm), Weak (+: 2-5 mm), or Inactive (-: No inhibition zone)

Table 3. Molecular identification of isolate B10 via 16S rRNA gene sequence analysis

Subject description	Max score	Query cover	E-value	Percent identity	Accession number
Bacillus paramycoides strain MCCC 1A04098 16S ribosomal RNA, partial sequence	1624	100 %	0.0	100.00 %	NR_157734.1

Note: The table showed top result from a BLASTn query against the NCBI database, confirming the isolate's identity based on maximum score, query coverage, and percent identity to the type strain

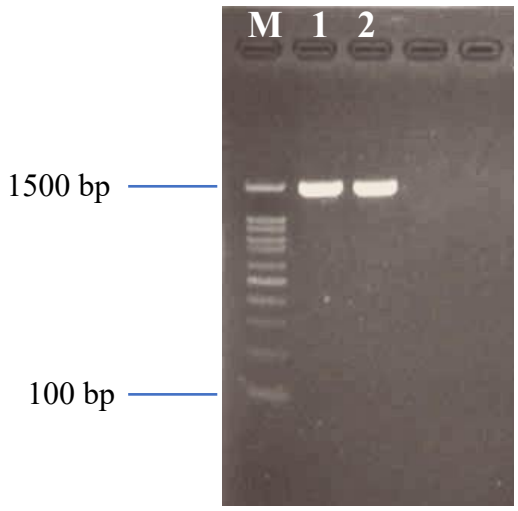

Figure 3. Agarose gel electrophoresis (2%) of the 16S rRNA gene PCR product from isolate B10. M: 100 bp DNA ladder, Lane 1: Positive control, Lane 2: A distinct band of approximately 1500 bp from isolate B10

Broad-spectrum antimicrobial activity against poultry pathogens

The antagonistic potential of 30 *Bacillus* isolates was evaluated against three key poultry pathogens. A remarkable prevalence of broad-spectrum antagonism was observed, with neutralized Cell-Free Supernatants (nCFS) from 28 of 30 isolates (93.3%) inhibiting the growth of all three pathogens. This finding demonstrates the widespread presence of antimicrobial activity within the native gut microbiome of Xiem ducks. As summarized in Table 2, inhibitory activity was particularly effective against the Gram-negative pathogens *E. coli* and *S. enterica*, with 96.7% of isolates showing activity. Notably, four isolates (B2, B10, B26, and B29) exhibited strong inhibition (zone ≥10 mm) against *E. coli*. Analysis of variance confirmed significant differences in the antimicrobial efficacy among the isolates ($F(29, 269) = 45.8$, $p<0.001$). Specifically, post-hoc analysis revealed that isolate B10 produced a significantly larger mean zone of inhibition across all three pathogens compared to all other isolates ($p<0.05$). Based on its consistently superior and potent activity, illustrated in Figure 2, isolate B10 was selected as the elite candidate for molecular identification.

Molecular identification of isolate B10

To determine the precise taxonomic identity, the B10 isolate underwent 16S rRNA gene sequencing. PCR amplification successfully yielded a single, clear amplicon of the expected size (~1500 bp), as confirmed by agarose gel electrophoresis (Figure 3). A BLASTn analysis of the assembled sequence revealed 100% identity and query coverage with the *Bacillus paramycoides* type strain (Table 3). To further validate this identification, a phylogenetic analysis was conducted. The resulting Neighbor-Joining tree (Figure 4) demonstrated that isolate B10 clustered definitively within a well-supported clade with the *B. paramycoides* type strain, clearly separating it from other members of the *B. cereus* group. This combination of molecular and phylogenetic evidence unequivocally identifies isolate B10 as *B. paramycoides*. The 16S rRNA gene sequence for this isolate has been deposited in the GenBank database under accession number PX112818.

Discussion

The indigenous xiem duck microbiome: A targeted source for autochthonous probiotics

The Gastrointestinal Tract (GIT) of indigenous poultry, such as the Vietnamese Xiem duck, represents a crucial yet under-explored ecological niche for bioprospecting novel probiotics. This study successful isolation of thirty distinct *Bacillus* strains from the Xiem duck gastrointestinal tract reinforces the principle that locally adapted animal breeds were valuable reservoirs for host-specific beneficial microbes. These animals co-evolve with their local environment, developing a gut microbiome uniquely optimized to neutralize regional pathogens and efficiently digest available diets (Evangelista et al. 2024).

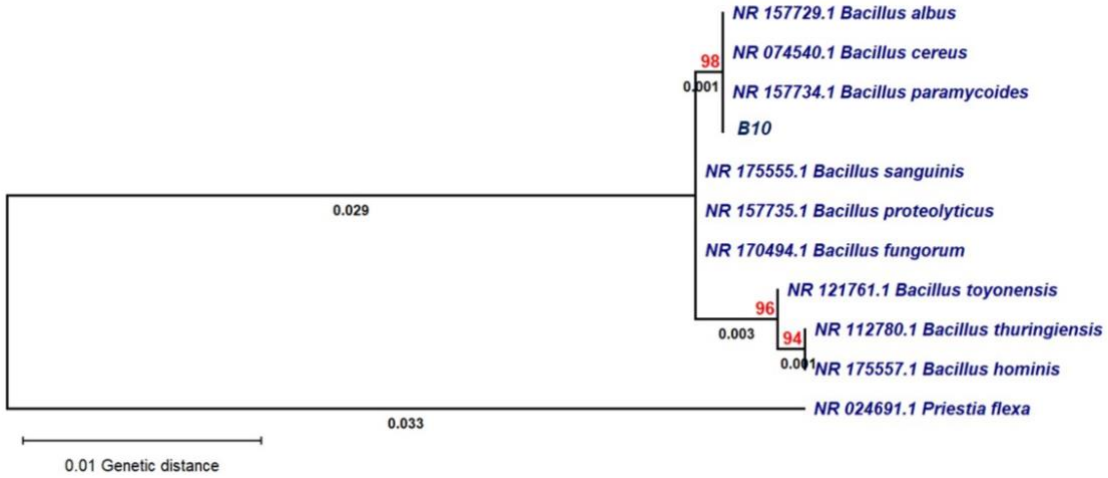

Figure 4. Neighbor-Joining phylogenetic tree based on 16S rRNA gene sequences, illustrating the taxonomic position of isolate B10. The analysis shows B10 forming a distinct and well-supported clade with the *Bacillus paramycoides* type strain. Bootstrap support values (from 1,000 replicates) are indicated at the nodes. The scale bar represents 0.005 nucleotide substitutions per site

Probiotics isolated from the target host animal, known as autochthonous probiotics, are theorized to possess superior colonization, persistence, and functional capabilities compared to allochthonous strains. This is because they are already adapted to the specific physiological conditions of the host's gut, including its pH gradients, bile salt concentrations, and native microbial community (Duraisamy et al. 2022). The findings of the current study align with research that identifies the GIT of diverse avian species from commercial broilers to wild birds as a rich source of potent *Bacillus* probiotics, highlighting the potential of this genus as a cornerstone for developing next-generation poultry feed additives (Penaloza-Vazquez et al. 2019; Ogbuewu et al. 2022).

A key methodological decision in the present study was the use of heat treatment (80°C) during the isolation phase. This step serves as a direct functional screen for thermostability, a trait conferred by the ability of *Bacillus* to form high-resistant endospores. This approach is well-supported in the literature of selectively isolating *Bacillus* species from complex environmental samples, such as the gut or soil, by eliminating heat-sensitive vegetative cells (Golnari et al. 2024; Hellany et al. 2024). The resilience of these endospores is not only a biological characteristic but also a paramount trait for industrial probiotic applications, as it allows the strains to survive the high temperatures (often exceeding 85°C) and mechanical pressures of the commercial feed pelleting process. This process eliminates non-spore-forming probiotics like many common Lactic Acid Bacteria (LAB) strains (Truong et al. 2021). This intrinsic durability contrasts sharply with the thermal sensitivity of non-spore-forming probiotic, such as many lactic acid bacteria strains, and ensures a viable and stable dose can be delivered in the final feed product (Ramlucken et al. 2020).

Further characterization confirmed the isolates' universal catalase-positive profile, which is particularly noteworthy from a functional standpoint. Catalase production allows the bacteria to neutralize reactive oxygen species like hydrogen peroxide, providing a critical survival advantage against oxidative stress generated by the host's own immune cells (e.g., macrophages) within the gut lumen (Golnari et al. 2024). The observed metabolic capacity to ferment glucose and sucrose but not lactose suggests a specific adaptation to the duck's omnivorous diet, which is naturally rich in plant-derived carbohydrates rather than milk-based sugars. This metabolic fingerprint indicates that these *Bacillus* strains occupy a different ecological niche than lactose-fermenting LAB, suggesting they may act synergistically with other beneficial gut microbes to enhance nutrient utilization and overall gut health (Fitri et al. 2023).

Broad-spectrum antimicrobial activity: Mechanisms and comparative efficacy

A primary finding was the potent and broad-spectrum antimicrobial activity demonstrated by the isolated *Bacillus* strains. Over 93% of the isolates inhibited the growth of both Gram-negative (*E. coli*, *S. enterica*) and Gram-positive (*S. aureus*) pathogens. This capacity to antagonize a wide range of taxonomically diverse pathogens is a highly desirable trait for a probiotic, suggesting the secretion of a versatile arsenal of bioactive compounds and offering a more robust solution for controlling co-infections common in poultry production (Nicolas 2025). The genus *Bacillus* is a well-documented producer of antimicrobial compounds, including bacteriocins and various families of lipopeptides like surfactins, iturins, and fengycins, which act by disrupting the integrity of pathogen cell membranes (Manhar et al. 2015; Xie et al. 2021). The results of strong inhibition zones suggest that isolates, particularly B10, likely produce multiple classes of these compounds.

Antimicrobial results showed that *B. paramycoides* B10 isolate has the potential to control the growth of pathogens. In the present study, isolate B10 showed moderate to strong activity (6 mm to ≥10 mm). Similar observations were

made for *B. paramycoides* B4J22-F from poultry litter, which reported inhibition zones of 10.2-10.6 mm against *E. coli* and *S. aureus* (Ainyakou-Sanga et al. 2023). Similarly, Ostad et al. (2024) demonstrated that *Bacillus coagulans* supernatant could effectively control *E. coli* and *Salmonella*. The strong inhibition against *E. coli* by four distinct isolates in the present study highlights the exceptional potency of strains sourced from the unique Xiem duck gut environment. This comparison is firmly situated in the global research context and underscores the significant therapeutic potential of isolate B10 as a novel antimicrobial agent. Beyond direct antagonism, the variation in inhibitory capacity among our isolates highlights the natural functional diversity within this ecosystem and reinforces the value of systematic screening to identify elite candidates for further development.

Bacillus paramycoides *B10: A promising candidate with critical safety considerations*

The consistent and potent inhibitory performance of isolate B10 marked it as the superior candidate, which was identified via 16S rRNA gene sequencing as *Bacillus paramycoides*. This identification is highly significant, as *B. paramycoides* belongs to the *Bacillus cereus sensu lato* group, a taxonomically complex group that includes both well-known pathogens like *B. anthracis* (the agent of anthrax) and *B. cereus* (a common cause of foodborne illness), alongside beneficial and commercially approved probiotic species such as *B. toyonensis* (Pohanka 2020; Jovanovic et al. 2021). This close taxonomic proximity necessitates a rigorous, multi-faceted safety assessment to differentiate beneficial strains from their pathogenic relatives a critical and non-negotiable step for regulatory approval and commercial viability.

Phylogenetic analysis revealed a safety screen, clearly separating isolate B10 from the *B. anthracis* and *B. cereus sensu stricto* clades. However, the high genetic similarity and horizontal gene transfer potential within the *B. cereus* group mean that a definitive safety evaluation cannot rely on 16S rRNA sequencing alone. The key distinction between pathogenic and non-pathogenic strains lies in the presence or absence of specific virulence factor genes. Pathogenic *B. cereus* strains typically carry genes encoding emetic toxins (the *ces* gene cluster, producing cereulide) or a suite of diarrheal enterotoxins (e.g., *nhe*, *hbl*, *cytK*) (Fatma et al. 2023), which are pore-forming proteins that disrupt intestinal epithelial cells. The promising antimicrobial activity of our B10 isolate must therefore be interpreted with this taxonomic caution. Our findings are supported by recent research that has increasingly validated the probiotic potential of *B. paramycoides* when properly characterized. For example, Firmani et al. (2023) found that a *B. paramycoides* strain from milkfish GIT improved fish growth performance and lacked hemolytic activity, a common proxy test for cytotoxicity. Similarly, a comprehensive genomic study by Magome et al. (2025) analyzed multiple *B. paramycoides* genomes and found that while some strains carried homologs of the *nhe* and *hbl* enterotoxin genes, they often lacked the complete gene clusters or key regulatory elements required for toxicity, designating them as safe. These studies provide crucial precedent, suggesting that non-pathogenic, beneficial strains of *B. paramycoides* exist and can be identified. Thus, while our in vitro results are highly promising, they must be followed by genomic analysis to confirm the absence of these specific virulence factors in isolate B10.

In conclusion, this research is the first to identify the gastrointestinal tract of the indigenous Vietnamese Xiem duck as a targeted source of *Bacillus* strains with potent, broad-spectrum antimicrobial properties. Isolate B10, unequivocally identified as *B. paramycoides*, emerged as a highly promising probiotic candidate due to its superior in vitro inhibitory activity against major poultry pathogens. However, its taxonomic position within the *B. cereus* group mandates a cautious and rigorous validation pathway forward. Future work must prioritize whole-genome sequencing to definitively confirm its genetic safety profile by screening for all known virulence-associated genes. Subsequently, controlled in vivo trials are essential to validate its efficacy and safety in the target host. This systematic, science-driven approach is critical to advance this promising isolate from a laboratory finding to a scientifically validated, regulatory-approved, and effective probiotic feed additive capable of enhancing the health and sustainability of the global poultry industry.

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