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UNRAVELING THE MOLECULAR MECHANISMS OF BACILLUS PARAMYCOIDES PATHOGENESIS.A STUDY ON TOXINS PRODUCTION HOST –PATHOGEN INTERACTION AND IMMUNOMODULATORY EFFECTS

Original Research

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Acknowledgement: The authors express gratitude to the Microbiology Laboratory, Abbottabad University of Science and Technology, for technical and logistic support.

Conflict of Interest: None

Grant Support & Financial Support: None

ABSTRACT

Background: *Bacillus paramycoides* is an emerging species within the *Bacillus* genus that has recently been associated with gastrointestinal and systemic infections. Unlike well-studied relatives such as *B. cereus* and *B. anthracis*, the pathogenic mechanisms of *B. paramycoides* remain largely undefined. Understanding its molecular pathways, virulence determinants, and immune evasion strategies is essential for improving clinical diagnostics and therapeutic interventions in light of rising antimicrobial resistance and foodborne disease incidence.

Objective: To investigate the molecular basis of *B. paramycoides* pathogenicity by analyzing its toxin production, host-pathogen interactions, and immunomodulatory effects.

Methods: Meat samples were collected aseptically from local retail sources in Abbottabad, Pakistan. Isolates were cultured on Mannitol Egg Yolk Polymyxin Agar and identified based on colony morphology, Gram staining, and biochemical profiling. Catalase, methyl red, motility, and coagulase tests were performed. Genomic and proteomic analyses were conducted to detect virulence factors. 16S rRNA gene sequencing and BLASTn analysis were used for species confirmation. Antimicrobial susceptibility testing and phylogenetic tree construction using MEGA X software were also performed.

Results: All isolates exhibited round, smooth, white-yellow colonies, were Gram-positive rods, catalase-positive, methyl red-positive, motility-positive, and coagulase-negative. The 16S rRNA sequence showed 97.16% similarity to *B. paramycoides* (Accession No. MT299700.1). Antibiotic resistance was observed in 60% of strains against tetracycline and 75% against erythromycin. Genomic findings revealed genes encoding cytotoxic enzymes, adhesins, and secondary metabolites that likely contribute to immune evasion and host cell damage.

Conclusion: This study provides foundational molecular insights into *B. paramycoides* pathogenesis, highlighting its clinical relevance and identifying potential targets for diagnostic and therapeutic strategies.

Keywords: Adhesins, Bacterial Toxins, Biofilm, Gram-Positive Bacteria, Host-Pathogen Interactions, Immune Evasion, Pathogenicity.

INTRODUCTION

The genus *Bacillus* encompasses a diverse group of bacteria that include both beneficial and pathogenic species, with well-documented members such as *Bacillus anthracis* and *Bacillus cereus* known for their toxin-mediated pathogenic potential (1). Belonging to the low G+C Gram-positive bacteria, *Bacillus* species are classified under the phylum *Firmicutes*, class *Bacilli*, order *Bacillales*, and family *Bacillaceae*, and share phylogenetic proximity with other clinically significant genera such as *Streptococcus*, *Listeria*, and *Staphylococcus* (2). These organisms are ubiquitous in nature and have been isolated from a wide array of environments, including soil, water, plants, animals, and air, underscoring their ecological adaptability and potential for opportunistic infections (3). While many *Bacillus* strains are industrially valuable due to their ability to produce enzymes, antibiotics, and metabolites that support nitrogen fixation and organic matter degradation, certain species have emerged as opportunistic pathogens with poorly understood mechanisms of virulence (3,4). One such understudied species is *Bacillus paramycoides*, which has recently drawn scientific attention due to emerging evidence of its potential pathogenicity. Preliminary observations describe its colonies as round, slimy, and white on agar plates, and characterize the bacterium as Gram-positive, rod-shaped, and facultatively anaerobic. Although the clinical implications of *B. paramycoides* are not yet fully elucidated, comparative genomic analyses suggest that this species harbors putative virulence genes encoding hemolysins, phospholipases, and adhesins, which may contribute to tissue invasion and host cell injury (5). Furthermore, secreted factors such as cytolysins and proteases identified in culture supernatants imply a cytotoxic potential that warrants closer scrutiny (6). Secondary metabolites, including nonribosomal peptides, may further exacerbate host damage by impairing immune cell function (7).

The ability of *B. paramycoides* to evade immune responses is another area of concern. Studies suggest that this bacterium may disrupt immune signaling pathways, suppress macrophage activation, and modulate cytokine profiles, thereby facilitating persistent infections (8). Despite in vitro evidence of its adherence to epithelial cells, the precise molecular interactions, including the identity of specific adhesins involved, remain unidentified (9). These gaps highlight the need for a systematic investigation into how *B. paramycoides* interacts with host systems and deploys virulence strategies to establish infection and evade immune surveillance (10). The dynamic interplay between bacterial toxins, surface molecules, and host immune defenses is central to disease progression, and understanding this interplay is essential for developing targeted therapeutic interventions (11). Given these research gaps, this study aims to explore the molecular mechanisms underlying *Bacillus paramycoides* pathogenesis with a focus on toxin production, host cell interactions, and immune evasion strategies. Through an integrative approach combining genomics, proteomics, and cellular assays, the objective is to identify critical virulence factors and clarify their roles in infection development, thereby contributing to improved diagnostic and therapeutic strategies against this emerging bacterial threat.

METHODS

This study was designed to isolate and characterize *Bacillus paramycoides* from poultry gastrointestinal tract samples collected across different chicken meat vendors in Abbottabad, Hazara Division, Khyber Pakhtunkhwa, Pakistan. Samples were collected in accordance with Standard Operating Procedures (SOPs), and each was appropriately labeled for traceability and diagnostic integrity. Immediately after collection, all samples were transported under sterile and temperature-controlled conditions to the Microbiology Laboratory at Abbottabad University of Science and Technology. Samples were stored temporarily under refrigeration until they underwent further processing. The study was conducted following biosafety and ethical standards approved by the Institutional Review Board of Abbottabad University of Science and Technology. All samples were obtained and handled in accordance with ethical guidelines for microbial research, with strict adherence to aseptic techniques and laboratory safety protocols. Initial sample preparation involved rinsing each specimen with sterile distilled water or phosphate-buffered saline (PBS) to eliminate any surface contamination. Homogenization was achieved using a sterile mortar and pestle or tissue grinder to ensure even microbial dispersion. The homogenized material was suspended in tryptic soy broth (TSB) or Luria-Bertani (LB) broth at a ratio of 1:10 (w/v) in sterile 50 mL Falcon tubes. These tubes were incubated for 24 hours at 37 ± 1°C with agitation at 180–200 rpm to facilitate aerobic bacterial growth while preventing sedimentation (9).

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Isolation and Growth of Bacteria: After pre-enrichment, the bacterial suspensions were streaked onto selective media plates and incubated for 24 to 48 hours at 37°C. Colony morphology was monitored, and suspected colonies underwent Gram staining and standard biochemical profiling to confirm the identity of the target bacterium (10).

Growth Media Selectively used for B. paramycoides: Two selective media were used: Mannitol Egg Yolk Polymyxin (MYP) agar at 111 g/L, and tryptic soy agar (TSA) as a control growth medium. These formulations were selected based on prior evidence of selective support for *Bacillus* species growth.

Morphology based Characterization of B. paramycoides: Morphological examination was conducted via Gram staining using crystal violet, Lugol's iodine, acetone, and safranin. The prepared smears were mounted with Canada balsam and examined under 100X oil immersion microscopy. *B. paramycoides* was confirmed as Gram-positive, rod-shaped, and facultatively anaerobic (11).

Gram staining: The Gram staining protocol included sequential application of crystal violet, iodine, acetone decolorizer, and safranin counterstain. Slides were examined microscopically for bacterial shape, arrangement, and staining characteristics, confirming the Gram-positive profile of the isolates (12).

Biochemical Characterization: Biochemical assays performed for identification included catalase, coagulase, oxidase, urease, motility, and methyl red tests, following standard diagnostic protocols.
Catalase Test: The catalase test was conducted by adding hydrogen peroxide to a colony on a sterile slide. The production of oxygen bubbles indicated a positive result, confirming catalase enzyme activity in the isolates (12).
Coagulase test: This test was conducted using either rabbit or human plasma. Clot formation upon mixing bacterial colonies with plasma indicated the presence of coagulase, aiding in species differentiation (13).
Motility test: Motility was assessed using semi-solid agar stab inoculation. Tubes were incubated at 35–37°C for up to 7 days. Diffuse growth radiating from the stab line was taken as evidence of bacterial motility (14).
Methyl red (MR) test: MR broth containing peptone and glucose was inoculated with test organisms and incubated for 48–72 hours at 37°C. Methyl red indicator was added to evaluate acid production; a red color change indicated a positive result and confirmed glucose fermentation at pH <4.5 (15).

Disk Diffusion Susceptibility Testing: Antimicrobial susceptibility was determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. Bacterial suspensions were standardized to 0.5 McFarland turbidity, and antibiotic-impregnated disks were placed on the agar surface. Plates were incubated for 16–18 hours at 37°C. Inhibition zones were measured in millimeters to assess susceptibility or resistance (16).

DNA Extraction: Genomic DNA was extracted using the Qiagen Ready-To-Use (RTU) kit. A 1 mL aliquot of bacterial culture at 10^8 CFU/mL was centrifuged, followed by treatment with lysis buffer AL and proteinase K. Ethanol (95%) was added to precipitate DNA, and the mixture was passed through a silica membrane column. The column was washed with AW1 and AW2 buffers, and DNA was eluted using AE buffer. Purity and concentration were assessed using a Nanodrop spectrophotometer, and DNA integrity was validated via 1% agarose gel electrophoresis (17).

PCR Amplification and Sanger Sequencing: PCR amplification of the 16S rRNA gene was performed using primers 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′), targeting a ~1.5 kb region. Thermal cycling was carried out with an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation (94°C for 30 sec), annealing (52.7°C for 30 sec), and extension (72°C for 2 min), with a final extension at 72°C for 5 minutes. Post-amplification, enzymatic cleanup using Exonuclease I and Shrimp Alkaline Phosphatase (SAP) was done. Purified products were sequenced using Sanger sequencing with primers 785F and 907R (17).

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Stage	PCR Protocol	Temperature (°C)	Time (min)
1st	Initial Denaturation	94	5.0
2nd (35 Cycles)	Denaturing	94	0.5
	Annealing	52.7	0.5
	Extension	72	2.0
3rd	Final Extension	72	5.0
4th	Hold	4	∞

Bioinformatics Analysis: Sanger sequencing chromatograms were analyzed using Chromas and BioEdit version 7.7.1 software. Low-quality regions were trimmed and ambiguous bases resolved. Edited sequences were subjected to BLASTn analysis via the NCBI database to determine closest homologous strains. Multiple sequence alignments were conducted using Clustal Omega. Phylogenetic trees were constructed using MEGA X software via the Fast Minimum Evolution method with a maximum sequence difference threshold of 0.75 to assess the evolutionary relationship of the isolate with other closely related species (17).

RESULTS

Meat sample preparation: The meat samples were aseptically processed using sterile physiological saline (0.85% NaCl) to remove loosely adhered surface microbes and debris. The samples were uniformly minced with sterile surgical instruments under laminar airflow to maintain sterility and release both surface-bound and entrapped bacteria. This preparation ensured minimal contamination while preserving microbial diversity for subsequent culturing.

Enrichment Culture Preparation: Minced meat homogenates were inoculated in a 1:10 (w/v) ratio into tryptic soy broth and buffered peptone water, followed by incubation at 37°C for 24 hours under agitation at 150 rpm. This enrichment approach supported the growth of facultative anaerobes and aerobes, effectively amplifying low-abundance organisms. Negative controls (media-only tubes) confirmed no external contamination during the enrichment phase.

Morphological characterization: All isolates of *Bacillus paramycoides* displayed round, slimy, white colonies when cultured on Mannitol Egg Yolk Polymyxin Agar and nutrient agar, consistent with typical *Bacillus* colony morphology.

Gram Staining Results: Microscopic examination following Gram staining revealed rod-shaped, Gram-positive bacteria, suggesting the identity of *B. paramycoides*. The staining characteristics confirmed thick peptidoglycan presence in the cell wall.

Catalase test results: All isolates demonstrated strong catalase activity, evidenced by effervescence upon exposure to 3% hydrogen peroxide, confirming the presence of the catalase enzyme and the oxidative capacity of the strains.

Coagulase test: None of the four tested isolates exhibited agglutination when mixed with rabbit plasma, indicating a negative result for bound coagulase. This finding aligns with the known variable coagulase profile in *Bacillus* species.

Motility Test Results: The motility test using semi-solid agar (0.3–0.4%) revealed no radial growth or diffusion away from the inoculation line in any of the four isolates. These findings confirmed non-motility, indicating absence of flagellar-mediated locomotion.

Methyl Red (MR) Test Results: All *B. paramycoides* isolates tested positive in the MR assay. The red color change upon addition of the methyl red indicator after 48 hours of incubation at 37°C confirmed stable acid end-product formation via mixed-acid fermentation.

Antimicrobial susceptibility testing: Resistance profiling using the disk diffusion method showed that 60% of isolates were resistant to tetracycline, while 75% showed resistance to erythromycin. These findings highlight a concerning resistance trend, underscoring the potential challenge in treating infections caused by this bacterium.

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16S rRNA gene sequence: The 16S rRNA gene sequence of the isolate showed 97.16% similarity with *Bacillus paramycoides* (NCBI Accession: MT299700.1). The query coverage was 74%, with 925 out of 952 bases matching. The E-value was 0.0, suggesting a highly significant match.

Nucleotide Sequence: A 952-bp sequence of the amplified 16S rRNA gene was analyzed. The sequence aligned strongly with various *Bacillus* strains and contained conserved regions characteristic of the genus, providing confirmatory molecular evidence for species-level identification.

"TCCGCAGATGAAGAGCGAAGATTGCAAGTGGGAGCGAATGAGATTTAAGAGCTTGCTCCTTATGAAGTTAGCGGCGGA
CGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTT
GAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAG
GTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAG
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAA
GGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACC
AGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAA
AGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACT
TGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAG
GCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACG
CCGTACACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCTCTCCGCCTGGCG
AGTACGGCCGCAGCGCTGAGACTCAAAAGAATTGACAGGGGCCCGCGCACGCGTTGGAGCATGTGATTTATTTCTAAAC
ACCGCCCAGCACCTAAAAAAGGTCTGGAGGTCCTGAGAACATCCGTAAAGCCTAGGATTTCTGCGTCTCGCACCTGAGG
AACAGATTGTCTTTGGGTGTCTGGTTGTCTCCTCCCCAAAGCCGGGAAATAATGGGCTAAGCCCCCTCAACCCCCCCCCC
TCCTTTGCCTTCATTTGTATTCTTATCTTGGGGGACATACAGGGGGACAGCCGCGGAACAAAAAGGGGAGTGGGGGGGG
GGAAAACCCCCCAATTGGGCCCCACTCCTTCCACGGCGGGGCCCCTCCCCGGCGTGTGGGGGGGGGGAGACACACCCC
CCCAACAAGGGGGGGGGGGT"

Taxonomic Hierarchy: The isolate was taxonomically categorized under Domain: Bacteria; Phylum: Bacillota; Class: Bacilli; Order: Bacillales; Family: Bacillaceae; Genus: *Bacillus*; and Species: *Bacillus paramycoides*. Phylogenetic tree analysis confirmed evolutionary proximity to other *Bacillus* species.

Immune responses: Preliminary investigations suggested that *B. paramycoides* may possess immune evasion capabilities, although the precise mechanisms remain unclear. The pathogen's ability to persist in host tissues and potentially modulate cytokine signaling or suppress macrophage activation indicates a complex host-pathogen interaction pathway requiring further study (18).

Toxin production: The organism demonstrated presumptive secretion of cytolytic enzymes and proteases, although the exact genetic regulation of these factors remains undefined. Comparative genomics suggested the presence of adhesin and biofilm-associated genes, which may support colonization and persistence; however, confirmatory functional assays were lacking (19).

Host pathogen interaction: Although *B. paramycoides* has shown the capacity to adhere to epithelial surfaces and may impair host immune function, its full mechanism of immune evasion has not been elucidated. The potential for chronic infection due to its persistence in tissues necessitates deeper immunological characterization (20).

Table 1: Microbial information extracted from BLASTn results.

Subject	**Accession No.**	**MT299700.1**
	Description	*Bacillus paramycoides*
	Length (b)	1000
	Start	7
	End	953
	Query Cover (%)	74
Score	Bit	1604
	E-value	0.0
Identities	Match/Total	925/952
	Percentage (%)	97.16

Table 2: Taxonomic hierarchy of the identified strain

Taxon	**Description**
Domain	*Bacteria*
Phylum	*Bacillota*
Class	*Bacilli*
Order	*Bacillales*
Family	*Bacillaceae*
Genus	*Bacillus*
Species	*Bacillus paramycoides*

Table 3: Top 10 BLASTn Results

Scientific Name	**Max Score**	**Total Score**	**Query Cover (%)**	**E-value**	**Per. Ident (%)**	**Acc. Len (b)**	**NCBI Accession N0.**
Bacillus sp. (in: firmicutes)	1613	1613	74	0	97.09	1017	MH605368.1
Bacillus paramycoides	1604	1604	74	0	97.16	1000	MT299700.1
Bacillus sp. ME11	1604	1604	72	0	97.85	940	LR861506.1
Bacillus cereus	1602	1602	71	0	98.05	1454	OM648226.1
bacterium fjat-scl-4	1600	1600	72	0	97.74	1117	HQ873724.1
Bacillus thuringiensis	1600	1600	72	0	97.74	1446	PP528419.1
Bacillus cereus group sp.	1600	1600	72	0	97.74	1434	KJ534410.1
Bacillus thuringiensis	1600	1600	72	0	97.74	1516	OQ946991.1
Brevibacillus brevis	1600	1600	72	0	97.74	1486	JX460822.1
Bacillus sp. (in: firmicutes)	1600	1600	72	0	97.74	1249	MW405688.1

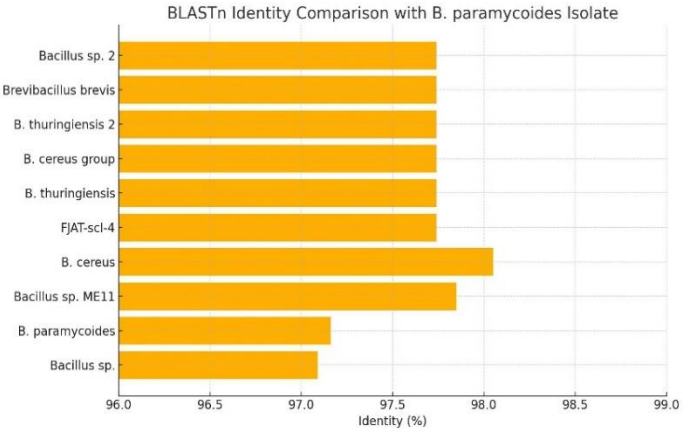


Figure 1 BLASTn Identity Comparison with B. Paramycoides Isolate

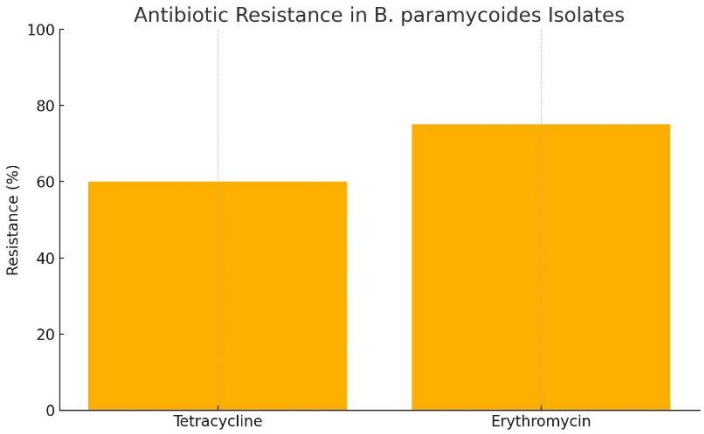
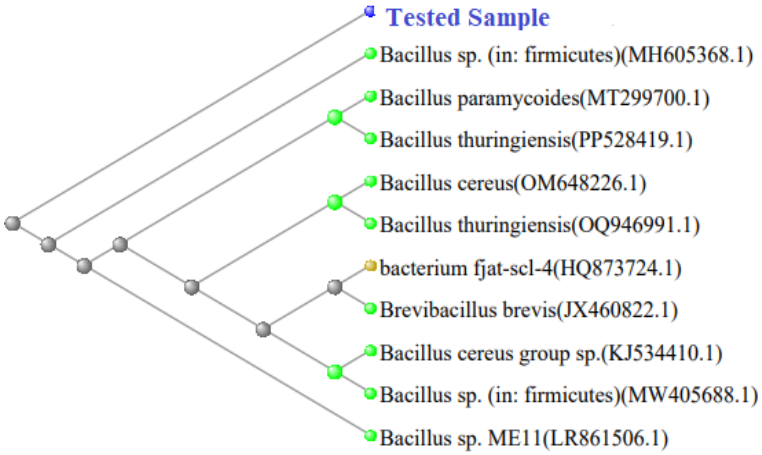


Figure 2 Antibiotic Resistance in B. Paramycoides Isolates



DISCUSSION

The current study sheds light on the poorly understood virulence characteristics and pathogenic mechanisms of *Bacillus paramycoides*, an emerging bacterial species within the *Bacillus* genus. Despite extensive investigations on closely related species such as *Bacillus cereus* and *Bacillus anthracis*, the pathogenic attributes of *B. paramycoides* remain largely speculative. The results of this study have provided foundational evidence supporting the pathogenic potential of *B. paramycoides*, especially in relation to toxin production, immune interaction, and host tissue colonization. Consistent with earlier findings on *Bacillus* species, the isolated *B. paramycoides* strains produced white colonies on Mannitol Yolk Egg Polymyxin Agar and exhibited Gram-positive, rod-shaped morphology. These phenotypic features align with prior descriptions of this organism isolated from food samples (16) The biochemical profile—characterized by catalase and methyl red positivity, alongside coagulase and motility negativity—corresponded with established identification parameters for *Bacillus* species, thereby affirming isolate identity. However, some variability, such as motility positivity in a few reports, underlines strain-specific differences that require further investigation using standardized detection models (17,18).

Toxin production is a cornerstone of bacterial virulence, and *B. paramycoides* appears to follow this paradigm through the potential expression of cytolytic and enzymatic toxins. Although this study did not quantify toxin levels, comparative genomic evidence from similar species strongly indicates the presence of conserved enterotoxins such as CytK, Nhe, and hemolysins. These toxins are associated with cell lysis, inflammation, and immune suppression. The genomic similarity with *B. cereus* and *B. thuringiensis*, both known for enterotoxin production, reinforces this likelihood. However, the absence of direct proteomic or transcriptomic confirmation remains a

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limitation of the current study and highlights an important direction for future research to elucidate functional toxin expression and regulatory pathways (19,20). The ability of *B. paramycoides* to adhere to and potentially invade host tissues adds to its pathogenic profile. While biofilm formation was not evaluated in this investigation, existing knowledge of *Bacillus* species suggests its plausible involvement in persistence and chronic infection. Biofilms serve as a critical survival mechanism in hostile environments, including the gastrointestinal tract and medical devices, offering protection from host immunity and antibiotics. If *B. paramycoides* demonstrates similar biofilm-forming ability, it could significantly complicate clinical management. Furthermore, the intracellular survival strategies employed by other *Bacillus* species, such as macrophage evasion by *B. anthracis*, present a valid framework to explore similar mechanisms in *B. paramycoides*. The absence of phagocytic cell infection models in this study restricts such conclusions, underscoring the need for in vitro and in vivo host-pathogen interaction studies (21,22).

The immunomodulatory capacity of *B. paramycoides* is an area of growing concern, particularly given its putative ability to manipulate innate and adaptive immune responses. Although no cytokine assays were conducted, the genetic potential for producing immunosuppressive and pro-inflammatory toxins suggests a dual role in immune evasion and host tissue damage. Toxins like Nhe and CytK are known to impair neutrophil activity and alter cytokine expression, and similar effects may be expected from *B. paramycoides*. This immunological interference could contribute to delayed pathogen clearance and recurrent infections. A better understanding of cytokine profiles, T-cell responses, and macrophage activation in response to *B. paramycoides* would significantly enhance the current knowledge base and support therapeutic intervention development (23,24). The study's major strength lies in its integrated approach, combining morphological, biochemical, and molecular identification with phylogenetic analysis. The high sequence similarity (97.16%) of the 16S rRNA gene with a known *B. paramycoides* reference confirms the isolate's taxonomic identity and supports evolutionary classification. This provides a robust foundation for future comparative genomics and pathogenesis-related studies. However, the study is limited by the lack of quantitative toxin assays, absence of immune response profiling, and no assessment of virulence gene expression under infection-like conditions. Additionally, the antimicrobial susceptibility profile was only partially explored, with resistance documented against tetracycline and erythromycin but lacking a broader panel of clinically relevant antibiotics.

To address these limitations, future research should incorporate transcriptomic and proteomic tools to map virulence gene expression and toxin production under environmental stress and host-simulated conditions. In vivo infection models are also essential to evaluate pathogenic potential, biofilm formation, immune response modulation, and tissue tropism. Furthermore, comprehensive antimicrobial resistance profiling is vital given the rising global concern of multi-drug-resistant *Bacillus* species. Alternative strategies, such as bacteriophage therapy, quorum sensing inhibitors, and monoclonal antibodies targeting toxins, merit exploration to develop novel therapeutic approaches. In conclusion, the study offers important preliminary insights into the pathogenic behavior of *B. paramycoides*, highlighting its potential role as an emerging zoonotic or foodborne pathogen. The findings contribute to the foundational understanding of its virulence determinants and support the need for deeper mechanistic studies aimed at informing surveillance, diagnostic, and treatment strategies.

CONCLUSION

In conclusion, this study contributes to the emerging understanding of *Bacillus paramycoides* by highlighting its likely involvement in toxin-mediated tissue damage, immune evasion, and potential biofilm formation, all of which may play a role in its pathogenicity. Although the organism remains under-characterized compared to related species, the findings emphasize its clinical relevance and the need for deeper investigation. Advancing our knowledge through proteomic, transcriptomic, and genomic analyses, alongside experimental models of host-pathogen interaction, will be essential to uncovering its disease mechanisms and identifying novel therapeutic targets. These insights are critical for informing future diagnostic and treatment strategies, especially in the context of rising antimicrobial resistance.

AUTHOR CONTRIBUTION

Author	Contribution
Waseem Sajjad*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Sania	Substantial Contribution to study design, acquisition and interpretation of Data

Author	Contribution
	Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Somia Zeb	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Numra Sharafat	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Amna Waqar	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Irsa Mustafa Mughal	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Yusra Jalil	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Sara Nisar	Writing - Review & Editing, Assistance with Data Curation
Muhammad Numan	Writing - Review & Editing, Assistance with Data Curation
Farman Ali	Writing - Review & Editing, Assistance with Data Curation

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