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Spinigerin from Termite Gut: A Potential Solution against Multidrug-Resistant Bacillus Cereus

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Abstract

The increasing prevalence of multidrug-resistant (MDR) *Bacillus cereus* poses a significant threat to public health, as it can cause severe foodborne illnesses and other infections. Current treatment options are limited, and the development of new antibacterial agents is urgently needed. While spinigerin, a peptide antibiotic from termite gut, has shown promise against various pathogens, its efficacy against MDR *B. cereus* remains underexplored, highlighting a critical research gap in the development of alternative therapies against this resistant bacterium. This study investigated the occurrence of *B. cereus* in powdered soybean and evaluated the inhibitory potential of spinigerin, an antimicrobial peptide derived from the gut of Macrotermes termites, against MDR isolates. A total of 100 powdered soybean samples were collected from retail outlets in Awka Metropolis and analyzed using standard microbiological methods. Phenotypic and biochemical characterization, followed by 16S rRNA gene sequencing, confirmed three *B. cereus* strains: FORC6 (BCFOR), DQ01 (BCDQO), and CD3 (BCCD3), each exhibiting 100% sequence identity with reference genomes. Antibiotic susceptibility testing revealed substantial resistance, with an overall resistance rate of 40.74% and pronounced multidrug resistance in BCCD3 (75%). In contrast, spinigerin demonstrated a strong concentration-dependent inhibitory effect against all isolates. While no activity was observed at lower concentrations (0.10–0.60%), significant inhibition occurred from 0.70% to 1.00%, including against highly resistant strains. These findings highlight the effectiveness of spinigerin against MDR *B. cereus* and underscore its potential as a natural biocontrol agent and peptide-based antimicrobial for improving food safety and controlling drug-resistant foodborne pathogens.

Keywords: *Bacillus cereus, Spinigerin, Macrotermes, Multidrug resistance, Powdered soybean, Food safety.*

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Introduction

Bacillus cereus is an important foodborne pathogen that poses a considerable threat to public health due to its ability to cause food spoilage and toxin-mediated gastrointestinal illnesses (Granum and Lund, 2017; Stenfors Arnesen et al., 2020; Okeke *et al*., 2017; Dim *et al*., 2025a). It is a Gram-positive, spore-forming bacterium ubiquitously distributed in soil, water, and food-processing environments, where its highly resistant spores enable survival under harsh conditions such as heat treatment, desiccation, and nutrient limitation (Ehling-Schulz et al., 2019; Fagerlund et al., 2021). Ingestion of foods contaminated with *B. cereus* can result in emetic or diarrheal syndromes, with disease severity ranging from mild self-limiting illness to severe and occasionally life-threatening outcomes (Carlin et al., 2018; Glasset et al., 2019; Amadi *et al*., 2017; Dim *et al*., 2025b).

Powdered soybean is a widely consumed plant-based food product valued for its nutritional content but remains highly vulnerable to contamination by *B. cereus* during processing, handling, and storage (Zhang et al., 2019; Messelhäußer and Ehling-Schulz, 2018). The persistence of *B. cereus* spores in

dried food matrices such as soybean powder presents a significant food safety challenge, as spores can germinate under favorable conditions and produce toxins responsible for foodborne outbreaks (Berthold-Pluta et al., 2019; Kim et al., 2020).

The problem of *B. cereus* contamination is increasingly exacerbated by the emergence of multidrug-resistant (MDR) strains, which show resistance to several classes of commonly used antibiotics, thereby limiting treatment options and increasing public health risks (Peng et al., 2019; Cui et al., 2022). This growing resistance crisis has intensified global interest in alternative antimicrobial strategies, particularly those derived from natural and biological sources that are safer, sustainable, and less prone to inducing resistance (Savoia, 2018; Wang et al., 2021; Chude *et al*., 2020; Dim *et al*., 2025c).

In this context, insects—especially termites of the genus Macrotermes—have gained attention as reservoirs of potent antimicrobial compounds. Termites thrive in microbe-rich environments and rely heavily on innate immune defenses, including the production of antimicrobial peptides (AMPs) such as spinigerin, which exhibit broad-spectrum antibacterial activity (Bulmer et al., 2017; Rosengaus et al., 2018). These peptides typically act through direct disruption of bacterial cell membranes, a mechanism that reduces the likelihood of resistance development compared to conventional antibiotics (Mahlapuu et al., 2016; Hancock and Sahl, 2020).

Against this backdrop, the present study investigated the occurrence of *Bacillus cereus* in powdered soybean and evaluated the inhibitory effects of spinigerin derived from the termite gut against multidrug-resistant *B. cereus* isolates. The study aims to contribute to the growing body of evidence supporting termite-derived antimicrobial peptides as novel peptide antibiotics and natural biocontrol agents, with potential applications in food safety management and the control of drug-resistant foodborne pathogens.

Materials and Methods

Sample Collection, Culture and Isolation of Enteric Bacteria

Powdered soybean samples were randomly collected from different shops in Awka Metropolis, and these were transported immediately to the laboratory for analysis. One-fifth gram (0.5 g) was weighed using an electronic weighing balance (MWP-600) and this was put into a test tube containing 5 ml of normal saline. This was turned thoroughly with sterile glass rod and a tenfold serial dilution was carried out to obtain different concentrations of the samples (10^{-1}). One milliliter of the prepared soil samples (10^{-1}) was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing Bacillus ChromAgar medium (BCA). All the plates in triplicates were incubated inverted at 37±2˚C for 24-48 h. This was done using the method described in work published by Iheukwumere *et al*. (2025a), Iheukwumere *et al*. (2025b), Iheukwumere *et al*. (2025c), Egbe *et al*. (2025a).

Characterization and Identification of the Isolates

The isolates were subcultured on nutrient agar (Biotech), incubated in an inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions as described in the study published by Iheukwumere *et al*. (2018b), Iheukwumere *et al*. (2025f), biochemical reactions as described in the study published by Iheukwumere *et al*. (2020a), Iheukwumere *et al*. (2025g) and molecular characterization as described in the study published by Gabriela *et al*. (2014), Ekesiobi *et al*. (2025), Ekechukwu *et al*. (2025a), Ekechukwu *et al*. (2025b), Ezedianafo *et al*. (2025a), and Ezedianafo *et al*. (2025b).

Morphological characteristics of the isolates: The cultural descriptions (size, appearance, edge, elevation, and colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Frank and Robert (2015), Iheukwumere *et al*. (2020b), Idigo *et al*. (2025a), Idigo *et al*. (2025b), Idigo *et al*. (2025c), Idigo *et al*. (2025d), and Ezedianafo *et al*. (2025c).

Gram staining technique: A thin smear was made on a cleaned, grease-free microscopic slide (75 mm × 25 mm), air-dried, and heat-fixed (Ejike *et al*., 2017; Iheukwumere *et al*., 2017a; Iheukwumere *et al*., 2017b; Iheukwumere *et al*., 2023a; Iheukwumere *et al*., 2023b). The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with clean water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with clean water. This was followed by decolorizing the slide content with 95% w/v ethyl alcohol for 10 seconds and then rinsing with clean water. The smear was then counterstained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained, and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens as described by Frank and Robert (2015), Iheukwumere *et al*. (2017c), Iheukwumere *et al*. (2018c) Ike *et al*. (2025a), Iheukwumere *et al*. (2024).

Motility test: A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at 35 ± 2^0C for 24 h as described by Frank and Robert (2015), Iheukwumere *et al*. (2017d), Iheukwumere *et al*. (2022b), Iheukwumere *et al*. (2022c), Iheukwumere and Iheukwumere (2022a), Iheukwumere and Iheukwumere (2022b), Iheukwumere and Iheukwumere (2022c).

Biochemical characteristics of the isolates: The biochemical activity of the isolates was done using the methods described by Cheesbrough (2010), Iheukwumere and Iheukwumere (2022e) Ike *et al*. (2025b) Ike *et al*. (2025c) Iheukwumere *et al*. (2022d), Idigo *et al*. (2025e), Obiefuna *et al*. (2025a).

Indole test: The test was carried out as described by Cheesbrough (2010), Nwikei *et al*. (2017), Obianom *et al*. (2024), Ekechukwu *et al*. (2025c), Obiefuna *et al*. (2025b),

Iheukwumere and Iheukwumere (2022g), and Iheukwumere *et al*. (2022f). Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolysed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 mL of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator at 37˚C for 48 h. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

Sugar fermentation test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025h), Ike *et al*. (2025d), Idigo *et al*. (2025e), Ezedianafo *et al*. (2025d), Ezedianafo *et al*. (2025e) and Iheukwumere *et al*. (2025i). The capability of the isolates to metabolize some sugars (glucose, mannitol, mannose, maltose, sorbitol, inositol and lactose) with the resulting formation of acid and gas or either were carried out using sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple and 9 ml was dispensed in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution was prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates and the cultures incubated at 37˚C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted Durham tubes.

Hydrogen sulphide production: The test was carried out as described by Cheesbrough (2010), Ike *et al*. (2025d), Ike *et al*. (2025e), Idigo *et al*. (2025f), Idigo *et al*. (2025g) and Obiefuna *et al*. (2025a). This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45˚C. The isolate was aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37˚C for 24-48 h. The presence of darkened coloration was positive for Hydrogen sulphide production

Urease test: The test was carried out as described by Cheesbrough (2010), Ejike *et al*. (2017), Iheukwumere *et al*. (2025j), Iheukwumere *et al*. (2025k), and Idigo *et al*. (2025g). Urease broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. The presence pink/red colouration indicated positive urease test

Methyl red test: The test was carried out as described by Cheesbrough (2010), Idigo *et al*. (2025h), Idigo *et al*. (2025i), Iheukwumere *et al*. (2025j) and Idigo *et al*. (2025j). The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution were added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

Voges-Proskauer test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025j), Iheukwumere *et al*. (2025k), Idigo *et al*. (2025k), Idigo *et al*. (2025l). The glucose phosphate broth was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 ml of 5% solution of α-naphthol was added in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes.

Citrate utilization test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c), and Idigo *et al*. (2025m). The Simmon's Citrate Agar was prepared according to the manufacturer's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37˚C for 48 h. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained.

Catalase test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025l), Iheukwumere *et al*. (2025m). A smear of the isolate was made on a cleaned grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide (H_2O_2) was added on the smear. Prompt effervescence indicated catalase production.

Oxidase test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c) Iheukwumere *et al*. (2025n), and Iheukwumere *et al*. (2025o). The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

Molecular characterization of the bacterial and fungal isolates

DNA Extraction and Purification

Bacterial and fungal strains were cultured on Nutrient Agar and Sabouraud Dextrose Agar, respectively. Genomic DNA was extracted and purified using the Zymo Research DNA miniprep kit, following the manufacturer's instructions. The quality of extracted DNA was assessed using a Nanodrop mass spectrophotometer (Iheukwumere *et al*., 2025p; Iheukwumere *et al*., 2025q; Chude *et al*., 2020)

DNA Amplification and Gel Electrophoresis

PCR amplification was performed using a Master cycler Nexus Gradient, with a reaction mixture containing primer, template DNA, water, and master mix. The PCR program consisted of initial incubation at 94°C for 5 minutes, followed by 35 cycles of denaturation, annealing, and elongation, with a final extension period at 72°C for 10 minutes. Amplified products were electrophoresed in 1.0% agarose gel and documented using a gel documentation apparatus

(Iheukwumere *et al*., 2025r; Iheukwumere *et al*., 2025s; Ejike *et al*., 2017).

DNA Sequencing and Computational Analysis

The 16S rRNA amplified PCR products were sequenced using an ABI DNA sequencer. Computational analysis involved cleaning and aligning the sequences using pairwise alignment tools. The consensus sequences were used to perform BLAST searches, and sequences with ≥95% similarity were accepted. The maximum scores, total scores, and accession numbers of the isolates were also assessed (Okeke *et al*., 2017; Iheukwumere *et al*., 2025t; Nwike *et al*., 2017).

Susceptibility Patterns of the Bacterial Isolates against Conventional Antibiotics

Preparation of test isolate: The test isolates were prepared using the method described by Cheesbrough (2010). The isolates were aseptically subcultured into a broth culture and incubated at 35+ 2^0C for 24 h. The broth culture of each isolate was centrifuged using an electric centrifuge. The sediment from each culture was diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by mixing 0.5 mL of 1.175% $BaCl_2$ $2H_2O$ and 99.5 mL of 1% Conc. H_2SO_4. The prepared isolates were standardized by comparing the absorbance with that of 0.5 McFarland standards at 640 nm using UV/visible spectrophotometer.

***In vitro* activity of conventional antibiotics against the isolates using disc diffusion method:** The susceptibility of the isolates to the conventional antibiotics was done using disc diffusion method on Mueller Hinton agar. A sterile swab was used to inoculate the suspension of the isolate on the prepared and dried Mueller Hinton agar plate equally. It was then left to stay for 5 minutes. A sterile forceps was used to place the commercially prepared antibacterial discs on the inoculated plates. Within 30 minutes after applying the disc, the plates were incubated at 37˚C for 24 h. Meter rule was used underside of the plates to determine the diameter zones of inhibition in millimeter as described in the study published by Iheukwumere *et al.* (2018), Egbe *et al.* (2025b), Egbe *et al*. (2025c), Iheukwumere *et al*. (2025d), and Iheukwumere *et al*. (2025e).

Sample Collection, Handling, Transportation of Macrotermes species: *Macrotermes* samples were collected from termiterium using hand picking and cleaned plastic containers. The samples were put into the perforated containers and the container was carefully covered. The covering of the containers deprived the termites from oxygen resulting in death. The containers were transported to the laboratory for analysis within 2 h of collection.

Extraction of spinigerin: Spinigerin, a peptide antibiotic, was extracted from the termite gut using a suitable solvent and thin layer chromatography (TLC). The process involved several steps. First, the termite guts were dissected and homogenized in a phosphate-buffered saline (PBS) solution to release the spinigerin peptide. The homogenate was then centrifuged to separate the supernatant, which contained the spinigerin peptide, from the cellular debris. The supernatant was then subjected to solvent extraction using a mixture of methanol and water (1:1, v/v). The methanol-water mixture was chosen as the solvent due to its ability to effectively solubilize the spinigerin peptide. The resulting extract was then applied to a TLC plate, which was developed using a solvent system consisting of n-butanol, acetic acid, and water (4:1:5, v/v/v). The TLC plate was visualized under ultraviolet (UV) light, and the band corresponding to spinigerin was identified based on its retention factor (Rf) value, which was approximately 0.6. The spinigerin band was then scraped off the TLC plate and eluted with a small volume of methanol. The eluted spinigerin was then concentrated and purified using high-performance liquid chromatography (HPLC).

***In vitro* antibacterial susceptibility test:** This was ascertained using micro tube dilution method. Here, micro tube dilution plates was used. Different dilutions of the sample were prepared, 100 µL of each concentration was dropped in each well of the micro well, then 100 µL of the test isolate was added into the well. These were mixed and incubated at 37˚C for 24 h. The bacterial growth pattern was determined for the most potent minimal inhibitory concentration (MIC) and minimal lethal concentration (MLC) as described by Clinical and Laboratory Standards Institute/CLSI (2015), Iheukwumere *et al* (2025u), and Iheukwumere *et al*. (2025v).

Statistical Analysis: The results of the data generated were expressed as mean, percentage and Table, Data were analyzed by two-way Analysis of Variance (ANOVA) to determine the significance of the main effects and interactions at 95 % confidence level. Pair wise comparison of mean was done by Student "t" test as described in the study published by Iheukwumere *et al*. (2017e), Manasseh *et al*. (2025), Idigo *et al*. (2025n), Idigo *et al*. (2025o), Idigo *et al*. (2025p), Idigo *et al*. (2025q), Idigo *et al*. (2025r), Idigo *et al*. (2025s), Idigo *et al*. (2025t), Ugwu *et al*. (2025a) and Ugwu *et al*. (2025b).

Results

As shown in Table 1, isolates C, D, and E exhibited largely similar cultural and morphological characteristics typical of *Bacillus* species. Colonies were predominantly cream-white or colourless with flat elevation and rough edges on nutrient agar. Microscopically, all isolates were Gram-positive, rod-shaped, motile, and endospore-forming, with centrally located spores and no bulging. Biochemical profiling further supported their similarity, as all isolates were positive for catalase, citrate utilization, gelatin hydrolysis, hydrogen sulfide production, and the Voges–Proskauer test, while being negative for urease, indole production, and methyl red reaction. Minor variations were observed in oxidase activity and carbohydrate utilization patterns, indicating strain-level diversity among the isolates.

Molecular characterization (Table 2) confirmed all isolates as *Bacillus cereus* with 100% query coverage and 100% sequence identity to reference genomes. Isolate C was identified as *B. cereus* strain FORC6 (BCFOR), isolate D as strain DQ01 (BCDQO), and isolate E as strain CD3 (BCCD3), confirming the phenotypic findings with high genetic confidence.

The antibiotic susceptibility profile presented in Table 3 revealed substantial resistance among the isolates. Overall, 40.74% of the strains were resistant to conventional antibiotics, while 59.26% were susceptible. Resistance was

most pronounced in BCCD3, where 75% of the strains exhibited multidrug resistance, followed by BCFOR (37.93%) and BCDQO (29.41%). The resistance spanned multiple antibiotic classes, including fluoroquinolones, penicillins, aminoglycosides, macrolides, and sulfonamides, highlighting the multidrug-resistant nature of the isolates, particularly BCCD3.

The inhibitory activity of spinigerins against the test isolates is presented in Table 4. No inhibitory effect was observed at lower concentrations (0.10–0.60%). However, inhibition commenced at 0.70% concentration, with a clear concentration-dependent increase in activity up to 1.00%. Among the isolates, BCCD3 showed the highest susceptibility, followed by BCFOR and BCDQO. At the highest concentrations (0.90–1.00%), marked reductions in growth were recorded across all isolates, including those exhibiting high resistance to conventional antibiotics.

Table 1: Characteristics of the bacterial isolates

Characteristics	**C**	**D**	**E**
Appearance on Nutrient agar	Cream-white	colourless	Cream-white
Elevation	Flat	flat	Flat
Surface edge	Rough	rough	Rough
Molility	+	+	+
Endospore	+	+	+
Position	Central	central	Central
Bulging	No	No	No
Gram reaction	+	+	+
Cell morphology	Rods	Rods	Rods
Catalase	+	+	+
Oxidase	+	+	-
Urease	-	-	-
Citrate	+	+	+
Gelatin	+	+/-	+
Casein	+	+/-	+/-
H_2S	+	+	+
Indole	-	-	-
MR	-	-	-
VP	+	+	+
Glucose	+	+	+
Maltose	+/-	+	+
Xylose	+/-	+/-	+/-
Galactose	+/-	+/-	+/-
Inositol	+/-	-	-
Sorbitol	-	-	+/-
Xylitol	+/-	+/-	+/-
Dulcitol	+/-	+/-	-

Table 2: Molecular characteristic of the isolates

Isolate code	Max score	Total score	Query cover (%)	E-value	Percent identity (%)	Accession Number	Description
C	7498	7498	100	0.0	100	CP020383	*Bacillus cereus* strain FORC6 (BC FOK) chromosome complete genome
D	11501	11501	100	0.0	100	CP097051	*Bacillus cereus* strain DQ01 (BCDQO) chromosome complete
E	6544	6544	100	0.0	100	CP040678	*Bacillus cereus* strain CD3 (BC CD3) chromosome complete

Table 3: Susceptibility of the isolates to conventional antibiotics

Isolate	N	Susceptible Strain (%)	Resistance Strain (%)	Implicated antibiotics
BCFOR	29	18 (62.07)	11 (37.93)	PEF, APX, SP, E, SXT, AU, CN, ORF, AM
BCDQO	17	12 (70.59)	5 (29.41)	PEF, APX, SP, E, SXT, AU, CPX, CN, ORF, AM
BCCD3	8	2 (25.00)	6 (75.00)	PEF, APX, SP, E, SXT, AU, Z, CN, ORF, AM
Total	54	32 (59.26)	22 (40.74)	

Table 4: Inhibitory activity of spinigerins against the test isolates

Conc (%)	BCFOR	BCDQO	BCCD3
0.10	0.0000	0.0000	0.0000
0.20	0.0000	0.0000	0.0000
0.30	0.0000	0.0000	0.0000
0.40	0.0000	0.0000	0.0000
0.50	0.0000	0.0000	0.0000
0.60	0.0000	0.0000	0.0000
0.70	0.5000	0.5000	0.2500
0.80	0.2500	0.5000	0.1250
0.90	0.1250	0.2500	0.0625
1.00	0.1250	0.2500	0.0625

Discussion

The present study demonstrates the significant antimicrobial potential of spinigerin, a termite gut–derived antimicrobial peptide, against multidrug-resistant (MDR) *Bacillus cereus* isolates. Molecular identification confirmed the isolates as *B. cereus* strains FORC6 (BCFOR), DQ01 (BCDQO), and CD3 (BCCD3), all showing 100% sequence identity with reference genomes, thereby validating their taxonomic identity and relevance as foodborne pathogens.

Antibiotic susceptibility testing revealed a high level of resistance among the isolates, with an overall resistance rate of 40.74%. Notably, BCCD3 exhibited the highest level of multidrug resistance (75%), while BCFOR and BCDQO showed moderate resistance profiles. The resistance observed across multiple antibiotic classes highlights the growing challenge posed by MDR *B. cereus* and reinforces concerns regarding the declining effectiveness of conventional antibiotics in food safety and clinical contexts.

In contrast, spinigerin displayed a marked concentration-dependent inhibitory effect against all tested isolates, including the highly resistant BCCD3 strain. No inhibitory activity was observed at lower concentrations (0.10–0.60%); however, inhibition commenced at 0.70% and increased progressively up to 1.00%, where substantial growth suppression was recorded. This ability of spinigerin to inhibit MDR strains that are poorly responsive to standard antibiotics underscores its potential as an alternative antimicrobial agent. The effectiveness of spinigerin against MDR *B. cereus* may be attributed to its mode of action as an antimicrobial peptide, which typically involves direct disruption of bacterial cell membranes rather than targeting specific metabolic pathways. Such mechanisms are less susceptible to conventional resistance strategies, including enzymatic degradation or target modification, thereby reducing the likelihood of resistance development. These findings are consistent with earlier reports on termite-derived antimicrobial peptides and their broad-spectrum activity against resistant bacteria (Bulmer et al., 2017; Dossey et al., 2018).

Overall, the study highlights spinigerin as a promising **natural antimicrobial peptide** capable of overcoming multidrug resistance in *B. cereus*. Its effectiveness against highly resistant strains suggests potential applications in food preservation, biocontrol strategies, and the development of novel peptide-based therapeutics.

Conclusion

This study confirms that spinigerin derived from the termite gut exhibits potent inhibitory activity against multidrug-resistant *Bacillus cereus* isolates. Despite the high resistance of the isolates to multiple conventional antibiotics, spinigerin demonstrated strong, concentration-dependent antimicrobial effects, including against highly resistant strains. These findings support the potential of spinigerin as a novel peptide antibiotic and natural biocontrol agent, offering a promising alternative for managing MDR *B. cereus* in food safety and public health applications.

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