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RESEARCH ARTICLE

Molecular identification and antibacterial activity of endophytic bacteria from *Bambusa vulgaris* leaves as antibacterial potential against phatogenic microoganism

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Abstract

The increasing prevalence of antibiotic-resistant bacteria poses a critical global health concern, necessitating the exploration of novel antibacterial solutions. Endophytic bacteria, which colonize plant tissues without causing harm, have gained attention as potential sources of bioactive metabolites. This study aimed to isolate and characterize endophytic bacteria from *Bambusa vulgaris* leaves and evaluate their antibacterial potential against pathogenic microorganisms. Leaf samples were collected from Bekasi, West Java, and subjected to a surface sterilization process prior to bacterial isolation. A total of 12 bacterial strains were successfully obtained and screened for antibacterial activity against *Bacillus subtilis*, *Propionibacterium acnes*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis* using the agar well diffusion assay. Three isolates exhibited notable inhibitory activity, with P8 demonstrating the strongest antibacterial effects against *B. subtilis*, *P. acnes*, and *S. epidermidis*. The two most potent isolates, P8 and K3, were characterized via 16S rRNA gene sequencing. Genomic DNA extraction was performed, followed by Polymerase Chain Reaction (PCR) amplification using the universal primers 27F (5′-AGAGTTTGATYMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). Sequencing and The Basic Local Alignment Search Tool (BLAST) analysis confirmed that isolate P8 exhibited 100% similarity to *B. subtilis* strain LZH-H1, whereas isolate K3 shared 99.85% similarity with *Pantoea stewartii* subsp. *indologenes* strain SR2-12. These findings suggest that endophytic bacteria from *B. vulgaris* endohytic bacteria hold promise as potential sources of antibacterial compounds. Further research is necessary to purify and characterize these bioactive metabolites for potential pharmaceutical applications.

1. INTRODUCTION

Bamboo is a highly versatile plant with applications spanning construction, agriculture, and traditional medicine. Among its many species, *Bambusa vulgaris* has attracted interest due to its diverse range of bioactive compounds, which have demonstrated antimicrobial, antioxidant, and anti-inflammatory properties (1,2). The leaves of *B. vulgaris* are particularly noted for their rich content of secondary metabolites, such as flavonoids, alkaloids, polyphenols, and tannins, which contribute to their antimicrobial potential (3,4). Various study indicate that *B. vulgaris* leaf extracts exert inhibitory effects against several pathogenic bacteria, including *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis*, and *Staphylococcus aureus*, making them promising candidates for antibacterial applications (5). However, the direct extraction of plant-based bioactive compounds presents sustainability concerns, as continuous harvesting may contribute to biodiversity loss and environmental degradation (6,7).

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To mitigate these challenges, researchers have shifted their focus to alternative sources of antibacterial compounds, particularly endophytic bacteria, which can produce similar bioactive metabolites and reside naturally within bamboo tissues.

Endophytic bacteria are microorganisms that inhabit plant internal structures without inducing disease (8). These microbial communities engage in symbiotic interactions with their host, often enhancing plant growth, stress tolerance, and natural defense mechanisms while synthesizing secondary metabolites with potential pharmaceutical applications (9). Several studies have highlighted endophytes as prolific producers of antimicrobial compounds, positioning them as promising alternatives to traditional plant-derived bioactive agents (10,11). Bacterial endophytes have been identified as producers of antibacterial substances comparable to those found in their host plant, underscoring their relevance in the discovery of natural product (12,13). By leveraging endophytes instead of whole plants, bioactive compounds can be obtained in a more sustainable and environmentally responsible manner.

The growing demand for novel antibacterial agents is particularly evident for treating skin infections caused by antibiotic-resistant bacteria. Dermatological infections can arise from various pathogenic microorganisms, including *S. epidermidis, Propionibacterium acnes, Bacillus subtilis*, and *P. aeruginosa* (14,15). These bacteria contribute to acne, abscesses, chronic wounds, and other skin-related ailments, posing significant challenges to dermatological care (16). For instance, *S. epidermidis* is a leading cause of hospital-acquired infections, frequently exhibiting multidrug resistance, thereby complicating treatment efforts (17,18). Similarly, *P. acnes* is a key contributor to acne vulgaris, a common skin disorder that affects millions of people worldwide (19). The rise of antibiotic resistance has rendered many conventional treatments less effective, reinforcing the urgent need for alternative therapeutic strategies that can efficiently target skin pathogens while reducing the risk of resistance development.

Bamboo-associated endophytic bacteria possess the capacity to produce antimicrobial compounds that are effective against skin-infecting pathogens (20,21). These bacteria generate bioactive metabolites that not only inhibit bacterial growth but also interfere with biofilm formation and attenuate virulence factors, making them valuable assets in antimicrobial research (22,23). Unlike synthetic antibiotics, endophyte-derived antibacterial compounds are less likely to induce resistance and often exhibit lower toxicity, making them attractive candidates for future therapeutic applications (24). By identifying and characterizing endophytic bacteria from *B. vulgaris*, the study can uncover novel bioactive compounds with potential use in treating drug-resistant skin infections.

To fully harness the potential of bamboo-derived endophytic bacteria, they must be identified and classified accurately using molecular techniques, particularly 16S rRNA gene sequencing (25). This technique provides precise taxonomic classification, enabling researchers to explore phylogenetic relationships among isolates and determine their biotechnological potential (26). In addition, laboratory-based antibacterial screening of endophytic isolates can help pinpoint the most promising strains for further development into potential therapeutic agents.

Given the pressing need for alternative antimicrobial solutions, this study aims to isolate and characterize endophytic bacteria from *B. vulgaris* leaves and evaluate their antibacterial properties against common skin pathogens. Through 16S rRNA gene sequencing, we aim to identify bacterial strains with high antimicrobial potential. The findings of this research could contribute to the discovery of novel antibacterial compounds derived from bamboo endophytes, offering a sustainable, plant-based alternative for combating antibiotic-resistant skin infections. This study may pave the way for the development of natural antimicrobial treatments by expanding our understanding of bamboo-associated endophytes, particularly in the fields of dermatology and infectious disease management.

2. MATERIALS AND METHODS

2.1. Equipment and Materials

Bamboo (*Bambusa vulgaris*) leaves, which served as the source for endophytic bacteria were the primary plant material used. The chemical reagents used in this study included agarose, distilled water, demineralized water (Brataco, Indonesia), sterile water for injection (Ikapharmindo Putramas, Indonesia), sulfuric acid (Merck, Germany), barium chloride (Merck, Germany), buffer solutions, ethanol (Merck, Germany), sodium hypochlorite (Merck, Germany), and nuclease-free water. A Genomic DNA extraction kit (Geneaid, Taiwan) was used for molecular analysis, while PCR mix and primers 27F (AGAGTTTGATYMTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) facilitated amplification. The microbiological media and staining agents included Mueller-Hinton agar (Himedia, India), Mueller-Hinton broth (Himedia, India), nutrient agar (Merck, Germany), nutrient broth (Himedia, India), crystal violet (Merck, Germany), Lugol's iodine solution (Merck, Germany), chloramphenicol, and safranin (Merck, Germany) for bacterial staining.

The bacterial cultures used in this study were pathogenic strains sourced from the Microbiology Laboratory, Faculty of Pharmacy, Universitas Singaperbangsa Karawang, including *Bacillus subtilis* ATCC 6633, *Propionibacterium acnes* ATCC 6919, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus epidermidis* FNCC 0048. The combination of specialized laboratory instruments, high-quality reagents, and well-characterized bacterial cultures ensured the accuracy and reliability of the experimental procedures conducted in this study.

2.2. Plant Collection and Surface Sterilization

The plant samples used for bacterial isolation were collected from the *B. vulgaris* leaves (Figure 1) in Bekasi, West Java. Whole plant specimens were identified at the Herbarium Jatinangor Laboratory, Plant Taxonomy Division, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Padjadjaran (UNPAD), confirming the species as *B. vulgaris* Schard. Ex. J.C. Wendl, commonly referred to as green bamboo (*Bambu Ampel Hijau*). To maintain freshness, sample collection was conducted in the mornings between February and March 2023. Only young and healthy leaves from the branch tips were selected for analysis. After collection, the leaves were thoroughly rinsed under running water to remove any external contaminants.

Sterilization was then carried out to ensure that the leaves were free of contaminant. This step was performed by immersing the leaves in 70% alcohol for 1 min, subsequently immersing them in a 2.5% sodium hypochlorite solution for 4 min, and finally immersing them in 96% ethanol for 30 s. Then, the leaves were rinsed with sterile distilled water. The sterile distilled water obtained from the final rinse was introduced into nutrient agar (NA) media (Merck, Germany) to verify the success of sterilization. After sterilization, the specimens were placed in an incubator at 37°C for 48 h. If no microbial growth appeared in the control plate after incubation, the sterilization procedure was assumed to have successfully eliminated all external contaminants (27).

2.3. Isolation, Purification, and Preservation of Endophytic Bacteria

The sterilized leaf samples were ground using a sterile mortar and diluted to a concentration of 10^3. From this dilution, a 100-µL aliquot was inoculated onto the surface of NA medium supplemented with nystatin using the spread plate method. Replication was performed 5 times. The plates were then incubated at 37°C for a period of 4 - 7 days to allow the growth of endophytic bacteria. Endophytic bacteria colonies were carefully collected from the surface of the NA medium using a sterile inoculation needle and sub cultured repeatedly until pure colonies were obtained. Purification was achieved by streaking the pure colonies onto fresh NA plates (28). After pure bacterial colonies had been obtained, they were stored in a solution containing 80% glycerol, stored at -80°C which acts as a cryoprotectant. This glycerol stock was utilized for subsequent experimental.

2.4. Fermentation and Antibacterial Activity Screening of Endophytic Bacteria

Pure bacterial isolates obtained from NA were inoculated into sterile nutrient broth (NB) (Merck, Germany) and incubated at 37°C for 2 days under stationary conditions. After incubation, the cultures were homogenized using a vortex mixer to ensure uniform distribution. The antibacterial potential of the fermented endophytic bacterial isolates was assessed against *Bacillus subtilis* ATCC 6633, *Propionibacterium acnes* ATCC 6919, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus epidermidis* FNCC 0048. Bacteria were cultivated in Mueller-Hinton Broth (Himedia, India) at 37°C for 24 h in a shaking incubator at 150 rpm. The bacterial suspension was then standardized to 0.5 McFarland turbidity to maintain uniform cell density across all assays (29,30).

Figure 1. Collection of *Bambusa vulgaris* leaves and example result of endophytic bacteria isolation

The screening of endophytic bacterial metabolites was performed using the agar well diffusion method. A 0.1 mL aliquot of the fermented endophytic bacterial culture was dispensed into wells on Mueller-Hinton agar plates, with chloramphenicol and sterile NB medium as the serving as the positive control and negative control, respectively. The plates were incubated at 37°C for 24 h, after which the antibacterial activity was evaluated using a caliper to measure the diameter of the inhibition zones (in mm). Clear zones around the wells indicated bacterial growth inhibition, which was recorded for further analysis (30,31).

2.5. Morphological Identification of Endophytic Bacteria

Identifying endophytic bacteria involves a comprehensive assessment of pure antibacterial isolates. This process includes both macroscopic and microscopic analyses. During the macroscopic examination, the colony morphology, including its shape, edges, elevation, and color was observed. Gram staining was used for microscopic analysis to identify and observe the cell shapes (32).

2.6. Molecular Identification of Selected Endophytic Bacterial Isolates Using 16S rRNA Gene Sequencing

A systematic molecular identification approach was employed to confirm the uniqueness and purity of the bacterial isolates under investigation. This process included selecting isolates based on antibacterial activity, extracting genomic DNA, amplifying the 16S rRNA gene via polymerase chain reaction (PCR), sequencing the amplified gene, and performing bioinformatics analysis to determine their taxonomic classification (33). Two isolates, P8 and K3, were selected for molecular identification due to their significant antibacterial activity against *Bacillus subtilis* ATCC 6633, *Propionibacterium acnes* ATCC 6919, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus epidermidis* FNCC 0048. All procedures were conducted under strict aseptic conditions to maintain accuracy and prevent cross-contamination, using only single-colony cultures for DNA extraction and subsequent molecular analysis.

Genomic DNA was extracted using the Geneaid Genomic DNA Extraction Kit (Geneaid Biotech Ltd., New Taipei City, Taiwan). The procedure started with sample preparation, where 200 µL of GT buffer and 10 µL of Proteinase K were added to the bacterial suspension, followed by vortexing to ensure uniformity. Then, the sample was incubated at 60°C for 15 min, with intermittent vortexing every 5 min. Bacterial lysis was facilitated by adding 200 µL of GBT buffer, followed by an additional 10-min incubation at 60°C, with occasional vortexing to enhance cell disruption. The DNA binding step involved adding 200 µL of absolute ethanol, vortexing the mixture, and transferring it to a GS column attached to a collection tube. The column was then centrifuged at 10,000 rpm for 2 min, and the flow-through was discarded. The washing step involved sequential washes with 400 µL of W1 buffer, followed by 600 µL of wash buffer, followed by centrifugation at 10,000 rpm for 30 s and subsequent removal of the supernatant. A final centrifugation at 10,000 rpm for 2 min ensured the complete drying of the column matrix. For DNA elution, the GS column was placed into a new 1.5 mL microtube, and 100 µL of preheated elution buffer was added directly to the center of the column. After incubation for 5 min, the samples were centrifuged at 10,000 rpm for 30 s. The extracted DNA was then stored at -20°C for subsequent analysis.

Amplification of the 16S rRNA gene was performed using PCR in a 20 µL reaction mixture containing 1 unit of Taq polymerase, buffer, 5 µL of template DNA, 1 µL of primer 27F, 1 µL of primer 1492R, 10 µL of 2× PCR mix, and 3 µL of nuclease-free water. The universal primers used for amplification were 27F (5′-AGAGTTTGATYMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The PCR conditions included an initial denaturation at 94°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 min, and extension at 72°C for 2 min, with a final extension at 72°C for 3 min. The PCR products were analyzed via gel electrophoresis, and the amplified DNA bands were visualized using an ultraviolet transilluminator to confirm successful amplification.

For 16S rRNA gene sequencing, 30 µL of unpurified PCR product and primers (at a concentration of 10 µM) were sent to PT Genetika Science Indonesia (Tangerang, Indonesia) for sequencing, performed by 1st BASE (Seri Kembangan, Malaysia). Sequencing data were analyzed using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) platform to determine sequence similarity with known bacterial species (34,35). To confirm the taxonomic classification, phylogenetic analysis was conducted using bioinformatics software, including SnapGene Viewer, BioEdit, and MEGA X, enabling the construction of phylogenetic trees and comparative sequence analysis. These analyses ensured the accurate identification of bacterial strains and provided insight into their evolutionary relationships (36–38). This study confirmed the identity of P8 and K3 as unique endophytic bacterial isolates by integrating antibacterial activity screening, genomic DNA extraction, PCR amplification, sequencing, and computational analysis. The findings contribute to a deeper understanding of the microbial diversity in *B. vulgaris* and highlight the potential applications of these isolates in biotechnology and pharmaceutical research.

3. RESULTS AND DISCUSSION

3.1. Isolation and Antibacterial Activities of Endophytic Bacteria

The isolation of endophytic bacteria from *B. vulgaris* leaves yielded 12 isolates, a relatively small number that can be attributed to various factors. The selected plant tissue, in this case, leaves, may inherently harbor fewer bacterial species than other tissues, such as roots or stems, which are known to support a more diverse microbial community (39,40). Furthermore, the isolation conditions, including the surface sterilization process and the selective growth media used, can significantly influence the diversity and number of endophytic bacteria recovered. Different media may favor certain bacterial groups while inhibiting others, leading to discrepancies in isolation outcomes across various plant species (41,42). Environmental factors, such as geographical conditions and climatic variations, can also affect the endophytic bacterial populations present in specific plant tissues (43,44). Isolation was conducted from 30 bamboo leaf samples, reflecting a moderate level of bacterial diversity within *B. vulgaris*. Macroscopic and microscopic examinations were performed to characterize these isolates and assess their morphological and cellular properties, with findings compiled in Table 1 and visually represented in Figure 2.

The diversity of endophytic bacteria associated with *B. vulgaris* is reflected in the wide range of colony morphologies observed, including size, shape, elevation, margin, surface texture, and pigmentation. This morphological diversity suggests a complex community of bacteria that exhibit metabolic versatility and adaptive capabilities, which are essential for their survival within the plant host. Endophytic bacteria can vary significantly based on the plant tissue type, with different bacterial strains exhibiting unique characteristics and functional traits (45,46). For instance, endophytes isolated from various plant parts have demonstrated capabilities such as producing plant growth-promoting substances and degrading environmental pollutants, indicating their potential roles in enhancing plant health and resilience (47,48). The presence of diverse bacterial taxa, including *Actinomycetota* and *Pseudomonadota*, underscores the ecological significance of these microorganisms in facilitating plant adaptation to environmental stresses (49). The observed pigmentation in bacteria, ranging from white to light brown, indicates the potential synthesis of secondary metabolites that may play crucial roles in bacterial defense, host interactions, and environmental adaptation. Various studies have highlighted the significance of pigments such as carotenoids and prodigiosin, which are known to exhibit antimicrobial properties and contribute to bacterial survival under stress conditions (50–52). For instance, carotenoid pigments have been linked to enhanced resistance against oxidative stress and UV radiation, serving as protective agents against bacteria in hostile environments (53,54). Additionally, pigments, such as pyomelanin, produced by certain marine bacteria acilitate adaptation to changing environmental conditions, further underscoring their ecological importance (55,56). The production of these pigments not only aids in defense mechanisms but also enhances microbial communities' competitive advantages (57,58). Overall, the diverse pigmentation observed in bacteria reflects their evolutionary adaptations and multifaceted roles in ecological interactions.

The diversity of colony morphology and pigmentation of endophytic bacteria is significantly influenced by host plant species and environmental conditions. Endophytic bacteria isolated from various plants exhibit distinct phenotypic traits, which can be attributed to the specific growth conditions and soil composition associated with each plant species (59,60). For instance, the morphological characteristics of endophytic bacteria can vary widely even when cultured on the same medium, indicating that the host plant's ecological niche is closely linked to genetic and physiological diversity (61). Environmental factors such as soil pH, nutrient availability, and moisture levels play a crucial role in shaping the community structure of these bacteria (62–64). The presence of diverse endophytic bacterial communities has been correlated with enhanced plant growth and resilience to stress, suggesting that these microorganisms adapt morphologically to optimize their survival and functionality within their specific plant hosts (65,66). Comparable variations in colony texture and pigmentation have been observed in endophytes from medicinal plants, where pigment-producing strains are often linked to antimicrobial and antioxidant properties (67–70). The presence of pigmented isolates in this study suggests that certain endophytic bacteria from *B. vulgaris* may possess bioactive potential, warranting further research into their biochemical properties, antimicrobial activities, and possible pharmaceutical applications.

A total of 12 endophytic bacterial isolates were screened for antibacterial activity against four humans pathogenic bacteria: *B. subtilis* ATCC 6633, *P. acnes* ATCC 6919, *P. aeruginosa* ATCC 27853, and *S. epidermidis* FNCC 0048. The diameters of the inhibition zones produced by each isolate were measured and analyzed (Table 2). The results revealed variability in antibacterial activity, with some isolates exhibiting significant inhibition against specific pathogens and others showing no inhibitory effects. Notably, none of the isolates inhibited *P. aeruginosa* ATCC 27853, which is consistent with previous findings that Gram-negative bacteria are generally more resistant to antibacterial compounds produced by endophytic bacteria due to their outer membrane, which acts as an additional barrier against antimicrobial agents (71,72).

Among the 12 isolates tested, only three isolates (P8, K3, and K4) exhibited antibacterial activity, specifically against Gram-positive bacteria. Isolate P8 displayed the strongest inhibition, effectively suppressing the growth of *S. epidermidis* FNCC 0048, *P. acnes* ATCC 6919, and *B. subtilis* ATCC 6633. Similarly, K3 demonstrated significant antibacterial activity against *P. acnes* ATCC 6919 and *B. subtilis* ATCC 6633 (Figure 3). In contrast, K4 exhibited moderate inhibition against *P. acnes*. The classification of strong and moderate antibacterial activity was based on the criteria established by David and Stout (59).

Isolates P8 and K3 (Figure 3) were selected for further molecular identification to determine their genetic composition and taxonomic classification. Molecular identification through 16S rRNA gene sequencing will provide insights into their phylogenetic relationships and potential novelty as antimicrobial-producing strains. The strong inhibitory effects observed in P8 and K3 suggest that these isolates may produce bioactive secondary metabolites, warranting further exploration of their pharmaceutical and biotechnological applications.

Table 1. Characteristics of isolated endophytic bacteria

Isolate	Colony shape	Color	Margin	Elevation	Size	Optical property	Gram	Cell shape
P1	Round	White	Entire	Raised	Small	Opaque	Positive	Coccus
P2P3	Round	Milky white	Undulated	Flat	Small	Opaque	Positive	Coccus
P4	Round	White	Entire	Flat	Small	Transparent	Negative	Coccus
P5	Round	Yellow	Entire	Raised	Small	Opaque	Negative	Coccus
P6	Round	White	Entire	Flat	Small	Opaque	Negative	Coccus
P7	Rhizoid	White	Irregular	Flat	Large	Opaque	Negative	Bacillus
P8	Rhizoid	White	Filamentous	Raised	Large	Transparent	Positive	Bacillus
K1	Round	Yellow	Entire	Raised	Small	Opaque	Negative	Coccus
K2	Round	Yellow	Curled	Flat	Small	Transparent	Negative	Bacillus
K3	Irregular	Yellow	Curled	Flat	Large	Transparent	Negative	Bacillus
K4	Round	Yellow	Entire	Flat	Small	Transparent	Negative	Coccus
K5	Punctiform	Yellow	Entire	Flat	Small	Transparent	Negative	Bacillus

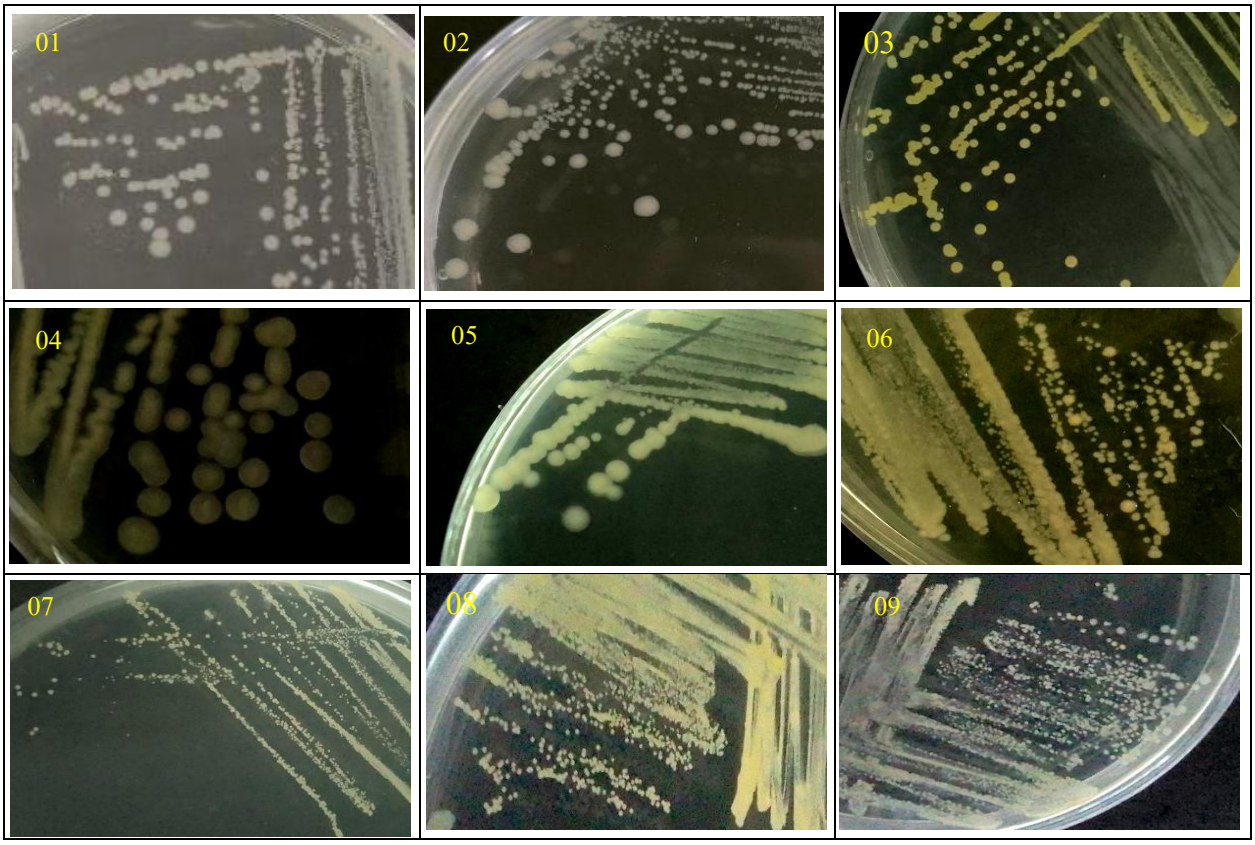

Figure 2. Colony morphology and color variations among the isolated bacterial isolates.

Table 2. Inhibition zone of endophytic bacteria against pathogenic bacteria

Isolate	Inhibition zone ± standard deviation (mm)*			
	Bacillus subtilis	*Staphylococcus epidermidis*	*Propionium acnes*	*Pseudomonas aeruginosa*
P1	-	-	-	-
P2P3	-	-	-	-
P4	-	-	-	-
P5	-	-	-	-
P6	-	-	-	-
P7	-	-	-	-
P8	17.13±1.5	16.27±1.7	16.76±1.2	-
K1	-	-	-	-
K2	-	-	-	-
K3	16.06±0.9	-	13.52±0.8	-
K4	-	-	9.20±0.77	-
K5				

*(-) : indicated zero inhibition zone

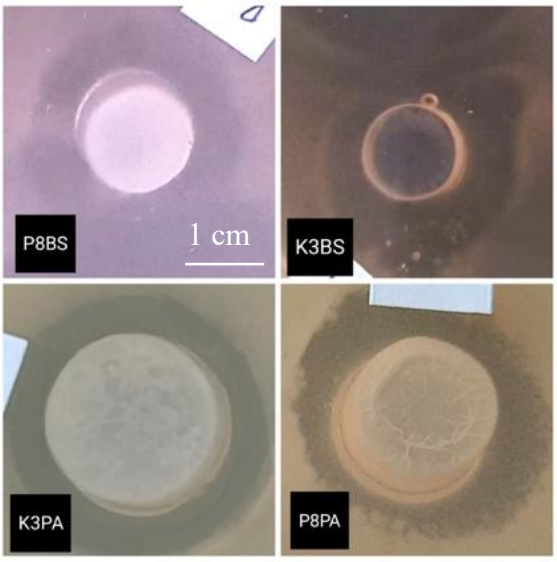

Figure 3. Representative results of antibacterial activity.
Isolate codes P8BS, K3BS, K3PA, and P8PA were isolated on MHA Medium.

3.2. Molecular Identification of Selected Endophytic Bacterial Isolates Using 16S rRNA Gene Sequencing

The isolates with the highest inhibition diameters, specifically P8 and K3, were selected for molecular identification. Genomic DNA was successfully extracted from P8 and K3 isolates using the Genomic DNA Mini Kit, a method chosen for its efficiency and reliability over conventional extraction techniques such as phenol-chloroform-based methods (59). The purity and integrity of the extracted DNA was assessed, followed by electrophoresis analysis to confirm its suitability for further molecular identification.

During gel electrophoresis, the extracted DNA was mixed with a loading dye, which increased its density to ensure proper settling in the gel wells. The dye also facilitated accurate placement within the wells and served as a visual marker for monitoring DNA migration through the gel matrix (60). To improve DNA yield, the elution buffer was preheated to 60°C for 2 min before elution, enhancing DNA recovery efficiency.

The electrophoresis results revealed distinct genomic DNA bands, confirming the successful extraction of high-quality DNA from the P8 and K3 isolates (Figure 4). The quality and integrity of genomic DNA extracted from the P8 and K3 isolates were evaluated according to established criteria. High-quality genomic DNA is defined by the presence of fragments exceeding 1000 bp, indicating minimal degradation; distinct, well-defined bands at the top of the electrophoresis gel, signifying intact large DNA fragments; and non-smearing bands, confirming the absence of contamination (61). These characteristics ensure that the extracted DNA is suitable for subsequent molecular analyses, including PCR amplification and sequencing.

The quality and concentration of genomic DNA extracted from P8 and K3 isolates were assessed, and the results are summarized in Table 3. The DNA concentrations ranged from 200 to 300 ng/µL, indicating a sufficient yield for downstream molecular applications. The A260/A280 purity ratios ranged from 1.8 to 2.0, meeting the quality benchmarks established by Sambrook et al. (59) and Kirby (60). These values confirm the absence of protein or phenol contamination, affirming the efficacy of the extraction method in producing high-purity DNA. The findings indicate that the DNA extracted from P8 and K3 met the quality requirements for subsequent PCR amplification, sequencing, and phylogenetic analysis.

The amplification process was conducted to increase bacterial DNA concentration, ensuring greater sequencing accuracy. Following PCR, gel electrophoresis confirmed the presence of single, well-defined bands, indicating the successful amplification and isolation of the 16S rRNA gene (Figure 5). These findings confirm the effectiveness of the primers specifically targeting the 16S rRNA region, demonstrating their suitability for subsequent sequencing and phylogenetic analysis.

These samples were further processed for sequencing using the Sanger sequencing method by 1st BASE in Malaysia through the service provided by PT Genetika Science Indonesia. Following the receipt of sequencing results, initial data analysis was conducted using three bioinformatics software tools to ensure accuracy and reliability. SnapGene Viewer was utilized to visualize nucleotide peaks and chromatograms and to trim low-quality nucleotide sequences (Figure 6). BioEdit was employed for generate consensus sequence, and MEGA X was used to construct phylogenetic tree to determine the evolutionary relationships of the isolates. After completing these analytical steps, BLAST analysis was performed using the National Center for Biotechnology Information (NCBI) online database to compare the obtained sequences with reference sequences for taxonomic identification.

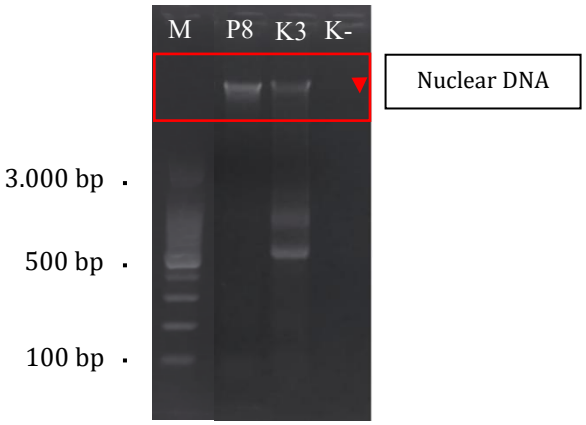

Figure 4. Gel Electrophoresis of Genomic DNA. M = 3kb DNA Marker; P8 and K3 = Samples.

Table 3. Concentration and Purity of Extracted Genomic DNA

Samples ID	DNA Conc (ng.µL-1)	DNA Purity (260/280)
P8	245,45	1,959
K3	267,23	1,979

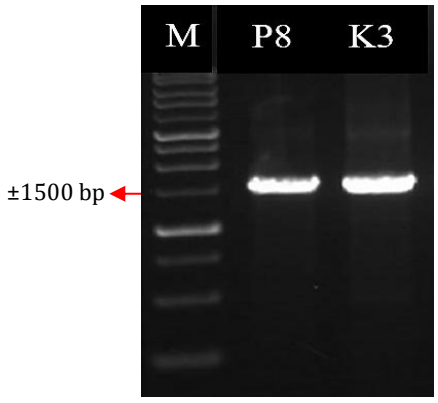

Figure 5. PCR amplification of 16s rDNA using 27F and 1492R universal primers. Amplicon length = ±1500 bp. M = DNA marker 1 kb, P8 and K3 = codes of isolates

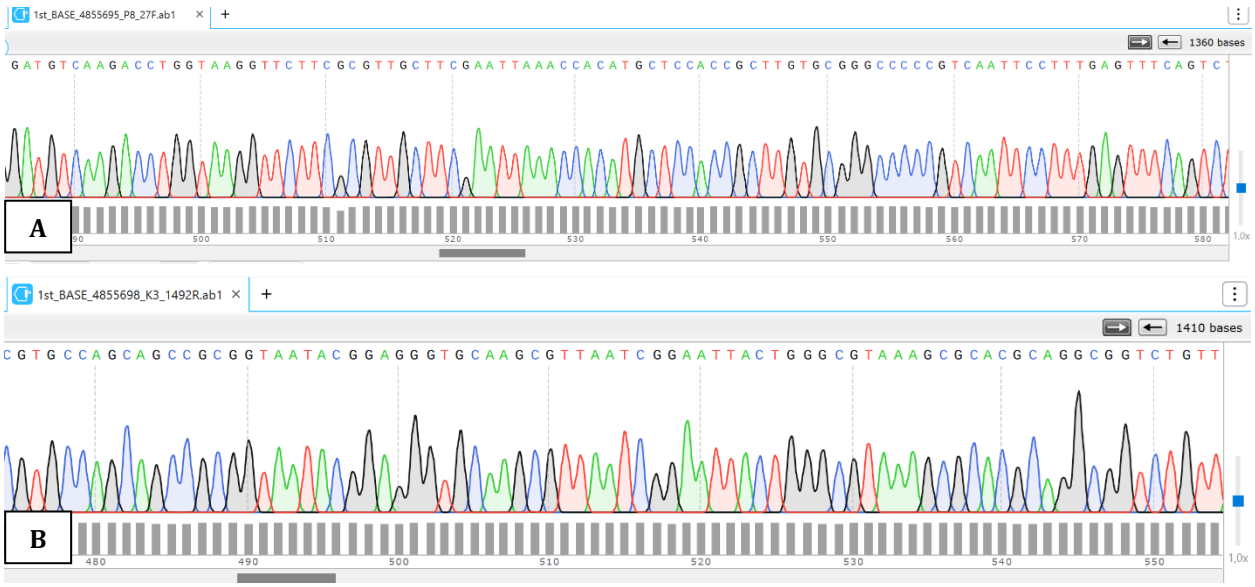

Figure 6. Electropherogram of the Partial 16S rRNA Gene Sequence Results for (A) Isolate P8 and (B) Isolate K3.

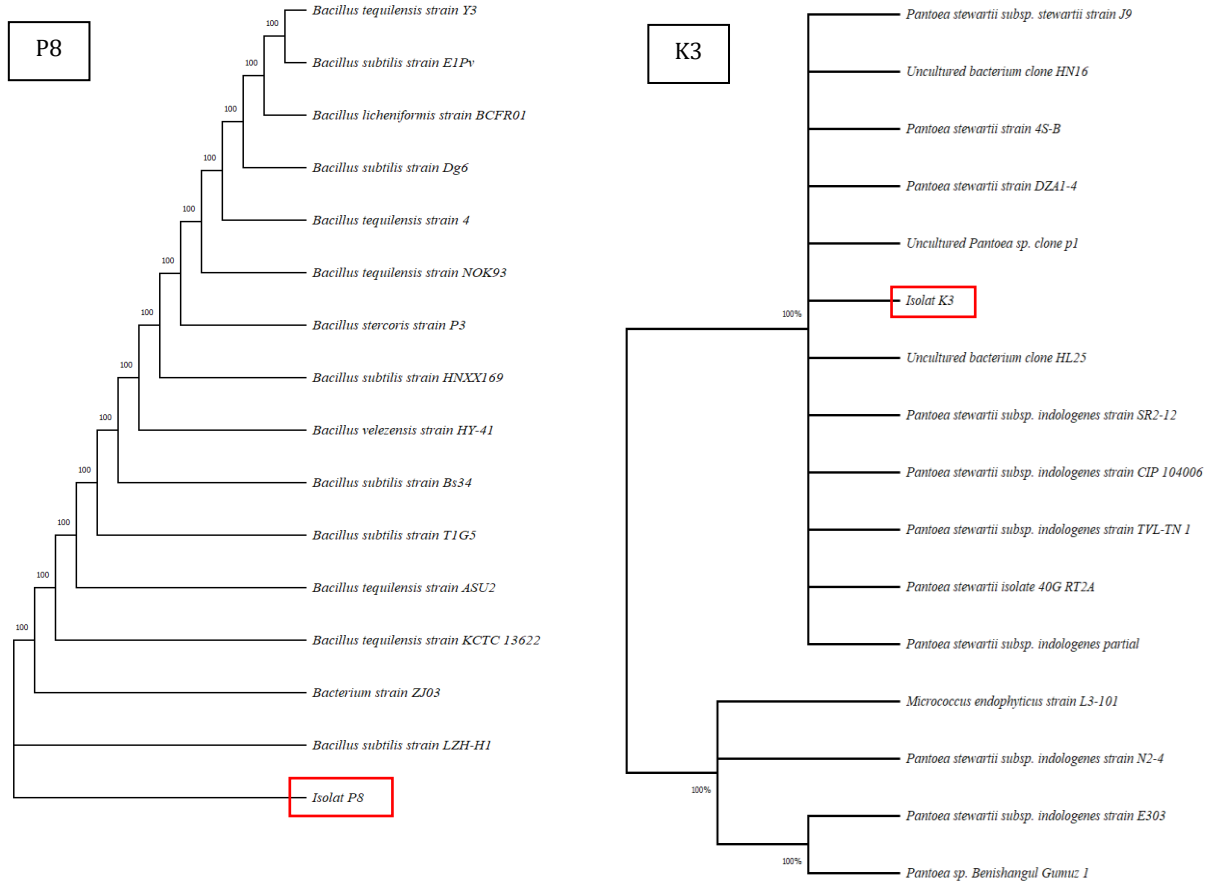

Figure 7. Phylogenetic tree of P8 and K3 isolates by neighbor-joining tree based on of 16s rRNA

The BLAST result of the sequencing data identified isolate P8 as *B. subtilis* strain LZH-H1 with 100% similarity, while K3 was identified as *P. stewartii* subsp. *indologenes* strain SR2-12 with 99.85% similarity. The phylogenetic analysis of isolate P8 demonstrates a close genetic relationship to species within the genus *Bacillus*, including the species *B. subtilis, B. tequilensis, B. stercoris, B. velezensis,* and *B. licheniformis*. Meanwhile, the phylogenetic tree of isolate K3 revealed a similarity to *P. stewartii* and *M. endophyticus*. These evolutionary relationships are visually represented in Figure 7.

These findings align with previous studies reporting that *B. subtilis* is a well-known endophytic bacterium with plant-growth-promoting and biocontrol capabilities (65,66). Conversely, *P. stewartii* is predominantly recognized as a plant pathogen, frequently associated with Stewart's wilt disease in crops (67,68). The identification of both beneficial and potentially pathogenic bacteria from *B. vulgaris* suggests a diverse microbial community, highlighting the need for further characterization to evaluate their ecological roles and potential biotechnological applications.

This study represents a significant advance in isolating and identifying endophytic bacteria from *B. vulgaris* leaves, with a particular focus on their potential applications in combating human pathogenic bacteria. Among the isolates, P8, identified as *B. subtilis*, exhibited strong antibacterial activity, confirming its potential use as a natural antimicrobial agent, consistent with previous studies (69). Additionally, isolate K3, identified as *P. stewartii*, exhbitied moderate antibacterial activity. This finding is particularly intriguing because *P. stewartii* is primarily known as a plant pathogen (70,71), thus revealing a novel potential application for this bacterium as an antibacterial agent.

These findings suggest that endophytic bacteria could be a valuable source of new antibacterial agents, especially against drug-resistant pathogens. However, limitations, such as the small sample size of 12 isolates, should be addressed in future studies. Expanding the sampling across regions and seasons may increase bacterial diversity, potentially identifying strains with stronger antibacterial properties (70,71). While P8 and K3 were effective against Gram-positive bacteria, none of them inhibited Gram-negative pathogens such as *P. aeruginosa*, highlighting the need for further research on overcoming Gram-negative resistance mechanisms (72). Future studies could explore a wider range of pathogenic bacteria beyond those affecting human skin, potentially leading to broader clinical applications. Nevertheless, this research highlights the untapped potential of bamboo plant endophytic bacteria as a source of novel antimicrobial agents, with important implications for combating antibiotic resistance.

4. CONCLUSIONS

In this study, 12 bacterial isolates were successfully isolated from *B. vulgaris* leaves. Among these isolates, 3 were proven to have antibacterial activity against pathogenic bacteria. The two isolates with the highest inhibition zones, namely P8 and K3, were then molecularly analyzed using the 16S rRNA method. P8, which had antibacterial activity against *B. subtilis, S. epidermidis,* and *P. acnes*, was found to have 100% similarity to *B. subtilis* strain LZH-H1. K3, which showed antibacterial activity against *B. subtilis* and *P. acnes*, had 99.85% similarity to *P. stewartii* subsp. *indologenes* strain SR2-12. In the future, the substances produced by each bacterial isolate are expected to be isolated and developed into antibacterial agents. Research can then focus on the purification and characterization of the produced antibacterial substances. This will also allow for the further elucidation of the mechanisms underlying the antibacterial properties of the bacteria. The continuous enhancement of efficacy and quality is needed because these compounds may undergo preclinical and clinical testing to serve as the foundations for the development of novel antibacterial compounds.

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REFERENCES

1. Abe AS, Amonkan KA, Jules Djetouan KM, N'Da KP. Phytochemical and acute toxicity study of aqueous extract of *Bambusa vulgaris* leaves on Wistar rats. Afr J Biol Sci. 2020;2(4):107-114. https://doi.org/10.33472/AFJBS.2.4.2020.107-114
2. Menchavez MT, Catipay JD, Espra AS, Castilo II PM. Antibacterial properties of *Bambusa vulgaris* (Bamboo) leaves and *Eryngium foetidum* (Culantro) leaves against *Staphylococcus aureus* and *Escherichia coli* bacteria. Glob J Med Plat Res. 2018;6(1):6-13. https://doi.org/10.22587/gjmpr.2018.6.1.2
3. Musyimi DM, Buyela DK, Kiema FM, Chebii TK. Phytochemical compounds and antimicrobial activity of extracts of bamboo against *Staphylococcus aureus* and *Escherichia coli*. Arch Ecotoxicol. 2023;5(2):45–51. https://doi.org/10.36547/ae.2023.5.2.45-51
4. Shanavas S, Duraimurugan J, Kumar GS, Ramesh R, Acevedo R, Anbarasan PM, et al. Ecofriendly green synthesis of ZnO nanostructures using *Artabotrys Hexapetalu* and *Bambusa vulgaris* plant extract and investigation on their photocatalytic and antibacterial

activity. Mater Res Express. 2019;6:105098. https://doi.org/10.1088/2053-1591/ab3efe

5. Haq N, Sjahril R, Rante H, Massi MN, Yustisia I, Pattellongi I. Molecular characterization of endophytic fungi from the leaves of beruwas laut (*Scaevola taccada* (Gaertn.) Roxb.) as antibacterial producer. Trop J Nat Prod Res. 2024;8. https://doi.org/10.26538/tjnpr/v8i1.20

6. Devi R, Nath T, Boruah RR, Darphang B, Nath PK, Das P, et al. Antimicrobial activity of bacterial endophytes from Chirata (*Swertia chirata* Wall.) and Datura (*Datura stramonium* L.). Egypt J Biol Pest Control. 2021;31(1):69. https://doi.org/10.1186/s41938-021-00410-9

7. Hashem AH, Al-Askar AA, Abd Elgawad H, Abdelaziz AM. Bacterial endophytes from *Moringa oleifera* leaves as a promising source for bioactive compounds. Separations. 2023;10(7):395. https://doi.org/10.3390/separations10070395

8. Narayanan Z, Glick BR. Secondary metabolites produced by plant growth-promoting bacterial endophytes. Microorganisms. 2022;10(10):2008. https://doi.org/10.3390/microorganisms10102008

9. Kaur P, Kumar V, Singh R, Dwivedi P, Dey A, Pandey DK. Biotechnological strategies for production of camptothecin from fungal and bacterial endophytes. S Afr J Bot. 2020;134:135–45. https://doi.org/10.1016/j.sajb.2020.07.001

10. Mohinudeen IAHK, Kanumuri R, Soujanya KN, Shaanker RU, Rayala SK, Srivastava S. Sustainable production of camptothecin from an *Alternaria* sp. isolated from *Nothapodytes nimmoniana*. Sci Rep. 2021;11(1):1478. https://doi.org/10.1038/s41598-020-79239-5.

11. Santos do Vale SML, Siviero A, Lessa LS, Mattar EPL, Almeida do Vale PA. Biotechnological potential of endophytic bacteria of bamboo *Guadua* sp. for promotion of growth of micropropagated yam plants (*Dioscorea rotundata* Poir). AIMS Agric Food. 2020;5(4):850–67. https://doi.org/10.3934/agrfood.2020.4.850

12. Figueroa-Bossi N, Balbontín R, Bossi L. Basic bacteriological routines. Cold Spring Harb. Protoc 2022;2022(10):pdb.prot107849. https://doi.org/10.1101/pdb.prot107849

13. Halim RA, Hasan N, Ramachandran K. Screening of endophytic bacteria as biocontrol agents against bacteria leaf blight (*Xanthomonas oryzae*). Hayati. 2020;27(3):215. https://doi.org/10.4308/hjb.27.3.215

14. Queiroz EG, Degenhardt J, Quoirin M, Silva K da. Endophytic bacteria associated with tissue culture and leaves of *Plinia peruviana*. Pesqui Agropecu Bras. 2020;55:e01844. https://doi.org/10.1590/s1678-3921.pab2020.v55.01844

15. Sabzikar A, Hosseinihashemi SK, Shirmohammadli Y, Jalaligoldeh A. Chemical composition and antimicrobial activity of extracts from thyme and rosemary against *Staphylococcus aureus* and *Candida albicans*. Bioresources 2020;15(4):9656–71. https://doi.org/10.15376/biores.15.4.9656-9671

16. Bhattarai S, Sharma BK, Subedi N, Ranabhat S, Baral MP. Burden of serious bacterial infections and multidrug-resistant organisms in an adult population of Nepal: A comparative analysis of minimally invasive tissue sampling informed mortality surveillance of community and hospital deaths. Clin Infect Dis. 2021;73:S415–21. https://doi.org/10.1093/cid/ciab773

17. Sebola TE, Uche-Okereafor NC, Mekuto L, Makatini MM, Green E, Mavumengwana V. Antibacterial and anticancer activity and untargeted secondary metabolite profiling of crude bacterial endophyte extracts from *Crinum macowanii* baker leaves. Int J Microbiol. 2020;2020:1–15. https://doi.org/10.1155/2020/8839490

18. Salo EN, Novero A. Identification and characterisation of endophytic bacteria from coconut (*Cocos nucifera*) tissue culture. Trop Life Sci Res. 2020;31(1):57–68. https://doi.org/10.21315/tlsr2020.31.1.4

19. Ren Z, Xie L, Okyere SK, Wen J, Ran Y, Nong X, et al. Antibacterial activity of two metabolites isolated from endophytic bacteria *Bacillus velezensis* Ea73 in Ageratina adenophora. Front Microbiol. 2022;13:860009. https://doi.org/10.3389/fmicb.2022.860009

20. Rahim R, Sultan S, Bhore SJ, Weber JFF. Molecular relationship and biosynthetic potential of endophytic bacteria. Open Conf Proc J. 2013;4:163–163. https://doi.org/10.2174/2210289201304010163

21. Mloka D, Sangeda RZ, Mwambete KD, Kamuhabwa AR. Magnitude of extended-spectrum beta-lactamase-producing gram-negative and beta-lactamase-producing Gram-positive pathogens isolated from patients in Dar es Salaam, Tanzania: A cross-sectional study. Cureus. 2022;14(4):e24451. https://doi.org/10.7759/cureus.24451

22. Kothalawala SG, Zhang J, Wang Y, Yu C. Submicron-sized vermiculite assisted oregano oil for controlled release and long-term bacterial inhibition. Antibiotics. 2021;10(11):1324. https://doi.org/10.3390/antibiotics10111324

23. Chukwu EE, Nwaokorie FO, Coker AO, Avila-Campos MJ, Ogunsola FT. 16S rRNA gene sequencing: A practical approach to confirming the identity of food borne bacteria. Ife J Sci. 2019;21(3):13. https://doi.org/10.4314/ijs.v21i3.2

24. Peker N, Garcia-Croes S, Dijkhuizen B, Wiersma HH, van Zanten E, Wisselink G, et al. A comparison of three different bioinformatics analyses of the 16S–23S rRNA encoding region for bacterial identification. Front Microbiol. 2019;10:620. https://doi.org/10.3389/fmicb.2019.00620

25. Jiang J, Yang B, Ross RP, Stanton C, Zhao J, Zhang H, et al. Comparative genomics of *Pediococcus pentosaceus* isolated from different niches reveals genetic diversity in carbohydrate metabolism and immune system. Front Microbiol. 2020;11:253. https://doi.org/10.3389/fmicb.2020.00253

26. Carrilero L, Dunn SJ, Moran RA, McNally A, Brockhurst MA. Evolutionary responses to acquiring a multidrug resistance plasmid are dominated by metabolic functions across diverse *Escherichia coli* lineages. MSystems. 2023;8(1):e0071322. https://doi.org/10.1128/msystems.00713-22

27. Das VA, Gautam B, Yadav PK, Singh S. Identification of conserved pathways in *Bacillus* strains known for plant growth-promoting behavior using a multifaceted computational approach. Agriculture. 2024;14(6):838. https://doi.org/10.3390/agriculture14060838

28. Singh AK, Sharma RK, Sharma V, Singh T, Kumar R, Kumari D. Isolation, morphological identification and *in vitro* antibacterial activity of endophytic bacteria isolated from *Azadirachta indica* (neem) leaves. Vet World. 2017;10(5):510–516. https://doi.org/10.14202/vetworld.2017.510-516

29. Yunita M, Manse Y, Wulandari MC, Sukmawati S, Ohiwal M. Isolation of endophytic bacteria from nutmeg plant as antibacterial agents against pathogenic bacteria. JPBIO (Jurnal Pendidikan Biologi). 2022;7(1):115–22. https://doi.org/10.31932/jpbio.v7i1.152

30. Chebotar VK, Chizhevskaya EP, Baganova ME, Keleinikova O V., Yuzikhin OS, Zaplatkin AN, et al.

Endophytes from halotolerant plants aimed to overcome salinity and draught. Plants. 2022;11(21):2992. https://doi.org/10.3390/plants11212992

31. Chagas MB de O, Prazeres dos Santos I, Nascimento da Silva LC, Correia MT dos S, Magali de Araújo J, Cavalcanti M da S, et al. Antimicrobial activity of cultivable endophytic fungi associated with *Hancornia speciosa* gomes bark. Open Microbiol J. 2017;11:179–88. https://doi.org/10.2174/1874285801711010179

32. Fatimah F, Millah AI, Fadilah RLA, Salsabila S, Ramly ZA, Sugiarti T, Nurhariyati T, Ni'matuzahroh N, Affandi M. Isolation and potency test of endophytic bacteria as nitrogen fixer from mangrove plant in Lamongan. J Ris Bio Apl. 2022;4(1):26–33. https://doi.org/10.26740/jrba.v4n1.p26-33

33. Liu Y-H, Wei Y-Y, Mohamad OAA, Salam N, Zhang Y, Guo J-W, et al. Diversity, community distribution and growth promotion activities of endophytes associated with halophyte *Lycium ruthenicum* Murr. 3 Biotech. 2019;9(4):144. https://doi.org/10.1007/s13205-019-1678-8

34. Fariska A, Fitri L, Ismail YS, Dita SF. The antibacterial and antioxidant activities of endophytic bacteria from cassava leaves (*Manihot esculenta* Crantz). Trop J Nat Prod Res. 2024;8(3):6617-6623. https://doi.org/10.26538/tjnpr/v8i3.21

35. Zhu X, Ni X, Waigi M, Liu J, Sun K, Gao Y. Biodegradation of mixed pahs by pah-degrading endophytic bacteria. Int J Environ Res Public Health. 2016;13(8):805. https://doi.org/10.3390/ijerph13080805

36. Gao L, Ma J-B, Huang Y, Muhammad M, Lian H-T, Shurigin V, et al. Insight into endophytic microbial diversity in two halophytes and plant beneficial attributes of *Bacillus swezeyi*. Front Microbiol. 2024;15:1447755. https://doi.org/10.3389/fmicb.2024.1447755

37. Singh S, Ghadge VA, Kumar P, Mathew DE, Dhimmar A, Sahastrabudhe H, et al. Biodiversity and antimicrobial potential of bacterial endophytes from halophyte *Salicornia brachiata*. Antonie Van Leeuwenhoek. 2021;114(5):591–608. https://doi.org/10.1007/s10482-021-01544-4

38. Naisi S, Bayat M, Salehi TZ, Rahimian Zarif B, Yahyaraeyat R. Antimicrobial and anti-biofilm effects of carotenoid pigment extracted from *Rhodotorula glutinis* strain on food-borne bacteria. Iran J Microbiol. 2023;15(1):79-88. https://doi.org/10.18502/ijm.v15i1.11922

39. Syaifudin A, Sutrisno S. Antibacterial activity and potential of natural textile dyes from sea water bacteria. Al-Hayat: J Biol Appl Biol. 2022;5(1):33–46. https://doi.org/10.21580/ah.v5i1.13446

40. Morgan S, Thomas MJ, Walstrom KM, Warrick EC, Gasper BJ. Characterization of prodiginine compounds produced by a *Vibrio* species isolated from salt flat sediment along the Florida Gulf Coast. Fine Focus. 2017;3(1):33–51. https://doi.org/10.33043/FF.3.1.33-51

41. Chaturwedi SB, Mainali S, Chaudhary R. Antibacterial activity of pigment extracted from bacteria isolated from soil samples. BMC Res Notes. 2024;17:169. https://doi.org/10.1186/s13104-024-06834-4

42. Azman A-S, Mawang C-I, Abubakar S. Bacterial pigments: The bioactivities and as an alternative for therapeutic applications. Nat Prod Commun. 2018;13(12):1747-1754. https://doi.org/10.1177/1934578X1801301240

43. Zeng Z, Cai X, Wang P, Guo Y, Liu X, Li B, et al. B iofilm formation and heat stress induce pyomelanin production in deep-sea *Pseudoalteromonas* sp. SM9913. Front Microbiol. 2017;8.:1822. https://doi.org/10.3389/fmicb.2017.01822

44. Marín-Sanhueza C, Echeverría-Vega A, Gómez A, Cabrera-Barjas G, Romero R, Banerjee A. Stress dependent biofilm formation and bioactive melanin pigment production by a thermophilic *Bacillus* species from Chilean Hot Spring. Polymers (Basel). 2022;14(4):680. https://doi.org/10.3390/polym14040680

45. Patel N, Dwivedi M, Jadeja S, Begum R. Antibacterial activity of marine bacterial pigments obtained from arabian sea water samples. J Pure Appl Microbiol. 2020;14(1):517–26. https://doi.org/10.22207/JPAM.14.1.54

46. Srilekha V, Krishna G, Seshasrinivas V, Charya MS. Antibacterial and anti-inflammatory activities of marine *Brevibacterium* sp. Res Pharm Sci. 2017;12(4):283-289. https://doi.org/10.4103/1735-5362.212045

47. Arzita A, Fitriani M, Fathia N, Nusifera S. Exploration of antibiotics-producing endophytic bacteria isolates from betel leaves in Jambi City Forest Park. Pak J Biol Sci. 2022;25(11):1001–1007. https://doi.org/10.3923/pjbs.2022.1001.1007

48. Wu W, Chen W, Liu S, Wu J, Zhu Y, Qin L, et al. Beneficial relationships between endophytic bacteria and medicinal plants. Front Plant Sci. 2021;12:646146. https://doi.org/10.3389/fpls.2021.646146

49. Viana TFC, Campelo APS, Baldani JI, Fernandes-Júnior PI, Baldani VLD, Silva WM, et al. Cultivable bacterial diversity associated with bromeliad roots from ironstone outcrops in Central Brazil. Braz J Biol. 2020;80(4):872–80. https://doi.org/10.1590/1519-6984.224982.

50. Mei Y-H, Li X, Zhou J-Y, Kong F-L, Qi S-S, Zhu B, et al. Both adaptability and endophytic bacteria are linked to the functional traits in the invasive clonal plant *Wedelia trilobata*. Plants (Basel). 2022;11(23):3369. https://doi.org/10.3390/plants11233369

51. Rizqoh D, Sipriyadi S, Suryani UH, Putri CN, Agustin M, Taurustya H, et al. Exploring the antibacterial activity of endophytic bacteria from Andaliman (*Zanthoxylum acanthopodium*) against *Bacillus subtilis*. Biodiversitas. 2024;25(2):700-707. https://doi.org/10.13057/biodiv/d250229

52. Liu J-Q, Zhang C-M, Gong Y, Xu M-J, Xing K, Gao J, et al. Diversity of endophytic bacteria associated with different cultivars of medicinal plant *Arctium lappa* L. and their potential for host plant growth promoting. Res Sq. 2021. https://doi.org/10.21203/rs.3.rs-1063781/v1

53. Mamangkey J, Mendes LW, Harahap A, Briggs D, Kayacilar C. Endophytic bacteria and fungi from indonesian medicinal plants with antibacterial, pathogenic antifungal and extracellular enzymes activities: A review. Inter J Sci Technol Manag. 2022;3(1):245–55. https://doi.org/10.46729/ijstm.v3i1.428

54. Chitranshi S, Gupta A, Sarkar N, Khare P. Bacterial endophytes of *Aloe vera* and their potential applications. Heri Sust Dev. 2022;4(1):32–41. https://doi.org/10.37868/hsd.v4i1.68

55. de Melo Pereira DÍ, Gurgel RS, de Souza ATF, Matias RR, de Souza Falcão L, Chaves FCM, et al. Isolation and identification of pigment-producing endophytic fungi from the Amazonian species *Fridericia chica*. J Fungi 2024;10(7):77. https://doi.org/10.3390/jof10010077

56. Sharma S, Gupta S, Dhar MK, Kaul S. Diversity and bioactive potential of culturable fungal endophytes of medicinal shrub *Berberis aristata* DC: A first report. Mycobiology 2018;46(4):370–81. https://doi.org/10.1080/12298093.2018.1538068

57. Reed DR, Nehmzow K, Essandoh MA, Ebqa'ai MA, Nelson TL, Lutter EI, et al. Relationship between cell envelope ultrastructure and the antibacterial properties of a novel hydrophobic eumelanin-inspired derivative. Front Bacteriol. 2023;2. https://doi.org/10.3389/fbrio.2023.1253097

58. Lotlikar G, Naik-Samant S. Bactericidal activity of endophytic bacteria isolated from *Acanthus ilicifolius*: a mangrove plant of Divar Island, Goa, against human pathogenic bacteria. Biotechnological Utilization of Mangrove Resources, Elsevier; 2020, p. 293–301. https://doi.org/10.1016/B978-0-12-819532-1.00012-3

59. Stout JD. Aspects of the microbiology and oxidation of wicken fen soil. Soil Biol Biochem 1971;3(1):9–25. https://doi.org/10.1016/0038-0717(71)90027-7

60. Djurkin Kusec I, Radisic Z, Komlenic M, Kusec G. Comparison of commercial DNA kits and traditional DNA extraction procedure in PCR detection of pork in dry/fermented sausages. Poljoprivreda/Agriculture. 2015;21(1 Supp):199–202. https://doi.org/10.18047/poljo.21.1.sup.47

61. Hall AC. A comparison of DNA stains and staining methods for agarose gel electrophoresis 2019. bioRxiv. https://doi.org/10.1101/568253

62. Levin RE, Ekezie F-GC, Sun D-W. DNA-Based technique: Polymerase chain reaction (PCR). Modern techniques for food authentication, Elsevier; 2018;527–616. https://doi.org/10.1016/B978-0-12-814264-6.00014-1

63. Kirby L. DNA Fingerprinting. vol. 16. London: Palgrave Macmillan UK; 1990. https://link.springer.com/book/10.1007/978-1-349-12040-6

64. Sambrook J, Fritsch E, Maniatis T. Molecular cloning: A laboratory manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press; 1989.

65. Xu W, Ren H, Ou T, Lei T, Wei J, Huang C, et al. Genomic and functional characterization of the endophytic *Bacillus subtilis* 7PJ-16 strain, a potential biocontrol agent of mulberry fruit sclerotiniose. Microb Ecol. 2019;77(3):651–63. https://doi.org/10.1007/s00248-018-1247-4

66. Riddech N, Ma NY, Ho PN, Sarin P. A plant growth promoting of rhizobacteria and endophytic bacteria in vegetable rhizosphere and root samples. J Pure Appl Microbiol. 2022;16:1909–21. https://doi.org/10.22207/JPAM.16.3.38

67. Doblas-Ibáñez P, Deng K, Vasquez MF, Giese L, Cobine PA, Kolkman JM, et al. Dominant, heritable resistance to Stewart's wilt in maize is associated with an enhanced vascular defense response to infection with *Pantoea stewartii*. Mol Plant Microbe Interact. 2019;32(12):1581–97. https://doi.org/10.1094/MPMI-05-19-0129-R

68. Cai L, Tian Q, Meng Q, Bao X, Xu P, Liu J, et al. Recombinase polymerase amplification assay for rapid field diagnosis of Stewart's wilt of corn pathogen *Pantoea stewartii* subsp. stewartii. Agric. 2023;13(10):1982. https://doi.org/10.3390/agriculture13101982

69. Qin Y, Wang Y, He Y, Zhang Y, She Q, Chai Y, et al. Characterization of subtilin L-Q11, a novel class I bacteriocin synthesized by *Bacillus subtilis* L-Q11 isolated from orchard soil. Front Microbiol. 2019;10:484. https://doi.org/10.3389/fmicb.2019.00484

70. Belahmadi MSO, Charchar N, Abdessemed A, Gherib A. Impact of petroleum refinery on aquatic ecosystem of Skikda Bay (Algeria): Diversity and abundance of viable bacterial strains. Mar Pollut Bull. 2023;188:114704. https://doi.org/10.1016/j.marpolbul.2023.114704

71. Kashfi R, Kelsey C, Gang DJ, Call DR, Gang DR. Metabolomic diversity and identification of antibacterial activities of bacteria isolated from marine sediments in Hawai'i and Puerto Rico. Front Mol Biosci. 2020;7:23. https://doi.org/10.3389/fmolb.2020.00023

72. Egan AJF. Bacterial outer membrane constriction. Mol Microbiol. 2018;107(6):676–87. https://doi.org/10.1111/mmi.13908