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Isolation, Molecular Characterization and Antimicrobial Potential of Bacteria Associated with Banana (*Musa* spp.) Pseudostem Water

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1. Abstract

Banana pseudostem is an underutilized agricultural by-product that contains nutrients and bioactive compounds and may serve as a potential source of microorganisms with biotechnological importance. The present study was undertaken to isolate and characterize bacteria associated with banana pseudostem water and to evaluate their antimicrobial potential. Banana pseudostem water was serially diluted and cultured on de Man–Rogosa–Sharpe (MRS) agar for bacterial isolation. The isolates were characterized based on colony morphology, Gram staining, biochemical tests, and molecular identification using 16S rRNA gene sequencing. Antibacterial, antifungal, and haemolytic activities were also evaluated. At a dilution of 10^{-3}, an average viable bacterial count of 3.2×10^5 CFU/mL was recorded, and two morphologically distinct isolates, designated Y1 and W1, were obtained. Both isolates were Gram-positive rods and exhibited distinct biochemical profiles. Molecular analysis identified Y1 as *Klebsiella pneumoniae*, while W1 was assigned to a *Bacillus*-related taxon based on 16S rRNA sequence analysis. The isolates showed variable antagonistic activity against the tested bacterial and fungal pathogens. No significant antibacterial inhibition was observed against *Staphylococcus aureus* and *Vibrio cholerae*, whereas slight inhibition was observed against *Salmonella* spp. Both isolates demonstrated antifungal activity against the tested fungal isolates. Haemolytic activity was also observed on blood agar, indicating the need for further safety assessment before potential food, probiotic, or therapeutic applications. Overall, the study demonstrates that banana pseudostem water represents a potential source of cultivable bacteria with antimicrobial properties and highlights the value of banana agricultural waste as a resource for microbial and biotechnological applications. Further studies involving comprehensive molecular characterization, metabolite profiling, mechanism-of-action studies, and biosafety evaluation are required to establish their practical applications.

2. INTRODUCTION

Banana is one of the most widely cultivated and consumed fruit crops worldwide and belongs to the family Musaceae. The genus *Musa* comprises more than 1,000 varieties that differ in size, shape, colour, texture, flavour, and chemical composition. Bananas are broadly classified into dessert bananas and cooking bananas or plantains. The crop is believed to have originated and been domesticated in Southeast Asia and subsequently spread to different tropical and subtropical regions of the world. India is one of the leading banana-producing countries, followed by China, Indonesia, Brazil, Ecuador, and the Philippines. In India, banana is cultivated extensively in states such as Tamil Nadu,

Maharashtra, Andhra Pradesh, Karnataka, and Gujarat. Important Indian cultivars include Grand Naine, Robusta, Rasthali, Poovan, and Nendran. Besides its nutritional and economic importance, banana has gained considerable scientific interest because different parts of the plant contain biologically active compounds with potential antimicrobial and antioxidant properties.

The banana pseudostem, commonly referred to as the banana stem, is a false stem formed by the tightly overlapping leaf sheaths. After fruit harvesting, large quantities of pseudostem biomass are generated as agricultural waste. However, the pseudostem contains considerable amounts of water, dietary fibre, minerals, phenolic compounds, and other bioactive constituents. Banana stem juice is traditionally prepared from the inner portion of the pseudostem and is consumed particularly in South India. Traditionally, it has been associated with digestive and urinary-health benefits. The presence of minerals such as potassium and magnesium and various antioxidant compounds may contribute to its biological properties. Banana-derived materials have also been investigated for antimicrobial activity due to the presence of compounds such as dopamine, gentisic acid, ferulic acid, lupeol, and other phytochemicals. These properties suggest that banana stem may have potential applications as a natural source of antimicrobial and antioxidant agents and in the development of value-added products.

Banana stem juice may contain a diverse population of microorganisms depending on the cultivar, geographical location, agricultural practices, harvesting, processing, storage, and hygiene conditions. Of particular interest are lactic acid bacteria (LAB), which are important microorganisms associated with plant materials and fermentation processes. Species such as *Lactobacillus plantarum*, *Lactobacillus paracasei*, and *Lactobacillus delbrueckii* have been reported from plant-associated environments. MRS (de Man, Rogosa and Sharpe) agar is widely used for the isolation and enrichment of LAB because its nutritional composition and acidic conditions favour the growth of lactobacilli and related bacteria. Some LAB strains may possess beneficial characteristics, including antimicrobial activity and the ability to metabolize certain organic compounds. However, these properties are strain-specific and therefore require experimental confirmation.

Considering the nutritional, phytochemical, antimicrobial, and microbial characteristics of banana pseudostem, banana stem juice represents a promising but comparatively underutilized biological resource. Investigation of its microbial diversity, particularly LAB and endophytic bacteria, together with evaluation of antimicrobial activity and calcium oxalate crystal inhibition, may provide useful scientific evidence for its potential applications. Such studies may also support the sustainable utilization of banana pseudostem waste and the development of value-added probiotic, antimicrobial, nutraceutical, and functional food products.

3. MATERIALS AND METHODS

3.1 Collection and Preparation of Banana Stem Water

Fresh banana pseudostems were collected after fruit harvesting and processed aseptically for the extraction of stem water. The outer fibrous sheaths were removed to obtain the tender inner portion, which was thoroughly washed with sterile distilled water to remove adhering soil and other surface contaminants. The inner stem was cut into small pieces and homogenized with a standardized volume of sterile distilled water. The homogenate was filtered through sterile muslin cloth to obtain banana stem water. The prepared sample was stored under refrigerated conditions until further microbiological analysis.

3.2 Serial Dilution and Isolation of Bacteria

Serial dilution was performed to obtain countable and well-isolated bacterial colonies. Briefly, 1 mL of banana stem water was transferred aseptically into 9 mL of sterile distilled water to obtain a 10^{-1} dilution. Serial ten-fold dilutions were continued up to 10^{-6} with thorough mixing at each step. Aliquots of the appropriate dilutions were inoculated onto selective and differential media for bacterial isolation.

For the isolation of lactic acid bacteria (LAB), 0.1 mL of the diluted sample was spread uniformly over de Man, Rogosa and Sharpe (MRS) agar plates using a sterile L-shaped spreader. The plates were incubated at 37°C for 24–48 h. Colonies showing characteristics typical of LAB were selected and purified by repeated streaking on fresh MRS agar plates. Pure cultures were maintained on MRS agar slants under refrigerated conditions for further characterization.

3.3 Morphological and Biochemical Characterization

The purified bacterial isolates were characterized by colony morphology and Gram staining. Gram staining was performed using crystal violet, Gram's iodine, alcohol decolourization, and safranin counterstaining, followed by microscopic observation under a compound microscope.

The isolates were further characterized using standard biochemical tests, including IMViC (Indole, Methyl Red, Voges–Proskauer and Citrate utilization), catalase, gelatin hydrolysis, carbohydrate fermentation using phenol red medium, and growth on MacConkey agar. These tests were used to obtain preliminary information regarding the physiological and biochemical characteristics of the isolates.

3.4 Molecular Identification of Bacterial Isolates

Genomic DNA was extracted from selected bacterial isolates using the NucleoSpin® Tissue Kit (Macherey-Nagel) according to the manufacturer's instructions. The quality of the extracted DNA was assessed by agarose gel electrophoresis. The bacterial 16S rRNA gene was amplified by polymerase chain reaction (PCR) using universal bacterial primers. The PCR products were separated on 1.2% agarose gel and visualized using a gel documentation system.

The amplified products were purified using ExoSAP-IT treatment and subjected to Sanger sequencing using the BigDye Terminator v3.1 Cycle Sequencing Kit. Sequencing was performed using an ABI 3500 DNA Analyzer. The quality of the obtained sequences was evaluated using Sequence Scanner Software, and sequence editing and alignment were performed using Geneious software. The resulting sequences were used for molecular identification of the bacterial isolates.

3.5 Antagonistic Activity

The antagonistic activity of the selected bacterial isolate against other bacterial isolates obtained from banana stem water was evaluated by the agar-based antagonism assay. Test organisms were inoculated onto nutrient agar plates, and the selected isolate was streaked centrally on the agar surface. Following incubation at 37°C for 24–48 h, the plates were examined for the formation of clear zones around the test isolate. The presence of an inhibition zone was considered indicative of antagonistic activity.

3.6 Hemolytic Activity

Hemolytic activity of the bacterial isolates was evaluated using blood agar medium. The isolates were streaked onto blood agar plates and incubated at 37°C for 24–48 h. Following incubation, the plates were examined for visible changes surrounding the bacterial growth. The presence or absence of haemolysis was recorded based on the appearance of clear or altered zones around the colonies.

3.7 Statistical and Experimental Considerations

All microbiological experiments were performed under aseptic conditions, and appropriate controls were maintained wherever applicable. The bacterial isolates obtained from banana stem water were evaluated based on their morphological, biochemical, and molecular characteristics, followed by assessment of their antagonistic and hemolytic properties. The obtained results were compiled and interpreted to determine the microbial diversity and potential biological characteristics of bacteria associated with banana stem water.

4. RESULTS

4.1 Isolation and Characterization of Bacterial Isolates

Serial dilution followed by spread plating of banana stem water on MRS agar produced countable colonies at the 10^{-3} dilution, with an average count of 32 colonies corresponding to approximately **3.2 × 10^5 CFU/mL**. Two morphologically distinct colonies were obtained and designated **Y1 (yellow)** and **W1 (white/creamy)**. Both isolates produced small, round, convex and glistening colonies on MRS agar. The occurrence of distinct colony morphologies suggests the presence of more than one bacterial type in banana stem water.

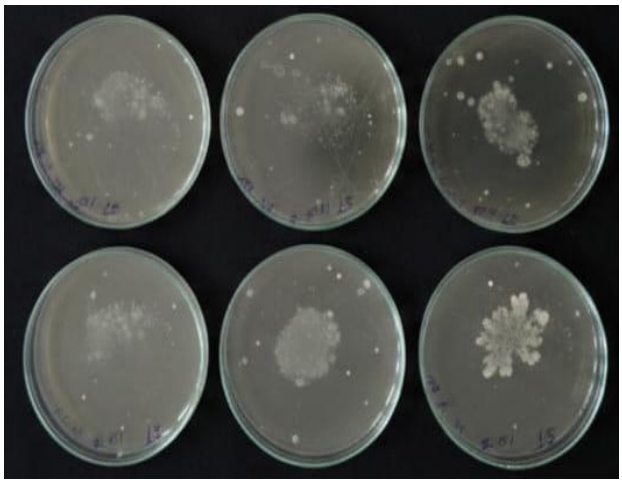

Figure 1 : Spread plate technique

Microscopic examination following Gram staining showed that both Y1 and W1 consisted predominantly of **Gram-positive rod-shaped cells**, occurring singly or in short chains. Biochemical characterization further demonstrated differences between the isolates. Y1 was positive for methyl red and citrate utilization but negative for indole and Voges–Proskauer tests, whereas W1 was negative for indole, methyl red and Voges–Proskauer tests but positive for citrate utilization. Y1 showed catalase activity, while W1 was catalase-negative. The catalase-negative nature of W1 is consistent with characteristics commonly observed among lactic acid bacteria, whereas the catalase-positive reaction of Y1 is compatible with *Bacillus* species.

Both isolates exhibited positive gelatin hydrolysis, indicating the production of extracellular proteolytic enzymes capable of hydrolysing gelatin. No growth was observed for either isolate on MacConkey agar, supporting their Gram-positive nature and indicating the absence of detectable Gram-negative, lactose-fermenting bacteria under the conditions tested. In the phenol red carbohydrate fermentation test, Y1 produced an acid reaction, whereas W1 showed no visible acid production, demonstrating differences in carbohydrate utilization among the isolates.

Figure 2 : Gelatin Hydrolysis - Formation of Rings

These morphological and biochemical characteristics indicate that banana stem water supports a diverse bacterial population containing isolates with different physiological and metabolic properties. Similar bacterial diversity, including LAB and *Bacillus* species, has been reported from banana pseudostem and other banana-associated materials.

4.2 Molecular Identification of Bacterial Isolates

Molecular identification was performed by amplification and sequencing of the bacterial **16S rRNA gene**. Genomic DNA extracted from the selected isolates produced suitable amplification products, which were subsequently purified and subjected to Sanger sequencing. The obtained sequences were edited and analysed using sequence-analysis software and compared with sequences available in the NCBI nucleotide database

using BLAST.

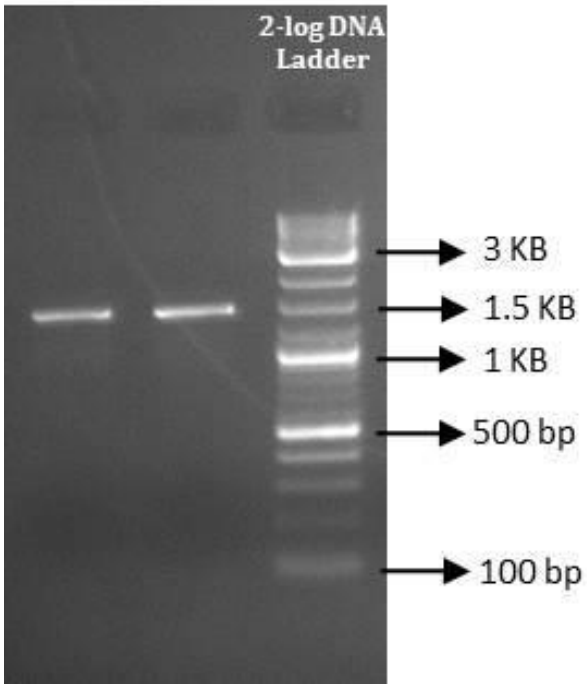

Fig 3: Gel electrophoresis

The **BY-1 isolate** showed a high degree of similarity with sequences belonging to the genus *Klebsiella*. The reported BLAST analysis showed approximately **98.8% sequence identity** over a 668-bp alignment with a *Klebsiella pneumoniae* reference sequence. Based on this similarity, BY-1 was identified as a *Klebsiella*-related isolate. However, because 16S rRNA similarity alone may not always provide definitive species-level discrimination within closely related taxa, further molecular confirmation would strengthen the identification.

>SR5638-BY1-RSF1-C07.ab1

TCAGAAATAAAAGGAGCTTGCTCTCGGGTGACG
AGCGGCGGACGGGTGAGTAATGTCTGGGAAACT
GCCTGATGGAGTTGGGATAACTACTGGAAACGG
TAGCTAATACCGCATAATGTCGCAAGACCAAAGT
GGGGGACCTTCGGGCCTCATGCCATCAGATGTGC
CCAGATGGGATTAGCTAGTAGGTGGGGTAACGG
CTCACCTAGGCGACGATCCCTAGCTGGTCTGAGA
GGATGACCAGCCACACTGGAACTGAGACACGGT
CCAGACTCCTACGGGAGGCAGCAGTGGGGAATA
TTGCACAATGGGCGCAAGCCTGATGCAGCCATGC
CGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGC
ACTTTCAGCGGGGAGGAAGGCGATAAGGTTAAT
AACCTCGTCGATTGACGTTACCCGCAGAAGAAGC
ACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATA
CGGAGGGTGCAAGCGTTAATCGGAATTACTGGG
CGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGG
ATGTGAAATCCCCGGGCTCAACCTGGGAACTGCA
TTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGG
GGGTAGAATTCCAGGTGTAGCGGTGAAAATGCG
TAGAGATCTGGAGGAATACCGGGTGGCCAAAGG
GGGCCCCCTTGGA

Figure 4 :FASTA format of Isolated Bacteria (BY-1)

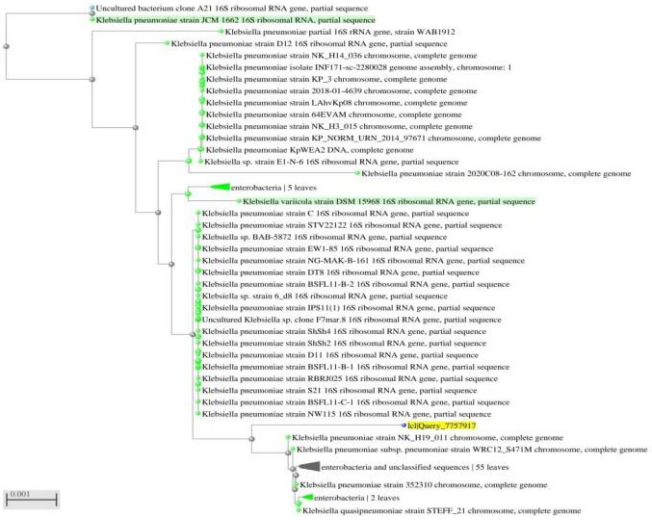

Figure 5: Phylogenetic tree of Isolated Bacteria (BY-1)

The **BW-1 isolate** showed similarity to members of the genus *Bacillus*. The BLAST results included matches to *Bacillus*/*Priestia* sequences, with the reported sequence identity being approximately 95% for one of the major alignments. The isolate was provisionally assigned to *Bacillus subtilis* based on the available sequence analysis. However, the relatively lower sequence identity and the reported *Priestia megaterium* match indicate that the species identification should be interpreted cautiously and confirmed using the complete sequence and

additional phylogenetic or molecular markers before publication.

>SR5638-BW1-RSR1-H08.ab1

CACTCAATCAGGGCGGGCCATCCGGATCCGCG

ATTACTAGCGATTCCAGCTTCACGCAGTCGAGT

TGCAGACTGCGATCCGAACTGAGAACAGATTT

GTGGGATTGGCTTAACCTCGCGGTTTCGCTGCC

CTTTGTTCTGTCCATTGTAGCACGTGTGTAGCC

CAGGTCATAAGGGGCATGATGATTTGACGTCA

TCCCCACCTTCCTCCGGTTTGTCACCGGCAGTC

ACCTTAGAGTGCCCAACTGAATGCTGGCAACT

AAGATCAAGGGTTGCGCTCGTTGCGGGACTTA

ACCCAACATCTCACGACACGAGCTGACGACAA

CCATGCACCACCTGTCACTCTGCCCCCGAAGGG

GACGTCCTATCTCTAGGATTGTCAGAGGATGTC

AAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAA

TTAAACCACATGCTCCACCGCTTGTGCGGGCCC

CCGTCAATTCCTTTGAGTTTCAGTCTTGCGACC

GTACTCCCCAGGCGGAGTGCTTAATGCGTTAGC

TGCAGCACTAAGGGGCGGAAACCCCCTAACAC

TTAGCACTCATCGTTTACGGCGTGGACTACCAG

GGTATCTAATCCTGTTCGCTCCCCACGCTTTCG

CTCCTCAGCGTCAGTTACAGACCAGAGAGTCG

CCTTCGCCACTGGTGTTCCTCCACATCTCTACG

CATTTCACCGCTACACGTGGAATTCCACTCTCC

TCTTCTGCACTCAAGTTCCCCAGTTTCCAATGA

CCCTCCCCGGTTGAGCCGGGGGCTTTCACATCA

GACTTAAGAAACCGCCTGCGAGCCCTTTACGCC

CAATATTCCGGACAACGCTGCCACTACGTATTA

CCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTT

TCTGATTAAGTACGGTCAGGTACGGCCTATCCG

AACCGTTACTTGTTTCTTTC

CGTAACAAACCGAGAGGCT

Figure 6: FASTA format of Isolated Bacteria (BW-1)

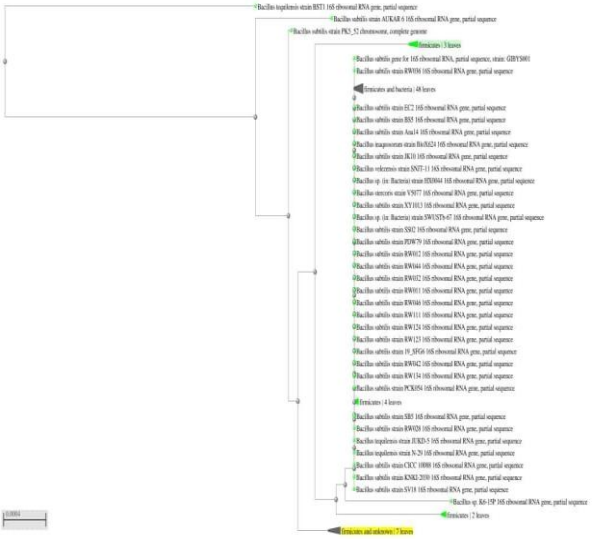

Figure 7: Phylogenetic tree of Isolated Bacteria (BW-1)

Overall, the molecular analysis supports the presence of both **Gram-negative *Klebsiella*-related bacteria and Gram-positive *Bacillus*-related bacteria** among the isolates obtained from banana stem water. The molecular findings should be considered together with the morphological and biochemical characteristics for reliable taxonomic identification.

4.3 Antibacterial Activity

The antibacterial activity of the isolated bacteria was evaluated against selected bacterial pathogens, including *Staphylococcus aureus*, *Vibrio cholerae*, and *Salmonella* spp. The isolates showed **no visible inhibition against *S. aureus* and *V. cholerae*** under the experimental conditions. A slight inhibition zone was observed against *Salmonella* spp., indicating limited and selective antibacterial activity.

The weak antibacterial activity observed in the present study may be associated with the production of low concentrations of antimicrobial metabolites or other strain-specific inhibitory compounds. The absence of inhibition against some test organisms does not necessarily indicate a complete absence of antimicrobial

potential, as activity can vary according to bacterial strain, culture conditions, incubation period, medium composition, and metabolite concentration. Further optimization and quantitative determination of inhibition zones would be useful for evaluating the antimicrobial potential of these isolates.

4.4 Hemolytic Activity

Both Y1 and W1 produced clear zones around their growth on blood agar, indicating **β-haemolytic activity**. The presence of complete haemolysis suggests that the isolates possess factors capable of disrupting erythrocytes. Although haemolytic activity can occur among environmental and plant-associated bacteria, it is an important safety characteristic that should be carefully considered when evaluating isolates for probiotic, food, or nutraceutical applications. Therefore, the haemolytic phenotype observed in the present isolates warrants further characterization before considering them for such applications.

4.5 Antifungal Activity

Both bacterial isolates demonstrated antifungal activity against the tested fungal isolates, as evidenced by the formation of distinct inhibition zones around the bacterial growth. The diameter of inhibition varied among the fungal isolates, indicating differential susceptibility to the metabolites produced by Y1 and W1.

The observed antifungal activity suggests that the isolates may produce extracellular or diffusible antimicrobial metabolites capable of suppressing fungal growth. Plant-associated *Bacillus* species are known to produce several bioactive compounds, including lipopeptides and other secondary metabolites, which may contribute to antifungal activity. Similar antifungal potential has also been reported for banana-derived extracts and microorganisms associated with banana tissues.

The antifungal activity observed in this study therefore indicates that banana stem water may harbour bacteria with potential biocontrol properties. However, identification of the specific antifungal compounds and determination of their mechanisms of action require further investigation.

5. DISCUSSION

The present study demonstrates that banana pseudostem water can serve as a source of diverse bacterial populations with distinct morphological, biochemical, and molecular characteristics. The isolation of bacterial strains from banana pseudostem is consistent with previous reports describing the presence of beneficial and endophytic bacteria associated with banana tissues, including lactic acid bacteria and *Klebsiella* spp. (Puranik et al., 2018; Luna-Pineda et al., 2019). The physicochemical and nutrient-rich nature of banana pseudostem may provide a suitable environment for the survival and growth of microorganisms.

The antibacterial and antifungal activities observed in the present study further support the reported biological potential of banana-derived materials. Previous studies have demonstrated antimicrobial activity of banana stem, sap, and pseudostem extracts against several pathogenic microorganisms, including *Streptococcus mutans*, *Enterococcus faecalis*, and fungal species such as *Candida albicans* and *Aspergillus niger* (Apriasari et al., 2013; Budi et al., 2020; Das & Velusamy, 2013; Waskito et al., 2020). Similarly, the phytochemical constituents and polyphenolic compounds present in banana pseudostem have been associated with antioxidant and antimicrobial properties (Kumar et al., 2014; Saravanan & Aradhya, 2011). The variable inhibition observed against the tested microorganisms in the present study may therefore be related to differences in bacterial strain, metabolite production, concentration, and susceptibility of the target organisms.

The molecular identification of the isolates indicates the occurrence of both Gram-negative and Gram-positive bacteria in banana pseudostem water. The detection of *Klebsiella pneumoniae* is noteworthy because *Klebsiella* species have previously been reported as endophytic or plant-associated bacteria in banana plants (Luna-Pineda et al., 2019). However, molecular identification based only

on 16S rRNA sequences should be interpreted cautiously, particularly when sequence similarity is relatively low or closely related bacterial taxa are involved. Additional molecular markers or phylogenetic analysis would provide greater confidence in species-level identification. The observed haemolytic activity of the isolates is an important finding from a safety perspective. Although banana pseudostem-associated microorganisms may have potential for biotechnological applications, haemolytic activity requires careful evaluation before any strain is considered for food, probiotic, or therapeutic applications. Therefore, antimicrobial potential should be considered together with appropriate biosafety and pathogenicity assessments.

Overall, the findings support the view that banana pseudostem is not merely an agricultural waste material but a potentially valuable biological resource. Earlier studies have reported its antioxidant, antimicrobial, wound-healing, anti-urolithiatic, and other functional properties (Boominathan et al., 2016; Adithya et al., 2014; Aishwarya et al., 2021; Dikshit et al., 2016). The present findings extend this knowledge by demonstrating the presence of cultivable bacteria with measurable antagonistic activity. Further studies involving comprehensive genomic identification, metabolite profiling, purification of bioactive compounds, mechanism-of-action studies, and safety evaluation are required to establish the practical applications of banana pseudostem-derived microorganisms and metabolites.

6. CONCLUSION

The present study highlights the biological and biotechnological potential of banana pseudostem (*Musa* spp.), an underutilized agricultural by-product. Banana stem water was found to support the isolation of bacterial strains with distinct morphological, biochemical, and molecular characteristics, demonstrating its potential as a source of microbial diversity. The identified bacterial isolates, including *Klebsiella pneumoniae* and a *Bacillus*-related strain, exhibited variable antibacterial and antifungal activities, indicating their possible role in the production of biologically active metabolites.

The observed microbial activities, together with the previously reported antimicrobial, antioxidant, wound-healing, anti-urolithiatic, and nutritional properties of banana stem, suggest considerable potential for pharmaceutical, nutraceutical, food, and environmental applications. However, the haemolytic activity observed in the isolates emphasizes the need for careful safety evaluation before considering their use in food, probiotic, or therapeutic applications.

Overall, banana pseudostem represents a valuable and sustainable biological resource for microbial isolation and value-added applications. Further studies involving complete molecular characterization, metabolite profiling, antimicrobial mechanism analysis, toxicity assessment, and controlled in vitro and in vivo investigations are necessary to establish its practical applications. Such research may contribute to the sustainable utilization of banana agricultural waste and the development of environmentally friendly biotechnological products.

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