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Exploring Antibacterial Properties of Bioactive Compounds Isolated from *Streptomyces* sp. in Bamboo Rhizosphere Soil

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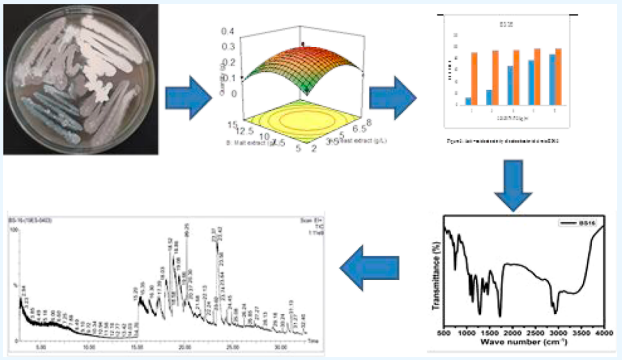
ABSTRACT: The increasing concern over multidrug resistance in pathogens has led to an ongoing search for novel antibiotics derived from soil actinobacteria. In this current investigation, actinobacteria were isolated from the rhizosphere of bamboo plants collected within the Megamalai forest of the Western Ghats in the Theni zone of Tamil Nadu, India. These actinobacteria were subjected to characterization, and their growth conditions were optimized to enhance the production of bioactive compounds. To assess antibacterial properties, the isolated Actinobacteria underwent testing using the agar plug method. The strain exhibiting notable antibacterial activity underwent further characterization through 16s rRNA gene sequencing and subsequent phylogenetic analysis. Employing response surface methodology (RSM), cultural conditions were fine-tuned. Bioactive compounds were extracted from the culture medium using ethyl acetate, and their antibacterial and antioxidant effects were evaluated through disc diffusion and DPPH radical scavenging methods, respectively. Ethyl acetate extracts were analyzed by using FT-IR and GC−MS techniques. In total, nine strains of Actinobacteria were isolated from the rhizosphere soil of bamboo. Among these, strain BS-16 displayed remarkable antibacterial activity against three strains: *Staphylococcus aureus* (19 mm), *Bacillus subtilis* (12 mm), and *Streptococcus pyogenes* (10 mm). This strain was identified as *Streptomyces* sp. The optimal conditions for bioactive compound production were determined as follows: malt extract (10 g), yeast extract (5 g), dextrose (5 g), pH 6.5, and temperature 30 °C. After a 7-day incubation period, the results showed a 6% increase in production. The ethyl acetate fraction derived from strain BS-16 exhibited dose-dependent antibacterial and antioxidant activities. FT-IR and GC−MS analyses revealed the presence of active compounds with antibacterial effects within the extract. Consequently, further investigation into the BS-16 strain holds promise for scaling up the production of bioactive compounds possessing antibacterial and antioxidant properties.



1. INTRODUCTION

In the 21st century, antimicrobial resistance (AMR) stands out as a paramount global health issue, presenting significant threats to humanity's well-being.[1] Recognizing this, the World Health Organization (WHO) pinpointed AMR as one of the foremost 10 global health risks in 2019. Consequently, the pursuit of novel antibacterial compounds has become even more urgent, with the aim to counter the proliferation of AMR infections within clinical environments.

Actinobacteria show great potential in creating secondary compounds that are being studied for various bioactive molecules such as antibiotics, anticancer, and antivirals for the treatment against different ailments.[2] In addition, actinomycetes are considered a source of agroactive compounds, plant growth-promoting agents, biocontrol molecules, and bioremediation.[3] Actinomycetes are responsible for nearly 70% of all known microbial secondary metabolites.[4] Macrolides, β-lactams, aminoglycosides, angucyclinone, phenazines, anthracyclines, benzoxazolophenanthridines, and tetracyclines are the antimicrobial substances isolated from actinomycetes.[5] About 75% of actinomycetales compounds were from *Streptomyces* and 25% were from rare actinomycetes.[5] Most of these compounds show antibiotic and antioxidant activity. Actinobacteria are present widely in soil, plant, animals, freshwater, and marine ecosystem. In these environments, actinobacteria play vital role in host nutrient assimilation and recycling as well as in defense.[1,6] Actinobacteria's adaptation and interaction in such harsh conditions has given them the ability to synthesize a diverse range of physiologically active metabolites through different

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biosynthetic pathway.[7] The filamentous actinobacteria with either moderate (~5−7.9 MB) or large genome (>8 MB) contains many natural products-biosynthetic gene cluster.[8] Recently, actinobacteria are focused for bioprospecting campaigns for discovering new bioactive molecules.[9] Bamboo is considered as a valuable resource for forests since it is both economically and environmentally beneficial. Bamboo depends on the rhizome and is a fast-growing plant. There are 116 genera and 1400 species of bamboo in the globe, except for Europe and Antarctica; they are mostly found in tropical and subtropical climates.[10] The advantages of bamboo are manifold. In the realm of traditional medicine, their leaves and roots have found utility. Research has demonstrated the presence of antioxidant, anticancer, and antibacterial qualities in bamboo roots and leaves.[11] An instance of note involves the isolation of *Streptomyces gramineus* JR-43T from the rhizosphere soil of a specific bamboo plant, *Sasa borealis*. Within *Sasa borealis* litter, this strain of *Streptomyces* stood out as the predominant actinobacterial isolate, constituting over 97% of the total composition.[12]

The soil microbiome serves as a valuable indicator of soil quality due to its exceptional responsiveness to even minor shifts in the environment caused by environmental stressors.[13] Within the rhizosphere soil, a multitude of microorganisms play a pivotal role in fostering both plant growth and potential bioactivity. The interaction between a plant and a microbe yields significant effects on the plant's development, encompassing factors like growth and microbial metabolism. Notably, the bacteria thriving in the rhizosphere of the soil exhibit an impressive capacity to break down intricate compounds present in plant secretions. Through the release of root exudates, plants stimulate microbial and nutritional processes in the rhizosphere, thus contributing to the accumulation of carbon originating from organic matter in the soil.[14] The plant rhizosphere constitutes the area between plant roots and the soil, inhabited by a diverse array of living organisms that regulate plant growth, encompassing both rhizosphere and endophytic bacteria.[15]

Among the countries of the Asia-Pacific region, China has the highest bamboo diversity, followed by India (approximately 148 species in 29 genera of bamboos), Japan, Vietnam, and South Korea.[10] India's deciduous and semievergreen regions, along with the tropical moist deciduous forests in both the northern and southern parts of the country, harbor the largest number of species.[16] Bamboo limits the amount of carbon dioxide. Also, in comparison to other trees, bamboo produces 35% more oxygen. Bamboo is known for various biological properties such as melanin synthesis inhibition, antimicrobial activity, tyrosinase inhibition activity, antioxidant activity, prevention of infectious diseases, anti-inflammation, and protection against various diseases (cardiovascular, metabolic, and neurological/neuropsychiatric diseases).[17] Bacteria are more diverse in rhizosphere (area adjacent to plant roots) than other areas without roots.[18] Hence, rhizosphere of various plants such as wheat, paddy, yam, *Barringtonia racemosa*, and *Calotropis procera* is explored for the Actinomycetes.[19,20] Keeping all these facts in view, in the present study, we aimed to isolate actinobacteria from the least explored environment such as bamboo rhizosphere and screening for the presence of antimicrobial activity.

2. MATERIALS AND METHODS

2.1. Materials. 2, 2-diphenylpicrylhydrazyl (DPPH) was purchased from Sigma, USA. Mueller Hinton Agar (MHA), yeast extract malt extract (YEME), agar, dextrose, yeast extract, and malt extract were purchased from Himedia, India. All the solvents of the highest purity were purchased from Merck, India.

2.2. Soil Sample Collection and Pretreatment. The soil samples were taken in sterile cover from the rhizosphere of bamboo plants [Megamalai forest (9° 41′ 59.99″ N, 77° 23′ 59.99″ E), Western Ghats in Theni zone, Tamil Nadu, India]. Collected samples were air-dried at room temperature for 48 h to reduce the level of bacterial contamination. All sediment samples were heated at 55 °C for 6 min to promote actinomycete isolation. The samples were gathered in sterile zip-lock bags.

2.3. Isolation of Actinobacteria Inhabiting Bamboo Rhizosphere. A 1 g soil sample was serially diluted in sterile saline solution at a ratio of 1:10 to 1:1000. The YEME medium supplemented with cycloheximide and nystatin was covered with an aliquot of 0.1 mL of each dilution. For 7 days, plates were incubated at 28 °C. As stock cultures, the purified colonies were kept in 20% glycerol at −80 °C on YEME agar slants.

2.4. Morphological Characterization and Screening of the Actinobacteria. Strain BS-16, the chosen isolate, was introduced into the ISP2 medium for the purpose of undergoing culture characterization. The Petri dishes containing the medium were subsequently placed in an incubator, where they were allowed to cultivate for a duration of 7 days at a temperature of 28 °C. The sterilized ISP2 was prepared and poured into the Petri plate. After solidification, about 2 cm square of the ISP2 agar was cut into the surface, and spore of the selected strain was added and incubated at room temperature. After incubation, the spore was observed using a scanning electron microscope.

2.5. Molecular Characterization of the Actinobacteria. The genomic DNA was isolated from the strain BS-16. 16S rRNA gene was amplified using the PCR method with Taq DNA polymerase and primers 518F (5′ CCAGCAGCCGCGCTAA-TACG 3′) and 800R (5′ TACCAGGGTATCTAATCC 3′). The conditions for thermal cycling were as follows: denaturation of the target DNA at 95 °C for 4 min, followed by 30 cycles at 94 °C for 1 min, primer annealing at 50 °C for 1 min, and primer extension at 62 °C for 1 min. At the end of the cycling, the reaction mixture was held at 72 °C for 10 min and then cooled to 4 °C (Eppendorf Mastercycler personal, USA). The PCR product was sequenced by an automated sequencer (AB13730, Eppendroff Master Cycler personal). The sequence obtained was compared for the similarity level with the reference species of *Streptomyces* contained in genomic database banks, using the "NCBI Blast" (https://www.ncbi.nlm.nih.gov/) web site. After BLAST analysis, a data set of potential orthologs was prepared by considering those database sequences which had >99% sequence identity with the query sequence of strain BS-16.[21]

A phylogenetic and molecular evolutionary analysis of BS-16 was conducted using software included in the MEGA version 418 package. The 16S rRNA sequence of the strains BS-16 was aligned (http://www.ebi.ac.uk/clustalw) against corresponding nucleotide sequences of representatives of the genus *Streptomyces* retrieved from GenBank.[22−24] Evolutionary distance matrices were generated as described, and a phylogenetic tree was inferred by the Neighbor joining method. Tree topologies were evaluated by bootstrap analysis based on 1000 resembling of the neighbor joining data set.[25,26]

2.6. Screening for Antibacterial Activity Using the Agar Plug Diffusion Method. YEME agar plates with actinobacterial strains were inoculated, and the plates were then incubated at 28 °C for 7 days. Agar plugs containing test

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organisms (*Staphylococcus aureus*, *Bacillus subtilis*, and *Streptococcus pyogenes*) were placed over MHA. All of the plates were incubated at 37 °C for 24 h. The zone of inhibition was measured in millimeters.

2.7. Optimization by Response Surface Methodology. The Box-Behnken design (BBD) was used to evaluate the significance of factors such as yeast extract, malt extract, dextrose, pH, and temperature, and the medium composition was optimized for the maximum production of bioactive compounds and antimicrobial activity. The response surface methodology (RSM) data were subjected to an analysis of variance (ANOVA) test. To determine the significance of the regression coefficient, the *F*-test and *p*-value were used, and the coefficient of determination (R^2) and adjusted R^2 were used to express the model's suitability. The suitability of the fitted model was evaluated by comparing the experimental and predicted values.

2.8. Production and Extraction of Bioactive Compound. Spores of the BS-16 strain were inoculated into ISP2 for 48 h at 28 °C in a rotary shaker at 120 rpm. The inoculum was then mixed with 100 mL of production medium (ISP2) and shaken for 7 days at 120 rpm and 28 °C in a rotary shaker. The supernatant was obtained at the end of the incubation period by centrifuging the production medium at 10,000 rpm for 30 min at 4 °C.

2.9. Antibacterial Activity of Ethyl Acetate Extract of BS-16. *2.9.1. Disc Diffusion Method.* The disc diffusion method was employed to assess the antimicrobial susceptibility against bacterial pathogens such as *S. aureus*, *B. subtilis*, and *S. pyogenes*. The positive controls for this evaluation included gentamicin and nystatin discs. Sterile discs, with a diameter of 6 mm from Himedia, were impregnated with 30 μL of a diluted crude extract in a solution of 0.5% DMSO. As a reference, discs coated with 0.5% DMSO from Qualigens Fine Chemicals Pvt. Ltd. (San Diego, USA) were utilized as solvent controls. After impregnation, the plates were maintained at a temperature of 4 °C for 30 min to facilitate the diffusion of the extract, followed by incubation at 37 °C for a span of 24 to 48 h. The presence of a distinct zone of inhibition encircling the disc indicated antagonistic activity against the test organism, with the diameter of this inhibition zone subsequently being measured to determine the degree of antagonism.[27]

2.9.2. Determination of Minimum Inhibition Concentration. The determination of the minimum inhibitory concentration (MIC) of the extract against each pathogen was carried out using a modified method.[28] In this procedure, 2 mL of sterile nutrient broth was prepared, and different concentrations of the extract (ranging from 0.313 to 20 mg/mL) were added to separate tubes. To each of these tubes, 0.1 mL of the respective test pathogen was introduced. Subsequently, the tubes were placed in an incubator set at 37 °C for 24 h. As a reference, an additional set of identical tubes containing 25 μg/mL chloramphenicol was utilized as a control. The evaluation involved detecting microbial growth through the assessment of the turbidity in the tubes following the incubation period. Specifically, the level of cloudiness observed in the tubes indicated the extent of the microbial growth. Among the tubes with various extract concentrations, the one displaying the least discernible signs of growth was identified as the tube possessing the lowest inhibitory concentration of the extract.

2.10. DPPH Radical Scavenging Activity. The scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was evaluated by dissolving it in 3.3 mL of methanol in a test tube. The test tube was wrapped in aluminum foil to block light. After 150 μL of the DPPH solution was added to 3 mL of methanol, the absorption at 517 nm was immediately measured and confirmed. Standard chemical (ascorbic acid) and actinomycete solvent (stock concentrations, i.e., 1 mg/1 mL) fractions were collected at concentrations of 100, 200, 300, 400, and 500 μL. 3 mL of methanol and 150 L of DPPH were then added to each reaction vessel. After 15 min at 517 nm, the absorbance was measured using a Cyber lab.[29]

The free radical scavenging activity of DPPH was determined using the formula

$$\text{scavenging activity (\%)} = \left[\frac{\text{absorbance (control)} - \text{absorbance (sample)}}{\text{absorbance (control)}}\right] \times 100$$

2.11. Fourier Transform Infrared Spectral Analysis. Using the potassium bromide (KBr) pellet method, the spectra were produced with a resolution of 1.0 cm^{-1} in the region of 4000 to 400 cm^{-1}. KBr pellet was made using 100 mg of KBr and 1 mg of the ethyl acetate fraction. The intensity versus wavenumber of the spectrum was displayed.

2.12. Gas chromatography−Mass Spectrometry Analysis. GC−MS analysis of the ethyl acetate extract obtained was carried out using the silica capillary column (ELITE- 5MS) 30 × 0.25 mm I.D., film thickness 0.25 μm to record the mass spectrum. An aliquot of 1.0 μL of the sample was administered. The compounds were identified based on comparisons of the mass spectra obtained with those from the Wiley and NIST 2008 libraries.

2.13. Statistical Analysis. Every experiment was carried out in duplicate, ensuring the reliability of the results. The mean value and standard deviation were calculated to provide a comprehensive overview of the data's central tendency and dispersion. To ascertain the significance of discrepancies in the mean values of inhibitory zones formed by the crude extracts compared with the control antibiotic when tested against the target organisms, OriginLab software was employed. This software facilitated the statistical assessment of variations between these values.

3. RESULTS

Colonies with actinobacterial morphology were observed on ISP2 media. In the preliminary antimicrobial screening, the strain BS-10 showed zone of inhibition against *S. aureus* (11 mm), as shown in Table 1. However, the strain BS-16 showed zone of inhibition against all the three strains [*S. aureus* (19 mm), *B. subtilis* (12 mm), and *S. pyogenes* (10 mm)] studied. Hence, BS-16 was selected as potential strain for further studies. The morphology of the BS-16 strain grown in YEME agar is shown in Figures 1 and 2. The SEM image of BS-16 Actinobacterial spores showed that the morphology is smooth and straight (Figures 1 and 2).

The BLAST analysis of the BS-16 actinobacteria 16s rRNA gene sequence revealed 99% similarity to *Streptomyces* sp. (Figure 3) and depicts the phylogenetic relationship of the strain BS-16 and related taxa. Sequence similarities of 99.67% were found between the potential strain BS-16 and various *Streptomyces* species. The sequencing of the 16s rRNA gene from strain BS-16 was successfully deposited in GenBank under the accession number MT525339.1.

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Table 1. Antibacterial Activity of Actinobacterial Strains by the Agar Plug Method[a]

s. no	strain	zone of inhibition (diameter in mm)		
		Staphylococcus aureus	Bacillus subtilis	Streptococcus pyogens
1	BS-2			
2	BS-4			
3	BS-5			
4	BS-10	11 ± 0.86		
5	BS-13			
6	BS-16	19 ± 1.44	12 ± 1.05	10 ± 1.26
7	BS-17			
8	SM-1			
9	SM-4			

[a]Each value is the mean ± SD of triplicate analysis.

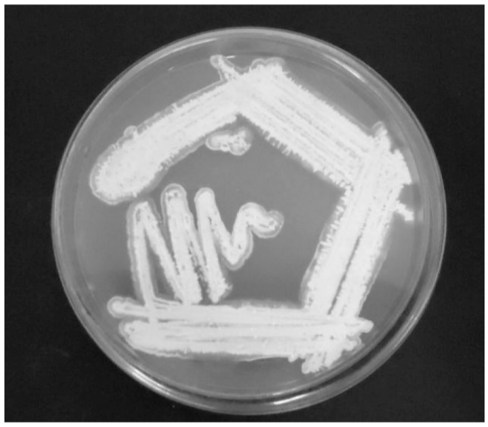

Figure 1. (a) Isolate BS-16 cultured on ISP-2 medium.

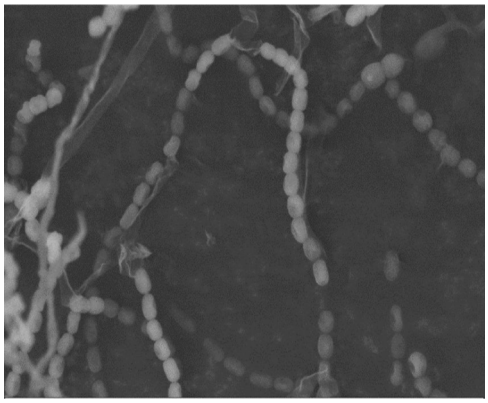

Figure 2. SEM image of the potential isolate.

The most accurate predicted R^2 values for bioactive compounds were 0.7872 and 0.6616 in the cubic model. Lack of fit had the highest P value (0.2316), indicating that it is not significant for zone of inhibition (Tables 2 and 3). The three-dimensional surface graphs were created to forecast the interaction between the five independent variables and the response (Figures 4 and 5).

The antibacterial effect of ethyl acetate extract of BS-16 culture was studied using a disc diffusion method. In comparison to *B. subtilis* (10 mm) and *S. pyogenes* (15 mm), the zone of inhibition against *S. aureus* (25 mm) was found to be higher for ethyl acetate extract of BS-16 culture media (Table 4).

Each value is the mean ± SD of triplicate analysis. MIC values of ethyl acetate extract of BS-16 culture media against *S. aureus*, *B. subtilis*, and *S. pyogenes* were found to be 0.661, 0.987, and 0.986 μg/mL, respectively (Figure 6). In vitro antioxidant activity of the ethyl acetate extract of BS-16 was described as DPPH radical scavenging activity (Figure 7). The IC_{50} value of the ethyl acetate extract of BS-16 against DPPH radical scavenging was found to be at 56.62 μg/mL.

The functional groups present in active components of the ethyl acetate extract of BS-16 were determined using Fourier transform infrared (FT-IR) (Figure 8). The observed peaks at 2982.21, 1736.25, 1372.64, 1233.38, 1041.02, 845.69, and 604.37 cm^{-1} correspond to CH alkane, C═O of aldehyde, NO_2 in the aliphatic nitro group, C−O−C in esters, CH−O−H in cyclic alcohol, R−NH_2 in primary amines, and C═O of amide, respectively.

The compounds identified in BS-16 are listed in Table 5. The major compounds identified were eicosanoic acid, 1,2-denzenedicarboxylic acid, diisooctyl ester, *N*-hexadecanoic acid, pentadecanoic acid, 9-(*E*)-eicosene, phthalic acid, monoethyl ester, and phthalic anhydride.

4. DISCUSSION

Actinomycetes, in particular, *Streptomyces* sp., continue to be the microbes with the greatest economic and biotechnological benefits, producing most of the antibiotics used in medicine. Rhizosphere creates an ideal habitat for microorganism growth due to availability of nutrient and organic materials derived from root exudates.[30] Various Actinobacteria (*Streptomyces bambusae* sp. *nov*., *Streptomyces. gramineus* sp. *nov*., *Streptomyces sasae* sp. *nov*) from bamboo rhizosphere have been isolated from various regions globally, including China and Korea.[31,32] Actinobacteria are common in rhizome soils and can produce a variety of useful secondary metabolites and substances with different properties. Actinobacteria isolated from bamboo rhizosphere soil exhibited unusual culture morphology and soluble pigment, indicating the potential of new bioactive compounds widely used in the field of biomedicine. Nonetheless, there has been limited exploration of actinobacterial species within the bamboo rhizosphere in India. This current research conducted bioprospecting on actinobacteria derived from the rhizosphere of bamboo plants situated in the Megamalai forest of Theni district, Tamil Nadu, India. In this study, a total of nine distinct actinobacteria strains were successfully isolated from soil samples collected from the bamboo plant rhizosphere. All of the isolated actinobacteria displayed unique morphologies. Initial screening for antibacterial activity revealed the strain BS-16's potency against various test organisms including *S. aureus*, *B. subtilis*, and *S. pyogenes*. Subsequent molecular and phylogenetic analyses confirmed the similarity of BS-16 to *Streptomyces* sp., and the 16S rRNA sequence was deposited under the accession number MT525339.1. For a long time, *Streptomycetes* has been considered as free-living soil inhabitants; however, the beneficial relationship of *Streptomyces* sp. with host plants as endophytes has been understood.[33] Exudates from root systems, which are rich in different organic compounds created by the plant in the vicinity of the roots, determine the diversity and concentration of soil microorganisms.[34] Recently, rhizosphere inhabiting Actinobacteria, in particular, *Streptomyces* sp. of various other plants such as *C. procera*, *Caesalpinia pyramidalis Tul*, cassava, and olive were reported for antimicrobial activities.[35,36] In concordance with these previous reports, the BS-16 (*Strepto-*

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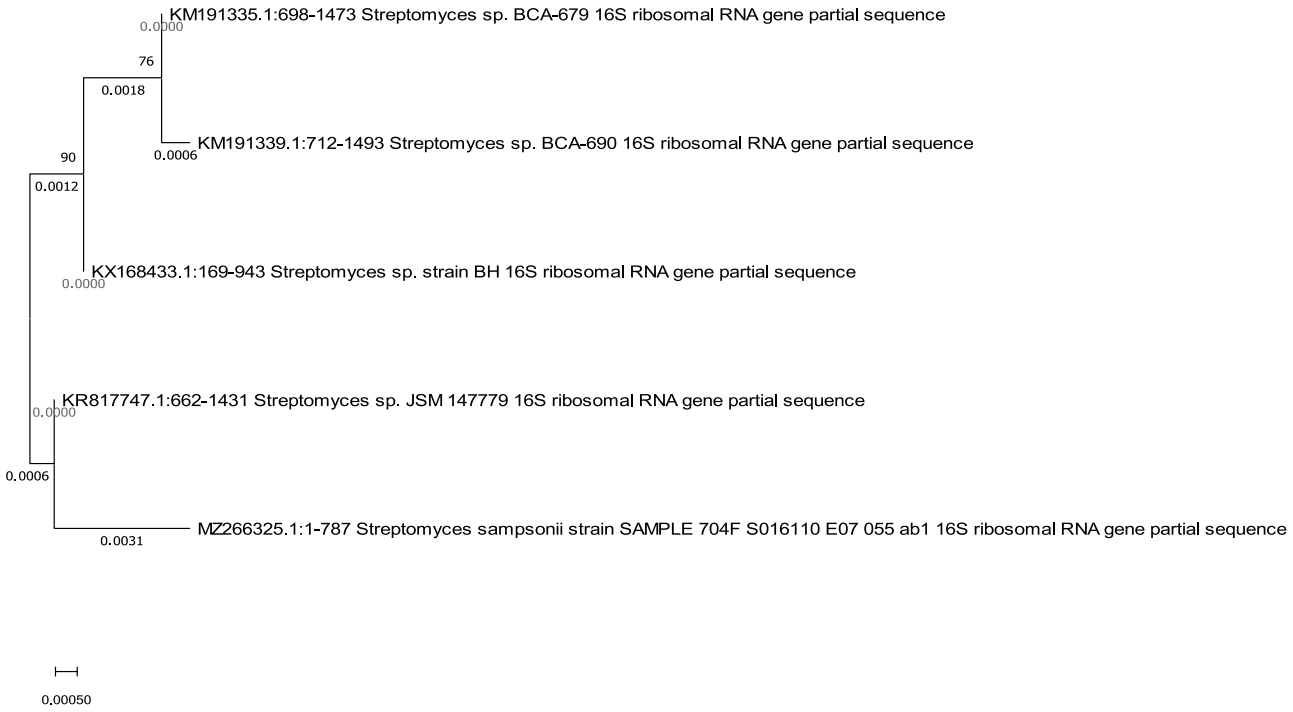

Figure 3. Neighbor-joining approach was used to construct a phylogenetic tree displaying the relationships between *Streptomyces* sp. BS-16 and related species as revealed by 16S rRNA gene sequencing.

Table 2. ANOVA Results for the Coefficient of the Cubic Model for Bioactive Compound Production

source	sum of squares	df	mean square	*F*-value	*p*-value	
model	0.4481	20	0.0224	20.63	<0.0001	significant
A-yeast extract	0.0000	1	0.0000	0.0230	0.8806	
B-malt extract	0.0121	1	0.0121	11.14	0.0026	
C-dextrose	0.0149	1	0.0149	13.70	0.0011	
D-pH	0.0005	1	0.0005	0.4456	0.5106	
E-temperature	0.0020	1	0.0020	1.82	0.1891	
AB	0.0006	1	0.0006	0.5754	0.4552	
AC	0.0002	1	0.0002	0.2071	0.6530	
AD	0.0001	1	0.0001	0.0921	0.7641	
AE	0.0016	1	0.0016	1.47	0.2362	
BC	0.0029	1	0.0029	2.68	0.1139	
BD	0.0007	1	0.0007	0.6223	0.4376	
BE	0.0030	1	0.0030	2.78	0.1076	
CD	0.0042	1	0.0042	3.89	0.0598	
CE	0.0009	1	0.0009	0.8285	0.3714	
DE	0.0000	1	0.0000	0.0230	0.8806	
A^2	0.0912	1	0.0912	84.00	<0.0001	
B^2	0.0854	1	0.0854	78.61	<0.0001	
C^2	0.0675	1	0.0675	62.10	<0.0001	
D^2	0.3044	1	0.3044	280.19	<0.0001	
E^2	0.1854	1	0.1854	170.67	<0.0001	
residual	0.0272	25	0.0011			
lack of fit	0.0242	20	0.0012	2.06	0.2162	not significant
pure error	0.0029	5	0.0006			
cor total	0.4753	45				

myces sp) strain isolated from bamboo rhizosphere has shown antibacterial activity.

The metabolites generated by actinobacteria are predominantly extracellular. Typically, these bioactive metabolites are isolated using the moderately polar solvent ethyl acetate.[37] Similarly, within this current investigation, the ethyl acetate fraction from the growth medium of BS-16 was subjected to further analysis regarding its antimicrobial potential. Notably, this fraction exhibited strong antibacterial activity against *S. aureus*, *B. subtilis*, and *S. pyogenes*. Additionally, the ethyl acetate fraction demonstrated in vitro antioxidant activity. The ethyl acetate extract of various actinomycetes species isolated from various habitats such as coastal, mangrove soil, and humus soil shows antioxidant activity.[38] Actinomycetes are known for the

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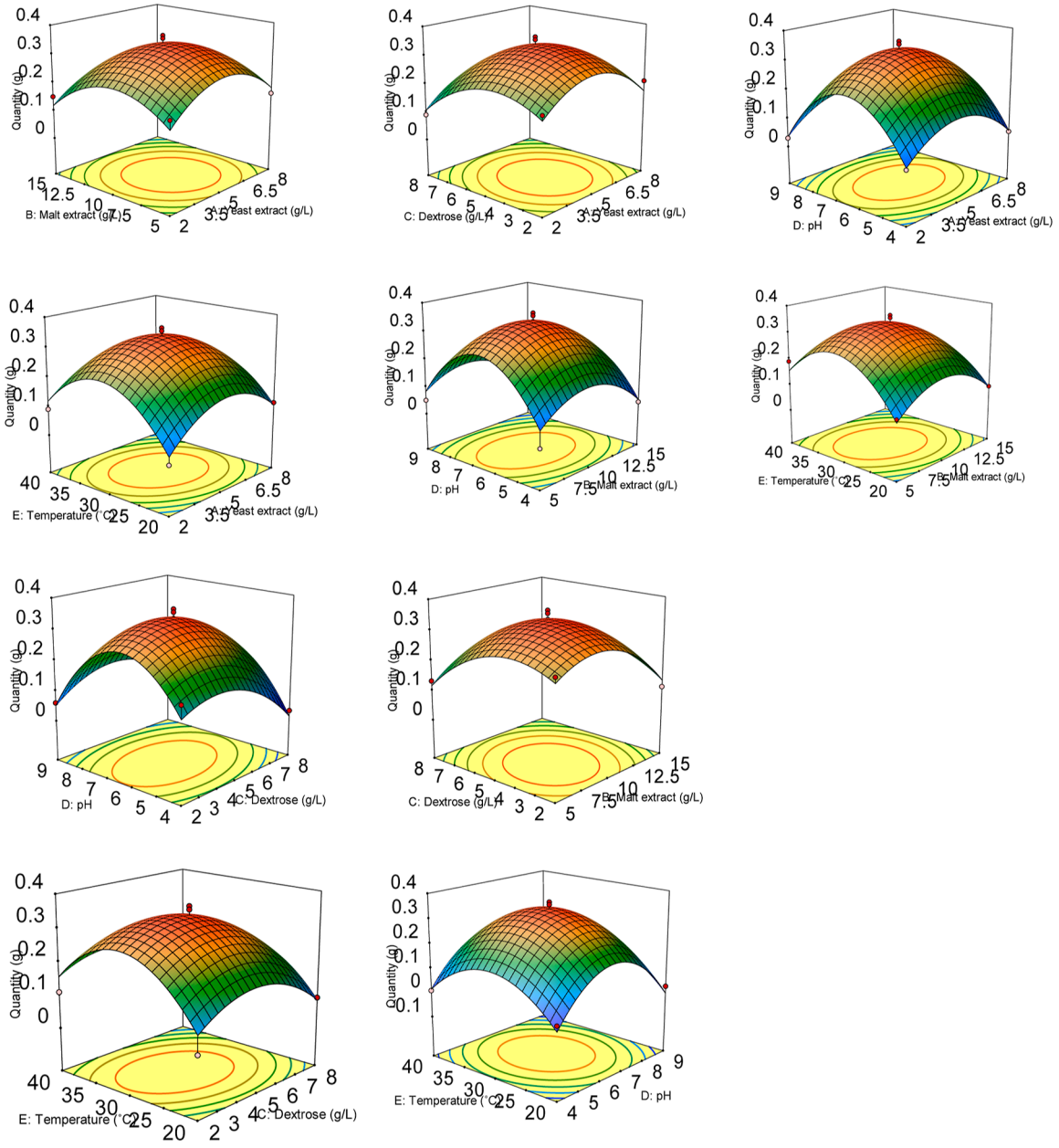

Figure 4. Response surface plots are three-dimensional graphical representations of the interacting effects of various selection variables on quantity by BS-16 against pathogens: (AB) yeast extract and malt extract, (AC) yeast extract and dextrose, (AD) yeast extract and pH, (AE) yeast extract and temperature, (BC) malt extract and dextrose, (BD) malt extract and pH, (BE) malt extract and temperature, (CD) dextrose and pH, (CE) dextrose and temperature, and (DE) pH and temperature.

production of phenolics and flavonoids compounds; therefore, they show antioxidant activity.[39] Also, the presence of active compounds with antioxidant activity would expand the application of BS-16 for the isolation of compounds to treat diseases related to oxidative stress.

The biological activity of ethyl acetate fraction of BS-16 was standardized using RSM. Previous reports have shown that the production medium composition and its concentration would influence the secondary metabolite production. The presence of glucose, which is an easily metabolizable carbon source, has repressed the secondary metabolite production. However, dextrose has been found to increase the production of compounds with antimicrobial activity. Similar to the carbon source, the presence of complex nitrogen sources such as malt extract, yeast extract, and soybean meal due to its slow decomposition has enhanced the antibiotics production in *Streptomycetes*.[40] Hence, the presence of yeast extract, malt extract, and dextrose in the medium has enhanced the antimicrobial metabolite production in BS-16. Also, the production was found to be optimum in slightly acidic conditions at pH 6.5. The isolated strain BS-16 might be neutrotolerant acidophiles, which had shown growth in neutral media; however, under acidic conditions or stress conditions at pH 6.5, enhanced production of antimicrobial compounds was observed.[41]

The ethyl acetate fraction of BS-16 was further characterized by FT-IR and GC−MS analysis. Through FT-IR, it was found that the phthalate compounds 1,2-benzenedicarboxylic acid, diisooctyl ester, phthalic anhydride, phthalic acid, and monoethyl ester were reported for antimicrobial activity.[42]

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temperature, (DE) pH and temperature.

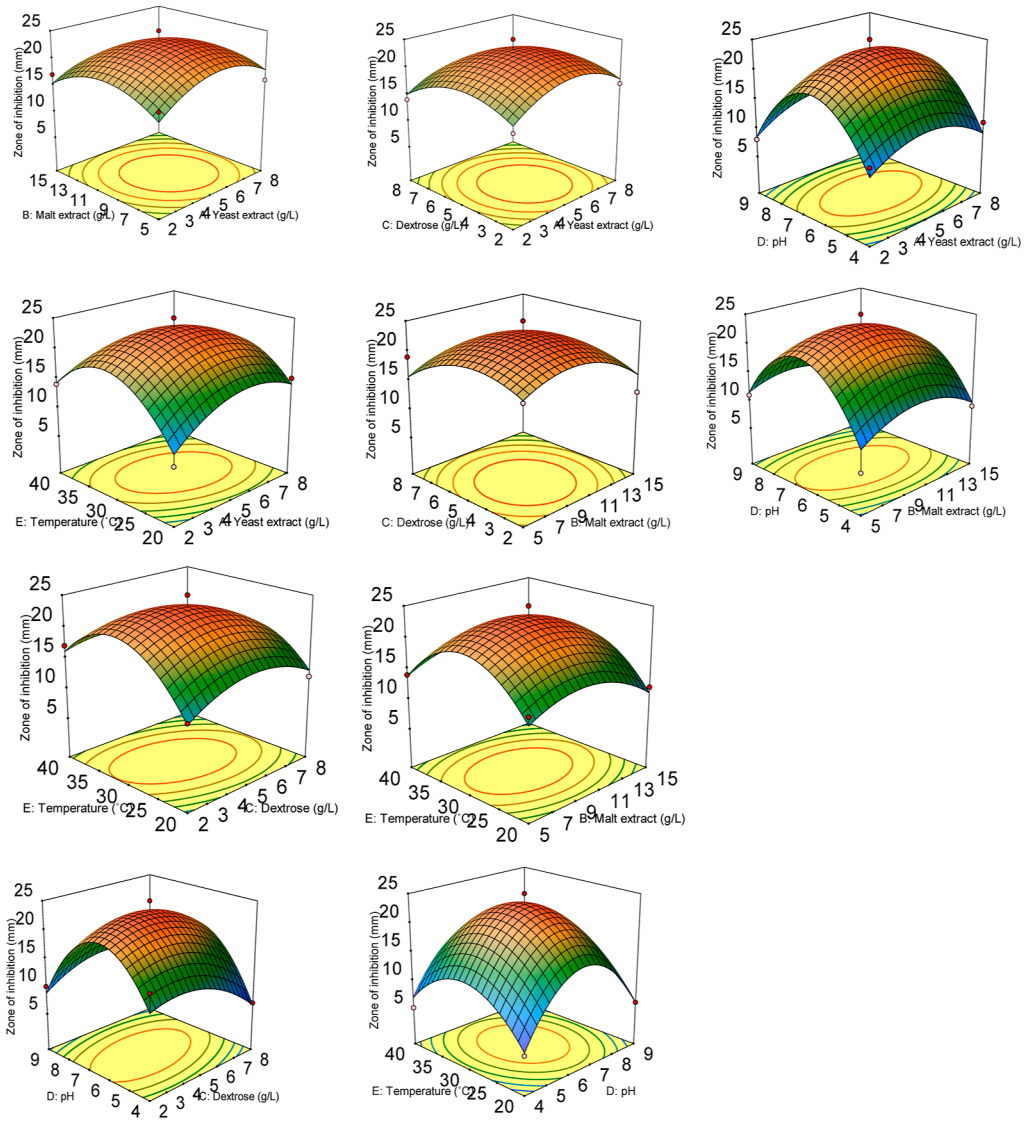

Figure 5. Response surface plots exhibit in three-dimensional views the interaction effects of various variables on the zone of inhibition (mm) resulting from bioactive compounds being produced by BS-16 against pathogens: (AB) yeast extract and malt extract, (AC) yeast extract and dextrose, (AD) yeast extract and pH, (AE) yeast extract and temperature, (BC) malt extract and dextrose, (BD) malt extract and pH, (BE) malt extract and temperature, (CD) dextrose and pH, (CE) dextrose and temperature, and (DE) pH and temperature.

The closely related compound called dimethyl phthalate was found to deform the cell membrane and disrupt the membrane channels to induce antimicrobial activity.[43] Similarly, the presence of these compounds in the BS-16 ethyl acetate extract could have disrupted the bacterial cell membrane. The antibacterial and antioxidant activity of *n*-hexadecanoic acid has been reported.[44] In addition, *n*-hexadecanoic acid was found to have anti-inflammatory activity through inhibition of phospholipase A_2 activity.[45] Pentadecanoic acid was found in the ethyl acetate extract of actinomycetes isolated from mangrove soil, which was found to have an antibacterial effect.[46] Also, pentadecanoic acid was reported for antibiofilm activity against organisms such as *Staphylococcus epidermidis*, *Candida albicans*, and *Klebsiella pneumoniae*.[47,48] Tetradecanoic acid (myristic acid) changed the bacterial morphology and membrane structure of food-borne pathogenic microorganism; *Listeria monocytogenes* inhibited its growth.[49] Myristic acid mixed system antibacterial activity against *S. aureus*.[50] Altogether, these previous reports show that the presence of all these compounds in the ethyl acetate fraction of BS-16 would have synergistically acted to show antioxidant and antibacterial activity.

5. CONCLUSIONS

A bioprospecting investigation was conducted within the rhizosphere of bamboo trees collected from the Megamalai forest in Tamil Nadu, India. Among a group of nine isolated Actinobacteria, BS-16 was singled out due to its notable antibacterial attributes. This strain was accurately identified as a strain of *Streptomyces* sp. using both 16S rRNA sequencing and phylogenetic analysis. The active compounds produced through the fermentation process of BS-16 were effectively extracted and subjected to characterization by using GC−MS analysis. On the whole, the BS-16 strain, extracted from the bamboo plant rhizosphere, displays considerable potential for future explora-

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Table 3. ANOVA Results for the Coefficient of the Cubic Model for the Zone of Inhibition

source	sum of squares	df	mean square	*F*-value	*P* value	
model	1139.61	20	56.98	12.46	<0.0001	significant
A-yeast extract	1.56	1	1.56	0.3417	0.5641	
B-malt extract	14.06	1	14.06	3.08	0.0917	
C-dextrose	20.25	1	20.25	4.43	0.0456	
D-pH	0.0156	1	0.0156	0.0034	0.9538	
E-temperature	2.64	1	2.64	0.5776	0.4544	
AB	4	1	4	0.8749	0.3586	
AC	1	1	1	0.2187	0.6441	
AD	1	1	1	0.2187	0.6441	
AE	12.25	1	12.25	2.68	0.1142	
BC	0.25	1	0.25	0.0547	0.817	
BD	5.06	1	5.06	1.11	0.3027	
BE	0.0625	1	0.0625	0.0137	0.9079	
CD	16	1	16	3.5	0.0731	
CE	12.25	1	12.25	2.68	0.1142	
DE	0.25	1	0.25	0.0547	0.817	
A^2	128.24	1	128.24	28.05	<0.0001	
B^2	117.33	1	117.33	25.66	<0.0001	
C^2	101.88	1	101.88	22.28	<0.0001	
D^2	880.02	1	880.02	192.48	<0.0001	
E^2	422.56	1	422.56	92.42	<0.0001	
residual	114.3	25	4.57			
lack of fit	101.47	20	5.07	1.98	0.2316	not significant
pure error	12.83	5	2.57			
cor total	1253.91	45				

Table 4. Antimicrobial Activity of the Crude Extract BS-16 Against the Bacterial Pathogens

s. no.	name of the pathogen	concentration of the compound (μg/mL)	zone of inhibition (mm)
1	*Staphylococcus aureus*	**25 μg**	25 ± 0.5
2	*Bacillus subtilis*	25 μg	10 ± 9
3	*Streptococcus pyogens*	25 μg	15 ± 1.2

tion in the extensive production of bioactive substances possessing antibacterial and antioxidant properties.

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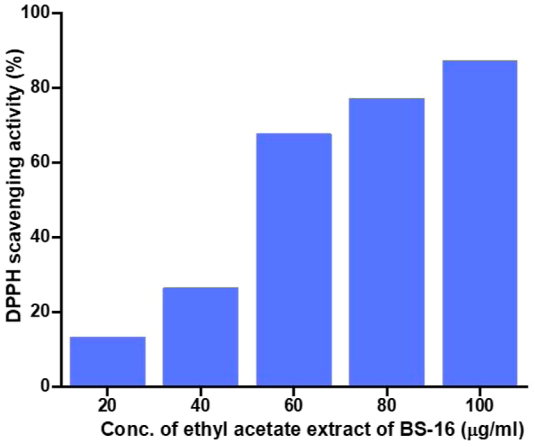

Figure 7. Antioxidant DPPH radical scavenging activity of the compound.

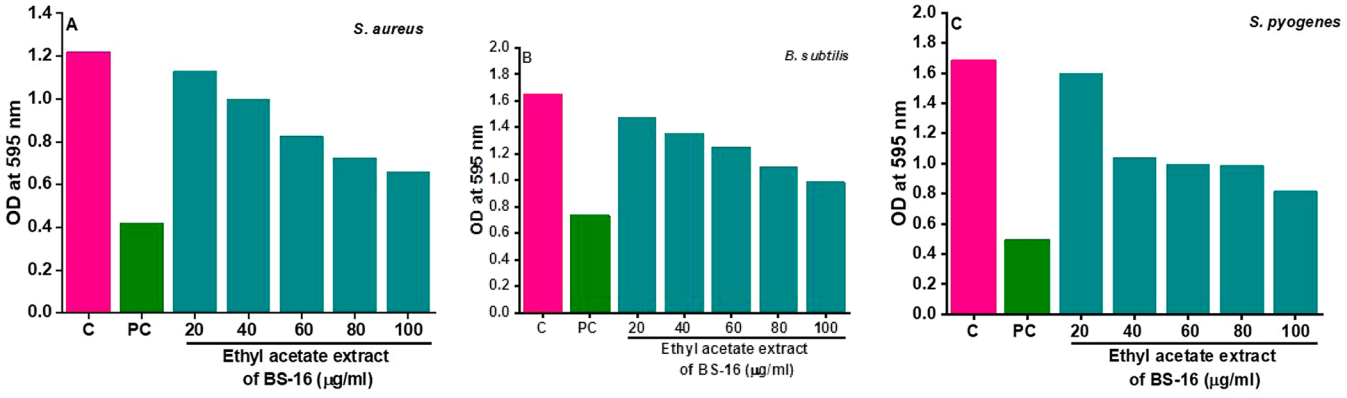

Figure 6. Minimum inhibitory concentration of actinomycetes isolated from rhizosphere soil against (a) *S. aureus*, (b) *B. subtilis*, and (c) *S. pyogenes*.

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Table 5. Compounds Identified in Ethyl Acetate Extract of BS-16 in GC−MS Analysis

si. no.	retention time	compounds	area (%)	molecular formula	molecular weight
1	15.348	phthalic anhydride	5.683	$C_8H_4O_3$	148
2	16.334	phthalic acid, monoethyl ester	6.084	$C_{10}H_{10}O_4$	194
3	17.389	tetradecanoic acid	2.337	$C_{14}H_{28}O_2$	228
4	18.094	pentadecanoic acid	10.665	$C_{15}H_{30}O_2$	242
5	18.520	1-docosene	3.147	$C_{22}H_{44}$	308
6	18.865	eicosanoic acid	17.624	$C_{20}H_{40}O_2$	312
7	19.450	*N*-hexadecanoic acid	11.716	$C_{16}H_{32}O_2$	256
8	20.010	11-tricosene	1.657	$C_{23}H_{46}$	322
9	20.245	9-eicosene, (E)-	6.496	$C_{20}H_{40}$	280
10	20.480	oleic acid	3.607	$C_{18}H_{34}O_2$	282

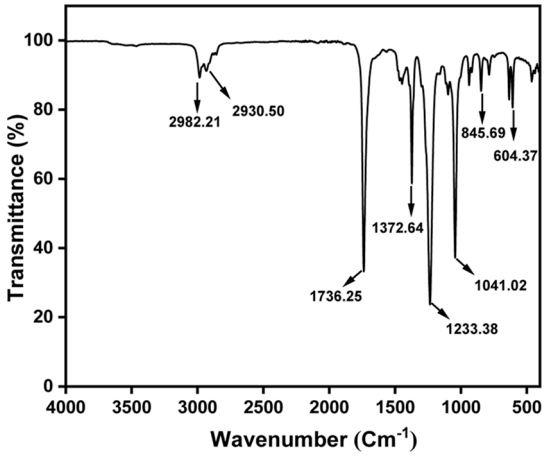

Figure 8. Fourier transform-infrared spectroscopy analysis of a crude extract of potential strain BS-16.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Goel, N.; Fatima, S. W.; Kumar, S.; Sinha, R.; Khare, S. K. Antimicrobial resistance in biofilms: Exploring marine Actinobacteria as a potential source of antibiotics and biofilm inhibitors. *Biotechnol. Rep.* **2021**, *30*, No. e00613.

(2) Salwan, R.; Sharma, V. Molecular and biotechnological aspects of secondary metabolites in Actinobacteria. *Microbiol. Res.* **2020**, *231*, 126374.

(3) Sharma, M.; Dangi, P.; Choudhary, M. Actinomycetes: source, identification, and their applications. *Int. J. Curr. Microbiol. Appl. Sci.* **2014**, *3*, 801−832.

(4) Subramani, R.; Aalbersberg, W. Marine Actinomycetes: an ongoing source of novel bioactive metabolites. *Microbiol. Res.* **2012**, *167*, 571.

(5) Bérdy, J. Bioactive microbial metabolites. *J. Antibiot.* **2005**, *58*, 1−26.

(6) Mohammadipanah, F.; Wink, J. Actinobacteria from arid and desert habitats: diversity and biological activity. *Front. Microbiol.* **2016**, *6*, 1541.

(7) Sivakala, K. K.; Gutiérrez-García, K.; Jose, P. A.; Thinesh, T.; Anandham, R.; Barona-Gómez, F.; Sivakumar, N. Desert environments facilitate unique evolution of biosynthetic potential in Streptomyces. *Molecules* **2021**, *26*, 588.

(8) Sayed, A. M.; Hassan, M. H. A.; Alhadrami, H. A.; Hassan, H. M.; Goodfellow, M.; Rateb, M. E. Extreme environments: microbiology leading to specialized metabolites. *J. Appl. Microbiol.* **2020**, *128*, 630.

(9) Goodfellow, M.; Nouioui, I.; Sanderson, R.; Xie, F.; Bull, A. T. Rare taxa and dark microbial matter: novel bioactive Actinobacteria abound in Atacama Desert soils. *Anton Leeuw Int. J. G.* **2018**, *111*, 1315.

(10) Bystriakova, N.; Kapos, V.; Lysenko, I.; Stapleton, C. M. A. Distribution and conservation status of forest bamboo biodiversity in the Asia-Pacific Region. *Biodivers. Conserv.* **2003**, *12*, 1833.

(11) Zhang, Y.; Jiao, J.; Liu, C.; Wu, X.; Zhang, Y. Isolation and purification of four flavone C-glycosides from antioxidant of bamboo leaves by macroporous resin column chromatography and preparative

https://doi.org/10.1021/acsomega.3c04954
ACS Omega 2023, 8, 36333−36343

high-performance liquid chromatography. *Food* **2008**, *107*, 1326−1336.
(12) Lee, H. J.; Han, S. I.; Whang, K. S. Streptomyces gramineus sp. nov., an antibiotic-producing actinobacterium isolated from bamboo (Sasa borealis) rhizosphere soil. *Int. J. Syst. Evol. Microbiol.* **2012**, *62*, 856−859.
(13) Sharma, S. K.; Ramesh, A.; Sharma, M. P.; Joshi, O. P.; Govaerts, B.; Steenwerth, K. L.; Karlen, D. L., Microbial community structure and diversity as indicators for evaluating soil quality, In *Biodiversity, Biofuels, Agroforestry and Conservation Agriculture*; Springer: Dordrecht, 2010; pp 317−358.
(14) Prabha, J.; Kumar, M.; Tripathi, R. Opportunities and challenges of utilizing energy crops in phytoremediation of environmental pollutants: a review. *Bioremediation. Environ. Sustainability* **2021**, 383−396.
(15) Pathan, S. I.; Ceccherini, M. T.; Sunseri, F.; Lupini, A., Rhizosphere as hotspot for plant-soil-microbe interaction, In *Carbon and Nitrogen Cycling in Soil*, 2020; pp 17−43.
(16) *Bamboo Diversity of India: An Update*: Sharma, M. L.; Nirmala, C., Eds.: World Bamboo Organization, 2015. https://www.researchgate.net/publication/342345120_Bamboo_Diversity_of_India_An_update.
(17) Ashour, A.; Elbermawi, A.; Amen, Y.; Allam, A. E.; Ikeda, H.; Nagata, M.; Kumagae, K.; Azuma, T.; Taguchi, A.; Takemoto, T.; et al. melanin synthesis inhibition activity of compounds isolated from Bamboo shoot skin (Phyllostachys pubescens). *Molecules* **2022**, *28*, 23.
(18) Hartmann, A.; Rothballer, M.; Schmid, M. Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant Soil* **2008**, *312*, 7−14.
(19) Silva-Lacerda, G. R.; Santana, R. C. F.; Vicalvi-Costa, M. C. V.; Solidônio, E.; Sena, K.; Lima, G. M. S.; Araújo, J. Antimicrobial potential of Actinobacteria isolated from the rhizosphere of the Caatinga biome plant Caesalpinia pyramidalis Tul. *Genet. Mol. Res.* **2016**, *15*, 15017488.
(20) Álvarez-Pérez, J. M.; González-García, S.; Cobos, R.; Olego, M. A.; Ibañez, A.; Díez-Galán, A.; et al. Use of endophytic and rhizosphere Actinobacteria from grapevine plants to reduce nursery fungal graft infections that lead to young grapevine decline. *Appl. Environ. Microbiol.* **2017**, *83*, No. e01564.
(21) Shirling, E. B.; Gottlieb, D. Methods for characterization of Streptomyces species. *Int. J. Bacteriol* **1966**, *16*, 313.
(22) Takahashi, Y.; Omura, S. Isolation of new Actinomycete strains for the screening of new bioactive compounds. *J. Gen. Appl. Microbiol.* **2003**, *49*, 141.
(23) Thompson, J. D.; Higgins, D. G.; Gibson, T. J. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **1994**, *22*, 4673−4680.
(24) Enmozhi, S. K.; Ganesan, V.; Kaari, M.; Selvaraj, B.; Venugopal, G.; Joseph, J.; Manikkam, R. Anti-infective potential of marine Actinobacteria against carbapenem resistant Klebsiella pneumoniae ATCC 13882. *Res. J. Pharm. Technol.* **2020**, *13*, 3653−3660.
(25) Boudjella, H.; Bouti, K.; Zitouni, A.; Mathieu, F.; Lebrihi, A.; Sabaou, N. Taxonomy and chemical characterization of antibiotics of Streptosporangium Sg 10 isolated from a Saharan soil. *Microbiol. Res.* **2006**, *161*, 288.
(26) Felsenstein, J. Phylogenies and the comparative method. *Am. Nat.* **1985**, *125*, 1−15.
(27) Yilmaz, E. I.; Yavuz, M.; Kizil, M. Molecular characterization of rhizospheric soil Streptomycetes isolated from indigenous Turkish plants and their antimicrobial activity. *World J. Microbiol. Biotechnol.* **2008**, *24*, 1461−1470.
(28) Akinjogunla, O. J.; Eghafona, N. O.; Enabulele, I. O.; Mboto, C. I.; Ogbemudia, F. O. Antibacterial activityofEthanolicextracts of PhyllanthusAmarus against ex- tended spectrum β-Lactamase producing E. coli isolated from stool samples of HIV sero-positive patients with or with out diarrhea. *Afr. J. Pharm. Pharmacol.* **2010**, *4*, 402−407.
(29) Patel Rajesh, M.; Patel Natvar, J. In vitro antioxidant activity of coumarin compounds by DPPH, Super oxide and nitric oxide free radical scavenging methods. *J. Adv. Pharm. Educ. Res.* **2011**, *1*, 52−68.
(30) Bais, H. P.; Weir, T. L.; Perry, L. G.; Gilroy, S.; Vivanco, J. M. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* **2006**, *57*, 233−266.
(31) Xing, Y.; Shi, W.; Zhu, Y.; Wang, F.; Wu, H.; Ying, Y. Screening and activity assessing of phosphorus availability improving microorganisms associated with bamboo rhizosphere in subtropical China. *Environ. Microbiol.* **2021**, *23*, 6074.
(32) Nguyen, T. M.; Kim, J. Streptomyces bambusae sp. nov., showing antifungal and antibacterial activities, isolated from bamboo (Bambuseae) rhizosphere soil using a modified culture method. *Curr. Microbiol.* **2015**, *71*, 658.
(33) Seipke, R. F.; Kaltenpoth, M.; Hutchings, M. I. Streptomyces as symbionts: an emerging and widespread theme? *FEMS Microbiol. Rev.* **2012**, *36*, 862.
(34) Sasse, J.; Martinoia, E.; Northen, T. Feed your friends: do plant exudates shape the root microbiome? *Trends Plant Sci.* **2018**, *23*, 25−41.
(35) Yun, T. Y.; Feng, R. J.; Zhou, D. B.; Pan, Y. Y.; Chen, Y. F.; Wang, F.; Yin, L. Y.; Zhang, Y. D.; Xie, J. H. Optimization of fermentation conditions through response surface methodology for enhanced antibacterial metabolite production by Streptomyces sp. 1−14 from cassava rhizosphere. *PLoS One* **2018**, *13*, No. e0206497.
(36) Gonzalez-Franco, A. C.; Robles-Hernández, L. Antagonist activities and phylogenetic relationships of Actinomycetes isolated from an Artemisia habitat. *Rev. Argent. Microbiol.* **2022**, *54*, 326.
(37) Saddiq, A. A.; Tag, H. M.; Doleib, N. M.; Salman, A. S.; Hagagy, N. Antimicrobial, Antigenotoxicity, and Characterization of Calotropis procera and Its Rhizosphere-Inhabiting Actinobacteria: In Vitro and In Vivo Studies. *Molecules* **2022**, *27*, 3123.
(38) Sivarajan, A.; Shanmugasundaram, T.; Sangeetha, M.; Radhakrishnan, M.; Balagurunathan, R. Screening, production, and characterization of biologically active secondary metabolite (s) from marine Streptomyces sp. PA9 for antimicrobial, antioxidant, and mosquito larvicidal activity. *Indian J. Mar. Sci.* **2019**, *48*, 1319−1326.
(39) Almuhayawi, M. S.; Mohamed, M. S. M.; Abdel-Mawgoud, M.; Selim, S.; Al Jaouni, S. K.; AbdElgawad, H. Bioactive potential of several Actinobacteria isolated from microbiologically barely explored desert habitat, Saudi Arabia. *Biology* **2021**, *10*, 235.
(40) Pandey, A.; Shukla, A.; Majumdar, S. K. Utilization of carbon and nitrogen sources by Streptomyces kanamyceticus M 27 for the production of an Anti-bacterial antibiotic. *Afr. J. Biotechnol.* **2005**, *4* (9), 909−910.
(41) Poomthongdee, N.; Duangmal, K.; Pathom-aree, W. Acidophilic Actinomycetes from rhizosphere soil: diversity and properties beneficial to plants. *J. Antibiot.* **2015**, *68*, 106.
(42) Banda, G. C.; Monjerezi, M.; Sumani, J.; Mpeketula, P. M. G. Phytoconstituents and Antimycobacterial activities of root extracts and fractions from Vernonia glabra, (Steetz) Vatke. *J. Chem.* **2022**, *2022*, 7003809.
(43) Huang, L.; Zhu, X.; Zhou, S.; Cheng, Z.; Shi, K.; Zhang, C.; Shao, H. Phthalic acid esters: Natural sources and biological activities. *Toxins* **2021**, *13*, 495.
(44) Ganesan, T.; Subban, M.; Christopher Leslee, D. B.; Kuppannan, S. B.; Seedevi, P. Structural characterization of n-hexadecanoic acid from the leaves of Ipomoea eriocarpa and its antioxidant and antibacterial activities. *Biomass Convers. Biorefin.* **2022**, 1−12.
(45) Aparna, V.; Dileep, K. V.; Mandal, P. K.; Karthe, P.; Sadasivan, C.; Haridas, M. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. *Chem. Biol. Drug Des.* **2012**, *80*, 434.
(46) Sangkanu, S.; Rukachaisirikul, V.; Suriyachadkun, C.; Phongpaichit, S. Evaluation of antibacterial potential of mangrove sediment-derived actinomycetes. *Microb. Pathog.* **2017**, *112*, 303−312.
(47) Ricciardelli, A.; Casillo, A.; Corsaro, M. M.; Tutino, M. L.; Parrilli, E.; van der Mei, H. C. Pentadecanal and pentadecanoic acid

https://doi.org/10.1021/acsomega.3c04954
ACS Omega 2023, 8, 36333−36343

coatings reduce biofilm formation of Staphylococcus epidermidis on PDMS. *Microb. Pathog.* **2020**, *78*, ftaa012.
(48) Galdiero, E.; Ricciardelli, A.; D'Angelo, C.; de Alteriis, E.; Maione, A.; Albarano, L.; Casillo, A.; Corsaro, M.; Tutino, M.; Parrilli, E. Pentadecanoic acid against Candida albicans-Klebsiella pneumoniae biofilm: Towards the development of an anti-biofilm coating to prevent polymicrobial infections. *Res. Microbiol.* **2021**, *172*, 103880.
(49) Chen, X.; Zhao, X.; Deng, Y.; Bu, X.; Ye, H.; Guo, N. Antimicrobial potential of myristic acid against Listeria monocytogenes in milk. *J. Antibiot.* **2019**, *72*, 298−305.
(50) Okukawa, M.; Yoshizaki, Y.; Yano, S.; Nonomura, Y. The Selective Antibacterial Activity of the Mixed Systems Containing Myristic Acid against Staphylococci. *J. Oleo Sci.* **2021**, *70*, 1239−1246.

https://doi.org/10.1021/acsomega.3c04954
ACS Omega 2023, 8, 36333−36343